

COMPARATIVE STUDY OF INTESTINAL PARASITES OF *Heterobranchus longefilis* and *Clarias gariepinus* SOLD IN LOKOJA MARKET, LOKOJA, KOGI STATE.

Egbunu, A.A. and Abdullahi, M.D.

Kogi State Polytechnic, Lokoja, Nigeria

ABSTRACT

*Intestinal parasites cause a great economic problem to both humans and life stock worldwide. They have been ranked as neglected diseases. This study aims at determining the prevalence of these parasites in some of the commonly sold and consumed fish species in Lokoja Kogi State Nigeria. Twenty 20 each of the different species of *Heterobranchus longefilis* and *Clarias gariepinus* were purchased from the two popular markets, Kpata and International markets in Lokoja and transported to the laboratory of the Kogi State Polytechnic, Lokoja. They were weighed and their lengths measured. Fish sex was determined using method. Each was dissected and intestinal contents recovered using sodium chloride flotation technique. Result showed that in both *Heterobranchus sp* and *Clarias sp* the males had higher incidence of parasitic infections (83.3% and 70%) than the females (16.7% and 30%) respectively. However the male of *Heterobranchus sp* were more infected than the male of *Clarias sp*. Whereas the female *Clarias sp*. were more infected than the female *Heterobranchus sp*. The respective incidence of intestinal parasites in *Heterobranchus longefilis* and *Clarias gariepinus* were 71.1% and 62.5% *Diphylobothrium latum*, 18.4% and Nil *Capillaria philippinensis*, Nil and 15.6% *Ascaris lumbricoides* 10.5% and 15.6% *Bacephalus polymorphus*, and 2.6% and 6.3% *Pomphorhynchus laevis*. These findings call for thorough cleaning and cooking of these fishes before consumption and the encouragement of aquaculture to monitor the feeding and sanitary environment of the breeding space of fish sold to consumers. Also there is a need to be extra careful in cleaning and cooking *Heterobranchus longefilis*, avoiding eating raw fish especially the intestine to prevent zoonotic infections.*

Keywords:

*Intestinal parasites, prevalence, *Heterobranchus longefilis*, *Clarias gariepinus**

1.0 Introduction

Intestinal parasites are among the leading causes of global health problems especially among the poorest and deprived communities. Intestinal parasites cause a great economic problem to both humans and life stock worldwide(Ekanem et. al, 2011). They have been ranked as neglected diseases.

Intestinal parasites still remains a notable health problem in resource-limited countries due to difficulties in the implementation of control measures. They are considered among the neglected tropical diseases (NTD). They could also cause zoonotic diseases because parasites such as tapeworm which are present in fish can also infect people and other fish eating mammals. (Sadauki et.al., 2022).

2.0 Background of Study

Fishes act as intermediate hosts for many digeneans and cestodes and also as definitive hosts for many helminths. These parasites therefore compete with the fish host with the fish for the needed essential nutrients absorbed by the fishes. (Frantz, A. 2018)

Subsequently, intestinal parasites hamper the growth of fish leading to morbidity and mortality making susceptible to surrounding predators. (Azadikhah et al.,2014 and Omeji et al., 2015)

3.0 Methodology

Study Area

This study was carried out in Lokoja Kogi State, Nigeria. Lokoja is located on the confluence of River Niger and River Benue. It is the capital city of Kogi State and can be found between latitude 7° 45' 27.56" - 7° 51' 04.34" N and longitude 6°41' 55.64" - 6°45' 36.58" E of the equator with a total land coverage of about 63.82 sq. km. (Adeoye, 2012).

Lokoja lies about 7.8023 degrees North of the Equator and 6.7333degrees East of the Meridian. It is about 165km Southwest of Abuja, Federal Capital Territory of Nigeria as the crow flies, and 390km Northeast of Lagos by same measure. It is located on the confluence of River Niger and River Benue, with a total land coverage of about 63.82 sq. km

Sample Size

Twenty (20) each of the different species of *Heterobranchus longefilis* and *Clarias gariepinus* were purchased from the two popular markets in Lokoja (Kpata and International markets)

Collection Of Samples

Samples were put in a transported bucket with water, covered and transported to the laboratory. They were weighed and their lengths measured. Fish sex was determined using method of

Iman et al. (2010) Each fish was separately dissected on a clean dissecting board and intestinal content recovered and washed into a beaker containing Sodium Chloride (Normal Saline) and adult parasites were collected using the normal saline flotation technique.

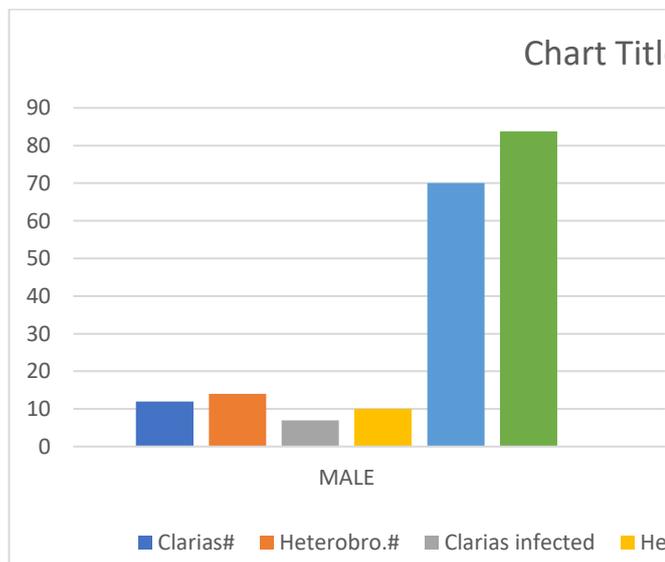
4.0 RESULTS

Table 1: Prevalence of intestinal parasites of (*Clarias gariepinus*) and *Heterobranchus longifilis* in relation to sex

Sex	No of <i>Clarias gariepinus</i> Examined	No of <i>Heterobranchus longifilis</i> Examined	No of Infected <i>Clarias gariepinus</i>	No of infected <i>Heterobranchus longifilis</i>	Percentage (%) <i>Clarias gariepinus</i> Infection	Percentage (%) <i>Heterobranchus longifilis</i> infected
Male	12	14	7	10	70	83.3
Female	8	6	3	2	30	16.7
Total	20	20	10	12	100	100

Fig 1. Prevalence of intestinal parasites of *Clarias gariepinus* and *Heterobranchus longifilis* in relation to sex

Table2: The prevalence of gastrointestinal helminth parasites identified in *Heterobranchus longifilis* and *Clarias gariepinus*



Species Identified Taxonomic Group	No of Parasite in <i>Heterobranchus longefilis</i>	Percentage (%)	No of Parasites in <i>Clarias gariepinus</i>	Percentage (%)
<i>Diphyllobothrium latum</i> (Cestode)	27	71.05	20	62.5
<i>Capillaria Philippiness</i> (Nematode)	7	18.42	NIL	NIL
<i>Ascaris lumbricoides</i> (Nematode)	NIL	NIL	5	15.6
<i>Bacephalus polymorphus</i> (Trematode)	4	10.53	5	15.6
<i>Pomphorhynchus laevis</i> (Acanthocephela)	1	2.63	2	6.25
Total	38	100	32	100

5.0 DISCUSSIONS

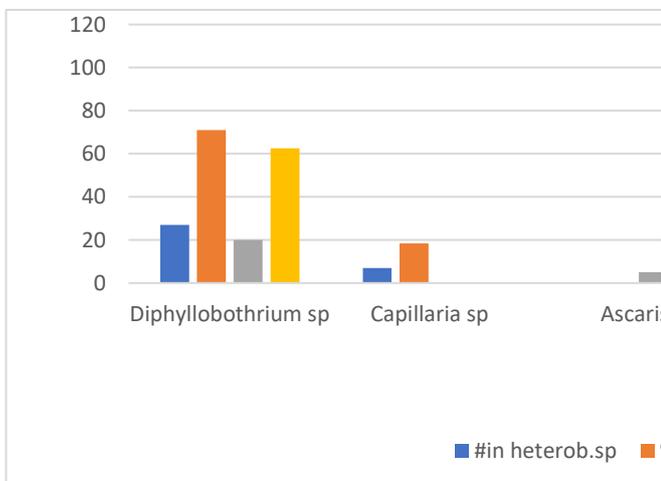


Fig 2. The prevalence of gastrointestinal helminth parasites identified in *Heterobranchus longefilis* and *Clarias gariepinus*

Result showed that in both *Heterobranchus* sp and *Clarias* sp the males had higher incidence of parasite infections than the females respectively. This agrees with the findings of Nnatuanya et. al., (2023) and Sadauki et.al.(2022) where the males had higher parasite intensity than the female These differences were however reported to be insignificant as observed in this study. However the male *Heterobranchus* sp were more infected than the male *Clarias* sp. Whereas the female *Clarias* was more infected than the female *Heterobranchus* sp.

Result showed the respective incidence of intestinal parasites in *Heterobranchus longefilis* and *Clarias gariepinus*. This study

shows a highest prevalence of cestodes in the two commonly eaten fishes prevalence of intestinal parasites in *Heterobranchus longefilis* and *Clarias gariepinus*. This agrees with the work of Abdel-Gaber et. al., (2015). Iyaji, et.al (2014), who observed that the higher infection of cestode parasites in *Clarias gariepinus* could be due to ingestion of eggs, copepods and molluscs which serve as intermediate hosts of the larva stages of cestodes.

6.0 CONCLUSION AND RECOMMENDATION

These findings call for thorough cleaning and cooking of these fishes before consumption and the encouragement of aquaculture to monitor the feeding and sanitary environment of the breeding space of fish sold to consumers. This will also recommended that the control of molluscs and copepods along the river banks be intensified to eradicate cestode and helminthic infections.

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FEATURE EXTRACTION TECHNIQUES FOR PALM-PRINT RECOGNITION SYSTEM

Oluwaseyi .I. oluwabukola

Oluwabukolabello@gmail.com

08068441825

Funso Bukola Omolara

ajengbeomolara@gmail.com

07038123222

Audu Lucy

08030900552

Kogi State Polytechnic, Nigeria,

Department of Computer Science, Lokoja, Nigeria.

Abstract

This paper presents a novel system for person identification, leveraging palm-print features extracted through Principal Component Analysis (PCA) and Local Binary Patterns (LBP). The approach treats the palm-print image as a texture image and introduces the use of the Fisher Palm method by considering the entire palm as the Region of Interest (ROI) for feature extraction. This distinctive methodology sets our research apart from prior studies, as it allows us to effectively address distortion issues from the pre-processing stage, leading to improved palm-print images. The developed system was evaluated using the PolyU Database, and the experimental results demonstrate that the combination of the two algorithms significantly increases accuracy. The efficacy of the system relies on the complementary nature of the employed algorithms. All algorithms were implemented in MATLAB and executed on a standard PC with an Intel CORE i3 processor, 4 GB of RAM, and Windows 7.

Keywords: Principal Component Analysis, Local Binary Pattern, Region of Interest, PolyU Database.

1. Introduction

The domain of biometric systems has emerged as an active area of research. This recognition approach involves several stages: image acquisition, preprocessing, feature extraction, and identification. Among various biometric traits, palmprint features have shown great potential for person recognition. Depending on the application field of palmprint systems, two types of features can be distinguished. The first type encompasses principal lines and wrinkles, which can be extracted from low-resolution images (less than 100 dpi). These features are commonly employed for identification purposes in commercial applications. On the other hand, the second type comprises singular points, ridges, and minutiae points, which are extracted from high-resolution images (greater than 100 dpi). These features find utility in forensic applications, including law enforcement (OLUWASEYI Inubiwon Olubukola, AREMU Dayo Reuben , AJIBOLA Folake, 2019). Palm print recognition is a biometric identification technique that utilizes the unique patterns and features present in an individual's palm for authentication and identification purposes. One

crucial step in palm print recognition is feature extraction, where distinctive characteristics of the palm are extracted and represented in a compact form. In recent years, Principal Component Analysis (PCA) and Local Binary Patterns (LBP) have emerged as effective feature extraction approaches for palmprint traits. This introduction will explore the application of PCA and LBP in palmprint recognition, highlighting their benefits and discussing relevant references.

PCA (Principal Component Analysis) is a dimensionality reduction technique widely used for feature extraction in various biometric modalities. In palmprint recognition, PCA can effectively capture the principal components of palm images, reducing their dimensionality while preserving the discriminative information. The extracted principal components, known as eigenpalm features, encode the most significant variations in the palmprint patterns. By representing palmprints in a lower-dimensional space, PCA facilitates efficient palmprint matching and classification.

LBP (Local Binary Patterns), on the other hand, is a texture descriptor that characterizes the local

structure and texture patterns in an image. In palmprint recognition, LBP operates by partitioning the palmprint image into smaller regions and extracting binary codes based on the comparison of pixel intensities within each region. The resulting LBP histogram represents the distribution of texture patterns across the palmprint image. LBP-based features capture fine-grained details and texture variations, which are valuable for distinguishing between different individuals.

The combination of PCA and LBP in palm print recognition has shown promising results in terms of accuracy and robustness. By applying PCA prior to LBP, the dimensionality of the palm print data can be reduced, making the LBP computation more efficient. Moreover, the combination of global palm print features (PCA) and local texture features (LBP) provides a comprehensive representation of the palm print traits, capturing both the overall structure and finer texture details.

The problem addressed in this study is the need for effective feature extraction approaches for palm print recognition. Palm print recognition is

a biometric identification technique that relies on extracting distinctive features from palm images for authentication and identification purposes. However, existing feature extraction methods face challenges in capturing the discriminative information from palm prints accurately, leading to decreased recognition performance. Therefore, there is a demand for novel feature extraction approaches that can enhance the representation of palmprint traits and improve the accuracy and robustness of palmprint recognition systems.

The aim of this study is to conduct an evaluation on Feature Extraction Techniques For Palm-Print Recognition System through individual and fusion of feature extraction techniques. The objectives are to:

- (i) Design feature extraction models using Principal Component Analysis (PCA), and Local Binary Pattern (LBP) techniques;
- (ii) classify the individual extracted features and the fused features using Weighted Majority Voting;
- (iii) implement each of the designed Technique using matlab; and

(iv) Evaluate the implemented feature extraction techniques in terms of False Acceptance Rate (FAR), False Rejected Rate (FRR), Equal Error Rate (ERR) and Recognition Rate (Accuracy).

The combination of Principal Component Analysis (PCA) and Local Binary Patterns (LBP) using a majority weighted voting method can be a powerful technique for feature extraction from palm print traits. Here's the significance of this approach:

Dimensionality Reduction: PCA is a widely used technique for dimensionality reduction. It projects the high-dimensional feature space into a lower-dimensional subspace while preserving the most important information. By applying PCA to palm print traits, the method can reduce the feature space and remove redundant or irrelevant information, leading to more efficient and effective feature extraction.

Texture Analysis: LBP is a texture descriptor that characterizes the local patterns in an image. It computes binary codes by comparing pixel intensities in a local neighbourhood. LBP is particularly effective in capturing texture

variations and patterns in palm print traits. By combining LBP with PCA, the method can leverage the texture analysis capability of LBP to capture fine-grained details in the palm print.

Complementary Information: PCA and LBP capture different aspects of the palm print traits. PCA focuses on the overall variance and structure of the data, while LBP emphasizes local texture patterns. By combining these two methods, the resulting feature set can provide complementary information, enhancing the discriminative power of the feature extraction process.

Majority Weighted Voting: The majority weighted voting method combines the features extracted from PCA and LBP using a voting scheme. Each feature contributes to the final classification decision based on its individual strength. This approach allows for an ensemble-like combination of features, reducing the risk of relying too heavily on a single feature extraction method and enhancing the robustness of the feature representation.

Yan, H., Huang, Z., Wang, W., & Lei, Z. (2019). Improved Recognition Performance: The combined PCA-LBP with majority weighted voting approach can lead to improved recognition performance in

palm print trait analysis. By leveraging the strengths of both PCA and LBP, it can capture important spatial and textural information from the palm print, leading to more accurate and reliable palm print recognition and authentication systems. In summary, the significance of combining PCA and LBP using the majority weighted voting method lies in their complementary nature, which enhances the feature extraction process for palm print traits, leading to improved recognition performance and robustness.

2. Literature Review

Review of a Related Works

(Ali, S. H., El-Khamy, S. E., & Lee, J. 2022)

This study emphasizes the limitations of traditional feature extraction methods for palmprint recognition, including difficulties in capturing complex palmprint patterns and handling variations in palm images. It introduces a deep learning-based approach, PalmNet, which combines feature extraction and classification networks to address these challenges. The citation provides evidence that the existing feature extraction approaches in

palmprint recognition need improvement to enhance recognition accuracy and handle variations in palmprint patterns effectively.

(Manisha.mAmbekar;S.N.Kakawal;S.S

Khedgikar, 2020) proposed Design, Acquisition and Collection of Various Multimodal Databases to Increase Accuracy and Level of Security. The researchers review that Biometric identification and verification technique is an automated sample detection and recognition system that make use of physiological or behavioral characteristics of individuals. Physical biometric traits which include face, fingerprints, palm prints, ear specimens, eye patterns such as iris and retina. Behavioral biometric traits include gait(behaviour), signature, typing speed/patterns, voice patterns etc. Unimodal biometric systems developed for each of these biometric features are not sufficient for gaining accuracy and security. To overcome this problem, multimodal biometrics are developed that apply fusion strategy of multiple biometric modalities or features from two or more than two sources. When we compare Uni-modal biometric systems with multimodal biometric systems then

multimodal systems improves recognition accuracy, security issues and system reliability problems. Various unimodal biometric databases are freely available But researchers are facing problems that there are very few multimodal databases are available developing multi modal biometric. This paper presents, collection of various biometric modalities like face, fingerprint, palm print, ear patterns which are obtained in controlled environment using different sensors to develop multimodal biometric system.

(Mustafa S. Kadhmi, Hayder Ayad, Mamoun Jassim Mohammed, 2021) proposed Palmprint Recognition System Based On Proposed Features Extraction And (C5.0) Decision Tree, Knearest Neighbour (Knn) Classification Approaches. In the biometrics research area, palmprint recognition system is gaining the most popularity. Significant performances have been achieved in the state-of-the-art palmprint recognition systems. However, only particular scenarios such as a features types and extraction methods are considered in the existing systems. However, these systems cannot meet complex application requirements due to the security

challenges it terms of accuracy and reliability. Therefore, in this paper an accurate and reliable Palmprint Recognition System called (PRS) is proposed. The system used proposed features extraction and classification approaches using direction, Local Binary Pattern (LBP) features, C5.0 and KNearest Neighbour (KNN). The system used two palmprint images datasets which are College of Engineering Pune (COEP), Chinese Academy of Sciences (CASIA) and achieved very high recognition rate 99.7% with lower error matching rate 0.009%.

Feature Extraction Algorithm

Feature extractions are very important approach in biometric identification and verification. Once the vital part is segmented, features can be extracted for matching. A feature extraction starts from an initial set of measured data and builds derived values (features) intended to be informative and non-redundant, facilitating the subsequent learning and generalization steps, and in some cases leading to better human interpretations (Kumar & Bhatia, 2014). Feature extraction is related to dimensionality reduction. Feature extraction is to define a palm print in a

wavelet feature set other than the original image. A feature with good discriminating ability should show a great variance between persons and small variance between samples from the same person (Nalamothu & Kalluri, 2012).

Among the many features that are obtainable on a palm, some of the key features are defined as palm geometric features (Krishneswari & Arumugam, 2010): This feature includes width, length and area of palm's shape. Principle line features: This feature includes important lines such as the line of head, life and heart in a palm print (Esther Rani & Shanmugalakshmi, 2014). They are mostly having features like stability and uniqueness thus are an important physiological characteristic to identify individuals' identification. Palm texture feature: This feature includes wrinkles of palm, they are very lighter and more zigzag thus are difficult to separate uniquely compare to the principle lines and are also less important (Yuvaraj, 2018). Data point features: This feature includes delta-like central region of a palm print, which is usually situated in the bottom area of finger and in outer region of the palm print. The stage is responsible for

the extraction of required lines and ridges, which are needed for classification. Extracted features play a significant role in the classification step (Kaur & Rajput, 2013). They are several feature extraction techniques: subspace-based approaches and structure-based approaches.

Subspace-Based Techniques

Subspace based approaches are proposed to regard a palm print image as a high-dimensional vector and map to a lower-dimensional vector (Kour et al., 2013). Different distance measures and classifier are used to contrast the features. They used principal Component analysis (PCA), linear discriminant analysis (LDA), and independent component analysis (ICA). The main thought of principal component analysis (PCA) is to reduce the dimensionality of a data set consisting of large number of interrelated variables, while keeping maximum variation present in the data set. In vector space, PCA identifies the major directions by computing the eigenvectors and eigenvalues of the covariance matrix of the data.

(i) Principal Component Analysis (PCA)

It is a linear technique that is referred to as Karhunen-Loeve Transformation (KLT) that optimally reduces redundancy (Singh, Sharma & Rao, 2011; Karamizadeh, Abdullah, Manaf, Zamani, & Hooman, 2013). PCA is employed for feature extraction and data representation in computer vision and pattern recognition such as palm print, face recognition and fingerprint (Yang, Zhang, Frang & Yang, 2004.; Bahurupi & Chaudhari, 2012). It searches for a set of representative projection feature vectors such as that the projected samples retain most information about original samples (M. Bala, Singh, & Meena, 2016). The PCA method applies a vector space transform to reduce the dimensionality of large database.

PCA Analysis Applications

1. PCA is used in machine learning to view multidimensional data.
2. Explore the factors in healthcare data that are thought to be extremely important in increasing the risk of any chronic condition.
3. PCA aids in image resizing.

4. PCA is employed in the analysis of stock and forecasting data.

5. When dealing with high-dimensional data sets, you can also utilize Principal Component Analysis to analyze patterns.

Advantages of Principal Component Analysis

1. Easy to compute and calculate.
2. Increases the speed of machine learning computer processes and algorithms.
3. Prevents data over fitting difficulties in predictive algorithms.
4. Increases performance of ML algorithms by eliminating unnecessary correlated variables.
5. Principal Component Analysis produces large variance while improving visualization.
6. Aids in the reduction of noise that cannot be disregarded automatically.

(ii) Linear Discriminant Analysis (LDA)

It is a powerful technique for data reduction and feature extraction used for the development of biometric systems (Malik & Bansal, 2016). The technique produces a robust representation that linearly transforms the original data space into a low-dimensional feature with focus on most

discriminant features (perform dimensionality reduction while preserving as much of class discriminatory information as possible) (Bhele & Mankar, 2012). This method gives significance to those feature vectors in underlying space that best describe the best discriminate among classes rather than best describing data. LDA projects an image data from high dimensional image space to a low-dimensional image by computing transformation that maximizes the between class scatter while minimizing the within class scatter.

(iii) Kernel Principal Component Analysis (KPCA)

The KPCA obtains features of the data non linearly. It selects the principal components in which is a high dimensional feature space that is related to the feature spaces non linearly (Zheng, Zou, & Zhao, 2005). The main idea of KPCA is to map the input data to the feature space F first using a non linear mapping Φ . when input data have non linearly been mapped, the Principle Component Analysis (PCA) will be performed on the mapped data (Hajian & Damavandinejadmonfared, 2014). The PCA only allows linear dimensionality reduction.

However, if the data has more complicated structures which cannot be properly represented in a linear subspace, the standard PCA will not be very useful (Kusban & Yogyakarta, 2016). Fortunately, kernel PCA allows us the generalization of standard PCA to non-linear dimensionality reduction. The kernel PCA is an unsupervised technique that is based on the simple idea of performing Principal Component Analysis in the feature space of a kernel (Ekinici & Aykut, 2008). Principal Component Analysis is a method of transforming a number of correlated variables into a smaller number of uncorrelated variables (Ekinici & Aykut, 2008b). PCA can be applied to palm print recognition by converting the pixels of an image into a number of eigen-palm feature vectors, which can then be compared to measure the similarity of two palm image (Dhanotiya & shrivastava, 2016). The PCA based features significantly minimize the noise, minimizes the memory usage and computational time of authentication. The main merit of PCA is that the Eigen palm approach helps in reducing the size of database required for testing images for its recognition (Koul & Alaria, 2018). The trained palm images are not stored

directly as raw images rather they are stored as their weights.

(iv) Independence Component Analysis (ICA)

It is a computational method for revealing hidden factors that underlie sets of random variables, signals and measurements (Dangi & Ahmad, 2009). It is a generalization of PCA, which attempts to find much relevant information contained in high-order relationship among the image pixels (reduces the second order and higher order dependencies in input samples) (Alyasseri, 2015). The technique has been successfully applied to several problems such as locating hidden features in financial data and face recognition (Deniz, Castrillon & Hernandez, 2003).

5.2.2 Structured-Based Techniques

The approach is accomplished by the manipulation of distance, angles, and the area measurements of the visual features derived from an image (Sharif, Mohsin & Javed, 2012). The method uses some simple measures to describe features of palm print image and their various relationships.

Images are detected by the application of these measures to locate features, these relationships which exist between features can be represented by their relative distances and positions. These techniques include:

(i) Local Binary Pattern (LBP)

This technique remains the simplest and powerful method used in the extraction texture feature as well as the shape gray image (Tembhare & Dongre, 2017). It tags the pixels representation by using threshold of the pixels of the local neighbour around each pixel and considers the result as binary numbers (Raghavendra & Busch, 2015). The operator assigns a label to every pixel in an image by thresholding the eight neighborhood pixels by the gray value of its centre. The feature vector is formed by concatenating the threshold binary bits in the anti-clockwise direction as shown in

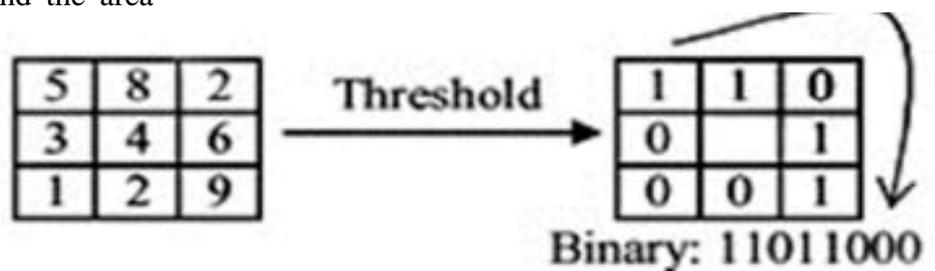


Figure 17.

Figure 17: Original LBP Operator (Ali, Yannawar, & Gaikwad, 2017)

The LBP code is invariant to any monotonic transformation on the pixel values (Doost & Amirani, 2013). Therefore, it is very suitable for representing palms which are illuminated from a fixed distance but with varying light intensity (Shende, Tete, Chandravanshi, & Surjuse, 2018). An extension to LBP code can be made using a subset of LBP string. An LBP string is called uniform if it consists of at most 2 bit-wise transitions from 0 to 1 or vice-versa. For example, 11111110 and 11101111 are uniform LBP strings whereas 11101011 and 10110101 are non-uniform. LBP technique is extremely compelling for surface descriptor.

Advantages of LBP

The Local Binary Pattern has been widely employed to extract texture information from biometric images (Anitha & Radhakrishna Rao,

2016) ; it owns many advantages, such as it is simple to implement and fast to compute (Tembhare & Dongre, 2017a). LBP is a gray-scale texture operator that characterizes the local spatial structure of the image texture (Gupta, Namdeo, & Chourey, 2017). Given the central pixel in image, a pattern code is computed by associating it with its neighbours.

(ii) Local Binary Pattern Histogram

Local Binary Pattern (LBP) and Local Binary Pattern Histogram (LBPH) are texture descriptors used in computer vision and image processing. To compute LBP for a given pixel, you need to compare the pixel value with its neighbours and generate a binary code based on the comparison. LBPH is an extension of LBP that computes a histogram of LBP values over a region of interest.

(iii) Modified Finite Radon Transform (MFRT)

The radon transform is the integral transform which takes a function f defined on the plane to a function Rf defined on the two-dimensional space of lines in the plane (Rajagopal & Manoharan, 2015), whose value at a particular

line is equal to the line integral of the function over that line. The radon transform can be used for line detection (Al-seddek et al., 2014). Otherwise, it is the projection of an image along particular direction. The radon transform is widely applicable to tomography, the creation of an image from the projection data associated with cross-sectional scans of an object (Esther & Shanmugalakshmi, 2013). The radon transform is the integral transform which contains the integral of a function over straight lines (Rajagopal & Manoharan, 2015; SaiedFazii, 2016). This technique computes the line integral along parallel paths in a certain direction (Huang, Jia, & Zhang, 2008; Sawant, Talib, Jondhale, & Patil, 2013). It is the projection of an image along particular direction, which can be used for line detection (Al-seddek, Soliman, Morsy, & Kishk, 2014).

Statistical Techniques

The approach can be further divided into local and global statistical approaches (Sharma & Vasudeva, 2017). Local statistical approach converts images into another domain and then splits the converted images into small sections.

Local statistics such as means and variances of each small region are computed and referred to as features. The small regions are commonly square but some of them are elliptical and circular. According to the collected papers, so far, no one investigates high order statistics for this approach. In addition to local statistics, researchers also use global statistics, which are computed from whole transformed images. Moments, centres of gravity and density are considered as the global statistical features.

Line-Based Techniques

A human palm has mainly three principal lines; life line, head line and heart line. Palm lines are obvious features in palm prints. This method builds edge detectors and applies the magnitude of the palm line. The magnitudes of the palm lines are projected in x and y coordinates forming histograms (Mane & Kalyankar, 2016). After this, the first and second order derivatives of the palm images are calculated. The first order derivative is used in the identification of the edge points and corresponding directions. The second order derivative is employed to find the magnitude of lines.

3. Methodology

In this section, we describe the methodology used to evaluate the performance of four different feature extraction techniques. To conduct these studies, we utilized the palm print dataset available in the PolyU database (R). The framework for our methodology, along with our objectives, is summarized in Figure 1.

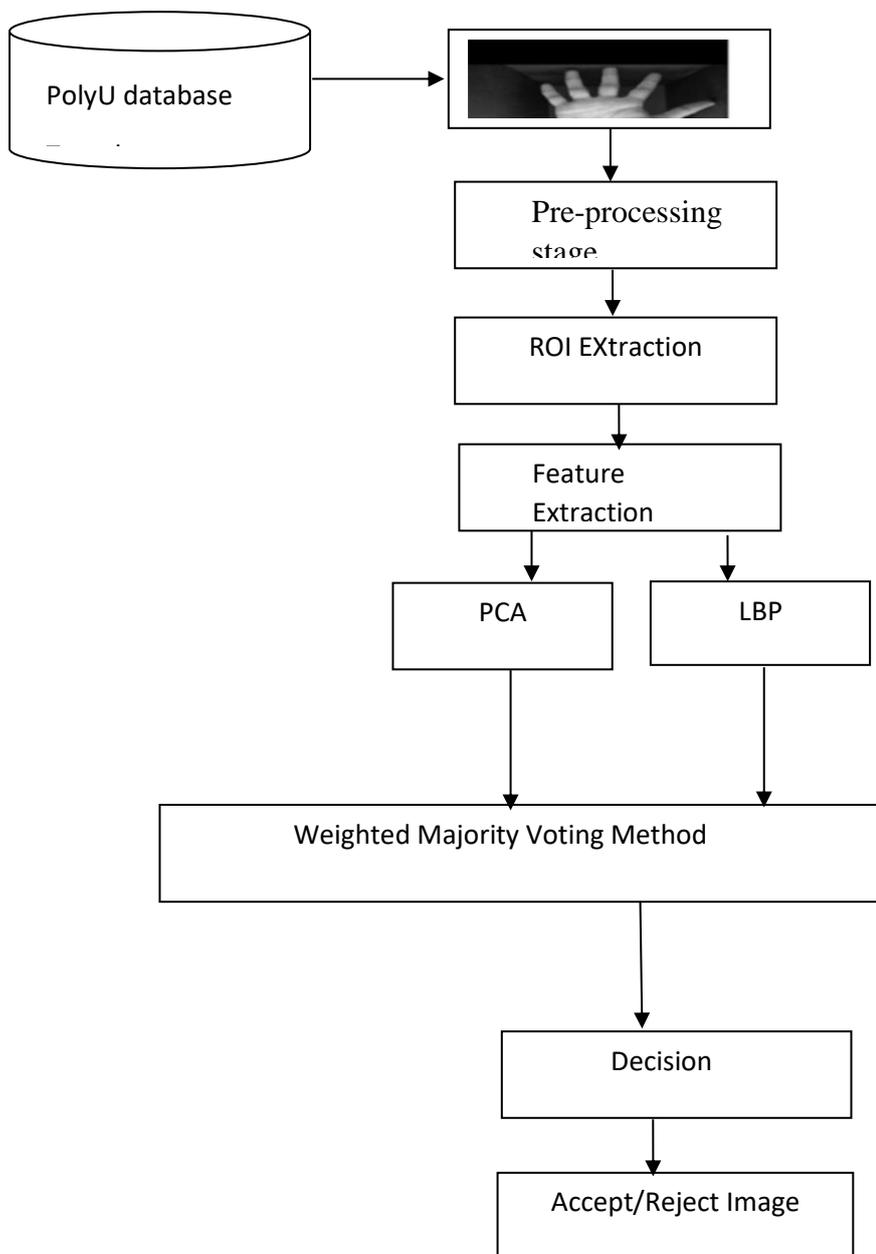


FIGURE 1: A BLOCK DIAGRAM OF DEVELOPED PALM PRINT RECOGNITION SYSTEM.

Acquisition of Palm print Dataset

The palmprint dataset used in this study was obtained from the PolyU Palm Print Database available at <http://www4.comp.polyu.edu.hk/~>. The database is available for research purposes and the sample of PolyU palmprint database is shown in Figure 2.

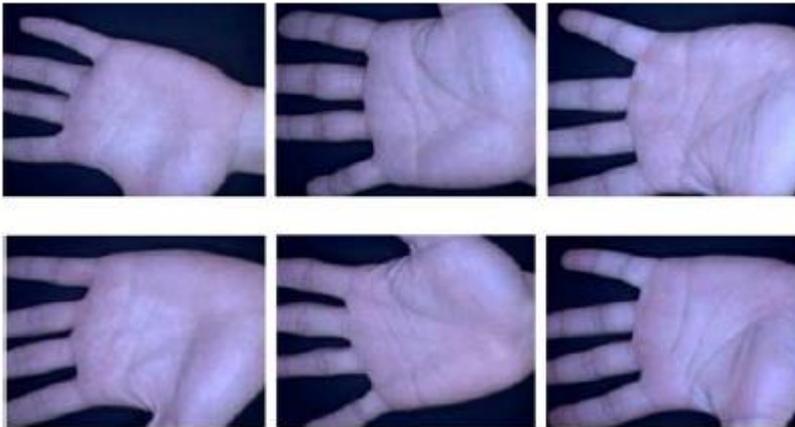


Figure 2: Sample of PolyU Palm print Database

Pre-processing Phase

The first step in the preprocessing of the palm print images was to download and save the PolyU palm print database onto the hard disk of the computer system. The images were then subjected to various preprocessing techniques to eliminate distortion, align the palm prints, and crop the region of interest (ROI). The extracted ROI was subsequently used for feature extraction. The following steps were employed to

achieve the preprocessing of the palm print images:

- a. Binarization of the palm image
- b. Hadamard multiplication
- c. Fisher method
- d. Bilateral interpolation
- e. Extraction of features from the palm trait (ROI)

Step 1: Binarization of a palm print images.

A total of 163 palm print images were downloaded, each consisting of 8 images captured for each palm. The images were stored in the computer memory as Gray level, with a maximum of 256 different Gray values ranging from 0 to 255. Each Gray value corresponds to a different color on the Gray scale palette. In cases where information needs to be extracted from the document image, the action must be processed

multiple times, leading to longer processing times. To overcome this, binary images are more efficient. Threshold techniques, also known as

Otsu binarization, are used to convert Gray scale images to binary images with pixel values of $\{0,1\}$.

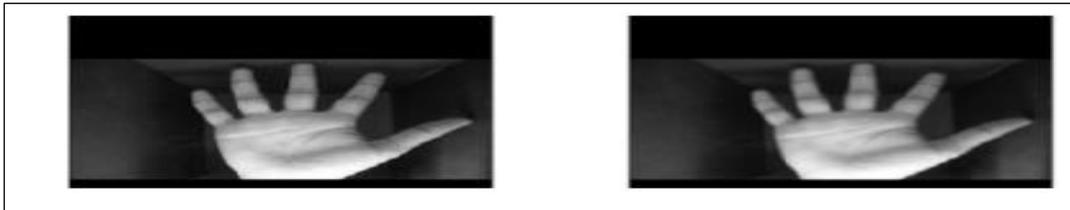


Figure 3: (a) Original sample

(b) Gray image

Original Image is blurred and mask is extracted using Otsu Binarization.(Right palmprint)

In Figure 22, the gray image is converted into a binary image, and then the Hadamard product is applied to the corresponding entries. This involves multiplying the pixel at position (i,j) in

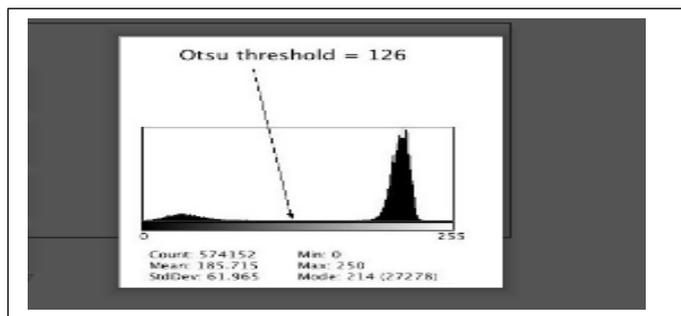
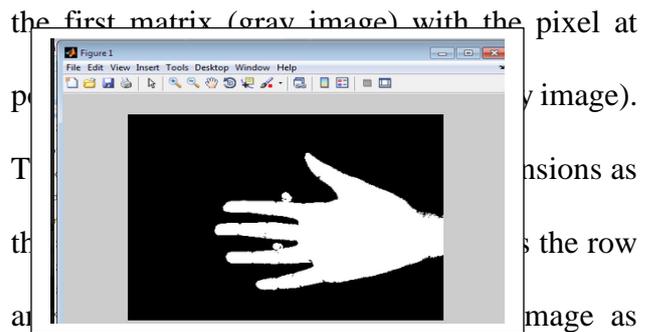


Figure 4: (a)Threshold value(T)

(b) Binary image

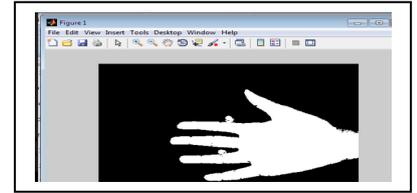


shown in Figure 5

Step2: Hadamard Multiplication.



Figure 5: Gray Image (g)



(b) Binary Image (f_i) g (Gray Pixel Value) * f_i
(Binary Pixel Value)

The multiplication process involves the gray pixel image and the binary pixel image, where the background of the binary image has a value of zero, and the white foreground has a value greater than zero. The multiplication is done for each corresponding pixel in both images, using the row and column indices of the image. The resulting matrix has the same dimensions as the initial matrices.

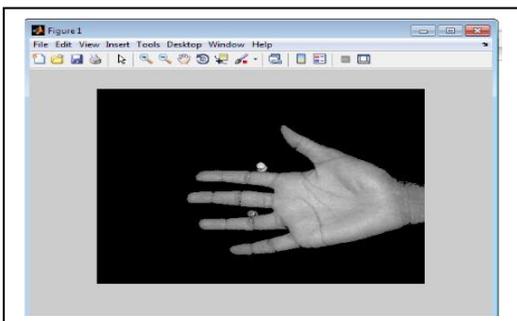


Figure 6: Palm Image after applying Hadamard method

Step3: Fisher palm method.

To process the image shown in Figure 24, we utilized the Fisher palm method and followed the subsequent algorithms/procedures:

a. Column by Column Transversal

Let r = Number of row

Let n_c = Number_of_Zeros in columns

$$if\ n_c = > \frac{r}{3}$$

Remove the column

End if

b. Row by Row Transversal

Let c = Number of column

Let n_r = Number_of_Zeros in the rows

$$if\ n_c = > \frac{c}{3}$$

Remove the row

End if

In order to prevent distortion, we employed the following method: first, we scanned through each column and removed any column that contains one third number of zeros as the row. Next, we scanned through each row (or width) and removed any row that contains one-third same number of zeros as the column. The resulting output can be seen in Figure 7.

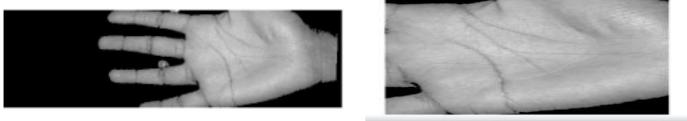


Figure 7: (a) After sweep row

(b) After sweep column

Step4: Bilinear interpolation.

Figure 25 of the palm print image was subjected to bilinear interpolation to resize it to 512 x 512 pixels. Increasing the size of the image results in higher resolution and brightness.

Step 5:Extraction of ROI: The resized image in Figure 7(b) was used to extract the Region of Interest (ROI). The resulting output is shown in

Figure 8(a), with the pixel corresponding to the palm print image highlighted in Figure 8(b). For this, the entire palm was considered as the ROI.

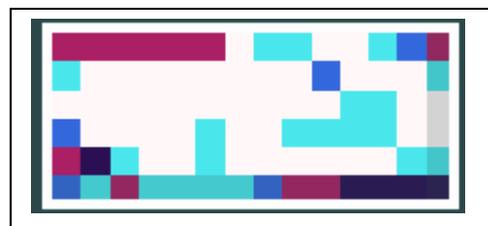


Figure 8: (a) Region of Interest (ROI)

(b) Pixel of ROI

Design of Feature extraction techniques

This section elaborated on the development of the two feature extraction techniques, namely:

A. PCA,

B. LBP

From Table 2 Matrices equation is given below

$$S = \begin{bmatrix} Px_1(1) & Px_1(2)\cdots & Px_1(N) \\ Px_2(1) & Px_2(2)\cdots & Px_2(N) \\ \vdots & \vdots & \vdots \\ Px_{17}(1) & Px_{17}(2)\cdots & Px_{17}(N) \end{bmatrix}$$

... (1)

Step 2: Standardized the Dataset

First, to standardize the dataset so we need to calculate the mean and standard deviation for each Pixel features.

a. Compute the mean and Standard Derivation of every dimension of the whole dataset.

$$\bar{X}_i = \sum_{i=0}^{40} \frac{x_i}{n_j}$$

... (2)

Where x_i is a dimensional vector.

Consider palm print dataset x_i where

$i = 1, 2, \dots, N$ along the row

N is the total number of the image matrix along the row is 40

n is the total number of the image matrix along the column is 17

$$\sigma^2_{ij} = \frac{(X_{ij} - \bar{X}_{i,j})^2 + \dots + (X_{ij} - \bar{X}_{i,j})^2}{N}$$

... (3)

Where x is the palm print feature and N is the number of person in the database

For each person N their palm feature x_i in the training set shown in Table 2 its

mean and Standard deviation is shown in Equation (3)

To find the mean and standard deviation mathematically, we need to compute them for each pixel position separately.

Let's take the first pixel position, which is the first digit of each number in the matrix. We have:

$$\begin{aligned} \text{Mean:} & (4 + 4 + 4 + 5 + 5 + 5 + 5 + 5 + 5 + 5 + 5 + 4 + 4 + 4 + 4 + 4 + 6) / 17 \\ & = 74 / 17 \\ & = 4.35294117647 \text{ (rounded to 11 decimal places)} \\ & = 4.353 \end{aligned}$$

$$\begin{aligned} \text{Standard deviation:} & \sqrt{(4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (5-4.353)^2 + (5-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (4-4.353)^2 + (6-4.353)^2) / 16 \\ & = \sqrt{0.111764 + 0.111764 + 0.111764 + 0.146764 + 0.111764 + 0.111764 + 0.111764 + 0.111764 + 0.111764 + 0.111764 + 0.397837 + 0.397837 + 0.111764 + 0.111764 + 0.111764 + 0.111764 + 0.111764 + 0.201961} / 16 \end{aligned}$$

Compute the covariance matrix of the standardized data. This matrix shows the relationships between the variables and how they vary together. The formula to calculate the

$$\begin{aligned} & = \sqrt{2.599968} / 16 \\ & = 0.322944 \text{ (rounded to 6 decimal places)} \\ & = 0.323 \end{aligned}$$

We can repeat this process for each pixel position to get the mean and standard deviation of the entire matrix. To standardize a dataset, we need to subtract the mean and divide by the standard deviation for each feature (column). Here's the standardized version of the dataset given above:

Standardization formula .

$$X_{new} = \frac{X_i - \bar{X}}{\sigma} \dots (10)$$

Where X_i is a dimensional vector along the column and row.

\bar{X} is a Mean while σ is the Standard Deviation

Step 3: Calculate the covariance matrix for the whole dataset

$$\begin{matrix} \text{covariance matrix:} & \text{COV}(X_1, X_1) \\ & \text{COV}(X_1, X_2) \dots \dots \dots \text{COV}(X_1, X_{40}) \\ & \text{COV}(X_2, X_1) & \text{COV}(X_2, X_2) \\ & \vdots & \dots \dots \dots \vdots & \dots \dots \dots \vdots \\ & & & & \text{COV}(X_{40}, X_1) & \text{COV}(X_{40}, X_2) & \dots \dots \dots \text{COV}(X_{40}, X_{40}) \\ & & & & \vdots & \vdots & \dots \dots \dots \vdots \end{matrix}$$

$$\begin{matrix} COV(X_{17},X_1) & COV(X_{17},X_2) \\ \dots\dots\dots & COV(X_{17},X_{40}) \end{matrix}$$

The property of standardized Normal distribution for mean is Zero and variance is equal to 1.

Since the dataset is Standardized the mean (\bar{X}) is 0 and the standard deviation is 1.

$$COV(X_1, X_1) = \frac{\sum (X_1 - \bar{X})^2}{N} \dots (4)$$

$$COV(X_1, X_2) = \frac{\sum (X_1 - \bar{X})(X_2 - \bar{X})}{N} \dots (5)$$

$$S = \begin{pmatrix} COV (X_1, X_1) & \dots & COV (X_1, X_{40}) \\ \vdots & \dots & \vdots \\ COV (X_{17}, P_1) & \vdots & COV (X_{17}, X_{40}) \end{pmatrix} \dots (6)$$

Step 4: Compute Eigenvectors and corresponding Eigenvalues

An eigenvector is a vector whose direction remains unaltered when subjected to a linear transformation. Now that we have the covariance matrix, we can simply compute eigenvalues and eigenvectors. Let S be a square

Eigenvector of λ_1

$$(S - \lambda I)V = 0$$

matrix, v a vector and λ a scalar that satisfies $Sv = \lambda v$, then λ is called eigenvalue associated with eigenvector v of S.

$$\text{Eigenvalue characteristics} = (S - \lambda I) \dots (7)$$

where λ is a constant while I is $\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$

$$\dots (8)$$

while Eigenvector is $(S - \lambda I)V$

Step 5: Eigenvalue, Eigen Vector. Normalized eigenvector.

$$\text{Eigen value formular is } \det(S - \lambda I) = 0 \dots (9)$$

$$\begin{pmatrix} COV (X_1, X_1) & \dots & COV (X_1, X_{40}) \\ \vdots & \dots & \vdots \\ COV (X_{17}, P_1) & \vdots & COV (X_{17}, X_{40}) \end{pmatrix} - \lambda \begin{pmatrix} 1 & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \dots & 1 \end{pmatrix} \dots (11)$$

Use equation (17) to find λ which is the first principal component

if $g_i \geq g_c = 1$

else

$g_i < g_c = 0$

Step 6: All the results are combined to get 8 bits value.

Binary Number generated which is 0011111.

Converting to decimal number is

$$0 * 2^7 + 0 * 2^6 + 1 * 2^5 + 1 * 2^4 + 1 * 2^3 + 0 * 2^2 + 0 * 2^1 + 0 * 2^0$$

=56(LBP code generated).It indicates that

all these pixels around the central value

equal to 56

Step 7: The decimal value of the binary is the LBP feature

633(LBP code generated)

Step 8: End

The resulting LBP matrix will have the same dimensions as the original pixel matrix:

6.3.4 Threshold Array Algorithm

Accuracy is a crucial factor in authentication systems and is determined by the reference threshold selected. The reference threshold is a value that distinguishes between genuine and impostor persons. To set the reference threshold,

the threshold array is used. The threshold array is obtained by extracting features using PCA, LBPI, LBP, and RT techniques and represented as q_i . In the training sample G1, each person's image features are grouped into seven samples, and 162 persons' feature extractions are used. In contrast, in the authentication sample G2, one sample per individual is used. M represents the number of samples per person, and N represents the total number of persons.

In G1, each hand feature vector in P1 is compared to all other seven hands feature vectors using the k-nearest neighbour method. The matching values are stored in the threshold array. Thus, for all 162 hand image samples,

In the first step, we group the 163 individuals into two groups: G1, which has 162 individuals with seven hand image samples each, and G2, which has one hand image sample for each individual.

For each of the Algorithm q_i

Let G1 = Training sample

G2 = Authentication sample

G1,7 samples per person(7*6)*162

$$\begin{aligned}
 P_1 &= (I_1, I_2, I_3, \dots, I_{M-1}) \\
 P_2 &= (I_1, I_2, I_3, \dots, I_{M-1}) \\
 P_3 &= (I_1, I_2, I_3, \dots, I_{M-1}) \\
 &\vdots \\
 P_N &= (I_1, I_2, I_3, \dots, I_{M-1}) \\
 \dots &\text{ (16) }
 \end{aligned}$$

group G2, 1 sample per each individual

$$\begin{aligned}
 P_1 &= [I_M], P_2 = [I_M], \dots, P_N = [I_M] \\
 \dots &\text{ (17) }
 \end{aligned}$$

M = Number of samples/person

N = Total number of person.

Next, in group G1, each hand feature vector in P1 is compared with all other seven hands feature vectors using the k-nearest neighbour method, and the matching values are stored in a threshold array. For all 162 hand image samples, a total of 42 x 162 matching values are stored in the threshold array TA.

$$\begin{aligned}
 TA(q) &= TA_1(q) + TA_2(q) + \dots + TA_{162}(q) \\
 \dots &\text{ (18) }
 \end{aligned}$$

The minimum and maximum matching values for each individual are found from the threshold arrays (TA_i) = (TA₁, TA₂, TA_N) and stored in the database as the threshold array

In step 4, we generate the threshold array TA_i for each individual i in group G1 using the k-nearest

neighbour method as the matching score. The total number of threshold values among P1 is 42 for 7 users in group P1, and the matching among person P1 in group G1 is tabulated in Table 3. From the table, we find the minimum and maximum thresholds of the individual of the training sample.

Table 3: MATCHING IN GROUP (G1) AMONG PERSON P₁

No of Sample	I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇
I ₁	X	I ₁ , I ₂	I ₁ , I ₃	I ₁ , I ₄	I ₁ , I ₅	I ₁ , I ₆	I ₁ , I ₇
I ₂	I ₂ , I ₁	X	I ₂ , I ₃	I ₂ , I ₄	I ₂ , I ₅	I ₂ , I ₆	I ₂ , I ₇
I ₃	I ₃ , I ₁	I ₃ , I ₂	X	I ₃ , I ₄	I ₃ , I ₅	I ₃ , I ₆	I ₃ , I ₇
I ₄	⋮	⋮	⋮	X	⋮	⋮	⋮
I ₅	⋮	⋮	⋮	⋮	X	⋮	⋮
I ₆	⋮	⋮	⋮	⋮	⋮	X	⋮
I ₇	I ₇ , I ₁	I ₇ , I ₂	I ₇ , I ₃	I ₇ , I ₄	I ₇ , I ₅	I ₇ , I ₆	X

The total number of threshold values among P1 is (7x6=42, for 7 users in group P1). The matching among person P1 in group G1 is tabulated in Table 7. From the table we find the minimum and maximum threshold of the Individual of the Training sample.

$T\Delta_{MAX} \rightarrow$ Overall Maximum of the Mpersons
 $T\Delta_{MIN} \rightarrow$ Overall Minimum of the Mpersons
 Number of samples in G1 is (162 * 42)

To find the overall total of maximum and minimum thresholds, we combine all the threshold values computed with a predetermined number n, also called the heuristic.

$$T\Delta_i = T\Delta_1 + T\Delta_2 + T\Delta_3 + \dots + T\Delta_N$$

combined all the $T\Delta_i$

Classifier	Accuracy	Threshold value	FAR	FRR	EER
PCA	0.9416	10.125 * 10 ¹	0.0431	0.0730	0.0584
LBPI	0.8171	6.163 * 10 ³	0.1073	0.2585	0.1829

$$(StepThreshold)h = \frac{T\Delta_{MAX} - T\Delta_{MIN}}{q}$$

... (19)

$$\text{Compute } \Delta = T\Delta_{MIN} + nh$$

We compute this value for n = 1, 2, 3, ..., 49, and analyze all the Δ_{NTH} values.

$$\begin{aligned} \Delta_1 &= T\Delta_{min} + h \\ \Delta_2 &= T\Delta_{min} + 2h \\ &\vdots \\ \Delta_{NTH} &= T\Delta_{min} + (n + 1)h \end{aligned}$$

... (20)

These 49 threshold values are tested with group G2 on group G1 images, and a reference threshold value for the system is chosen based on two criteria:

- (1) where FAR and FRR are equal, and
- (2) where FAR is minimum.

We use the performance evaluation given above to determine our reference threshold (Δ_{NTH}) values.

4. Result and Discussion

The tables below shown the summary of the training and the authentication data for two features vectors (classifier)

Table 4: Training Data

Based on the provided table, we have the following information:

Classifier: PCA and LBPI Accuracy: The accuracy of the PCA classifier is 0.9416, while the accuracy of the LBPI classifier is 0.8171.

Threshold value: The threshold value for the PCA classifier is 10.125 * 101, and for the LBPI classifier is 6.163 * 103.

FAR: The false acceptance rate (FAR) for the PCA classifier is 0.0431, and for the LBPI classifier is 0.1073.

FRR: The false rejection rate (FRR) for the PCA

classifier is 0.0730, and for the LBPI classifier is 0.2585. **EER:** The equal error rate (EER) for the PCA classifier is 0.0584, and for the LBPI classifier is 0.1829.

Based on these values, we can draw the following conclusions:

Accuracy: The PCA classifier has a higher accuracy (0.9416) compared to the LBPI classifier (0.8171). This indicates that the PCA classifier performs better in correctly classifying the samples. **Threshold value:** The threshold value for the LBPI classifier ($6.163 * 10^3$) is higher than that of the PCA classifier ($10.125 * 10^1$). This implies that the LBPI classifier may have a stricter threshold for accepting samples as positive.

FAR: The PCA classifier has a lower false acceptance rate (0.0431) compared to the LBPI classifier (0.1073). This means that the PCA classifier has a lower probability of incorrectly

Classifier	FAR	FRR	EER	Accuracy
PCA	0.0429	0.0736	0.0583	0.9417
LBPI	0.0956	0.2209	0.1582	0.8417

accepting a negative sample as positive. **FRR:** The PCA classifier has a lower false rejection

rate (0.0730) compared to the LBPI classifier (0.2585). This indicates that the PCA classifier has a lower probability of incorrectly rejecting a positive sample as negative. **EER:** The EER represents the point at which the FAR and FRR are equal. The PCA classifier has a lower EER (0.0584) compared to the LBPI classifier (0.1829). This suggests that the PCA classifier achieves a better balance between the false acceptance and false rejection rates.

In summary, the PCA classifier outperforms the LBPI classifier in terms of accuracy, FAR, FRR, and EER. However, the LBPI classifier has a stricter threshold value, which may impact its

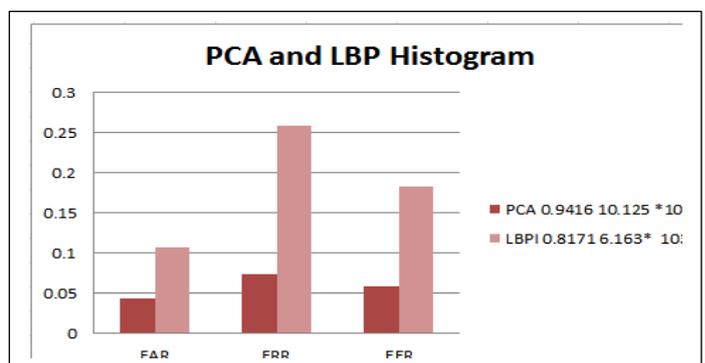


Figure 10: showcases the performance evaluation of Principal Component Analysis (PCA) and Local Binary Pattern (LBP) techniques.

Table 5: Authentication Data

Based on the provided information, it seems to be a comparison of two different classifiers (PCA

and LBPI) based on their performance metrics, such as False Acceptance Rate (FAR), False Rejection Rate (FRR), Equal Error Rate (EER), and Accuracy.

For the PCA classifier:

FAR (False Acceptance Rate) is 0.0429, which indicates the proportion of impostor (incorrect) samples that are incorrectly accepted as genuine. FRR (False Rejection Rate) is 0.0736, representing the proportion of genuine (correct) samples that are incorrectly rejected as impostors. EER (Equal Error Rate) is 0.0583, which is the point where the FAR and FRR are equal. It signifies the trade-off between accepting impostors and rejecting genuine samples.

Accuracy is 0.9417, indicating the overall correctness of the classifier in distinguishing between genuine and impostor samples.

For the LBPI classifier:

FAR (False Acceptance Rate) is 0.0956, suggesting a higher proportion of impostor samples being incorrectly accepted. FRR (False Rejection Rate) is 0.2209, indicating a higher

proportion of genuine samples being incorrectly rejected.

EER (Equal Error Rate) is 0.1582, representing the point where the FAR and FRR are equal for the LBPI classifier. Accuracy is 0.8417, which suggests a lower overall correctness compared to the PCA classifier. In summary, based on the provided metrics, the PCA classifier appears to outperform the LBPI classifier in terms of accuracy, FAR, FRR, and EER. It achieves higher accuracy and lower error rates, indicating better performance in distinguishing between

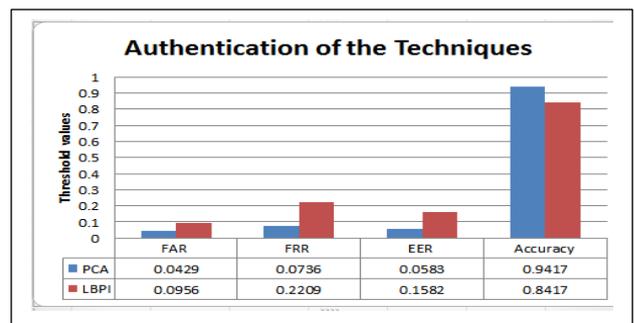


Figure 11. presents the accuracy results of the classifiers.

Table 6:G1(Fusing algorithms through the use of training data.)

Classifier	FA R	FR R	EE R	Accur acy	Improve ment
PCA+	0.02	0.08	0.05	0.944	Yes
LBP	50	68	59	1	

In the given interpretation, we are discussing the performance of a classifier using a specific feature extraction technique called PCA+LBP. The performance of the classifier is evaluated

using several metrics, including FAR, FRR, EER, Accuracy, and Improvement.

FAR (False Acceptance Rate): It is the rate at which the classifier incorrectly accepts a non-matching input as a match. In this case, the FAR is 0.0250, which means that the classifier has a relatively low rate of falsely accepting non-matching inputs. **FRR (False Rejection Rate):** It is the rate at which the classifier incorrectly rejects a matching input as a non-match. Here, the FRR is 0.0868, indicating that the classifier has a relatively higher rate of falsely rejecting matching inputs. **EER (Equal Error Rate):** It is the point at which the FAR and FRR are equal. In this case, the EER is 0.0559, which represents the overall performance of the classifier. Lower EER values indicate better performance. **Accuracy:** It is the proportion of correct classifications made by the classifier. The accuracy is 0.9441, suggesting that the classifier has a high level of correct classifications. **Improvement:** The "Yes" in this column signifies that there has been an improvement in the classifier's performance compared to a previous evaluation or a baseline. The extent of improvement is not specified. Overall, the

PCA+LBP classifier demonstrates a good level of accuracy (94.41%) and achieves a relatively low FAR (0.0250), but there is room for improvement in reducing the FRR (0.0868) to enhance the overall performance.

Table 7: G2(Authentication Data)

Classifier	FAR	FRR	EER	Accuracy	Improvement
PCA + LBP	0.0232	0.0798	0.0515	0.9485	Yes

In Table 7, labeled as "G2 (Authentication Data)," we have the performance metrics of a classifier using the PCA + LBP (Authentication Data) technique. The table includes the following metrics: FAR, FRR, EER, Accuracy, and Improvement. **FAR (False Acceptance Rate):** The FAR value for the ACD classifier is 0.0232, indicating a relatively low rate of falsely accepting non-matching inputs as matches. **FRR (False Rejection Rate):** The FRR value is 0.0798, which suggests a relatively higher rate of falsely rejecting matching inputs as non-matches. **EER (Equal Error Rate):** The EER value is 0.0515, serving as an overall measure of the classifier's performance. A lower EER indicates better performance. **Accuracy:** The accuracy of the PCA + LBP classifier is 0.9485, indicating a high

level of correct classifications. Improvement: The "Yes" in the Improvement column signifies that there has been an improvement in the classifier's performance compared to a previous evaluation or a baseline. The extent of improvement is not specified. To summarize, the ACD classifier demonstrates a low FAR (0.0232) and achieves a relatively low EER (0.0515), indicating good performance in terms of accepting non-matching inputs and the overall classification accuracy. However, there is room for improvement in reducing the FRR (0.0798) to enhance the classifier's performance further.

5. Conclusion

Several feature extraction techniques have been proposed by researchers in palm print recognition for the extraction of palm features template from palm images. This study employed two techniques of feature extraction: PCA and LPB combined features in which a comparative performance analysis was conducted of these extraction methods based on different evaluation metrics. The developed system was evaluated with PolyU Database. The experimental results showed that combine two increase the accuracy. The Accuracy depends on how they complement

each other. All algorithms were developed in the MATLAB software and run on a PC with an Intel CORE i3 processor, 4 GB of RAM and Windows 7.

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COST OF SPENDING ON FOREIGN EDUCATION AND ITS ECONOMIC IMPLICATIONS.

BY

Ibrahim Murtala & Olukotun Taiwo Hassan

School of Preliminary Studies, Art and Social Sciences, Itakpe Campus

Kogi State Polytechnic, Lokoja

Abstract

This study examines the cost of spending on foreign education and its economic implications. It relies on literature to descriptively analyse the implications. The study finds that the speed at which Nigerian students migrate abroad in pursuit of foreign education has continually been on an exponential rise. It also reveals that each year, there is a significant rise in the number of students moving abroad for education despite the huge costs of financing such moves. Nigerian students migrating to foreign countries for their undergraduate and postgraduate studies will negatively weaken the country's currency via exchange rate. Therefore, the study advises Budgetary Allocation to the education sector must be such that it is above average. Funds must be made available in order to put in place the necessary paraphernalia for a sound education. Structures that engender peaceful atmosphere, laboratories and technical equipment for practical, research grants, scholarships are all necessary for an all-round upliftment in the educational sector and should be provided.

1.0 Introduction

Education is necessary for the economic growth and political stability of any nation. However, due to the lack of resources, Nigeria has not paid necessary attention to education (Mukkadis 2019). This in effect has lowered the rate of accessibility to quality and affordable education. Lack of motivated and qualified tutors, incessant strikes and unstable calendars among others are the major fallouts of negligence of post-secondary education by the government. Education is a strategic part of any nation's functions because it determines the kind of jobs obtained, the amount of money earned and lifelong economic well-being. It is also the principal instrument for preparing an individual for later professional training, adjusting to his/her environment, and also a foundation of good citizenship. Therefore, to deny any individual the opportunity for education implies denying, not only the individual to succeed, but also denying the country the expected tax revenues and economic growth. The effect of

education on economic growth as relevant to human capital in a certain region or an individual country has been found in previous researches. But thus far, there have been little attempts to examine the effects of education expenditure on per capita GDP in developing countries, and whether the effects differ from that of SubSaharan African countries.

The perennial challenges of the Nigerian education sector include infrastructural decay and deficit, inadequate funding of research endeavours and poor remuneration of the university workers. The failure of successive Nigerian governments to remedy the situation has led to intermittent industrial disputes between the government and the organized university workers' unions. The implication of this is that the workers (teaching and non-teaching) became demoralized with attendant consequences on the academic pursuit of students. Recent decades have witnessed a surge in the demand for foreign education by

Nigerians as Nigerians' craze for higher education abroad is common knowledge. In 2012, a non-governmental organization, Exam ethics international said that Nigeria loses over N1.5 trillion to foreign education annually with neighbouring Ghana alone getting about N160 Billion as tuition fees for the over 71,000 students studying there. The Central Bank of Nigeria (CBN) has disclosed that foreign education has cost Nigeria a whopping sum of \$28.65 billion between 2010 and 2020 (10 years). This amount was not sent abroad but was part of the apex bank's Foreign Exchange reserves that could have helped the Naira to be much stronger today. If we were able to avoid a significant portion of this demand, the Naira would be much stronger today. All these, according to the apex bank, add pressure on the Nigerian Naira and cause the Dollar exchange rate to increase.

WHY THE CRAZE FOR FOREIGN EDUCATION?

This in essence is a form of importation if viewed from the service angle. Some of the factors responsible for this are as discussed below:

1. Poor government funding and infrastructural deficit
2. Persistent industrial disputes between the government and academic unions
3. Prolonged academic programmes
4. Job/Career prospects after graduation
5. General Living Conditions

These factors are further explained below:

1. **Poor government funding and infrastructural deficit:** Poor funding of education by successive governments in Nigeria has made higher education unattractive in Nigeria. The lack of support from the Nigerian government due to policy inconsistency, discriminatory treatment of students from private and public universities as well as pervasive corruption in the disbursing agencies have all serve to reduce the quality of education

in Nigeria. As at 2015, Nigeria had about 125 Universities, yet, most of the students seeking admission could not have access or admission to higher education. As posited by the Brookings Institute, Nigeria has one of the lowest levels of social spending, resulting in poor school infrastructure, lack of school maintenance, inadequate learning materials and ill-qualified teachers.

2. Persistent industrial disputes between the government and academic unions: Incessant strikes by various academic unions such as ASSU, NASU, SSANU and others have become the motivations that lead to the search for alternative opportunities elsewhere. The consequence of government's refusal to hearken to the demands of the lecturers as well as other tertiary institution workers is passed to the students. Apart from the incessant industrial actions that do truncate academic process, whenever the academic activities resume, students are made to bear the brunt of the failed negotiation.

3. Prolonged academic programmes: a direct fallout of the incessant academic strikes is distortion in the academic calendar which has prolonged the stay of students in schools. The overstretched programmes affect the career growth of students and more often than not affect them psychologically.

4. Job/Career prospects after graduation: the availability of jobs after graduation contributes in no small measure to the craze in foreign education. Remunerations, health insurance, leisure hours and pensions are some of the motivating factors with jobs after graduation. The National Bureau of Statistics (NBS) data witnessed an increase in the labour force as well as an increase in the number of unemployed people in Nigeria. The statistic puts the number of the labour force at over 66.8 million people in 2022 while the number of unemployed people stood at 22.45 million. This posts a bad and scary precedence for students in Nigeria and the country as a whole. This is another reason why Nigerian students who have

the means to study abroad gladly take the opportunity rather than study in Nigeria where the chances of securing good jobs after graduation is relatively low. As many Nigerians study abroad, there is the tendency that they will get jobs after school which gives rise to repatriated funds to the country.

5. General Living Conditions: countries with generally moderate to advanced living conditions have often attracted students from. Generally, living conditions would include the standard and cost of living, weather and climate, good transportation and road networks, portable water, good health care, power supply and a high life expectancy.

EXPENDITURE ON FOREIGN EDUCATION AND ITS IMPLICATION ON THE NIGERIAN ECONOMY.

Directly or indirectly, the economic implication of expenditure on foreign education are typical of importation of any other item or service. It has a far reaching effect on exchange rate, the

stability of local currency and the general price level in the economy.

1. Pressure on scarce Foreign Exchange
2. Brain Drain
3. Capital Flight
4. Decrease in the Productive Capacity of Tertiary Institutions in Nigeria
5. Decline in quality of Education

1. Pressure on scarce foreign exchange: Nigeria has witnessed a decline in its foreign exchange earnings due partly to the fact that its earnings from crude oil sales has dwindled in recent years. Nigeria therefore cannot but ration the available foreign currencies to more productive sectors of the economy. The demand for forex for foreign education exacerbates the precarious situation the country has found itself. The CBN data, according to a report by local newspaper *Punch*, showed that, in December 2021, Nigerians spent US\$90.67 million on foreign education. In January 2022, a total of US\$60,202,730.84 was spent on foreign

education, while US\$69.9 million was spent in February 2022. These are but a drop out of the amount been expended on foreign education. Alternatively, these figures would have been available for other more productive sectors of the economy to access.

2. Brain Drain: The battered economy as well as society that have come to characterize the Nigerian situation is not an appealing sight. So, one wonders whether it is possible for the diasporas to come back to work in the state of the country. This is more so that the fears of the diasporas cannot be allayed given the enormity of challenges they would face if they come home. Some of these challenges include, but are not limited to, corruption, political patronage, security risks, lack of access to productive inputs, lack of sufficient government stimulus, inconsistent government policies, high rate of unemployment, low wages and poor state or lack of infrastructure. The consequences of brain drain include; drastic fall in the standard of education, tremendous reduction in the

quality of skilled manpower in tertiary institutions in Nigeria (particularly the universities), depletion of Nigeria's natural supply of intellectual and professional manpower and rise in the Gross Domestic Product (GDP) of the receiving countries.

3. Capital Flight: Capital flight refers to migration of capital from one country to other countries of the world. In Nigeria, Foreign Education and Medical Expenses and Looted Funds are the major channels through which huge capital leave the country. Capital flight has been reported to exert detrimental effects on both the short and long run growth of the economy. The first notable effect is the fact that capital transferred abroad cannot contribute to domestic investment and thus it is regarded as diminishing possibilities for economic development. Necessary imports are limited by the foreign exchange drain from both the flight itself and the fact that earnings on such flight are not repatriated. The rate of capital formation is greatly reduced by capital flight and this

adversely affects the country's current and future growth aspects. If this capital had been invested in production of domestically produced export goods that could finance imports, the import constraint on growth could have been relaxed. Nigeria is currently faced with infrastructural deficit that has impaired the development of the country and its transformation into an industrial economy. The crux of the argument is that capital flight reduces domestically available investible capital. It represents forgone investment in manufacturing plants, infrastructure, and other productive capacity. Furthermore, much of the capital that flees the country is not taxed, thus depriving the country of revenues capable of contributing to fiscal deficits and constraining expenditures on social welfare programs, security challenges in the country, and infrastructural development. This hinders economic growth because investment has been diverted abroad and also because necessary imports are limited by foreign exchange drain

from both the flight itself and the fact that earnings on such assets are often not repatriated as observed by Pastor (1990).

4. Decrease in the Productive Capacity of Tertiary Institutions in Nigeria: Indeed, Nigeria's public tertiary institutions remain in perpetual deteriorating condition in spite of the efforts at increasing capacity by building more universities. The institutions are severely overcrowded with sky-rocketing student to teacher ratios and chronic faculty shortages. Overcrowding at institutions and inadequate funding resources are contributing factors to the decline in the quality of higher education. The system has far outgrown the resources available for it to continue offering high-level quality education. Other factors contributing to the decline in quality are the unstable environment due to frequent strikes by students or staff, the quality of students admitted to programs, and the quality of the academics recruited. These factors need to be taken into consideration in rethinking quality promotion.

5.0 RECOMMENDATIONS

As part of efforts to stem the tide of increasing demand for foreign education by Nigerians, this paper recommends the following:

1. Staff Motivation including annual bonuses, leave allowances and other incentives particularly to the academic staff of universities will go a long way in improving the attitude to work of lecturers thereby improving their value addition to education and an all-round improvement in service delivery.

2. Regular Payment of Salaries and Allowances to both academic and non-academic staff of universities will reduce the frequency of the incessant strikes being experienced in recent years. In order to engender industrial harmony, academic unions as well as the government must also be honest and hold-up their own ends of negotiations or bargains. Government must be seen to exhibit the will to improve on the standards of education in the country while

academic unions must complement these efforts by being alive to their responsibilities.

3. Training and Re-training of staff in tertiary institutions will go a long way in keeping them up-to-date and in shape to deal with the educational demands of the 21st century. Staff could be sponsored abroad where students flood to, to understand why students are attracted to such destinations in order for such factors to be replicated in tertiary institutions back home.

4. Budgetary Allocation to the education sector must be such that it is above average. Funds must be made available in order to put in place the necessary paraphernalia for a sound education. Structures that engender peaceful atmosphere, laboratories and technical equipment for practical, research grants, scholarships are all necessary for an all-round upliftment in the educational sector and should be provided.

6.0 CONCLUSION

Like a leaking roof, the adverse effects of foreign education tourism are gradually eating deep into the Nigerian economy and the purses of the citizenry. The speed at which Nigerian students migrate abroad in pursuit of foreign education has continually been on an exponential rise. Each year, there is a significant rise in the number of students moving abroad for education despite the huge costs of financing such moves. Nigerian students migrating to foreign countries for their undergraduate and postgraduate studies will negatively weaken the country's currency via exchange rate. This portends a grave consequence for the economy and country at large if left unchecked.

Like it is said, it all begins in the heart, looking at the way forward, it is the position of this paper that the first approach to arresting this ugly trend is to have a paradigm shift towards our educational system. We should begin to think outside the box to find solutions to this biting problem. We should not even think of stopping

our students from traveling abroad for further studies because that will amount to reducing the scope of learning amongst our citizens. Rather we should go out there, learn what these advanced countries are doing to promote their education system and come back and develop our system. The education managers should work on the education system and make it attractive. Furthermore, a major way to get this done is through partnership programmes with the educationally advanced countries, then the knowledge or results be transferred or incorporated into the Nigerian education system.

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MACROECONOMIC POLICIES AND GROWTH: WHAT DOES GROWTH THEORY SAY? EXPECTATIONS AND WEAKNESSES

BY

Ibrahim Murtala & Olukotun Taiwo Hassan

School of Preliminary Studies, Art and Social Sciences, Itakpe Campus

Kogi State Polytechnic, Lokoja

Abstract

This article examines the relationship between macroeconomic policies and economic growth from the perspective of growth theories. The objective of this article is to review and analyze what the growth theories postulate and to determine the expectations from these theories as the frameworks for building sound macroeconomic policies. The article also analyses the weaknesses of the growth theories in building efficient macroeconomic policies that deliver sustainable economic growth. The article presents a comprehensive review of various growth theories including resources-based growth theory, export-led growth theory, Schumpeterian growth theory, dual economy growth theory, stages of economic growth theory, Harro-Domer growth theory, neoclassical growth theory, and endogenous growth theory. The article concludes that while these theories offer important insights into the relationship between macroeconomic policies and economic growth, they also have weaknesses that need to be addressed in building efficient macroeconomic policies that deliver sustainable economic growth. This article provides a useful guide for policymaker

and researchers interested in the development of effective macroeconomic policies that promote economic growth.

1.0 Introduction

One of the most important goals of a country's economic policy is to build and to continuously improve its economic system so that it can be able to generate high rates of growth which are economically, socially and ecologically sustainable. The realization of this goal would enable its citizens to reach the standard of living in the developed market economies within a reasonable period of time. Economic growth, in the world or in a particular region or country, depends to a large extent on the nature and quality of economic policy. For example, if there is a good environment for households and firms to save and invest in the developing world, economic growth is generally observed. The International Monetary Fund (2000) also claims that where sound macroeconomic policies have been sustained, they have raised growth (Collier and Dollar, 2001).

While economic theory is largely mute on the question of whether macroeconomic policies affect economic growth, an examination of the experience of different countries over various periods and the policies they pursued, lends strong support to the idea that macroeconomic policies do play a role in the growth process.

A macroeconomic policy framework conducive for growth can be characterized by five features: a low and predictable inflation rate; an appropriate real interest rate; a stable and sustainable fiscal policy; a competitive and predictable real exchange rate; and a balance of

payments that is regarded as viable. Countries with these macroeconomic characteristics tend to grow faster than those without them, though there are many individual cases of both developing and developed countries suggesting that satisfying only some of these conditions does not sustain strong growth. It is also important to recognize that the direction of causation is somewhat ambiguous: while good macro outcomes should be conducive for growth, strong growth is also conducive for good macroeconomic outcomes.

This paper presents a wide-range examination of both theoretical and empirical evidence on the many ways macroeconomic policies may influence economic growth. Given monetary policy's crucial role in determining the inflation rate in the long run, there is a particular emphasis on the relationship between inflation and growth.

The purpose of macroeconomic policy is to ensure that, the economy achieves non-inflationary, stable growth of price level control or inflation, promoting stability or moderating fluctuations in the level of output and appropriate living standard of the people or conducive long-term growth.

Targeting economic growth, both developed and developing countries employ different macroeconomic policies on the frameworks of various growth theories. However, the growth performance of many the developing countries over the past two decades has been unsatisfactory and uneven, often accompanied by sharp declines

in rates of investment and domestic saving rates, fallen even more dramatically, thereby contributing to unsustainable external payment positions. In Nigeria for instance, despite the recovery of demand for oil post COVID-19 in 2021 and the subsequent rises in oil prices to above \$120 per barrel, Nigeria's oil revenue remained poor leading to galloping exchange rates, rising negative balance of payment, awkward growth till date. The GDP grew from 0.15% to 5.01% first and second quarters of 2021 respectively but started nosediving from the third quarter the same year and down to 2.25% third quarter 2022 (NBS, 2023) in spite of various macroeconomic policies employed by both monetary and fiscal policy makers.

Therefore, the growth theories, as frameworks for macroeconomic policies what do they say and postulate? What are the expectations from these theories? What are their weaknesses? What therefore, is the role of macroeconomic policies in achieving sustainable growth? What is the relationship between macroeconomic policies and growth? Why are there gaps between what the growth theories say and the empirical experiences of various countries?

The main objective of this paper is to review a number of the prominent growth theories, their expectations and their weaknesses.

The specific objectives of this paper include:

- i. to review what the growth theories say and postulate

- ii. to determine the expectations from the growth theories as the frameworks for building sound macroeconomic policies
- iii. to analyze the weaknesses of the growth theories in building efficient macroeconomic policies that deliver sustainable economic growth

2.0 Conceptual Review

Economic Theory

Economic theory refers to a set of ideas and principles that attempt to explain how the economy works. It is a body of knowledge that guides economists and policymakers in understanding economic behaviour, analyzing economic problems, and designing solutions. Economic theories provide a framework for analyzing economic phenomena, making predictions, and testing hypotheses. These theories help us understand how individuals, firms, and governments make economic decisions, how markets function, and how economic policies affect the economy. They help economists and policymakers understand the behaviour of different economic agents, such as consumers, firms, and governments, and predict how changes in the economy will affect them. Theories also provide insights into the workings of different markets and the distribution of resources. (Mankiw, 2019)

2.2 Macroeconomic Policy

Macroeconomic policy refers to the actions taken by governments and central banks to manage the

overall performance of the economy. It is designed to achieve specific macroeconomic objectives such as low inflation, high employment, and stable economic growth. Macroeconomic policies can be either expansionary or contractionary, depending on the state of the economy and the goals of policymakers. Expansionary policies, such as fiscal stimulus and monetary easing, aim to stimulate economic activity and boost growth, while contractionary policies, such as fiscal austerity and monetary tightening, aim to reduce inflation and control excessive growth (Blanchard et al., 2017). Macroeconomic policies are crucial in shaping economic growth and the welfare of a nation.

2.3 Economic Growth

Economic growth is the increase in the production and consumption of goods and services in an economy over time. It is typically measured by the annual change in real gross domestic product (GDP), which is the value of all goods and services produced in an economy adjusted for inflation. Economic growth is essential for improving living standards, reducing poverty, and creating new opportunities for businesses and individuals. Achieving sustainable and inclusive economic growth is a key goal of policymakers around the world, and understanding the drivers of economic growth is critical for designing effective economic policies (Acemoglu et al., 2020). Sustainable economic growth is critical in achieving the economic and social goals of a nation.

2.4 Models of Economic Growth

Since the process of economic growth is based on great variety of factors that change over time, models of economic growth necessarily apply some simplifications. These simplifications consist in classification and aggregation of the causes of economic growth. Models of economic growth also can be divided according to the criterion of time. Long-term models are primarily used to determine the path of sustainable growth. They characterize a pattern according to which the economy should grow (Woźniak 2008, pp. 145-146) Mostly, in models of economic growth a depreciation rate for capital and growth of population are exogenous. In the case of the savings rate, the models can be divided into two groups.

3.0 Methodology

In line with the objective of this study, the study adopts qualitative methodology. It reviews and analyses the existing theories of economic growth to infer their tenets, their objectives or expectations and spotlight their weaknesses.

4.0 Presentation of the Theories, Review and Analysis

4.1 Resources-Based Growth Theory

Resource-based Growth Theory is a macroeconomic theory that emphasizes the role of natural resources in promoting economic growth and development. The theory posits that countries with abundant and well-managed natural resources are more likely to experience

sustained economic growth than those without. This theory emphasizes the importance of efficient resource use, technological innovation, and sound macroeconomic policies. It suggests that countries with abundant natural resources, such as oil, minerals, and agricultural land, can achieve sustained economic growth by effectively managing and utilizing these resources.

One of the key tenets of the resource-based growth theory is that natural resources play a vital role in driving economic growth. According to this theory, natural resources such as minerals, oil, gas, and timber are critical inputs for economic activity. These resources can be used to produce goods and services, and they can also be exported to generate foreign exchange earnings. The exploitation and management of natural resources can, therefore, provide a significant source of revenue for governments and contribute to overall economic growth. For example, a country that has significant oil reserves can use the revenue generated from oil exports to invest in infrastructure, education, and technology. This, in turn, can lead to higher levels of productivity, job creation, and economic growth. Hence, countries must invest in the efficient use of natural resources to ensure long-term economic growth and development.

Harold Hotelling was one of the earliest proponents of Resource-based growth theory. In his 1931 paper titled "The Economics of Exhaustible Resources," Hotelling introduced the concept of "nonrenewable resources" and

argued that the optimal rate of extraction of such resources should be governed by the expected future value of the resource. Hotelling's theory was based on the idea that natural resources are finite and that their depletion could lead to a decline in economic growth in the long run. In other words, resources should be managed in a way that maximizes their long-term value rather than being exploited in a way that only provides short-term gains.

Other scholars such as Albert Hirschman and Gunnar Myrdal in the mid-twentieth century also emphasized the importance of natural resources in promoting economic growth and development. In the 1970s and 1980s, economists such as Richard Auty and Jeffrey Sachs further developed the theory, arguing that effective management of natural resources could lead to sustained economic growth.

Jeffrey Sachs and Andrew Warner are two economists who have also made significant contributions to the development of Resource-based growth theory in recent years. In their 1995 paper titled "Natural Resource Abundance and Economic Growth," Sachs and Warner studied 98 countries and found that those with abundant natural resources tended to experience slower economic growth than those without. They suggested that the "resource curse," or the tendency of resource-rich countries to experience political instability and economic stagnation, could be avoided through sound economic policies and effective resource management.

4.1.1 Expectations of Resource-Based Growth Theory

One of the key expectations of resource-based growth theory is that natural resources can provide a significant source of income and export earnings for countries, which can promote economic growth and development. For example, countries with abundant oil reserves can generate significant revenue from oil exports, which can be used to fund investments in physical and human capital, research and development, and infrastructure.

The theory provides valuable insights into the role of natural resources in promoting economic growth and development. By understanding the complex relationships between natural resources, technological innovation, and sound economic policies, policymakers can make informed decisions about resource management and economic policy to ensure sustained economic growth and development.

Countries that are rich in natural resources have the potential and are expected to experience sustained economic growth and development, provided that they manage their resources effectively and invest in human capital and technology.

4.1.2 Weaknesses of Resource-Based Growth Theory

There are a number of weaknesses and drawbacks with this theory. These include issues such as corruption, political instability, and

environmental degradation, which can hinder economic growth and development and lead to a vicious cycle of poverty and underdevelopment. The extraction and use of natural resources particularly in the developing countries have negative environmental and social impacts. This is called resource curse, the tendency of resource-rich countries to experience political instability and economic stagnation, a very pronounced risk associated with relying on natural resources for economic growth.

That is, countries with abundant natural resources are more often prone to economic and political instability, corruption, and inequality. This is because natural resources can create a concentration of wealth and power in the hands of a few, which can lead to social and political tensions and undermine economic growth and development.

Dutch Disease is another peril of Resource-based Growth Theory. The Dutch Disease occurs because the increase in revenue from natural resource exports can lead to an appreciation in the value of the country's currency. This can make other industries, such as manufacturing and agriculture, less competitive on the global market as their goods become more expensive in relation to foreign currencies. The windfalls of foreign currency can lead to inflation, which can further reduce the competitiveness of other industries.

Another consequence of the Dutch Disease is that the sudden influx of revenue can lead to a neglect of other industries and sectors of the economy.

Governments may become overly reliant on natural resource exports and neglect other areas such as education, infrastructure, and human capital development. This can lead to a lack of economic diversification and can leave the economy vulnerable to fluctuations in natural resource prices.

Lastly, natural resource depletion is drawback to this theory. The natural resources can be depleted and that will lead to declining economic growth in the long run.

4.1.3 Macroeconomic Policy Recommendations by the Theory

These policies include efforts to promote economic diversification, industrialization that encourage investment in physical and human capital, promote technological progress and innovation, and ensure that resource revenues are used effectively to promote economic development.

Also, macroeconomic policies that ensure effective management of natural resources is crucial for adopting this theory to achieve sustained economic growth. This includes managing resource extraction and production in a way that maximizes economic benefits while minimizing negative environmental and social impacts. It also involves investing in complementary sectors such as manufacturing, services, and agriculture to diversify the economy and reduce reliance on natural resources.

That is, the growth theory puts emphasis on macroeconomic policies directed at efficient resource use, technological innovation, and sound economic policies. One way to promote efficient resource use is to implement policies that encourage sustainable resource management practices. This could include implementing regulations to reduce the negative environmental impacts of resource extraction and promoting the use of renewable resources.

Technological innovation is another critical factor in promoting economic growth. Innovation can improve the efficiency of resource use and help countries to transition to more sustainable and diversified economic activities. By investing in research and development and promoting entrepreneurship, governments can foster an environment that encourages innovation and entrepreneurship.

Sound economic policies are critical for promoting sustained economic growth. This includes policies that promote macroeconomic stability, such as sound fiscal and monetary policies, as well as policies that encourage investment in human capital, such as education and healthcare.

Additionally, policies that promote trade and investment can help countries to leverage their natural resources and promote economic growth.

To mitigate the negative effects of the Dutch Disease that Resource-based Growth Theory can lead to, governments can implement policies that promote economic diversification, such as

investing in education, infrastructure, and research and development. Additionally, some countries have established sovereign wealth funds to invest a portion of their natural resource revenue into other sectors of the economy, helping to create a more balanced and stable economic environment.

Resource-based Growth Theory has been further developed and refined by a wide range of economists and researchers. And till today it remains an important framework for understanding the role of natural resources, human capital, and technology in promoting economic growth and development.

4.2 Export-led Growth Theory

Export-led growth theory is a macroeconomic theory that is centre on the role of international trade and exports in promoting long-term economic growth and development. This theory suggests that countries can achieve sustained economic growth by specializing in the production of goods and services for export and that exports can be a key driver of productivity gains, increased investment, technological progress and competitiveness. That is country's economic growth can be accelerated by increasing its exports, which can lead to increased export earnings and investment in other sectors of the economy.

It postulates that countries should focus on export-oriented industries and that countries can increase their economic growth and development by producing goods and services that are in

demand in other countries and by exporting these goods and services. This means that countries can benefit from international trade by exporting goods and services that they produce efficiently and importing goods and services that they do not produce efficiently.

Raúl Prebisch was the first to articulate the idea in the 1950s that developing countries could promote economic growth by focusing on import substitution and export-oriented industrialization. Prebisch's work laid the foundation for later economists to develop the theory further. Albert O. Hirschman developed the theory further in the 1960s. Hirschman argued that developing countries could achieve sustained economic growth by promoting export-oriented industries, reducing dependence on imports, and focusing on the development of domestic industries. He also emphasized the importance of balancing export-oriented growth with domestic demand and promoting technological innovation.

Paul Krugman also made important contributions to the development of the theory. In the 1970s and 1980s, Krugman used mathematical models to demonstrate the potential for export-led growth. His work emphasized the importance of economies of scale, increasing returns to scale, and imperfect competition in generating sustained economic growth through exports. Michael Porter (1990) in his book "The Competitive Advantage of Nations" argued that countries could achieve sustained economic

growth by specializing in industries where they have a competitive advantage, and exporting those goods and services to other countries. He identified four factors that contribute to a country's competitive advantage: factor conditions, demand conditions, related and supporting industries, and firm strategy, structure, and rivalry

4.2.1 Expectations of Export-Led Growth Theory

The expectation of export-led growth theory is that exports can provide a source of external demand that can grow foreign exchange earnings, drive economic growth and development.

The theory also leads to learning by exporting, whereby firms that engage in exporting can gain access to new markets, technologies, and knowledge that can improve their competitiveness and productivity. This means that policies that promote exports can also have positive spillover effects on domestic firms and industries, leading to further productivity gains and economic growth.

Exports can generate significant positive externalities, or spillover effects, that can contribute to sustained economic growth. For example, by exporting, firms can gain access to new markets and customers, which can lead to greater specialization and economies of scale.

Development of domestic firms and industries, exporting firms are more likely to adopt new

technologies and innovative production processes in order to remain competitive in global markets.

The theory is expected to deliver efficient economies of scale, whereby the benefits of increased production volume lead to lower costs and increased productivity. This means that countries that specialize in export-oriented industries can achieve significant gains in productivity and economic growth by increasing their production volume and taking advantage of economies of scale.

4.2.2 Weaknesses of Export-Led Growth Theory

The one demerit of this theory is lack of productive economic base for especially the less developed countries with low-skilled and labour-intensive industries to successfully compete in global markets.

In addition, countries may face challenges in ensuring that the benefits of export-oriented growth are distributed equitably and that there is not a negative impact on the environment or local communities.

It can be difficult to maintain export-led growth over the long term, as countries may face increasing competition and diminishing returns to export-oriented industries.

It can be difficult for countries to shift their economic focus towards export-oriented industries, particularly in industries where they face significant international competition.

Over-dependence on exports can lead to long-term economic vulnerabilities and neglect of other sectors of the economy.

4.2.3 Macroeconomic Policy Recommendation by Export-Led Growth Theory

Export-led growth theory has important implications for macroeconomic policy, it suggests policies that promote trade liberalization and export promotion and promote foreign investments

It recommends policies that promote competitiveness of export-oriented industries, such as investing in infrastructure, technology, and human capital, as well as policies promote entry into free trade agreements.

The theory also has application in formulation of international trade facilitation policies such that reduce trade barriers, improve infrastructure, logistics, port decongestion and provide support for export-oriented firms and industries.

Export subsidies, incentives, pioneer status and other investment in export-oriented industries which can have significant positive effects on economic growth and development should be pursued by countries whose economies have productive economic base for manufacturing export goods and services.

It is important for countries to carefully consider the potential benefits and risks of export-led growth policies and to tailor these policies to their specific economic context.

However, there are empirical experiences of countries like China, Japan and South Korea who have successfully pursued and implemented export-led growth strategies and have achieved sustained economic growth and development over the last few decades in line with the basic tenets of export-led growth theory.

4.3 Schumpeter's Theory of Economic Growth

Schumpeter's theory of development assigns paramount role to the entrepreneur and innovations introduced by him in the process of economic development. According to Schumpeter, the process of production is marked by a combination of material and immaterial productive forces. The material productive forces arise from the original factors of production, viz., land and labour, etc., while the immaterial set of productive forces are conditioned by the 'technical facts' and 'facts of social organization'. The Schumpeterian production function can, therefore, be written as –

$$Q = f [k, r, I, u, v] \dots (1)$$

Where, Q stands for the output, k for the Schumpeterian concept of "produced means of production", r for natural resources, l for the employed labour force. The symbol u represents the society's fund of technical knowledge and v represents the facts of social organization, i.e., the socio-cultural milieu within which the economy operates.

The above function shows that the rate of growth of the output depends upon the rate of growth of productive factors, the rate of growth of technology and the rate of growth of investment friendly socio-cultural environment. Schumpeter held that the alterations in the supply of productive factors can only bring about gradual, continuous and slow evolution of the economic system.

On the other hand, the impact of technological and social change calls for spontaneous, discontinuous change in the channels of output flow. Thus taking into account these two types of distinct influences Schumpeter distinguished two components in the dynamic evolution of the economy – (a) the “growth component” which brings about gradual, continuous and slow evolution due to the changes in the factor availability, (b) the “development component” which brings about spontaneous and discontinuous change in the channels of output flow due to changes in the technical and social environments.

Schumpeter regarded land to be constant. The growth component will, therefore, include only the effects of changes in population and of increase in the producer goods. But Schumpeter further maintains that there does not exist any a priori relationship between the changes in population and the changes in the flow of goods and services. In other words, Schumpeter considers the population growth to be exogenously determined. Now, the increase in producer goods results from a positive rate of net savings.

The major part of savings and accumulations are attributed by Schumpeter to profits. But, according to him, the profits can arise if innovations such as new techniques of production are employed or if new product is introduced. Hence ultimately it is the change in the technical knowledge (i.e., variable u) which is responsible for any change in the stock of producer goods, i.e., the rate of capital accumulation directly depends on the rate of technical change.

Regarding the historical development, Schumpeter subscribed to Marx’s materialistic interpretation of history and he maintained that the economic state of people emerges only from the preceding total situation. However, the most important point of Schumpeter’s theory is that

the expansion of output depends upon the history of technological development. In simple words, we can say, according to Schumpeter, the growth of output is geared to the rate of innovations. No doubt, Schumpeter holds that the trend of economic growth shall be fixed by the exogenous variable of population growth, yet according to him, the process of economic development is synonymous with discontinuous technical change, i.e., innovations. The agent which brings about innovations is called by Schumpeter as entrepreneur. Thus, entrepreneur becomes the pivot of Schumpeter’s model.

4.3.1 Relevance of Schumpeter’s Theory for Developing Countries:

The conditions obtaining in Western Europe and America after the First World War presented a capitalist system in full swing, wherein the innovator acted as the initiator and controller of economic development. Schumpeter’s observant eye got the clue to formulate a theory of development presenting a unified view of the whole economic process. Schumpeter viewed “development” as a distinct phenomenon which, he says, “is spontaneous and discontinuous change in the channels of flow, disturbance of equilibrium, which forever alters and displaces the equilibrium state previously existing.”

This springs from changes in the economic life due to endogenous factors (initiated from within) and not exogenous factors which are forced upon it. Explaining his contention further, he holds that “Should it turn out that there are no such changes arising in the economic system itself, and that the phenomenon that we call economic development is in practice simply founded upon the fact that the data change and the economy continuously adapts itself to them, then we should say that there is no economic development.” This concept wherein endogenous changes in the economy act as the sole prime mobile of development restricts the relevance of Schumpeter’s theory to the growth problems of developing economies.

Rigid and outmoded socio-economic institutions, low saving potential and laggard technology are completely incapable to generate developmental impulses from “within” in the underdeveloped countries. They have to take recourse to imported capital, technology and skill to initiate and propel their developmental wheels. For instance, India made a big stride forward in growth and it has sought foreign capital to help in its economic development. It has also gone for foreign collaboration in terms of loan, equipment, skill and technical know-how. Since factors from ‘without’ are responsible for initiating and operating development projects, they cannot, according to Schumpeter, be regarded as embodiments of India’s genuine process of economic development. This contention of Schumpeter is unsustainable and unconvincing.

It cannot be gainsaid that every such plant has generated a developmental wave in the Indian Economy. Thus, Alfred Bonne remarks, “Exclusion from Schumpeter’s definition would not make the new plant cease to be a case of development, having in view precisely those goods which are the essential objectives of development activities in economically backward countries.” In this view, therefore, Schumpeter’s theory of development is incongruent with the conditions prevailing in the developing world.

Further, Schumpeter’s preoccupation with only the endogenous factors and his insistence on development as embodying only the spontaneous and discontinuous changes makes him oblivious of the role of population growth as an economic force in the developmental process. He regarded population as exogenously determined and held that there does not exist any deterministic a priori relationship between population growth and variations in the flow of goods and services. But it is precisely the excessive population pressure that is responsible for revolutionising the methods and techniques of agricultural

production in the presently overpopulated developing countries.

In fact, some of the post-Keynesian theories regard population growth as a stimulant for autonomous investment. By failing to take proper cognisance of one of the most vital phenomena operating in the presently underdeveloped economies, Schumpeter rendered his theory almost ineffectual to such countries.

Further, the existence of a business elite, i.e., the entrepreneurial class, is fundamental to Schumpeter’s theory of economic development. The carrying out of innovations and using new production functions is the prerogative of this elite group of private entrepreneurs. However, there are serious doubts about the effectiveness of this social group in the development of the developing countries. The contemporary history of economic development of these countries provides ample evidence to reveal that it is not only the private entrepreneurial class, but also the national governments that are responsible for preparing and launching programmes of industrialisation.

With the development process of these countries being rapidly imbued with the socialistic hues, their governments have increasingly assumed the role of a national entrepreneur. Not the innovations of the private entrepreneur but the “government action and mass impulses today seem to be the most characteristic motive forces of economic development.” So much so that even in the private sector of these economies the entrepreneurs cannot fulfill their functions without the active and substantial assistance from the government and semi-public bodies. Moved by such a un-Schumpeterian economic landscape in the developing countries, Prof. Gunnar Myrdal remarks that “it represents, indeed, an attempt at a complete reversal of what once happened in the now developed countries as described by the Schumpeterian model.”

In developing economies, a number of factors such as the outmoded socio-economic institutional framework, tradition-ridden investment horizon and unreliable attitude for undertaking of new ventures, have all contributed in denigrating the pivotal role assigned to the Schumpeterian entrepreneur in his functional aspects. The governments of these countries under such conditions cannot afford to remain an idle and passive spectator. It is incumbent for them to come forward and become the herald of industrialization by playing the role of a unified national entrepreneur.

Furthermore, the governments of the developing countries are committed to the rapid creation of 'social overheads' or what is now called infrastructure in order to fulfill the popular demand for higher standards of living. The private capital fails to come forward because of the lumpy nature of such investments and the long gestation periods involved. On the other hand, an agency like the government has sufficient means to mobilize the capital resources of the economy through various fiscal and monetary measures and by borrowing from abroad.

The very exigency of the situation in the developing economies compels their governments to shoulder the responsibility of initiating and steering the gigantic task of economic development. Thus, the Schumpeterian model of development which assigns the primary and central role to the private entrepreneur and only a secondary and passive function for the government is a misfit to the conditions obtaining in the developing countries.

Besides, the entrepreneurial innovation so pivotal to the working of Schumpeter's model has no significance to the process of development in the developing countries. Henry C. Wallich and H.W. Singer have held that due to the demonstration effect on an international plane, the businessmen in the developing countries are

prone to import and assimilate the already known technology and methods of production from the developed countries rather than undergo the risks of innovating anew (some of which in any case may prove to be abortive). Hence the development process in the developing countries is increasingly becoming a process of derived development, being based on assimilation of existing innovations made elsewhere rather than on the Schumpeterian type of indigenous innovations.

In the Schumpeterian model, by its very nature and approach, inflationary pressures are bound to operate as the development process gathers momentum. The entrepreneurs' innovational activity being financed by the credit-creating banking system, credit-creation assumes a vital role in his model. The creation of credit leads to a rise in purchasing power of the community without a corresponding increase in production. Increased purchasing power results in an increased demand for production services and consumer goods. The increased demand coupled with the increased volume of money in circulation results in a general price rise.

But in the consumption-oriented development process of a developing economy, the inflationary tendencies are very powerful, persistent and cumulative in nature. "It is not only development and associated investment that are responsible for inflationary tendencies, but the entire social climate of demand-oriented economy." They become a serious drag on the development process itself. Thus, the production-oriented Schumpeterian vision of development process fails to realise the hurdles like secular inflation that characterise the consumption-oriented development of the developing economies. What in fact is needed is a totally different framework of analysis and theory that is realistic to the circumstances of these economies.

However, certain aspects of Schumpeter's model retain universality of application. Irrespective of

the type of economy and its stage of development, the importance of innovations as one of the major factors in economic development remains unassailable. 'Technological possibilities are an uncharted sea', and in this Apollo age, we can safely assume that the developing countries can hardly afford to remain mere imitators and assimilators.

Even if mere transfer of ready-made and proven techniques of production is sought, there remains the problem of adaptation of foreign technology in the domestic economy. It calls for a certain amount of pioneering spirit and entrepreneurial skill in so far it is new to the country in which it is to be adapted. Further, the risks of transplanting such technology in underdeveloped economies would be considerable. Hence the entrepreneurs in these countries should possess at least some of the basic qualities of the Schumpeterian entrepreneur.

From the point of view of successful development in developing countries Schumpeter's theory highlights the urgency of bringing about drastic transformation of the tradition-ridden socioeconomic institutions and reshaping of the inimical attitudes to develop a favourable climate for the growth of entrepreneurship. Adequate entrepreneurship is one of the prerequisites for sparking off a take-off stage in these countries.

Further, once the process of industrialisation sets apace in the developing countries, Schumpeter's theory can undoubtedly throw considerable light on the problems associated with the long-run increase in productivity. It shall also provide clues to the problem of absorption of 'surplus labour' in gainful employment as a result of innovations. In this way Schumpeter's theory of development can provide some valuable lessons to the countries for avoiding waste and extra hardships that are liable to attend an unplanned and uncoordinated development.

4.4 Arthur Lewis' Growth Theory or Dual-Sector Growth Theory

Another theory of economic growth was developed by Arthur Lewis. In his work he dealt with the problem of poor countries, but with a rich labour force. Lewis shared the overall vision of classical economists, but did not always agree with their diagnoses and methods. The theory explains how developing countries can achieve sustained economic growth and reduce poverty through the process of industrialization. The theory was developed by Arthur Lewis, a Saint Lucian economist who won the Nobel Memorial Prize in Economics in 1979.

The theory is based on the assumption that the economy is divided into two sectors: the traditional sector and the modern sector. The traditional sector is characterized by low productivity, low wages, and a surplus of labour, while the modern sector is characterized by high productivity, high wages, and a shortage of labour. The traditional sector is typically based on agriculture, while the modern sector is based on industry and services. The developing countries have a surplus labour force in the rural sector, which can be absorbed by the industrial sector, leading to increased productivity, higher wages, and economic growth. This surplus labour force is known as the "Lewisian Dualism" or "Lewisian Turning Point." In the early stages of development, the majority of the population in developing countries work in agriculture and are employed in small-scale, low-productivity, and low-wage activities.

4.4.1 Expectations of Lewis' Theory

The theory was designed to help developing countries achieve transfer of labour from the traditional sector to the modern sector as the economy grows, the demand for labour in the urban industrial sector increases, which leads to higher wages and a shift of labour from agriculture to industry.

Lewis' theory emphasizes the role of international trade in promoting economic growth. Developing countries should focus on exporting manufactured goods to developed countries, which have a high demand for these products. This can help to promote industrialization, increase foreign exchange earnings, and reduce dependence on primary commodity exports.

4.4.2 Macroeconomic Policy Recommendation of Lewis' Growth Theory

The key to achieving sustained economic growth, according to Lewis, is to ensure that the industrial sector is competitive and efficient. This can be achieved through policies that encourage investment in the industrial sector, such as providing infrastructure, access to credit, and tax incentives. These policies can help to reduce the cost of production and increase the competitiveness of domestic firms, leading to increased industrial output and employment.

4.4.3 Weakness of Lewis' Theory

It oversimplifies the process of economic development and ignores the complex financial

limitation, political, social, and institutional factors that affect the success of industrialization. The theory places too much emphasis on industrialization as the sole path to economic growth, neglecting the importance of other sectors such as services and agriculture.

4.5 Walt Rostow Theories of Growth or Stages of Economic Growth

Another theory of economic growth was created by Walt Rostow. Rostow, like Lewis, made economic development dependent on the accumulation of capital, and distinguished five stages of development (Rostow 1960, pp. 4-16). According to Rostow, the biggest problem for poor countries is to achieve the third stage, called "take off". Poor countries have a problem with the interruption of the "vicious circle" established through the years. Rostow proposed to break it by accumulating capital. However, he realized that in cases where there was an absence of opportunities to increase internal accumulation, external support would be necessary. In 1971 Rostow added a sixth stage of economic development, called "quality" - characterized by the continuous improvement in the quality of goods and services (Rostow 1971).

The theory, also known as the "Stages of Economic Growth" theory, was developed by American economist Walt Rostow in the 1950s. The theory attempts to explain how economies develop and grow over time, and it proposes that all economies go through five distinct stages on their path to modernization and industrialization.

Rostow in his book 1960, "The Stages of Economic Growth: A Non-Communist Manifesto" defined the five stages of growth as follows:

1. **Traditional Society:** This stage is characterized by subsistence agriculture, limited technology, and a largely rural population. Economic output is low, and there is little surplus wealth to be invested in economic growth.
2. **Preconditions for take-off:** This stage sees the emergence of some basic infrastructure and institutions that are necessary for economic growth, such as transportation systems, banks, and legal systems. Investment in physical capital and education also increases, leading to a growing economy.
3. **Take-off:** In this stage, the economy experiences sustained growth, as new industries emerge and the economy begins to shift away from agriculture. This is often accompanied by increased urbanization, industrialization, and international trade.
4. **Drive to Maturity:** At this stage, the economy is fully industrialized, and growth is driven by innovation, diversification, and specialization. The economy is able to sustain high levels of production and consumption, and there is a growing middle class.

5. **Age of High Mass Consumption:** The final stage is characterized by a high level of mass consumption, as the population has access to a wide range of consumer goods and services. The economy is driven by demand, and growth is sustained by high levels of consumer spending.

4.5.1 **Expectation of the Theory**

The expectation of the theory is that all economies as long as they adequately prepare for each stage and follow this path will eventually reach the final stage of high mass consumption.

4.5.2 **Macroeconomic Policy Recommendation of Rostow's Theory**

The policy makers should embark on policies that will target and deliver the requirements for each stage of the growth until they reach high mass consumption stage.

4.5.3 **Weakness of the Theory**

The theory is often criticized for its Eurocentric view of economic development, as it assumes that all countries should follow the same path to modernization as the Western industrialized nations. However, it remains a widely studied and influential theory in the field of economic development.

4.6 **The Harro-Domer Growth Model**

Harro-Domer models of economic growth are based on the experiences of advanced capitalist economy that attempt to analyze the

requirements of steady growth in such economy. The Harro and Domer models of economic growth are interested in discovering the rate of income growth and investment require for the smooth and uninterrupted working of the economy. The theory laid emphasis on the dual character of investment, stressing that, it creates income and argument the productive capacity of the economy by increasing its capital stock. Meanwhile, the former may be regarded as the 'demand effect' and the latter the 'supply effect' of investment. Hence so long as net investment is taking place, real income and output will continue to expand. However, for maintaining a full employment equilibrium level of income from year to year, it is necessary that both real income and output should expand at the same rate at which the productive capacity of the capital stock is expanding.

Otherwise, any divergence between the two will lead to excess of idle capacity, thus forcing entrepreneurs to curtail their investment expenditures. However, the Domer model link between aggregate supply and aggregate demand through investment while Harro model tries to show in his model how steady (i.e., equilibrium) growth may occur in the economy.

The theory has some assumptions including, full employment equilibrium level of income, absence of government interference (capitalist based theory) – fiscal policy and foreign trade (external policy), no changes of interest rate (monetary policy) and so on.

But, the Harro and Domer models of economic growth suffered some weakness, as follows:

The propensity to save (α or s) and the capital-output ratio (σ) are assumed to be constant. In actuality, they are likely to change in the long run and thus modify the requirements for steady growth. A steady rate of growth can, however be maintained without this assumption.

The assumption that labor and capital are used in fixed proportions is untenable. Generally, labor can be substituted for capital and the economy can move more smoothly towards a path of steady growth.

The two models also fail to consider changes in the general price level (inflation). Price changes always occur over time and may stabilize otherwise unstable situations. According to Meier and Baldwin, "If allowance is made for price changes and variable proportions in production, then the system may have much stronger stability than the Harrod model suggests.

Finally, the assumption that there are no changes in interest rates is irrelevant to the analysis. Interest rates change and affect investment. A reduction in interest rates during periods of overproduction can make capital-intensive processes more profitable by increasing the demand for capital and thereby reduce excess supplies of goods.

The Harrod-Domar models ignore the effect of government programs on economic growth. If,

for instance, the government undertakes programs of development, the theory analysis does not provide us with causal (functional) relationship.

It also neglects the entrepreneurial behaviour which actually determines the warranted growth rate in the economy. This makes the concept of the warranted growth rate unrealistic.

4.7 Neoclassical Growth Theory or Solow-Swan Model

Neoclassical growth theory or Solow-Swan model is a macroeconomic theory that explains long-term economic growth through the accumulation of capital and technological progress. The theory was first developed in the 1950s by economists Robert Solow and Trevor Swan, and it has been refined and expanded upon by subsequent scholars.

The Solow-Swan model starts with the assumption that economic growth is driven by two main factors: the accumulation of capital and technological progress. In this model, capital refers to the stock of physical goods and assets used in production, such as machines, factories, and infrastructure. Technological progress refers to improvements in the efficiency and effectiveness of production, such as new inventions or better organizational practices.

The Solow-Swan model assumes that the accumulation of capital and technological progress both contribute to economic growth, but at different rates. Specifically, the model

suggests that as a country accumulates more capital, the rate of economic growth will eventually slow down, because the marginal returns on investment will diminish. At this point, technological progress becomes the primary driver of economic growth.

The Solow-Swan model also predicts that economic growth will eventually converge to a steady state, in which the rate of technological progress and the rate of population growth determine the long-run growth rate of the economy. In other words, if a country has a higher rate of population growth or technological progress, it will experience faster economic growth in the long run.

Solow-Swan model is illustrated with production function, which describes the relationship between inputs (such as capital and labor) and outputs (such as goods and services). The production function can be written as:

$$Y = F(K, AL)$$

Where Y is output, K is capital, L is labor, and A is a measure of technological progress. F is a function that describes how inputs are transformed into output.

According to the Solow-Swan model, the rate of economic growth can be expressed as the change in output over time, or:

$$g = dY/Y$$

This rate of growth can be decomposed into two components: the growth rate of capital (k) and the growth rate of technology (a):

$$g = s/v + ga$$

Where s is the savings rate (i.e., the proportion of income that is saved and invested), v is the capital-output ratio (i.e., the amount of capital needed to produce one unit of output), and ga is the rate of technological progress.

4.7.1 Expectation of Neoclassical Growth Theory

The Solow-Swan model is expected to influence and has been influential in shaping understanding of economic growth, and it has been used to formulation of macroeconomic policy decisions related to investment, education, and innovation.

4.7.2 Weakness of Neoclassical Growth Theory

However, the model has also been criticized for its simplifying assumptions and its failure to account for important factors such as institutional quality, human capital, and environmental degradation.

4.8 Endogenous Growth Theory

Endogenous growth theory is a macroeconomic theory that explains long-term economic growth through factors within the economic system itself, rather than external factors such as capital accumulation or technological progress alone. The theory was first developed in the late 1980s by economists Paul Romer and Robert Lucas Jr., and it has since been expanded and refined by subsequent economists. It emphasizes the role of internal factors such as investment in research and development (R&D), human capital, and

knowledge spillovers in promoting economic growth. Unlike Neoclassical growth theory, which sees technological progress as an exogenous factor that is determined outside of the economic system, endogenous growth theory suggests that technological progress can be influenced by economic policies and individual decisions.

The key idea behind endogenous growth theory is that economic growth is driven by the creation and dissemination of knowledge and ideas, which are produced through a variety of channels within the economy. Unlike Neoclassical growth theory, which assumes that technological progress is exogenous (i.e., driven by factors outside the economic system), endogenous growth theory suggests that technological progress is endogenous (i.e., driven by factors within the economic system).

Endogenous growth theory also challenges the idea that returns to capital diminish over time, as classical growth theory suggests. Instead, endogenous growth theory suggests that returns to investment in knowledge and ideas can actually increase over time, leading to sustained long-term economic growth.

One of the key drivers of endogenous growth is human capital, which refers to the knowledge, skills, and abilities of individuals in an economy. According to endogenous growth theory, investments in education and training can lead to increases in human capital, which in turn lead to increases in productivity and economic growth.

This process can also create positive feedback loops, in which higher levels of economic growth lead to increased investment in education and training, which leads to even higher levels of economic growth.

Another key driver of endogenous growth is innovation, which refers to the creation and dissemination of new knowledge and ideas. Endogenous growth theory suggests that innovation can be spurred by a variety of factors, including investment in research and development (R&D), intellectual property protection, and competition. For example, firms may invest in R&D in order to develop new products or services, or to improve existing ones. Intellectual property protection, such as patents or copyrights, can incentivize firms to invest in innovation by allowing them to capture the benefits of their research. Competition can also spur innovation by encouraging firms to seek out new and better ways of producing goods and services.

4.8.1 Expectation of Endogenous Growth Theory

Endogenous growth theory has contributed to our understanding of the complex interactions between economic growth, technological progress, and investment in knowledge and innovation. By emphasizing the importance of internal factors in promoting economic growth, this theory has broadened our perspective on the

potential for sustained economic growth and the role of economic policies in promoting it.

In order to model endogenous growth, a variety of mathematical models that incorporate the various channels through which knowledge and ideas are created and disseminated within the economy. These models can be complex and highly specialized, and they often require significant amounts of data to estimate and test.

Endogenous growth theory is expected and has been having a significant impact on macroeconomic thinking, and it has been used to formulate macroeconomic policy decisions related to education, innovation, and intellectual property protection.

4.8.2 Weakness of Endogenous growth theory

However, like any economic theory, it has its limitations and critiques. Some economists argue that endogenous growth theory overemphasizes the role of knowledge and ideas, and underemphasizes the importance of physical capital and natural resources. Others argue that the theory is too focused on the role of the private sector in driving economic growth, and that it neglects the role of government in creating the conditions for growth.

5.0 Discussions and Recommendations

5.1 Policy Implications of the aforementioned Model

This theory suggests that convergence of growth rates per capita of developing and developed

countries can no longer be expected to occur. The increasing returns to both physical and human capital imply that the rate of return to investment will not fall in developed countries relative to developing countries. In fact, the rate of return to capital in developed countries is likely to be higher than that in developing countries. Therefore, capital need not flow from the developed to the developing countries and actually the reverse may happen.

Another implication is that the measured contribution of both physical and human capital to growth may be larger than suggested by the Solow residual model. Investment on education or research and development of a firm has not only a positive effect on the firm itself but also spillover effects on other firms and hence on the economy as a whole. This suggests that the residual attributed to technical change in the Solow growth accounting may be actually much smaller.

One of the important implications is that it is not necessary that economies having increasing returns to scale must reach a steady state level of income growth, as suggested by the Solow-Swan model. When there are large positive externalities from new investment on research and development, it is not necessary for diminishing returns to start. So the growth rate of income does not slow down and the economy does not reach steady state. But an increase in the saving rate can lead to a permanent increase in the growth rate of the economy.

5.2 Growth Effects on Macroeconomic Policies

Empirical studies linking macroeconomic policies to the growth rate of output and income are extremely important for several reasons. First, it is necessary to determine empirically the extent to which the theoretical models relationships with macroeconomic policies involved in the above mentioned theories stand up against the observed growth performance of developing countries. Second, the empirical studies should help in formulating growth-oriented adjustment programs and in establishing priorities in their implementation. And, finally, empirical investigations usually provide suggestions for further research aimed at understanding how macroeconomic policies influence the rate of growth in potential output and national income.

5.3 Growth effects of fiscal policies

The results for the growth effects of the ratio of total government expenditure to GDP have been inconclusive, which is not altogether surprising, since different expenditure compositions for a given level of the total have diverse effects on output growth, this is because several studies focus on the components of government expenditure.

5.4 Growth effects of monetary and interest rate policies

The effects of monetary and interest rate policies on the rate of economic growth are transmitted

through the effects of the rate of inflation and real interest rates on the level of private investment and its productivity and high rates of monetary growth usually lead to inflationary episodes.

5.5 Growth effects of external policies

It was argued that a sustained expansion of exports supported by appropriate external policies would raise total factor productivity and should thus be included as a separate factor of production. Several cross-country estimates of the effect of growth of exports on the rate of growth of real GNP for developing countries are available.

6.0 Summary and Conclusion

Resource-based growth theory suggests that natural resources can be used to promote economic growth and development, provided that they are properly managed and invested in. Export-led growth theory proposes that countries can promote economic growth by specializing in the production of goods for export, which can help to increase productivity and generate foreign exchange. Schumpeterian growth theory emphasizes the importance of innovation and entrepreneurship in driving economic growth. This theory suggests that economic growth is driven by the creation of new technologies, products, and industries. Arthur Lewis' growth theory suggests that developing countries can achieve economic growth and development by transferring surplus labour from the agricultural sector to the industrial sector, where labour productivity is higher. Rostow's

theory postulates that countries should know the stages of growth and prepare adequately for each. The Harrod-Domar growth model suggests that economic growth is driven by investment, and that an increase in investment leads to an increase in output, which in turn leads to economic growth. Neoclassical growth theory emphasizes the importance of capital accumulation in driving economic growth, and suggests that economic growth can be sustained through investments in human capital and technological innovation. Endogenous growth theory proposes that sustained economic growth comes from within a country, rather than from external factors. It suggests that a country can achieve sustained economic growth by investing in education, research and development, and promoting innovation, technological progress and that policies that promote innovation and entrepreneurship can have a significant impact on economic growth.

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ANALYSIS OF MEASLES CASES IN NIGERIA USING ARIMA MODEL

¹OBAFAIYE ADEGBOYEGA BENEDICT ²AHIABA MATHIS ³MICHAEL SUNDAY

Department of Statistics, Kogi State Polytechnic, Lokoja

¹Corresponding authors' email: adbenedictad@yahoo.com

Abstract

Measles is a vaccine-preventable, highly transmissible viral infection that affects mostly children under five years. Numerous studies on modelling measles cases using different methodologies were documented in literature. In this study, the modelling of measles cases in Nigeria was done using ARIMA model. Measles data were collected on a yearly basis from KNOEMA, from 1988 to 2022. Different models from the time series data on measles framework were explored. Result showed stationarity in the data after first differencing. Based on various candidate models, ARIMA (1,1,1) emerged as the best model using BIC as model selecting criteria. The selected model was adequate using positive value of stationary r square (0.771). From R square calculated, 72.2% estimate of the proportion of the total variation is in the series (measles cases $_1$) is explained by the model. However, the forecasting accuracy based on the selected model ARIMA (1, 1, 1), was assumed to be adequate.

Keywords: Measles, Box-Jenkins, ARIMA, Forecast

1.0 INTRODUCTION

Measles is a vaccine-preventable, highly transmissible viral infection that affects mostly children under five years. It is a cause of mortality and morbidity world-wide. Despite achieving and sustaining global measles vaccination coverage of about 80% over the past decade, measles remains the fifth leading cause of mortality among children aged less than 5 years. It accounts for more than 30 million cases annually and 0.9 million deaths every year, with approximately half of these occurring in Africa.

The World Health Organization (WHO) defines measles elimination as “the absence of endemic [measles virus](#) transmission in a defined geographic area for a period of at least 12 months in the presence of a well performing surveillance system”.

Nigeria is the most populous nation in Africa and the seventh most populous in the world. The country occupies an area of 923,768 square kilometers. In terms of land mass and population size, the northern part of the country is larger than the southern part. Nigeria is affected by four climate types (e.g., Tropical rainforest, Savannah, tropical dry/Sahel and Highland climate). These climate types are different from the southern part to the northern part of Nigeria through the country's middle belt. The tropical

rainforest climate can be found in the south of Nigeria, while the dry climate can mostly be seen in north of the country. In Nigeria, the peak season for measles transmission begins in January and runs through May; the transmission peak is generally attained in the dry season in Sub-Saharan Africa.

Nigeria introduced [measles vaccination](#) into the routine immunization program in 1978 for children aged 9 months. Also during the early measles control period, case-based measles surveillance was initiated in 2006. In 2011, the country endorsed the 2020 elimination goal of reduction of measles incidence to less than 1 case / 0.000001 population per year, and achievement of at least 95% MCV1 coverage in routine immunization and during campaigns at both national and district levels. Measles surveillance performance indicator targets should be maintained and include obtaining a blood specimen from ≥ 1 suspected measles case in at least 80% of districts annually, and investigating 2 or more cases of non-measles febrile rash illness per 100,000 population annually. During the early stages of implementing measles control activities, Nigeria conducted an initial “catch-up” campaign (target age: 9 months to 15 years; Administrative coverage: 96.0%) in late 2005, and a “follow-up” campaign (target age: 9 months to 4 years; Administrative coverage: 112.0%) in 2008. With routine MCV1 coverage of less than 50.0%, high incidence rate and the persistence of measles outbreaks, the country has been conducting nationwide [mass](#)

[vaccination](#) campaigns every 2 years. The National MCV1 coverage was 33% in 2000, 44% in 2006, and 41% in 2007; the country saw its measles vaccination coverage slightly increasing from 53% in 2008 to 56% in 2010. The incidence of confirmed measles was 2 cases per million in 2006 and increased to 16 cases per million in 2007 and 68 cases per million in 2008 as more cases were captured by the recently-introduced system.

Previous studies have described progress toward measles elimination in Nigeria during 2005–2008 and 2012–2016.

This research work is embarked upon in order to predict for the likely future occurrence of measles cases in Nigeria. Measles is one of the most deadly diseases that ravage the health status of young ones and even the adult are not spare from it.

The aim of this research work is the application of ARIMA model to measles cases in Nigeria

Objectives of the research work includes:

- a. To test for data stationarity.
- b. To fit a time series model that best describes measles data.
- c. To forecast for the expected measles cases in Nigeria
- d. To give recommendation based on findings

The study will cover primarily measles cases in Nigeria. Stationarity was observed before model selection. ARIMA (1,1,1) model was selected as the best fitted model for the collected data (yearly measles cases). Bayesian Information Criterion

(BIC) was also considered. Other parameters from the resulted output includes: Stationary r square, and Mean absolute Error.

2.0 LITERATURE REVIEW

This chapter present the review of the theoretical and empirical literature as it relates to measles cases recorded in Nigeria.

Empirical review

This research study applied ARIMA modeling also known as Box- Jenkins approach to analyze and forecast measles cases in Nigeria. ARIMA is the method first introduced by Box and Jenkins (1976) and until now has become the most popular models for forecasting univariate time series data (Harris *et al*, 2012). The primary principle of methodology involves forecasting the future values of a particular variable using the past or lagged values of the same variable. The ideology is termed “let the data speak for themselves” according to (Gujarati, 2004). Procedures involved in ARIMA modeling is explained below.

The Box-Jenkins ARIMA model has evolved from the combination of AR (Autoregressive) and MA (Moving Average), the ARMA models.

Theoretical Review

Richmond (1957), defined time series as a set of observation on the same variable, such that the observations are ordered in time, the successive observation differ among themselves, not only because of sampling variations and other chance or random effects, but also because the true value

of the variable being measured-the parameter, is changing over time.

Raymond and Tse (1997) suggested that the following two questions must be answered to identify the data series in a time series analysis: (1) whether the data are random; and (2) have any trends? This is followed by another three steps of model identification, parameter estimation and testing for model validity. If a series is random, the correlation between successive values in a time series is close to zero. If the observations of time series are statistically dependent on each another, then the ARIMA is appropriate for the time series analysis.

Meyler et al (1998) drew a framework for ARIMA time series models for forecasting Irish inflation. In their research, they emphasized heavily on optimizing forecast performance while focusing more on minimizing out-of-sample forecast errors rather than maximizing in-sample 'goodness of fit'.

Stergiou (1989) in his research used ARIMA model technique on a 17 years' time series data (from 1964 to 1980 and 204 observations) of monthly catches of pilchard (*Sardina pilchardus*) from Greek waters for forecasting up to 12 months ahead and forecasts were compared with actual data for 1981 which was not used in the estimation of the parameters.

Contreras et al (2003) in their study, using ARIMA methodology, provided a method to predict next-day electricity prices both for spot markets and long-term contracts for mainland Spain and Californian markets. In fact a plethora

of research studies is available to justify that a careful and precise selection of ARIMA model can be fitted to the time series data of single variable (with any kind of pattern in the series and with autocorrelations between the successive values in the time series) to forecast, with better accuracy, the future values in the series.

3.0 RESEARCH METHODOLOGY

The data used for this study is secondary data on yearly measles cases in Nigeria. The data is from 1988 to 2022 and was obtained from online statistical database www.knoema.com

ARIMA was used to model and forecast the yearly measles cases in Nigeria.

ARMA model

ARMA models are the combination of the simple auto regressive moving average model of order (p, q) called the Autoregressive moving Average (ARMA) model. The p represents the order of the autoregressive process and the q represents the order of the moving average process. The general form of the model is given by

$$y_t = \mu + \phi_1 y_{t-1} + \phi_2 y_{t-2} + \dots + \phi_p y_{t-p} + \varepsilon_t + \theta_1 \varepsilon_{t-1} + \theta_2 \varepsilon_{t-2} + \dots + \theta_p \varepsilon_{t-p}$$

3.1

Autoregressive (AR) Models

A time series is said to be autoregressive, if based on its past values, future values can be predicted. An autoregressive process of order one tells us that based on the lag one (1) variable of the time

series, the future values of the series can be predicted. It simply tells what will happen in the future depends on what is happening today. The AR (1) model is given by the equation

$$y_t = \phi_0 + \phi_1 y_{t-1} + \varepsilon_t,$$

3.2

where ε_t is assumed to be a white noise process. AR (2) model is also given as

$$y_t = \phi_0 + \phi_1 y_{t-1} + \phi_2 y_{t-2} + \varepsilon_t.$$

3.3

Generally, an autoregressive model of order p is given by the equation below

$$y_t = \phi_0 + \phi_1 y_{t-1} + \phi_2 y_{t-2} \dots \phi_p y_{t-p} + \varepsilon_t,$$

3.4

where ε_t is assumed to be a white noise,

y_t = Response variable at time t,

y_{t-1} = Observation (predictor variable) at time t-k,

ϕ_i = Regression coefficients to be estimated

Moving Average (MA) Models

A time series y_t is said to follow moving average process if the current value of the observation is in terms of the past shocks or residuals. This means that based on past values of the residuals, the future values of the series can be predicted. The moving average models are always stationary because the moments are time invariant. A simple MA (1) model is given by

$$y_t = \mu + \varepsilon_t + \theta_1 \varepsilon_{t-1},$$

3.5

where ε_t is the white noise process. An MA model of order 2 written as MA (2) is also given as;

$$y_t = \mu + \varepsilon_t + \theta_1 \varepsilon_{t-1} + \theta_2 \varepsilon_{t-2}$$

3.6

Generally a moving average of order q is a regression model with the dependent variable, y_t depending on the previous values of the error rather than the variable itself.

$$y_t = \mu + \varepsilon_t + \theta_1 \varepsilon_{t-1} + \theta_2 \varepsilon_{t-2} + \dots + \theta_p \varepsilon_{t-p}$$

3.7

Where y_t = response variable at time t, μ = constant mean of the process, $\theta_1, \theta_2, \dots, \theta_p$ = Coefficients of regression to be estimated and ε_{t-p} = the error in time t-p.

Autoregressive Integrated Moving Average (ARIMA) Model

One approach, advocated in the landmark work of Box and Jenkins, (1976) is the development of systematic class of models called Autoregressive integrated moving Average (ARIMA) models to handle time-correlated modeling and forecasting. It is the generalization of the ARIMA (p, q) models to provide adequate models for a non-stationary time-series variables.

The ARIMA models are generally referred to as ARIMA (p, d, q) models, where p is the order of AR (autoregressive Process), q is the order of the moving average process and the d is the order of

the integration of the series (that is the number of times the series has to be differenced in order to make it stationary). If the series is differenced once, then we say the series is integrated of order one and if the series is differenced d times to make it stationary, then we say the series is integrated of order d .

Stationarity test of Time Series Data

ARIMA model is generally applied for stationary time series data after differencing. A time plot of the data can suggest whether the time series needs any differencing before performing formal tests. Also, the stationarity and non-stationarity properties are checked by applying sequence plot that shows when the time series is said to be stationary if both the mean and the variance are constant over time. The ADF statistic is a negative number and the more negative it is, the stronger the rejection of the hypothesis that there is a unit root at some level of confidence i.e., the time series is nonstationary. If the time series is non-stationary, we do the first differencing or a higher order differencing till the time series becomes stationary. The times of differencing the series is indicated by the parameter d in the ARIMA (p,d,q) model.

Forecasting

After the Box-Jenkins approach, the selected model will be used in forecasting the future values of the variable under consideration.

4.0

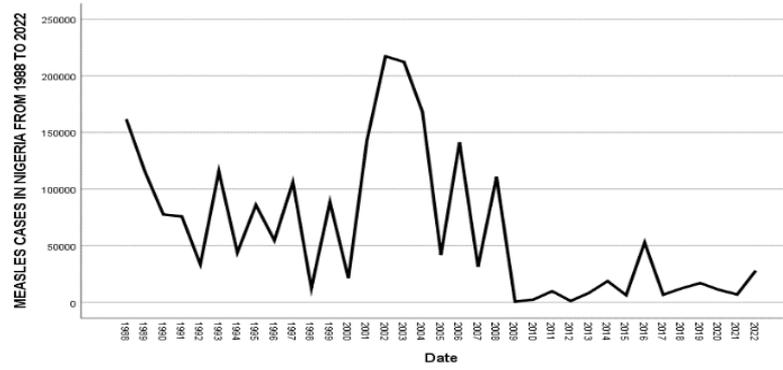


Figure 1

As shown in Figure 1. The time series plot above has shown how the measles cases dataset behaves through equally spaced time interval. It can be observed that the yearly measles cases recorded exhibit a form of upward and downward though not stationary over time. Therefore, there will be need for differencing in order to attain stationarity. They also depicts that the measles yearly dataset does not have a constant mean and variance over time. Therefore, there is need to adjust them to form a stationary series, so that the values vary more or less uniformly about a fixed level over time.

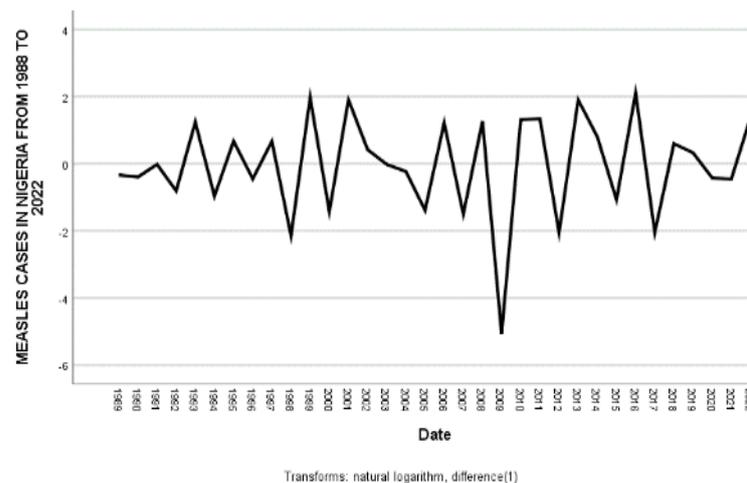


Figure 2

From figure 2, time series is said to be stationary if both the mean and the variance are constant

over time. A time plot of the data in figure 2 can suggest that the time series is stationary and does not need any further differencing before performing formal tests.

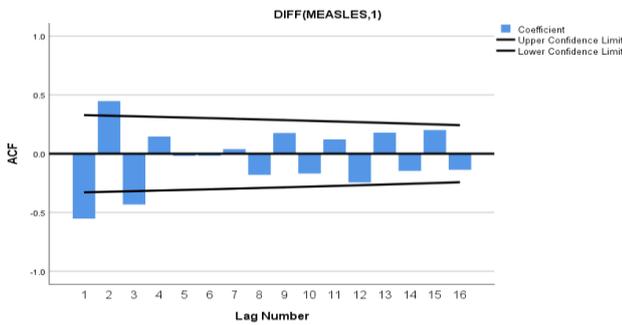


Figure 3

Figure 3 shows the autocorrelation plot

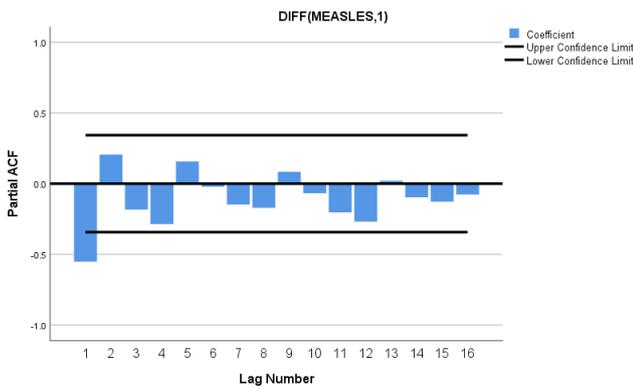


Figure 4

Figure 4 above shows the partial autocorrelation plot Model identification, which is determined by the order of AR and MA. The order of AR and MA component is suggested by the sample ACF and PACF plots based on the Box-Jenkins approach. The significant lag of the PACF suggests the order of AR while the significant lag of the ACF suggests the order of MA.

The ACF and PACF plots respectively suggest that $p=0$, $d=1$ and $q=0$ ARIMA (0,1,0) was needed to describe this data set autoregressive, integration order and moving average process respectively. The principle of parsimony was upheld.

Table 4.2 shows the model description used for the data analysis

Table 4.1

Model Description

			Model Type
Model ID	DIFF(MEASLES,1)	Model_1	ARIMA(1,1,1)

Table 4.2 show the result of the fitted model for measles cases from 1988 to 2022

Fit Statistic	Mean
Stationary R-squared	.753
R-squared	.728
RMSE	56307.205
MAPE	255.606
MaxAPE	2731.593
MAE	37615.925
MaxAE	156299.988
Normalized BIC	22.195

Table 4.3

Model	Model Fit statistics					Ljung-Box Statistics
	Stationary R	R-squared	MAPE	MAE	Normalized B	
DIFF(MEASLES,1)	0.753	0.728	255.606	37615.925	22.195	9.518

Table 4.4

Forecast

Model		2023	2024	2025
DIFF(MEASLES,1)- Model_1	Forecast	-9935	6731	-1624
	UCL	101094	130583	126333
	LCL	-120963	-117121	-129581

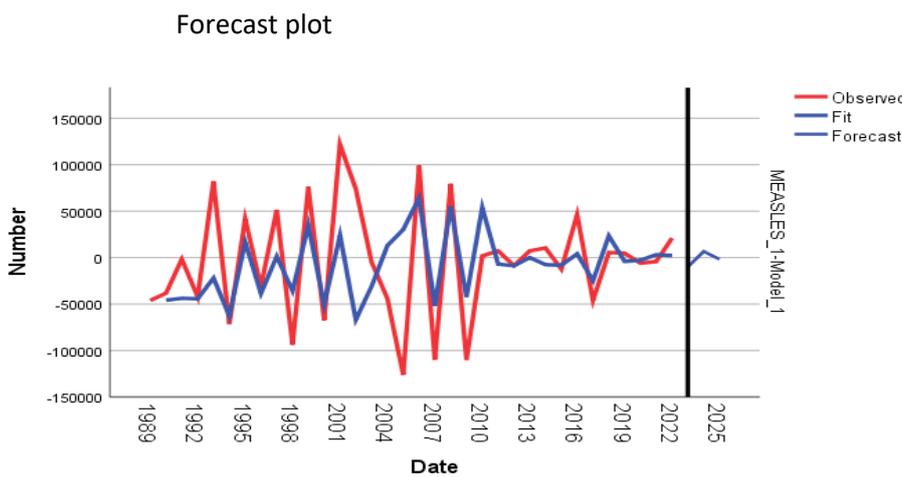


Figure 5

Discussion of findings

Figure 1 and 2 shows the sequence plot and stationarity (differencing) plot. Auto regressive integrated moving average ARIMA (1, 1, 1) was used in this research work. Table 4.1 and 4.2 shows the selected model and model summary (measles cases _1). Minimum average percentage error (MAPE) is 288.14, the Maximum average percentage error (MaxPE) is 2557.864, the goodness of fit measures (measles cases_1) with r squared is 0.72, mean absolute error is 40080.15, and normalized BIC is 22.195.

From the autocorrelation and partial autocorrelation plot of lags, the ACF lag plot shows that $AR = p = 1$, with difference of 1. The Partial ACF plot of lags, also show that $MA = q = 1$ with difference of $1 = I$.

Therefore $AR = 1$, $DIFF = I = 1$, $MA = q = 1$ Which indicate that ARIMA (1,1,1) process can be used for the model prediction.

Conclusion

It was observed that, fitted model have a good measure that compares the stationary part of the measles cases _1 to a simple mean model. Positive value of stationary r square (0.771) shows that the model under consideration is better than a baseline model. From r square calculated, 72.2% estimate of the proportion of

the total variation is in the series (measles cases $_1$) is explained in the model. The research study has found out that measles cases in Nigeria could be best modeled with ARIMA (1,1,1).

Recommendation

The result of this research work can be used as a guide to prepare for future occurrence of meningitis in Nigeria.

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ANTI-DIABETIC PROPERTY OF ETHANOL EXTRACT OF *Coccinia indica* LEAVES AGAINST ALLOXAN INDUCED HYPERGLYCEMIA ALBINO RATS

Akuba, B.O¹, Ojade, F.E¹ and Balogun, S. G.**.

¹Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic Lokoja.

Corresponding Author: akubabarnabas32@gmail.com

Abstract

Diabetes mellitus is a complex disease which is marked with distinctive hyperglycemia, lipoprotein abnormalities, defect in reactive oxygen species scavenging enzymes, raised basal metabolic rate and altered intermediary metabolism of major food substances. The hyperglycaemia in diabetes mellitus can evolve from an absolute under secretion of insulin (type 1 DM), insulin action (type 2 DM) or both. Hyperglycaemia is the hallmark of diabetes mellitus responsible for its cardinal symptoms. This study was aimed at investigating the anti-diabetic property of ethanol extract of Coccinia indica leaves (CODILE) on sugar levels and bodyweight of hyperglycaemic rats. The percentage yields of the ethanol extract of Coccinia indica leaves (CODILE) was found to be 6.4%w/w. The qualitative phytochemical screening revealed that CODILE contain alkaloids, terpenoids, tannins, flavonoids and saponins. The lethal dose (LD₅₀) of the extract was estimated to be above 5000mg/kg body weight. Hyperglycaemia was induced in albino rats by administration of a single dose (150mg/kg) of alloxan intraperitoneally. Rats having fasting blood glucose (FBG) \geq 200mg/dl after 72h was considered hyperglycaemic. Anti-diabetic study was investigated for 28 days with four groups of hyperglycaemic rats. Four groups of hyperglycaemia rats and one group of normal rats were used for the study. Groups 1 and 2 served as non-diabetic and diabetic controls respectively. Groups 3 and 4 received 160 and 320 mg CODILE /kg body weight respectively, groups 5 received metformin (MEN) (150 mg/kg) alone. Weekly fasting blood glucose levels and body weight change were monitored for 28 days. The results revealed that there is a significantly ($p < 0.05$) reduction of blood glucose level of all the treated groups on day 7, 14, 21 and 28 when compared with diabetic control group. It also revealed significantly ($p < 0.05$) decreased of blood sugar level of the metformin (MEN) treated group than the untreated group. No distinctive difference was observed across the body weight of treated rats when compared to diabetic control. This study revealed that CODILE possesses anti-diabetic activity and can be used in the management of type 2 diabetes.

Keywords: *Coccinia indica*, Metformin, Fasting blood sugar, Hyperglycaemia, Body weight.

Introduction

Diabetes mellitus is a disease characterized by hyperglycaemia caused by under secretion of insulin, insulin action or both (Khan *et al.*, 2009). It affects the metabolism of macromolecules such as carbohydrates, fats and proteins in the body, leading to multiple complications including neuropathy, nephropathy and retinopathy (Afolayan & Sunmonu, 2010). In 2013, according to the International Diabetes Federation, people living with diabetes were 382 million worldwide and are projected to rise to 592 million by 2035. The management of hyperglycemia is the hallmark of treatment in diabetes. Current data revealed that diabetes is on the rise from 143 million persons to 300 million persons by 2025 (Mitra *et al.*, 2007). Many therapeutic approaches have been deployed for treatment of diabetes including the use of oral hypoglycaemic agents. Despite numerous orthodox medications available for the management of hyperglycemia but there has been elevated demand for the use of plant products with anti-diabetic potentials.

Coccinia indica belongs to the family Cucurbitaceae commonly called little Kanduri ki bel in Hindi, gourd or Rantondli in Marathi and Bimba in Sanskrit. It is indigenous to Bengal and other parts of India. *C. indica* grows abundantly all over India, tropical Africa, Australia, and Fiji and throughout the oriental countries. Different parts of the plant are used in India and tropical Africa for folk medicine for treatment of number of ailments including diabetes, inflammation, wounds, ulcers, in eruptions of skin, cough, fever and asthma. Scientific investigation of *C. indica* revealed that the crude extract has antioxidant, hepatoprotective, anti-diabetic, anti-inflammatory and antinociceptive, hypolipidemic, anti-bacterial, and anti tussive activities has been in vogue from ancient times (Kuma *et al.*, 2010). This study was aimed at investigating the antidiabetic property of ethanol extract of *Coccinia indica* leaves against alloxan induced hyperglycaemia rats.

Methodology

Collection and identification of plant materials

Coccinia indica was gotten from Ejule, Ofu LGA of Kogi State and identified by Mr. Akanni Theophilus Gbenga Department of Biological Science, Federal University Lokoja, Kogi State, Nigeria.

Chemicals and drugs

Metformin (Glucobay® Bayer), Methanol (BDH, England), Alloxan monohydrate (Sigma-Aldrich, UK), were purchased from the country representative of Sigma Chemical, St. Louis USA. A digital glucometer and its corresponding test strips (Fine Test®, Infopia Co., Ltd. USA) were purchased from a Dave mercy pharmacy store in Lokoja Local Government Area, Kogi State, Nigeria. All other chemicals used were of analytical standard.

Animals

Healthy rats weighing 120-190g of either sex, purchased from Kwara State, Nigeria were used for this investigation. They were maintained in stainless steel cages at normal and standard laboratory conditions. They were maintained on clean water and pelleted rat feed. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985).

Extract Preparation

The plant leaves were pulverized and coarse into powder in an electric hammer mill. The 1000g of the plant powdered was extracted with ethanol by cold maceration with occasional shaking for 72 h. Then filtered using Whatman filter paper (No 1) to obtain the filtrate. The filtrates were evaporated to dryness on a hot water bath at 45°C to obtain the concentrated extract. The percentage yield of the extract was calculated using the relation:

$$\begin{aligned} & \text{Percentage Yield (\%)} \\ &= \frac{\text{Weight of extract (g)}}{\text{Weight of pulverized stem bark (g)}} \\ & \times 100 \end{aligned}$$

The percentage yield of the extract is 4.9% and the extract was henceforth referred to as CODILE.

Phytochemical screening

The phytochemical composition of *Coccinia indica* extract was evaluated using methods as described by Ejikeme *et al.* (2014).

Median lethal dose study

The oral median lethal dose (LD₅₀) of the extract was determined in rats according to procedures documented by Lorke (1983). The study was evaluated in two phases. First phase, nine rats were randomized into three (3) groups of three (3) rats which graded dose of 10, 100, and 1000mg/kg body weight of CODILE was administered. The rats were kept under the same conditions and observed for signs of toxicity, but were not limited to paw-licking, stretching, respiratory distress and mortality for 24h. Based on the findings of the first phase, the following graded dose 1600, 2900 and 5000mg/kg body weight of CODILE were administered to another set of three (3) groups of three rats in the second phase. These rats were also monitored closely for 24 hours after treatment for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD₅₀. The LD₅₀ was calculated as the geometric means of the maximum dose producing 0 % mortality (D₀) and the minimum dose that produced 100 % mortality (D₁₀₀) and mathematically expressed as:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Induction of hyperglycaemia

The procedures as documented by Dunn and McLetchie (1943) were adopted for these investigations. Experimental rats were fasted overnight; freshly prepared alloxan (150mg/ kg

body weight) was administered intraperitoneally. The animals were thereafter allowed food and water *ad libitum*. After 5 days, the blood glucose levels in the rats were monitored using a Fine Test[®] digital glucometer and the corresponding test strip following an overnight fast. Rats having fasting blood glucose (FBS) level ≥ 200 mg/ dl were considered hyperglycaemic.

Animal experimental design

Twenty (20) rats divided into five groups of four (4) rats each. Group 1 and 2 served as the normal and diabetic controls respectively, received 1ml distilled water/kg body weight. Rats in group 3 and 4 received 160 and 320 mg /kg CODILE respectively. Rats in group 5 rats were treated with 150 mg / kg Metformin (MEN) body weight only. FBS (fasting blood sugar) and body weight was estimated on weekly bases.

Statistical Analysis

All data obtained was expressed as Mean \pm deviation and analysed using one- way analysis of variance (ANOVA) followed by Duncan *post-hoc* test for multiple comparison tests. Statistical tool used is statistical package for social science (SPSS) version 20. Values were considered significant at $p \leq 0.05$.

RESULTS

Phytochemical Screening

The present study reveals preliminary phytochemical analysis of the ethanol extract of *Coccinia indica* leaves which tested positive for alkaloids, terpenoids, tannins, flavonoids and saponins, except reducing sugar, steroid and anthraquinones flavonoid and terpenoid (table 1).

Table 1: The Phytochemical screening of the Ethanol leaf Extract of *Coccinia indica* (CODILE)

Phytochemicals	Ethanol leaf extract of <i>Coccinia indica</i>
Tannin	++
Alkaloid	+++
Terpenoid	+
Flavonoid	+++
Saponin	+++
Steroid	ND
Reducing sugar	ND
Anthraquinones	ND

Key: + slightly detected, ++ moderately detected, +++ highly detected and - Not detected

Table 2: LD ₅₀ Study of Ethanol Leaf Extract of <i>Coccinia indica</i> (CODILE)		
Group	Treatment (mg/kg)	ND/NT
FIRST PHASE		
1	10	0/3
2	100	0/3
3	1000	0/3
SECOND PHASE		
1	1600	0/3
2	2900	0/3
3	5000	0/3
ND/NT: Number of rats deaths/number of rats treated		

Median lethal dose study

Table 2 reveals that, the ethanol leaf extract of *Coccinia indica* did not show any sign of toxicity or mortality after the first and second phases, during the monitoring period at the doses administered orally. Therefore, the oral median lethal dose (LD₅₀) of the extract was estimated to be above 5000 mg/kg according to method documented by (Lorke’s, 1983).

Outcome of Ethanol Leaf Extract of *Coccinia indica* (CODILE) and Metformin (MEN) on FBS of Rats

The FBS of the various hyperglycaemic groups are contained in table 3. The mean blood sugar level of all animals in the treated groups with the exception of group 1 (Normal control) were all diabetic. However, the mean blood sugar levels of groups 3, 4 and 5 showed statistically significant (p< 0.05) decrease in blood sugar on day 14, 21 and 28 respectively when compared with diabetic control (group 2). The mean blood sugar level of group 3 (160mg/kg CODILE) and group 4 (320mg/kg CODILE) showed a

statistically ($p < 0.05$) decrease in blood sugar on day 7, 14, 21 and 28 respectively when compared to the diabetic control (group 2).

The mean blood sugar level of group 5 (150mg/kg MEN) showed a statistically ($p < 0.05$) decrease in blood sugar on day 14, 21 and 28 respectively when compared to diabetic control (group 2), but showed no significant ($p < 0.05$)

difference when compared to group 3 (160mg/kg CODILE) and group 4 (320mg/kg CODILE) on day 14, 21 and 28 respectively.

Table 3: Outcome of Ethanol Leaf Extract of *Coccinia indica* (CODILE) and Metformin (MEN) against FBS of Hyperglycaemic albino Rats.

Treatment	Post- Treatment Time (Day)				
	0	7	14	21	28
Group 1	80.3±4.04 ^a	79.6±4.91 ^b	80.3±4.04 ^a	79.6±2.08 ^a	77.6±2.08 ^a
Group 2	191.3±7.50 ^b	197.6±9.01 ^c	203.0±11.53 ^c	207.0±14.42 ^c	210.6±16.21 ^d
Group 3	191.3±3.51 ^b	186.3±2.08 ^b	181.3±1.15 ^b	159.6±6.80 ^b	152.3±2.08 ^c
Group 4	201.3±12.05 ^b	187.6±2.51 ^b	174.3±10.78 ^b	156.3±5.13 ^b	130.3±7.6 ^b
Group 5	196.3±4.04 ^b	190.00±2.00 ^{bc}	179.00±8.54 ^b	163.3±13.31 ^b	141.3±16.04 ^{bc}

Data are expressed as mean ± S.D of FBS. Data was analyzed by one- way ANOVA followed by Duncan *post – hoc* test for multiple comparisons, (n=4). Values with different lowercase letters as superscripts denote level of significance ($p < 0.05$) down the column.

Group 1: Receive 1 ml distilled water (Normal control)

Group 2: Received 1 ml distilled water (Diabetic control)

Group 3: Received CODILE (160mg/kg body weight)

Group 4: Received CODILE (320mg/kg body weight)

Group 5: Received MEN (150mg/kg body weight)

Outcome of Ethanol Leaf Extract of *Coccinia indica* (CODILE) and Metformin (MEN) on Average Body Weight of Hyperglycaemic Albino Rats.

Table 4 revealed that no significant difference in the mean body weight of rats in the hyperglycaemic groups on day 7, 14, 21 and 28 when compared to day 0 across the groups.

Table 4: Outcome of Ethanol Leaf Extract of *Coccinia indica* (CODILE) and Metformin (MEN) on Average Body Weight of Hyperglycaemic Albino Rats.

Treatment	Post-treatment time (Day)				
	0	7	14	21	28
Group 1	194.2±2.43 ^a	192.8±1.90 ^a	192.4±3.13 ^a	193.1±4.42 ^a	193.7±3.48 ^a
Group 2	175.2±17.37 ^a	169.5±16.73 ^a	166.7±16.95 ^a	166.4±18.38 ^a	155.0±24.48 ^a
Group 3	163.2±52.10 ^a	155.07±49.16 ^a	150.0±49.11 ^a	147.4±52.05 ^a	142.9±52.23 ^a
Group 4	177.0±50.32 ^a	175.1±49.92 ^a	172.0±48.16 ^a	171.7±49.30 ^a	173.3±48.58 ^a
Group 5	128.9±57.55 ^a	127.0±58.41 ^a	126.7±58.06 ^a	126.9±56.56 ^a	126.0±54.53 ^a

Data are expressed as mean ± S.D of Average Body Weight. Data was analyzed by one- way ANOVA followed by Duncan *post – hoc* test for multiple comparisons, (n=4). Values with different lowercase letters as superscripts denote level of significance (p < 0.05) down the column.

Group 1: Receive 1 ml distilled water (Normal control)

Group 2: Received 1 ml distilled water (Diabetic control)

Group 3: Received CODILE (160mg/kg body weight)

Group 4: Received CODILE (320mg/kg body weight)

Group 5: Received MEN (150mg/kg body weight)

DISCUSSION

The main purpose of diabetes management is to decrease blood sugar levels to the normal range. Multiple drugs are available for the reduction of blood sugar level, but most of them are not without severe adverse effects. Consequently, some diabetics often resort to medicinal plants

and foods with hypoglycaemia property but with fewer or no adverse consequences. This study investigated the antidiabetic property of various doses of the ethanol extract of *Coccinia indica* Leaves (CODILE) against alloxan induced hyperglycaemic rats.

The median lethal dose (LD₅₀) study of the extract produced no physical signs of toxicity such as weakness, gasping, writhing, palpitation, anorexia, decreased respiratory rate or death and reduction in body weight up to the highest administered oral dose of 5000mg/kg within 24 hours of post-administration.

Hyperglycaemia of the animals was induced with the use of alloxan. Induction of diabetes by alloxan is expressed by damaging the insulin producing pancreatic beta- cells of the islets of Langerhans leading to reduced synthesis and release of endogenous insulin (Ghosh, 2005). The induction of diabetes by alloxan is a cytotoxic action mediated by reactive oxygen species with a simultaneous massive elevation in cytosolic calcium level, causing a drastic total destruction of beta- cells producing type 1-like diabetes mellitus (Szkudelski, 2001).

In this investigation, results obtained revealed that the ethanol leaf extract of *Coccinia indica* produced significant reduction in blood sugar level against alloxan induced hyperglycaemic rats in a fashion dose and time dependent style. This is totally in agreement with those of other researchers, who systematically demonstrated that extracts of various parts of plant (leaves, roots, stems, flowers and seeds) possess antidiabetic activities (Jaiswal *et al.*, 2009; Chinwe and Isitua, 2010). Its anti-diabetic activity might be as a result of the depression of gluconeogenic enzymes or inhibition of glucose-6-phosphatase activities in the liver by the extract, subsequent reduction in glucose release to the blood (Naik & Juvekar, 2003). Its anti-diabetic role could also be attributed to the synergistic role of the extract constituents (flavonoids saponins, steroids, alkaloids, terpenoids) present in *Coccinia indica*. Tannin and flavonoid have been documented to stimulate calcium, glucose uptake and cause regeneration of damaged pancreatic islets, (Tapas *et al.*, 2008). Most of the anti-diabetic plants show various mechanisms of antidiabetic activity like, insulin-like effect, alteration of glucose metabolism, reduction of absorption of glucose from intestine, improve glucose tolerance, enhancing insulin signal pathway, generation of beta cell in pancreas, hypoglycemia through increase glucose uptake and glycogen synthesis (Banshidhar & Deepmala, 2013). The anti-diabetic property of the extract was greater than of metformin compared to the hyperglycemic control. This suggested that *Coccinia indica* extract possess anti-diabetic property, therefore can play a significant role in managing diabetes.

The results revealed, decreased in body weights in first week of all rats in hyperglycaemic groups. This observation could be accrued to the rate conversion of storage fat and proteins to glucose (gluconeogenesis) (Champe *et al.*, 2005). However, steady increased of body weight was observed across the treated hyperglycaemic groups after the first week, probably due to improvement in glucose uptake by cells. The results of this study is in agreement with that of Kim *et al.* (1996) which determined the effect of leptin sensitivity in adult and aged rats and found

a slight increase in weight after an initial fall (in the first week) in adult rats given metformin. This study revealed slight difference in weights of the rats in treated groups compare with diabetic control and normal control suggesting that the use of *Coccinia indica* extract in diabetics will not bring about an increase in weight.

CONCLUSION

In conclusion, results from this investigation clearly demonstrated the anti-diabetic property of *Coccinia indica* via its significant reduction of hyperglycaemia in diabetic rats. Therefore, *Coccinia indica* could be potentially used as natural therapy for management of type 2 diabetes mellitus.

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Ameliorative Effect of Ethanol Leaf Extract of *Cassia tora* on Alloxan Induced Diabetic Rats.

Akuba, B.O¹, Ojade, F.E¹ and Balogun, S. G.^{1**}.

¹Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic Lokoja.

Corresponding Author: Akuba, Barnabas Ojochegebe, Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic, Lokoja.
Tel: +2348069616697, E-mail: akubabarnabas32@gmail.com

ABSTRACT

*Diabetes accounts for the leading cause of mortality and morbidity worldwide. Hyperglycaemia is the hallmark of diabetes mellitus responsible for its cardinal symptoms. Oxidative stress resulting from the hyperglycemic condition in Type 2 diabetes has been implicated in the impairment of the pancreatic β -cells and diabetes complications such as diabetes nephropathy (damage to the kidney), cirrhosis (damage to the liver), diabetes retinopathy (damage to the eye) The aim of this study is to evaluate the effect of ethanol leaf extract of *Cassia tora* (CASTOLE) on some serum biochemical parameters and bodyweight of diabetic rats. Four groups of diabetic rats and one group of normoglycaemic rats were used for the study. Groups I and II served as non-diabetic and controls respectively. Groups III and IV received 250 and 350 mg CASTOLE /kg body weight respectively, groups V received metformin (MET) (150 mg/kg). Weekly body weight changes were estimated for 28 days. The blood was collected for serum biochemical analysis. The result revealed that there is a significant decrease in the body weight of the animals during the first week and mark significant increase was observed in the body weight of treated rats when compared to diabetic control after 28 dyas. Also, liver biomarker enzymes (AST, ALT and ALP), Total bilirubin (TBIL) and Albumin (ALB) activities were significantly ($p < 0.05$) reduced and increased Plasma Total Protein (TP) in the treated groups compared with the diabetic control group indicating that the extract has an ameliorative impact on the liver. Rise in liver enzymes was almost restored to normal in animals treated with *Cassia tora* extract. This study revealed that CASTOLE possesses hepatoprotective activity and could be used to maintain the integrity of the liver during the management of type 2 diabetes.*

Key words: *Cassia tora*, Metformin, Serum biochemical parameters, Diabetes.

Introduction

Diabetes Mellitus (DM) commonly referred to as diabetes is a group of metabolic diseases characterized by hyperglycemia (High blood sugar levels over a prolonged period), either because the pancreas does not produce enough insulin, or because the cells do not respond to the insulin that is produced (David and Gardner, 2011). The main symptoms of high blood sugar include polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). If left untreated, this chronic disease can cause many complications (Cooke and Plotnick, 2008).

Type 2 diabetes is the most common form of diabetes (Shi and Hu 2014); it is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (David and Gardener, 2011), leading to hyperglycemia and ultimately malfunctioning of the pancreatic β -cells. Prolonged hyperglycemia results in increased generation of reactive oxygen species (ROS) and alteration of endogenous antioxidants (Ohkuwa *et al.*, 2005).

Oxidative stress resulting from the hyperglycemic condition in Type 2 diabetes has

been implicated in the impairment of the pancreatic β -cells and diabetes complications such as diabetes nephropathy (damage to the kidney), cirrhosis (damage to the liver) (Shukla *et al.*, 2003), diabetes retinopathy (damage to the eye) (Bears *et al.*, 2004; Hove *et al.*, 2004) and diabetes neuropathy (damage to the nerves of the body) (Seki *et al.*, 2004). Therefore, it is important to counteract diabetic pathophysiology through multi-target therapeutic agent. Multi-component plant extract (chemically standardized) could offer this multimodal therapeutic value.

Cassia tora is a legume belonging to the *caesalpinaceae* family. It grows wild mostly in the tropics and is considered a weed in many places (Kubmarawa *et al.*, 2011). The plant is an annual herbaceous herb, almost an under shrub up to 30-90cm high with pinnate leaves which is about 10cm long. Each leaf has three pairs of leaflets that are opposite and oblique at the base. The yellow coloured flowers are bearded in the axel of the leaves. The plant bears flowers in rainy seasons. Common names of *Cassia tora* are sickle pod (English), sickle senna (English), foetid cassia (English), chueh-mintsu (China), 'tapsa' (Nigeria). The leaves of *Cassia tora* is used for soup preparations in some parts of northern Nigeria (Kubmarawa *et al.*, 2011) and popularly consumed in Adamawa state of

Nigeria. This study investigated the evaluation of hypoglycaemic efficacy of ethanol extract of *Cassia tora* leaves to identify possible mechanisms of its blood glucose-lowering effects and glycemic control in diabetes.

Materials and methods

Collection and identification of plant material

The plant of *Cassia tora* was collected fresh from Kogi State and was identified by Mr. Akanni Theophilus Gbenga of Biological Science Department of Federal University Lokoja, Kogi State.

Preparation of the Extract

The leaves of the plant were pulverized to coarse powder in an electric hammer mill. The plant powdered material was extracted with ethanol by cold maceration with occasional shaking for 72 h. Then filtered using Whatman filter paper (No 1) to obtain the filtrate. The filtrates were concentrated and evaporated to dryness on a hot water bath at 45°C to obtain the extract. The percentage yield of the extract is 4.6% and the extract will henceforth be referred to as CASTOLE.

Phytochemical screening

The phytochemical composition of each extract was determined using methods variously

described by Trease and Evans (1996) and Sofowara (2006).

Animals

Healthy Wistar rats of either sex weighing 130-150g were used for the study. They were maintained in stainless steel cages at normal and standard laboratory conditions. They were maintained on clean water and pelleted rat feed. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985).

Acute toxicity study

The oral median lethal dose (LD₅₀) of the extract was determined according to the method described by Lorke (1983). The study was investigated in two phases. First phase, nine rats were randomized into three groups of three rats which were given CASTOLE at doses of 10, 100, and 1000mg/kg body weight. The rats were kept under the same conditions and observed for signs of toxicity which included paw-licking, stretching, respiratory distress and mortality for 24h. Based on the results of the initial phase, the following CASTOLE doses- 1600, 2900 and 5000mg/kg body weight were administered to another set of three groups of three rats in the

second phase. These rats were also monitored closely for 24 h post treatment for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD₅₀. The LD₅₀ was calculated as the geometric means of the maximum dose producing 0 % mortality (D₀) and the minimum dose that produced 100 % mortality (D₁₀₀) and mathematically expressed as:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Induction of hyperglycaemia

The Induction of hyperglycaemia was carried out according to method described by Dunn and McLetchie (1943). Experimental rats were fasted overnight; freshly prepared alloxan (150mg/ kg body weight) was administered intraperitoneally. The animals were thereafter allowed food and water *ad libitum*. The day which alloxan was administered was considered as day 1. After 4 days (day 5), the blood glucose levels in the rats were determined following an overnight fast, using a Fine Test[®] digital glucometer and the corresponding test strip. Rats having fasting blood glucose (FBS) level \geq 200mg/ dl were considered hyperglycaemic.

ANIMAL EXPERIMENTAL MODEL

Five (5) groups (4 groups of hyperglycaemic rats and 1 group of normoglycaemic rats) of four (4) animals each were used for the investigation. Groups III and IV rats received 250 and 350 mg /kg CASTOLE respectively. Groups V Rats were treated with 150 mg / kg Metformin (MET) body weight. Group I and II served as the normal and diabetic controls respectively, received 1ml distilled water/kg body weight.

Weekly body weight change

Rats in all groups were weighed weekly during the period of treatment and on the day before diabetic induction for changes in body weight.

Serum biochemistry

On the 28th day of the experiment, all the rats were euthanized by chloroform inhalation and blood samples were collected by cardiac puncture. The blood was collected into plain serum tubes, allowed to clot and centrifuged for 10 minutes at 3500 rpm. The homogenates were separated, stored in the refrigerator and used for evaluation of biochemical parameters, (aspartate transaminase (AST), alanine transaminase (ALT) levels, alkaline phosphatase (ALP) levels,

total protein, albumin, total bilirubin (TBIL) were determined using Randox diagnostic kits. Method described by Reitman and Frankel, (1957) was adopted.

Statistical Analysis

All data were expressed as mean ± SD. The data were analysed by one way analysis of variance (ANOVA) followed by Duncan *post –hoc* test using the Statistical Package for Social Sciences (SPSS), version 20. Differences of P < 0.05 were considered statistically significant.

RESULTS

Phytochemical Screening

In present study, table 1 reveals the preliminary phytochemical analysis of the ethanol extract of *Coccinia indica* leaves which tested positive for Tannin, Alkaloid, Flavonoid, Steroid, Saponin, Terpenoid and Reducing sugar except Anthraquinones using methods previously described by Trease and Evans (1996) and Sofowara (2006).

Table 1: Phytochemical analysis of the Ethanol Extract of *Cassia tora* Leaves (CASTOLE)

Phytochemicals	Ethanol extract of <i>Cassia tora</i> Leaves
Tannin	+++
Alkaloid	++
Terpenoid	+
Flavonoid	++
Saponin	++
Steroid	++
Reducing sugar	+
Anthraquinones	ND
Key: + slightly detected, ++ moderately detected, +++ highly detected, ND: Not detected	

D/T: Number of deaths/number of rats treated

Acute Toxicity Study

In the first phase of the experiment, the methanol extract of *Cassia tora* leaves did not show any sign of toxicity or mortality during the monitoring period at the doses administered orally. The second phase of the experiment at the dose of 5000mg/kg body weight, one sign of toxicity and mortality was recorded at the dose administered orally. Hence the oral median lethal dose (LD₅₀) of the extract was therefore estimated to be above 5000 mg/kg according to method described by (Lorke’s, 1983).

Table 2: Acute Toxicity Study of the Ethanol Extract of *Cassia tora* Leaves (CASTOLE)

Group	Treatment (mg/kg)	D/T
PHASE I		
1	10	0/3
2	100	0/3
3	100	0/3
PHASE II		
1	1600	0/3
2	2900	0/3
3	5000	0/3

Effect of Ethanol Extract of *Cassia tora* Leaves (CASTOLE) and Metformin (MET) on Average Body Weight of Rats.

Table 3 reveals a slightly statistically significant difference was found in the mean body weight of rats in the hyperglycaemic groups when compared with group 2(diabetic control), baseline group 1 (Non-diabetic control) and between treatment groups.

Effect of Ethanol Extract of *Cassia tora* Leaves (CASTOLE) and Metformin (MET) on some Serum Biochemical Parameters of Alloxan-induced Hyperglycaemic Rats.

Table 4 showed the serum biochemical analysis after 28 days of treatment that treated group 3 exhibited statistically significant ($p < 0.05$) difference in serum ALT, ALP, AST, TBIL, ALB and TP when compared to group 2 (diabetic control). The treated group 4 revealed a statistical significant ($p < 0.05$) decrease in AST, ALT, ALP and ALB when compared to group 3 and was found to be statistically significant ($p < 0.05$) decrease in AST, ALT, ALP and TBIL, higher in TP and ALB when compared to group 2 (Diabetic control).

The metformin (MET) treated group 5 shows a statistically significant ($p < 0.05$) decrease in AST, ALT, ALP and TBIL, higher in TP and ALB, when compared with group 2, 3, and 4.

Table 3: Effect of Ethanol Extract of *Cassia tora* Leaves (CASTOLE) and Metformin (MET) on Average Body Weight of Hyperglycaemic Rats.

Treatment	Post-treatment time (Days)				
	0	7	14	21	28
Group 1	116.5±33.09 ^a	116.0±11.31 ^a	117.7±3.54 ^a	117.0±5.41 ^a	117.5±5.30 ^a
Group 2	152.3±15.25 ^c	136.5±5.33 ^b	136.8±19.32 ^b	138.0±9.11 ^b	138.0±8.23 ^b
Group 3	154.0±17.33 ^c	148.3±21.11 ^c	147.7±10.44 ^c	148.5±16.07 ^c	148.7±21.11 ^c
Group 4	146.5±16.17 ^{ab}	145.0±3.09 ^c	144.1±6.17 ^c	146.0±4.21 ^c	146.5±7.31 ^c
Group 5	136.7±24.00 ^b	135.0±6.34 ^b	138.5±12.21 ^b	140.3±11.19 ^b	141.0±4.48 ^b

Data represented as mean ± S.D of Average Body Weight. Data was analyzed by one- way ANOVA followed by Duncan *post – hoc* test for multiple comparisons, (n=4).

Mean values having different lowercase letters as superscripts are considered significant ($p < 0.05$) down the column.

Group 1: Normal Control receive 1 ml distilled water

Group 2: Diabetic Control Received 1 ml distilled water

Group 3: Received 250mg/kg body weight of CASTOLE

Group 4: Received 350mg/kg body weight of CASTOLE

Group 5: Received 150 mg/kg body weight of MET

Table 4: Effect of Ethanol Extract of *Cassia tora* Leaves (CASTOLE) and Metformin (MEF) on some Serum Biochemical Parameters of Alloxan-induced Hyperglycaemic Rats.

Serum Biochemical Parameters						
Treatment	AST	ALT	ALP	TBIL	TP	ALB
Group 1	30.7±8.00 ^a	34.0±3.07 ^a	112.5±15.09 ^b	2.2±1.01 ^a	50.0±1.16 ^e	42.6±4.65 ^a
Group 2	221.5±13.15 ^c	234.0±12.22 ^c	217.0±11.21 ^d	3.4±13.21 ^c	26.5±4.31 ^a	135.0±3.38 ^e
Group 3	200.0±6.16 ^d	222.5±5.33 ^d	200.3±11.00 ^c	2.6±11.52 ^{ab}	33.0±8.77 ^b	119.5±2.56 ^d
Group 4	185.3±10.34 ^c	199.5±19.21 ^c	192.3±16.21 ^c	2.4±4.31 ^{ab}	49.6±1.45 ^c	82.6±5.73 ^c
Group 5	167.7±6.21 ^b	152.6±10.01 ^b	100.6±9.14 ^a	2.1±9.00 ^a	58.5±3.96 ^d	80.5±6.46 ^b

Data represented as mean ± S.D of concentration of serum biochemical parameters of TBIL (mmol/L), AST, ALT, ALP in (µmol/L), TP and ALB in (g/dl). Data was analyzed by one- way ANOVA followed by Duncan *post – hoc* test for multiple comparisons, (n=4).

Mean values having different lowercase letters as superscripts are considered significant (p < 0.05) down the column.

Group 1: Normal Control receive 1 ml distilled water

Group 2: Diabetic Control Received 1 ml distilled water

Group 3: Received 250mg/kg body weight of CASTOLE

Group 4: Received 350mg/kg body weight of CASTOLE

Group 5: Received 150 mg/kg body weight of MET

DISCUSSION

Diabetes mellitus is a major disease affecting nearly 10% of the population. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a major medical problem. Furthermore, insulin resistance or decreased level of insulin is a characteristic feature of cirrhosis (Mukhtar *et al.*, 2004). The acute hyperglycemic causes life threatening ketoacidosis and also chronic hyperglycemia leads to conditions like retinopathy, nephropathy and peripheral insufficiencies. Liver disease is one of the

leading causes of death in persons with diabetes (Keith *et al.*, 2004).

Alloxan produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in the animals (Mukhtar *et al.*, 2004). However, it is found that action is not specific to pancreas as other organs such as liver, kidney and haemopoietic system also affected by alloxan, administration as seen from the elevation of marker enzymes (Regi *et al.*, 2002). The aim of this study is to evaluate the hepatoprotective effect of ethanol leaf extract of *Cassia tora* on some serum biochemical parameters and bodyweight and of alloxan induced diabetic albino rats.

The acute toxicity study of the extract produced no significant physical signs of toxicity such as writhing, weakness, anorexia, gasping, and palpitation, reduction in body weight, decreased respiratory rate or death within 24 hours of post-administration. Hence the oral median lethal dose (LD₅₀) of the extract was therefore estimated to be 5000 mg/kg.

Experimental diabetes of the animals was induced with the use of alloxan. Alloxan induces diabetes by damaging the insulin producing pancreatic beta- cells of the islets of Langerhans resulting in reduced synthesis and release of endogenous insulin (Ghosh, 2005). The cytotoxic action of alloxan induced diabetes is mediated by reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration leading to a drastic total destruction of beta- cells producing type 1-like diabetes mellitus (Szkudelski, 2001).

The body weights of the rats in all the hyperglycaemic groups showed a decrease in the first week. This observation could be attributed to the increased conversion of storage fat and proteins to glucose (gluconeogenesis) (Champe *et al.*, 2005). However, after the first week, the body weights of both the treated hyperglycaemic groups showed a steady increase, probably with improvement in glucose uptake by cells, though with no significant difference throughout the course of the experiment. The

result of this study is in agreement with that of Kim *et al.* (1996) which determined the effect of leptin sensitivity in adult and aged rats and found a slight increase in weight after an initial fall (in the first week) in adult rats given metformin. This study reveal slightly significant difference in the weights of the rats in the treated groups compare with diabetic control and normal control suggesting that the use of *Cassia tora* extract by diabetics will not bring about an significant increase in weight. In an inflammatory situation, there is a leakage of cytoplasmic enzymes into circulation, in this study, increase in serum levels of AST and ALT was observed in alloxan-induced diabetic animals. The increased level of AST may be due to necrosis, Myocardial infarction and hepatocellular damage, while the increased serum level of ALT may be due to inflamed liver cells. In medicine, the presence of elevated values of ALT and AST is indicative of liver damage (Giboney, 2005). This is in agreement with the study by Derosa *et al.*, (2007) where there was an increased in serum AST and ALT levels after the administration

of alloxan to Wistar rats. It was also discovered by Recknagel, (1987) that there was an increased level of AST in gross cellular necrosis, as in Streptozotocin induced diabetes-damaged to the pancreatic cells of diabetic rats. It has also been observed by Jensen *et al.*, (2004) that liver which has been inflamed or undergoes cell death caused serum increased in ALT and liver with myocardial infarction and hepatocellular damaged induced increased level of AST. The observation that the activity of serum AST appeared to be more elevated than ALT, is also in agreement with the findings of Recknagel, (1987), Tanaka *et al.*, (1988) and Nanbara *et al.*, (1990) that AST rise high and had more activity than did ALT in the serum of diabetic mice. This study revealed that treatment of alloxan- induced diabetic rats with *Cassia tora* extract at various doses brought about decrease of the transaminases activity. The rate of decrease occurred in a dose- dependent fashion, reduced levels of ALT, AST and ALP in rats treated with the extract could also be attributed to the hepatoprotective ability of

some bioactive compounds of the extract to prevent the metabolism of alloxan into more toxic metabolite and minimized the production of free radicals. Some herbal plants possess hepatoprotective effects. This is in agreement with Lipkin, (1995) where humans were administered with variety of herbal plants that contains saponin and they proved to be potent against cancer and hepatic cell proliferation.

There was an observed increase in plasma bilirubin produced in the diabetic control when compared to treated groups. Treatment of alloxan-induced diabetic rats with graded doses of *Cassia tora* extract treated groups showed a significant reduction in serum total bilirubin produced compared to the untreated diabetic rats. According to Rana *et al.*, (1996), increase in plasma bilirubin (hyperbilirubemia) in alloxan-treated rats may be as a result of a decrease in liver uptake, conjugation or increased bilirubin production from hemolysis. Also, the plasma bilirubin elevation in diabetic rats indicates alteration in liver function as confirmed by the changes in the plasma enzymes activities. The

hepatoprotective potentials of the extract and extract and metformin administration were observed in the reversal of increase in serum bilirubin levels.

Serum total protein concentration in diabetic control group was significantly decreased when compared with the diabetic treated groups and normal control. A number of factors that might be responsible for this reduction include decrease due to proteinuria and albuminuria, which are important clinical markers of diabetic nephropathy (Mauer *et al.*, 1981), and due to increased protein catabolism (Almdal and Vilstrup, 1988) as a result of insulin deficiency from free radical generation due to alloxan induction, since it has been established that insulin stimulates the incorporation of amino acids into protein (Almdal and Vilstrup, 1988). Also, increased rate of amino acids conversion to glucose (Chu *et al.*, 2007), decreased amino acid uptake (Garber *et al.*, 1980), and increased conversion rate of glucogenic amino acids to CO₂ and H₂O (Ahmed, 2005). The results of this study showed that, the treatment of diabetic rats with

leaf extract of *Cassia tora*, brought about marked increase in serum total protein contents. The extract showed strong effect as total elevations of protein and decrease albumin level were observed in these groups. The elevation level could be as a result of high hepatic uptake of amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzes amino acids transamination.

CONCLUSION

This study revealed that *Cassia tora* extract possesses hepatoprotective activity and could play a significant role in the management of the symptoms associated with diabetes.

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Quality Evaluation of Yoghurt Enriched with Coconut Milk Using Chilli Stalks as Probiotic Starter Culture

Ohiare, R. O.* , Joshua, V. A. and Momoh, R.

Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic, Lokoja, Kogi State, Nigeria.

*Corresponding Author : raliat01ohiare@gmail.com

ABSTRACT

Yoghurt is the most popular and acceptable fermented dairy product consumed all over the world. Due to the increasing sensitization on consumption of dairy products with functional properties, the potential of producing acceptable yoghurt from full cream milk enriched with coconut milk was investigated in this study. Chilli stalks were used as a source of lactic acid probiotic bacteria in the fermentation of milk for yoghurt production. Five (5) formulations were made and labeled as sample A (100% full cream milk) which served as control, sample B (80:20), sample C (70:30), sample D (60:30) and sample E (50:50) full cream milk and coconut milk respectively. Yoghurts produced were analyzed for proximate, physico-chemical and microbial qualities using standard procedures. Results obtained showed that the proximate composition increased with the addition of coconut milk. Moisture content ranged from 70.0 – 81.0 %, crude protein (1.75 – 4.25 %) crude fat (2.00 – 5.00 %) and ash content (0.89 – 0.99 %) respectively. A reverse trend was observed for carbohydrate content (25.36 – 9.26 %), total solids (30.00 – 19.00 %) and total solid- nonfat (28.00 – 14.50 %). While total titrable acidity and pH ranged from 0.73 – 0.98% and 4.05 – 4.70, respectively. The microbial analysis showed that sample A had the highest bacterial count (6.5×10^4 cfu/ml). Total coliform and fungal counts were not detected in any of the samples. This study established that the inclusion of coconut milk improved the proximate and physicochemical properties of yoghurt. Chilli stalks removed as waste during food processing can also provide an easy access to healthy starter culture in yoghurt making, changing its status from waste to wealth.

Keywords: Enriched yoghurt, chilli stalks, coconut milk, physicochemical, microbiological

INTRODUCTION

There is an increasing demand for health-promoting foods and beverages, which is prompting the food and dairy industry to develop functional foods that meet the demand of its health conscious consumers. In addition to being nutritious, functional foods refer to processed foods containing ingredients that aid specific bodily functions which are developed specifically to promote health or reduce the risk of diseases (Ndife *et al.*, 2014). Yoghurt is a milk-based product manufactured by lactic acid fermentation enabled by symbiotic cultures (Nyanzi *et al.*, 2021). It is made by adding certain bacteria called probiotics to milk. These bacteria produce lactic acid, which decreases milk pH and coagulate milk proteins to give yoghurt its texture and characteristic tang. (Moore, 2014; Olatide *et al.*, 2019). Probiotic bacteria are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill *et al.*, 2014; Nyanzi *et al.*, 2021). Yoghurt is globally regarded as a functional food employed to deliver

probiotics and prebiotics to the consumer which combines unique nutritional values with the promotion of gut health, heart functions, and the natural immune defence in humans (Ogunyemi *et al.*, 2021). Traditionally, yoghurt is made from dairy milk but plant-based or non-dairy milk alternative like coconut milk, tigernut milk and soymilk are rising trend and fast growing segment in new food product development category of functional beverage across the globe. They can serve as an inexpensive alternate to poor economic group of developing countries and in places, where cow milk supply is insufficient. Coconut milk is rich in vitamin and minerals such as iron, calcium, potassium, magnesium and zinc. Also rich in antioxidants such as vitamin E which helps in fighting against ageing, coconut milk consumption is rarely associated with allergenic reactions (Sethi *et al.*, 2016).

For yoghurt preparation, the milk is allowed to ferment with the microbial inoculums called lactic acid bacteria (LAB) belonging to the general lactobacillus and bifidobacterium

used as a starter that determines the body, texture and flavor of final yoghurt under controlled environmental conditions. These bacteria ferment milk sugar lactose to lactic acid thus lowering the pH, and inhibiting the growth of pathogenic bacteria (Miah *et al.*, 2017). In recent time, the pure freeze-dried starter culture is scarce in the market, which makes people resort to store bought yoghurt as starter culture in yogurt making. Hence, the need for an alternative isolation of LAB from readily available sources becomes imperative. According to Olatide *et al.* (2019), there have been claims that the calyx of capsicums fruits such as chilli, are often rich in various lactobacilli. These natural bacteria create a starter for lacto-fermentation of milk. In addition, capsaicin from the chilli appears to increase the metabolic rate of the lactobacilli. This forms the thrust of this study to investigate the potential of producing acceptable yoghurt from full cream milk enriched with coconut milk and the effectiveness of using chilli stalks as a source

of lactic acid bacteria in the fermentation of milk for yoghurt production.

METHODOLOGY

Study Area

This study was carried out in Lokoja, Kogi State, Nigeria. Lokoja is located on the confluence of River Niger and River Benue. It is the capital city of Kogi State and can be found between latitude $7^{\circ} 45' 27.56'' - 7^{\circ} 51' 04.34''$ N and longitude $6^{\circ} 41' 55.64'' - 6^{\circ} 45' 36.58''$ E of the equator with a total land coverage of about 63.82 sq. km. (Adeoye, 2012).

Lokoja lies about 7.8023 degrees North of the Equator and 6.7333 degrees East of the Meridian. It is about 165km Southwest of Abuja, Federal Capital Territory of Nigeria as the crow flies, and 390km Northeast of Lagos by same measure. It is located on the confluence of River Niger and River Benue, with a total land coverage of about 63.82 sq. km.

Study Design

Five (5) different samples were formulated and coded as A, B, C, D and E. Various

percentages of the ingredients were combined where sample A served as control with 100% powdered milk.

Sample Collection and Preparation

Commercial powdered full cream (peak) milk, coconut and chilli pepper were purchased from International market, Lokoja, Kogi State. Coconut milk was prepared by adopting and modifying the method described

by Belewu *et al.* (2010) and Ndife *et al.* (2014). Chilli pepper with the stalk attached was washed thoroughly with clean water and stalks were detached whenever needed for the inoculation. The methods described by Belewu, (2005) and Olatide *et al.* (2019) were adopted and modified for the yoghurt production as shown in figure 1.

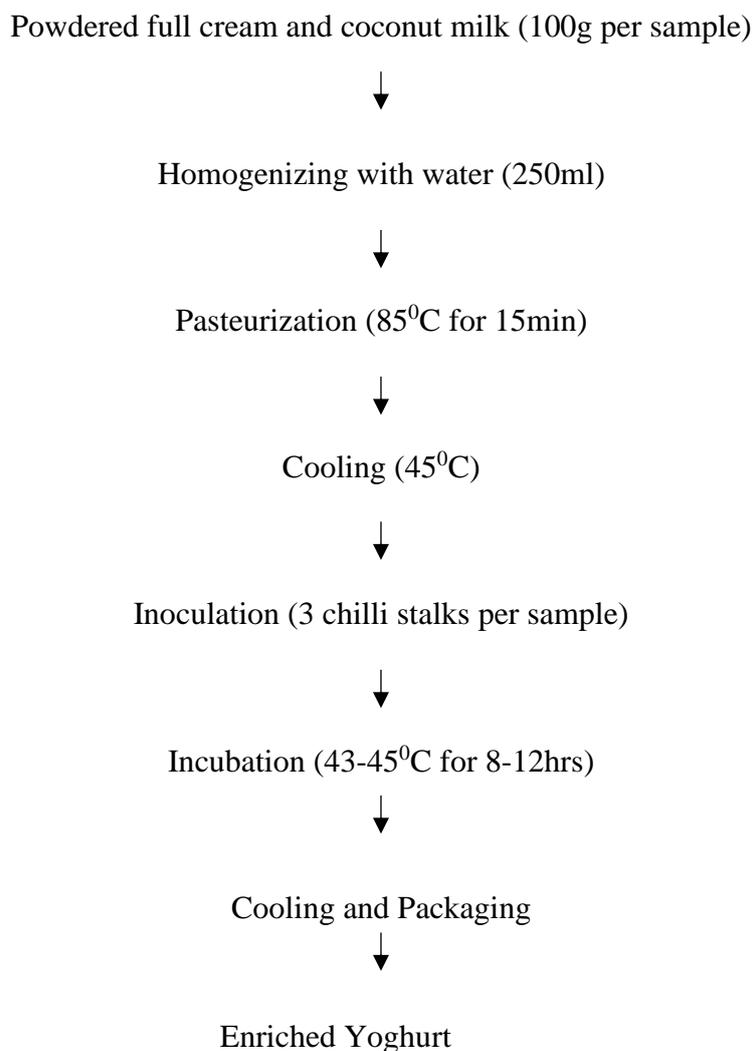


Fig 1. Flowchart for the production of enriched yoghurt

Source: Belewu, (2005) and Olatide *et al.* (2019) with slight modifications

Analyses of Samples

Chemical and Physico-chemical Analysis

The proximate composition of the yoghurt was determined according to AOAC (2019) and Ogunyemi *et al.* (2021). Total solid was determined as described by AOAC (2019). Total solid non-fat was obtained by taking the difference between % total Solids and % fat content (AOAC, 2005 and Igbabul *et al.*, 2014). The pH was determined using a pH meter as described by AOAC (2019) and total titrable acidity (TTA) was determined as described by AOAC (2019) and Mbaeyi (2019).

Microbiological Analysis

The determination of the microbial counts in the yoghurts was performed by using the nutrient agar (NA) for the total bacteria counts,

MacConkey agar (MAC) for the total coliform counts and potato dextrose agar (PDA) for the total fungal counts, as outlined in compendium of methods for the microbiological examination of foods (AMPH, 1992) and Matin *et al.* (2018) with some modifications. The colonies were counted and the result was expressed as colony forming unit per ml. (cfu/ml).

Statistical Analysis

Statistical analysis was performed on the results obtained using the Statistical Package for Social Sciences (SPSS) software, version 20.0. Data were evaluated using one-way ANOVA and Duncan multiple range test. A *P*-value of ≤ 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Table 1: %Proximate and Physicochemical Composition of Enriched Yoghurt

Sample	Moisture	Crude protein	Crude fat	Ash	CHO	Total solids(%)	Total solid non-fat(%)	TTA (g/100ml)	pH
A	70.00 ±0.69 ^e	1.75 ±0.00 ^d	2.00 ±0.00 ^e	0.89 ±0.07 ^a	25.36 ± 0.48 ^a	30.00 ±0.78 ^a	28.50 ±0.53 ^a	0.98 ± 0.02 ^a	4.05 ± 0.08 ^d
B	72.50 ±0.55 ^d	1.79 ±0.29 ^d	2.50 ±0.63 ^d	0.94 ±0.01 ^a	22.31± 0.05 ^b	28.00 ±0.61 ^b	25.50 ±0.77 ^b	0.93± 0.11 ^{ab}	4.25 ± 0.04 ^c
C	73.10 ±0.71 ^c	3.91 ±0.36 ^c	3.50 ±0.05 ^c	0.95 ±0.01 ^a	18.54 ± 0.12 ^c	26.90 ±0.51 ^c	23.40 ±0.86 ^c	0.85 ± 0.08 ^b	4.35 ± 0.06 ^b
D	81.00 ±0.34 ^a	4.20 ±0.29 ^b	4.50 ±0.17 ^b	0.98 ±0.05 ^a	9.32 ± 0.40 ^d	19.00 ±0.65 ^d	14.50 ±0.63 ^d	0.73 ± 0.19 ^d	4.40 ± 0.02 ^b
E	80.50 ±0.86 ^b	4.25 ±0.01 ^a	5.00 ±0.23 ^a	0.99 ±0.03 ^a	9.26 ± 0.05 ^e	19.50 ±0.92 ^d	14.50 ±0.00 ^d	0.75 ± 0.14 ^d	4.70 ± 0.01 ^a

Mean and standard deviation of three determinations. Mean value with different superscripts in a column are significantly ($p \leq 0.05$) different from each other. A= (100% full cream milk); B= (80:20 full cream milk and coconut milk); C= (70:30 full cream milk and coconut milk); D= (60:40 full cream milk and coconut milk); E= (50:50 full cream milk and coconut milk); CHO= Carbohydrate; TTA= Total titrable acidity.

Table 2: Microbial Count (cfu/ml) of Enriched Yoghurt

Sample	Total Bacterial Count (10^4)	Total Coliform Count (10^4)	Total Fungal Count (10^4)
A	6.5 ± 0.01 ^a	Nil	Nil
B	6.0 ± 0.04 ^b	Nil	Nil
C	3.4 ± 0.16 ^d	Nil	Nil
D	5.0 ± 0.06 ^c	Nil	Nil
E	6.0 ± 0.08 ^b	Nil	Nil

Mean and standard deviation of three determinations. Mean value with different superscripts in a column are significantly ($p \leq 0.05$) different from each other. A= (100% full cream milk); B= (80:20 full cream milk and coconut milk); C= (70:30 full cream milk and coconut milk); D= (60:40 full cream milk and coconut milk); E= (50:50 full cream milk and coconut mil

Proximate and Physicochemical Composition of Enriched Yoghurt

Table 1 shows the proximate and physicochemical composition of the different yoghurt samples evaluated in this study. There was significant ($p < 0.05$) difference in the moisture content value between the enriched samples and control. Sample D was the highest (81.00%) while sample A recorded the lowest (70.00%) respectively. The high moisture content could be attributed to the homogenization of milk with water prior to fermentation and the fact that yoghurt is a liquid food product. This is in agreement with previous studies (Mbaeyi and Ezeoke, 2019; Eze *et al.*, 2020). The protein, fat and ash content in the samples ranged from (1.75 – 4.25%), (2.00 – 5.00%), (0.89 – 0.99%) and increased as the proportion of the coconut-milk increased in the yoghurts. Similar observations were made by Ndife *et al.* (2014) in coconut- cake enriched yoghurt. The carbohydrate content ranged from 25.36% in sample A to 9.26% in sample E. The decrease in CHO content could probably be due to the increase in other constituents as the level

of enrichment increased. The low carbohydrate value of the yoghurt makes it an ideal food for lactose intolerance individuals (Igbabul *et al.*, 2014).

For total solids, the control yoghurt sample had the highest (28.50) value and differed ($p < 0.05$) significantly from the enriched yoghurt samples, while sample D and E showed no significant ($p < 0.05$) difference. The total solids non-fat of the yoghurt samples ranged from 28.50% in sample A to 14.50% in sample D and E respectively. Similar observation was made by Haneen, (2019). The total titratable acidity and the pH values also ranged from (0.73 – 0.98%) and (4.05 – 4.70) in the yoghurt samples. The enriched-yoghurt samples had lower acidity values than control yoghurt. Similar result of titratable acidity was reported by Mbaeyi and Ezeoke (2019). The pH values of the enriched yoghurt samples (except sample E) are within the range of Food Standard Code who requires that the pH of yoghurt be a maximum of 4.50 in order to prevent the growth of any pathogenic organisms (Ndife *et al.*, 2014).

Microbial Count of Enriched Yoghurt

The results obtained for the microbial counts (Table 2) showed that the total bacterial counts (TBC) did not show a particular pattern in relation to the enrichment of yoghurts. Sample A which served as control had the highest microbial load (6.5×10^4 cfu/ml) followed by sample B and E having the same microbial load (6.0×10^4 cfu/ml). Chilli stalks suspected to contain microorganisms (lactic acid bacteria) which served as starter culture in the yoghurt production may have contributed to the total bacterial count. The microbial status of the yoghurts is within the acceptable standard count of yoghurt ($10^6 - 10^7$ cfu/ml) (Mbaeyi and Ezeoke, 2019). The absence of yeast and mould (total fungal count) and total coliform count signifies that the yoghurt samples are free from contamination due the hygienic sanitary conditions employed during production.

Conclusion

Findings from this study revealed that the nutritional quality of the full cream yoghurt was improved by enrichment with coconut milk. The results have also demonstrated that chilli stalks

contain probiotic microorganisms that are responsible for lactose fermentation in yoghurt production as evident in the decreased pH and increased titrable acidity. Thus, fortifying yoghurt with coconut milk is of great interest to improve the functionality and create functional foods with health benefits. Isolation of microorganisms from chilli stalks will also serve as a cheap and easily available source of yoghurt starter and store of probiotics which is a desirable product, considering its immense health benefits. However, further research on sensory evaluation of the produced yoghurt should be carried out to determine the most preferred sample in terms of appearance, colour, taste, texture and overall acceptability.

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Bacteriological Assessment and Antimicrobial Resistance Profile of *Salmonella* spp and *Escherichia coli* isolated from Livestock farms in Lokoja, Kogi State, Nigeria

Usman, Z¹., Momoh, R.² and Ohiare, R.O.³

Department of Science Laboratory Technology, School of Applied Science, Kogi State Polytechnic
Lokoja, Kogi State

¹Corresponding Author: zainabusman855@Gmail.com, +2347069469873

ABSTRACT

This study was carried out to determine the Bacteriological Assessment of Antimicrobial Resistance Profile of Escherichia coli and Salmonella spp isolated from livestock farms in Lokoja, Nigeria. A cross-sectional survey was utilized in this study. A total of two livestock farms were randomly surveyed. Eight fresh fecal samples and environmental samples were collected in sterile universal bottles from each livestock farms and transported immediately to Microbiology Laboratory, Kogi State Polytechnic, Lokoja for isolation and identification of bacteria and antimicrobial susceptibility tests using standard method as described by Cheesborough and Clinical and Laboratory Standard Institute (CLSI). From the sixteen fecal and environmental samples collected from livestock farms in the study, 10(62.5%) were positive for E. coli and 15(93.75) were positive for Salmonella spp. The isolates showed high resistance to antibiotics such as Ampiclox (100%), Amoxicillin (70%) and Pefloxacin (70%). They were susceptible to Gentamicin (100%), Ciprofloxacin (90%) and Rocephin(100%). Escherichia coli and Salmonella spp are associated with livestock farms in Lokoja, Nigeria. This study has established resistance of these isolates to Ampiclox (100%), Amoxacillin(100%) and Pefloxacin(100%). However, Gentamicin, Ciprofloxacin and Rocephin are recommended as drugs of choice against Escherichia coli and Salmonella spp infections that may arise from these livestock farms based on the findings in this study. Improved hygienic practise among livestock farmers is strongly recommended, Public awareness and enlightenment on the dangers of indiscriminate use of antibiotics in livestock is needed and there is a need for monitoring, surveillance and routine antimicrobial susceptible profile assessment across the livestock farms in Nigeria.

Keywords: Bacteriological, Antimicrobial Resistance Profile, Susceptibility, Livestock farm

Introduction

Background of the study

Antimicrobial resistance is a major public health crisis, threatening the return of untreatable infections and deaths on a massive scale if appropriate actions are not taken. To reduce the problem of human infections caused by resistant bacteria transferred from animals, there is continuous pressure to restrict the use of antimicrobials in animals (FAO, 2015). The increasing trend regarding the emergence of bacteria resistant to antimicrobial agents and their potential for transmission to humans through animal production has led various authorities worldwide to implement measures to decrease antimicrobial use in livestock production. Studies relating to the antibiotic resistance in livestock production are therefore becoming relevant in both developed and developing nations of the world (FAO, 2015).

Antimicrobial agents are useful in livestock farming for a number of purposes such as therapeutic (treating sick animals), metaphylaxis (control treatment of whole herd in case of

disease outbreak), prophylaxis (preventive treatment), and growth promotion (Economou and Gousia, 2015). These antimicrobial agents are used broadly in veterinary practice in Nigeria and other parts of the world that have not banned the use of these agents to prevent or control infectious disease, minimize postsurgical infections and as growth promoters by adding them in feed at sub-therapeutic doses (Economou and Gousia, 2015).

The increasing demand for animal protein especially in developing parts of the world is causing an increase in animal production, and in connection with this, antimicrobial use in food-animal production was estimated to rise by 67% between 2010 and 2030 (Van Boeckele *et al.*, 2015). Despite the historical and the current positive contribution of antimicrobial use in animal health and production management, there exist a number of possible drawbacks associated with the use of antimicrobials in livestock. One of such drawbacks is the misuse of antimicrobials in animals which might be potentially threatening and cause the emergence of antimicrobial-resistant bacteria strains by

increasing selection pressure on bacteria to become resistant (Van Boeckel *et al.*, 2015). Other negative consequences associated with antimicrobial use are the occurrence of unacceptable level of drug residues in food of animal origin. The inappropriate use of antimicrobials in food and animals can result in accumulation of toxic and harmful residues in animal products that can further affect the health of consumers largely by causing allergic reactions (Baynes *et al.*, 2016; (Tajick and Shohreh, 2016).

It is estimated that 60 to 80% of all cattle, sheep, swine and poultry receive antimicrobials at some point in time and not more than 5 to 10% of antimicrobial was employed on proper clinical dosage resulting in a wide spread misuse of antimicrobial drugs (Tajick and Shohreh, 2016). Following the misuse of antimicrobials usage in food and animals by farmers, it will become a worldwide dispute relating to public health and food safety (Tajick and Shohreh, 2016). Apart from the public health impact, an increasing prevalence of antimicrobial resistance, particularly to frequently used antimicrobials in

livestock, could also lead to reduced treatment options and increased animal disease and production losses (Alhaji and Isola, 2018). Furthermore, infected animals may shed these bacteria, posing a threat to other farm animals, household pets, and humans, through direct contact or environmental contamination (Hartantyo *et al.*, 2018). Infected animals may also act as a reservoir for resistant bacteria, which might enter the food chain (Chang *et al.*, 2015).

Methodology

The Study Area

The study area Lokoja is Kogi state capital city which is located in the north central part of the country. It is sited at the confluence of the rivers Niger and Benue (www.britannica.com). Lokoja has an area of 3,180km² (1230sq mi), a population of 692,050. It lies on latitude 7°48'07"N and longitude 6°44'39"E. Temperatures are generally high in this area with the highest temperature usually recorded during the day. It shares common boundaries with Niger, Kwara, Nassarawa and the Federal Capital

Territory to the north. To the east the State is bounded by Benue state to the South by Enugu and Anambra states (www.nigeriagallery.com). Lokoja town is situated in the topical wet and dry savanna climate zone of Nigeria and temperature remain hot year round.

Study Design

A cross-sectional survey was utilized in the study. A total of two livestock farms were randomly surveyed.

Sample size

Four (4) samples each of stool and environment were collected from each livestock farm for Microbiological assessment, making a total of sixteen (16) samples collected from the livestock farms in Lokoja, Kogi State, Nigeria.

Sample Collection

Fresh fecal and environmental samples were collected in sterile universal bottles from the livestock farms. Samples were taken immediately to the Microbiology Laboratory, Kogi State Polytechnic, Lokoja for isolation,

identification of bacteria and antimicrobial susceptibility tests.

Laboratory analysis

The isolation and identification of bacteria were done using standard method as described by (, 2000) and Clinical and Laboratory Standard Institute (CLSI 2021). Nutrient agar, MacConkey Agar, Eosine Methylene Blue (EMB), Mannitol Salt Agar (MSA) and SS Agar were prepared and used for the isolation and enumeration of bacteria. These were prepared as specified in the manufacturer’s manuals.

Antimicrobial susceptibility tests were done using disc diffusion method (, 2000; CLSI, 2015).

RESULTS

Table 1: Morphological and Biochemical properties of the bacterial isolates from livestock farm samples.

Biochemical Properties	<i>E. coli</i>	<i>Salmonella Spp</i>
Gram-reaction	Positive (-ve)	Negative (-ve)
Colonial Morphology	Rods	Rods
Catalase	Positive (+ve)	Positive (+ve)

Oxidase	Negative (-ve)	Negative (-ve)
Citrate	Negative (-ve)	Negative (-ve)
Indole	Positive (+ve)	Negative (-ve)

Table 2: Prevalence of *E. coli* and *Salmonella spp* in livestock farm samples

Source code	No of samples	No. (%) of <i>E. coli</i> Isolates	No. (%) of <i>Salmonella spp</i> Isolates
CFS 1	1	1(6.25)	1(6.25)
CFS2	1	1(6.25)	1(6.25)
CFS3	1	1(6.25)	1(6.25)
CFS4	1	1(6.25)	1(6.25)
CFS5	1	1(6.25)	1(6.25)
CFS6	1	1(6.25)	2(12.5)
CFS7	1	1(6.25)	2(12.5)
CFS8	1	1(6.25)	1(6.25)
CES9	1	0(0.0)	0(0.0)
CES10	1	1(6.25)	0(0.0)
CES11	1	0(0.0)	1(6.25)
CES12	1	0(0.0)	1(6.25)
CEA13	1	0(0.0)	1(6.25)
CES14	1	0(0.0)	0(0.0)
CES15	1	0(0.0)	1(6.25)
CES16	1	1(6.25)	1(6.25)
Total	16	10(62.5)	15(93.75)

Key: CFS= Cattle Feecal Sample; CES= Cattle Environmental Sample

Table 3: Overall prevalence of *E. coli* and *Salmonella spp* from livestock farms investigated

Source	No of samples	No (%) of <i>E. coli</i> Isolates	No (%) of <i>Salmonella spp</i> Isolates
Feecal Samples	8	8(50.0)	10(62.5)
Environmental Samples	8	2(12.5)	5(31.25)

Table 4: Antibacterial Susceptibility test result for *Escherichia coli*

Iso lat code	P E F	C N	A P X	Z	A M	R	C P X	S	S T	E	Total
											16
EC 1	2	2	10	0	2	2	37	2	2	2	16
EC 2	0	2	0	0	8	2	34	2	0	0	10(62.5)
EC 3	2	2	0	0	6	2	37	2	0	5	15(93.75)
EC 4	2	2	10	5	2	2	36	2	2	2	16
EC 5	1	1	0	0	1	2	18	1	1	1	16
EC 6	1	1	0	1	1	2	37	1	1	1	16
EC 7	1	1	0	1	1	2	34	1	0	1	16
EC 8	1	1	0	1	1	2	35	1	1	2	16
EC 9	1	1	0	1	1	2	33	1	1	1	16
EC 10	1	1	0	1	1	2	33	1	1	1	16

Table 5: Antibacterial Susceptibility test results for *Salmonella spp* in millimeters

Iso lat code	P E F	C N	A P X	Z	A M	R	C P X	S	S T	E	Total
											16
SP 1	2	2	11	0	2	2	26	2	2	2	16

SP 2	2	2	0	1	8	2	24	2	0	0
2	4	5		4		4		3		
SP 3	2	2	0	1	6	2	27	2	0	1
3	5	5		4		5		5		2
SP 4	2	2	18	5	1	2	26	2	2	2
4	6	6			9	4		5	4	3
SP 5	1	1	0	0	1	2	27	1	1	2
5	7	6			2	4		7	0	5
SP 6	1	1	0	1	1	2	27	1	1	1
6	7	7		6	1	6		7	2	9
SP 7	2	1	0	1	1	2	24	1	1	1
7	6	6		7	3	5		5	3	7
SP 8	1	1	0	1	1	2	25	1	1	2
8	5	5		7	2	6		0	2	4
SP 9	1	2	0	1	1	2	23	1	1	1
9	7	6		4	5	6		7	2	6
SP 10	1	1	0	1	1	2	23	1	1	1
10	6	6		6	2	5		6	0	4

Key: S= Susceptible; I= Intermediately Resistant; R= Resistant

Discussion

The microorganisms isolated in this study were *Escherichia coli* and *Salmonella spp.* These organisms have been implicated in several human and animals' diseases. Therefore, they are of serious zoonotic importance and may constitute to serious public health threat as the study animals are reared for human consumption. This study agrees with the findings of Makolo *et al.*, 2019. The isolation of *Escherichia coli* (used as indicator organism) in this work indicated the

presence of fecal contamination. This could also result to serious public health challenges. Also, the observation of the high resistant rate of *E. coli* isolates call for surveillance to the commonly prescribed drugs of choice is a serious health concern.

This resistance might be due to *E. coli* ability to colonize different environments, including the gut of humans and animals. This attribute has provided it with the evolutionary advantage to acquire antibiotic resistance traits from other bacteria within its environment, as well as to be easily transmitted via the fecal-oral route. *E. coli* strains becomes resistant to beta lactam antibiotics by producing plasmid-borne extended-spectrum β -lactamases (ESBLs); and Ampiclox is an example of beta-lactam antibiotics. The isolation of *Salmonella spp* in this work is also an indication of poor environmental hygiene among the livestock farms (Chmielarczyk *et al.*, 2014).

Furthermore, this study revealed that *Salmonella spp* was resistance to most of the antibiotics tested. For many antibiotics, pre-existing highly efficient resistance mechanisms evolved in

antibiotic producers or their competitors and was easily acquired by pathogenic *Salmonella spp* by horizontal gene transfer of mobile genetic elements, for example, from organisms in soil (Nesme and Simonet, 2015).

The susceptibility of *Salmonella spp* to Gentamicin, Ciprofloxacin and Rocephin in this study may be as a result of their mechanisms of action which is inhibiting the basic protein synthesis that a bacterial cell requires for its survival (Hu *et al.*, 2013). This might also be due to uncommon usage of these antibiotics on the livestock within the study population.

Conclusion

This study has established that *E. coli* and *Salmonella spp* are associated with livestock farms in Lokoja, Nigeria. Their presence calls for serious attention as they have been implicated in several human and animals' diseases. This study has also established resistance of these isolates to Ampiclox, Amoxicillin and Pefloxacin. This is a public health threat as completely resistant strains of pathogenic bacteria might emerge due to the continued and indiscriminate use of

antibiotics on livestock for non-therapeutic purposes. However, Gentamicin, Ciprofloxacin and Rocephin are recommended as drugs of choice against *E. coli* and *Salmonella spp* infections that may arise from the livestock farms within the study area based on the findings of this study.

Recommendations

1. Improved hygiene among livestock farmers is strongly recommended.
2. Public awareness and enlightenment on the dangers of indiscriminate use of antibiotics in livestock is needed.
3. There is a need for monitoring, surveillance and routine antimicrobial assessment farms in Nigeria.

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DENSITY FUNCTIONAL THEORY(DFT) AND RESTRICTED HARTREE FORK (RHF) BASED
STUDY OF THE STRUCTURES AND THERMODYNAMIC PROPERTIES OF THE MOLECULAR
COMPONENTS OF DEOXYRIBONUCLEIC ACID(DNA)

BY

SALIHU ABUBAKAR, salbak2000@gmail.com, ESUGA-MOPAH NINMA MARY & OLATUNDE

OPEYEMI STEPHEN

Department of Science Laboratory Technology, Kogi State Polytechnic,

Lokoja

ABSTRACT

Deoxyribonucleic acid (DNA) is a molecule that contains the instruction an organism needs to develop, live and reproduce. These instructions are found inside every cell and are passed down from parents to their children. In this work, the DNA nucleobases i.e. Adenine (A), Guanine (G), Cytosine(C) and Thymine (T) were investigated using Gaussian 03 package which employs quantum chemical methods calculation. The optimized geometrical structures calculated are the bond lengths and bond angles while the thermodynamic parameters are the entropy, enthalpy, and heat capacity of the molecules were also calculated using Restricted Hartree–Fock (RHF) and Density Functional Theory (DFT) methods with 3-21G, 3-21+G,6-31G and 6-31+G basis sets. The values of optimized geometrical parameters of each of the molecules obtained by the different methods were compared with one another. It was found that there is a good agreement between the calculated values. The values of the thermodynamic properties of each of the molecules calculated by the different methods were in agreement with one another.

Keywords: Nucleobases,thermodynamics,quantum,optimised.

INTRODUCTION

Biophysics, as an interdisciplinary science, employs principles from physics to examine intricate macro-molecular systems within living organisms (Alberts et al., 2014). Living organisms operate as self-regulated chemical entities, guided by molecular signals, receptors, and transducers (Nelson et al., 2020). Nucleic acids, biopolymers, proteins, and enzymes constitute essential subsystems in this context (Phillips et al., 2012). Biophysicists delve into the investigation of intramolecular and intermolecular interactions, exploring electronic and structural alterations, as well as the transfer of electrons, protons, ions, and energy within biological systems (Nelson et al., 2020). Theoretical challenges arise from the inherent openness and far-from-equilibrium nature of living systems.

Historically, studies on biological systems were dominated by disciplines such as biochemistry, molecular biology, structural biology, and genetics. These fields have yielded precise information concerning the chemical compositions of organisms, reaction pathways, and the elucidation of genetic codes (Alberts et al., 2014). A potential shift towards a new era suggests an integral role for physics and mathematics in advancing biophysics (Nelson et al., 2020). Biochemistry, focusing primarily on direct atomic contact, encounters challenges in addressing subtle, weak forces contributing to various biological phenomena, including the solvation problem.

Biological function emanates from specific chemical reactions and cascades, wherein molecules rely on either quantum mechanical or classical interactions. The primary task for theoretical biophysicists revolves around scrutinizing the physical characteristics of biological molecules and simple systems, all while considering the openness of biological systems (Phillips et al., 2012). Routine exchanges of energy and matter transpire between biological systems and their

environment, with components like proteins undergoing continuous restructuring, showcasing stability timescales longer than their biological functions.

Modeling assumes a critical role in scientific progress. A biological model serves to illustrate interrelationships, a biochemical model portrays complex chemical reactions, and a computational model simulates processes. In contrast, physical models encompass theoretical descriptions with equations of motion, enabling quantitative predictions and emphasizing the necessity to move beyond mere reproduction of known results (Alberts et al., 2014).

NUCLEIC ACIDS

Nucleic acids, including DNA and RNA, play a crucial role in living organisms by preserving and transmitting genetic information. (Shen, 2019) Despite their one-dimensional nature, understanding the three-dimensional structure of nucleic acids is essential for governing their organization, functions, and interactions with proteins.

Each nucleotide, the basic unit of nucleic acids, comprises a phosphate group, a sugar group, and a nitrogen base. The specificity of nucleic acid units is determined by the organic base, related to either the purine or pyrimidine ring system. DNA contains four bases: Adenine (A), Guanine (G), Cytosine (C), and Thymine (T), while RNA replaces Thymine with Uracil (U) (Tropp, 2012)

James Watson and Francis Crick proposed a DNA structure model, emphasizing the anti-parallel arrangement of two strands held together by hydrogen bonds between nitrogenous bases. Hydrogen bonds that hold together the two strands of nucleotides in DNA have been the main scope of many experimental and theoretical investigations for three decades. The importance of this molecular interaction is due to its role in DNA replication and complementarity of nucleic acid bases which is the cornerstone of the genetic code (Espejo and Gonzalez, 2007).

Watson-Crick base pairing ensures complementary interactions, with Adenine pairing with Thymine (or Uracil in RNA) and Guanine pairing with Cytosine (Hartl and Ruvolo, 2012). This precise base pairing is crucial for DNA replication, preventing detrimental gene mutations (Friedberg et al, 2005).

AIM AND OBJECTIVES

Aim: The aim of this research is to study the molecular structure and thermodynamic properties of Guanine, Cytosine, Adenine and Thymine.

Objectives

The specific objectives of this research are:

- (i) To obtain the optimized parameters of the compounds (bond lengths and bond angles).
- (ii) To calculate the thermodynamic properties (enthalpy, entropy and heat capacity) of the molecules.

JUSTIFICATION

There have been exceptional developments of experimental methods to investigate the structure of nucleic acids at the atomic level. However there are many instances in which some information are not obtainable. As a result of the limitations of experimental techniques and recent development of computer hardware and simulation algorithms, we have witnessed an expansion of computational studies of nucleic acids in recent times. The current computational methods provide us with information complementary to the experimental techniques. A deeper investigation of the molecular structures and properties of the molecular components of the DNA is required because of their significance in cell life.

THEORETICAL BACKGROUND

Geometry optimization: Geometry optimization is a name for the procedure that attempts to find the configuration of minimum energy of a molecule. The procedure calculates the wave function and the energy at a starting geometry and then proceeds to search a new geometry of lower energy. This is repeated until the lowest energy geometry is found. The procedure calculates the force on each atom by evaluating the gradient (first derivative) of the energy with respect to atomic position. Geometrical parameters are: bond lengths, bond angles and dihedral angles. (Riahi et. al, 2010).

Bond length is the distance between the nuclei of two atoms joined by a covalent bond. Bond length depends on the particular atoms in the bond and on the bond order. The shorter the bond length, the larger is the value of bond energy. It is expressed in Angstrom units (Å) or picometers (pm). $1\text{Å} = 10^{-10}\text{m}$ and $1\text{pm} = 10^{-12}\text{m}$.

Bond angles are the angles made by the lines joining the nuclei of the atoms in a molecule. The bond angles of a molecule, together with the bond lengths define the shape and size of the molecule.

Restricted Hartree-Fork (RHF): In computational Physics and Chemistry, the Restricted Hartree-Fork (RHF) method is a method of approximation for the determination of the wavefunction and the energy of a quantum many-body system in a stationary state. It is used for closed shell atoms or molecules such as He, H₂O, NH₃, CH₃COOH etc. Most molecules fall in this group.

The most common type of *ab initio* calculations is performed in the framework of the Hartree-Fock (HF) method, which is based on the central field approximation. This means that the Coulombic electron-electron repulsion is taken into account by integrating the repulsion term. This gives the average effect of the repulsion, but not the explicit repulsion interaction. This is a variational calculation, meaning that the approximate energies calculated are all equal to or greater than the exact energy. One of the advantages of this method is that it breaks the many-electron Schrodinger equation into many simpler one-electron equations (below is an example for an electron "1"):

$$\hat{F}(1) \phi_i(1) = \epsilon_i \phi_i(1)$$

Each one-electron equation is solved to yield a single-electron wave function- $\phi_i(1)$, called an orbital, and an energy - ϵ_i , called an orbital energy. The orbital describes the behavior of an electron in the average Coulomb-exchange field of all the other electrons expressed in the Fock operator, \hat{F} . The Fock operator also contains the kinetic energy operator and the Coulomb interaction with nuclei.

Density Functional Theory (DFT)

The density functional theory is a computational quantum mechanical modeling method used in Physics, Chemistry and Material science to investigate the electronic structure (principally the ground state) of many-body systems, in particular atoms, molecules and the condensed phases (Robert et. al, 2002). It is presently the most successful (and also the most promising) approach to compute the electronic structure of matter. Its applicability ranges from atoms, molecules and solids to nuclei and quantum and classical fluids. DFT predicts a great variety of molecular properties: molecular structures, vibrational frequencies, atomization energies, ionization energies, electric and magnetic properties, reaction paths etc.

Beyond Hartree-Fock approximation, the great advantage of density functional theory stems from the inference of correlation effects. More exactly, the density functional approach is based on a strategy of modelling the electron correlation via general functionals of the electron density. Following the work by Kohn and Sham, the approximate functionals employed by current DFT methods separate the electronic energy into several terms:

$$E = E_T + E_V + E_J + E_{XC}$$

Where E_T is the kinetic energy term, E_V includes terms describing the potential energy of the nuclear-electron attraction and of the repulsion between pairs of nuclei, E_J is the electron-electron repulsion term, and E_{XC} is the exchange-correlation term and includes the remaining part of the electron-electron interactions.

The energy sum $E_T + E_V + E_J$ corresponds to the classical energy of the charge distribution ρ . The exchange-correlation term E_{XC} accounts for the exchange energy arising from the antisymmetry of the quantum wavefunctions and for the dynamic correlation in the motions of individual electrons.

Hohenberg and Kohn demonstrated that E_{XC} is entirely determined by the electron density:

$$E_{XC}(\rho) = \int f(\rho_\alpha(r), \rho_\beta(r), \nabla\rho_\alpha(r), \nabla\rho_\beta(r)) d^3(r)$$

Where ρ_α , ρ_β are referring to the corresponding α , β spin densities.

E_{XC} is usually divided into components, referred to as the *exchange* and *correlation* parts, but actually corresponding to the same-spin and mixed-spin interactions, respectively:

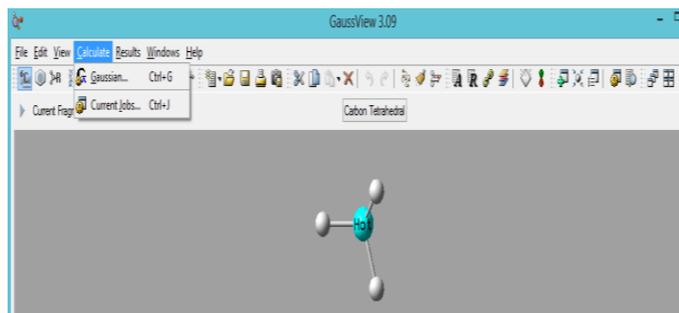
$$E_{XC}(\rho) = E_X(\rho) + E_C(\rho)$$

Pure DFT methods are defined by pairing an exchange functional with a correlation functional. For example, the well-known BLYP functional pairs Becke's gradient-corrected exchange functional with the gradient-corrected correlation functional of Lee, Yang and Parr. (Gang and Charles, 2007)

METHODOLOGY

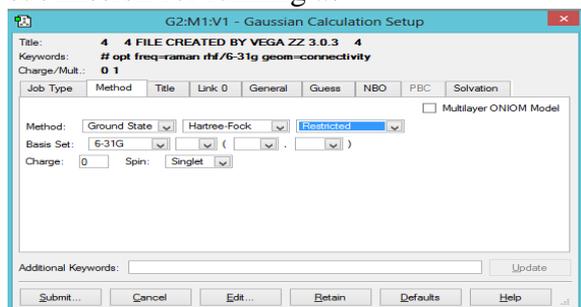
Materials Used: Some of the materials used include a 64-bit operating system HP laptop, Gaussian 03 application software and Open Babel software. The molecular structures of Guanine, Cytosine, Adenine and Thymine bases were obtained from Ligand expo database. Ligand expo (formerly Ligand Depot) is an online database which provides chemical and structural information about small molecules (so-called ligands) within the structure entries of the Protein Data Bank (PDB) Computational Procedure: This work employed computational methods to carry out all the computations. All calculations were performed using Windows Version Gaussian 03 Package. The Gaussian input file was obtained by using Open Babel Package to convert the file from PDB file format. Once this was completed, the Gaussian menu path was used to open the

Gaussian Calculation Setup dialog. As illustrated in the figure below.

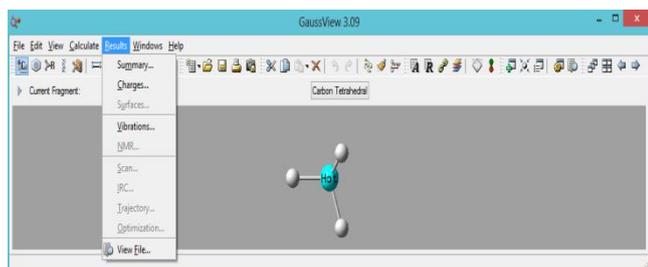


The Gaussian Calculation Setup Window

This dialog allows you to set up virtually all types of Gaussian calculations and to submit them from GaussView. Clicking on calculate menu displayed another dialogue box where the job type, in this case, optimization was chosen and then another dialog where the different types of calculations were set up before submission for running as



The results of the calculations were obtained by clicking on results menu as shown below.



The molecular structures of Adenine, Cytosine, Guanine and Thymine were optimized using RHF and DFT methods with the Gaussian 03 package program. Different basis sets such as 3-21G, 3-21+G, 6-31G and 6-31+G were used. Geometric parameters such as the

optimized bond lengths and bond angles were obtained. Some thermodynamic properties of each of the molecules such as enthalpy, heat capacity, and entropy.

RESULTS AND DISCUSSION

THERMODYNAMIC PROPERTIES OF THE NUCLEOBASES

Some of the thermodynamic properties of adenine, cytosine, guanine and thymine calculated in this work includes enthalpies, heat capacities and entropies. The properties as shown in Tables 1 and 2 were calculated at different levels of theory (RHF and DFT).

Enthalpy

It can be seen from Tables 1 and 2 that at each level of theory with the different basis sets, that guanine has the highest values of enthalpies while cytosine has the lowest values. The order is indicated as follows: G>T>A>C. This indicates that guanine is the most energetic of the molecules. There was also a very strong agreement between the values of enthalpies obtained by the different methods together with the basis sets used.

Heat Capacity

Guanine is also observed to have the highest values of heat capacities at each level of theory with all the basis sets used while cytosine has the lowest values. The order is as follows: G>A>T>C. This means that guanine is the largest of the molecules. Therefore, it is the most stable.

Entropy(S)

The molecule with the highest entropy is guanine while cytosine has the lowest entropy. There is also a strong agreement between the values of entropies obtained by the different methods.

Table 1 The Thermodynamic Properties Obtained By RHF Method

Molecules	E(kCal/Mol)			CV(Cal/Mol-kelvin)		
	3-21G	3-21G +	6-31G	3-21G	3-21G+	6-31G
Adenine	77.498	80.786	78.602	26.176	26.207	25.43
Cytosine	68.322	68.160	69.689	21.470	21.566	22.80
Guanine	83.844	83.783	84.761	27.305	27.313	27.08
Thymine	81.512	81.325	81.978	25.003	24.984	24.85

Table 2 The Thermodynamic Properties Obtained By DFT Method

Molecules	E(kCal/Mol)			CV(Cal/Mol-kelvin)			S(Cal/Mol-kelvin)		
	3-21 G	3-21G +	6-31G	3-21G	3-21G +	6-31G	3-21G	3-21G+	6-31G
Adenine	71.245	71.290	72.013	27.372	27.408	26.871	83.991	85.157	81.676
Cytosine	63.078	63.004	64.228	24.167	24.202	25.596	81.370	80.880	82.168
Guanine	77.740	77.768	78.437	30.291	30.266	30.067	87.756	88.129	86.898
Thymine	75.545	75.460	75.926	27.230	27.167	27.000	83.710	83.288	83.383

OPTIMIZED BOND LENGTHS

The optimized bond lengths obtained by different methods for the molecules are listed in Tables 3,4,5 and 6.

Table 3: Optimized Bond lengths of Adenine.

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3892	1.3899	1.3817	1.3979	1.3996	1.3926
R(1,6)	1.3673	1.3706	1.3655	1.3852	1.388	1.3841
R(1,7)	0.9951	0.9965	0.9902	1.0124	1.0138	1.0073
R(2,3)	1.2926	1.2979	1.2953	1.3252	1.3299	1.3259
R(2,14)	1.0634	1.0647	1.0645	1.0762	1.0775	1.0774
R(3,4)	1.3956	1.4015	1.3915	1.4002	1.407	1.3999
R(4,6)	1.3812	1.38	1.3824	1.4035	1.4019	1.4046
R(4,8)	1.3951	1.3944	1.3987	1.4075	1.4059	1.4105

Table 4: Optimized Bond lengths of Cytosine

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3528	1.3563	1.3569	1.3628	1.3682	1.3686
R(1,4)	1.4226	1.417	1.4117	1.458	1.448	1.4433
R(1,10)	1.0001	1.0015	0.9951	1.0178	1.0192	1.0126
R(2,6)	1.3489	1.355	1.3518	1.3611	1.3689	1.3659
R(2,8)	1.37	1.3729	1.3739	1.3893	1.3901	1.3914
R(3,4)	1.3744	1.3715	1.3722	1.3242	1.3848	1.3857

Table 5; Optimized Bond Length (Å) of Guanine Molecule

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3956	1.3973	1.3875	1.4036	1.4066	1.3981
R(1,3)	1.3553	1.3589	1.3549	1.3766	1.3792	1.3762

R(1,4)	0.99 52	0.9966	0.9902	1.0125	1.014	1.0074
R(2,5)	1.29 03	1.2946	1.2928	1.3211	1.3253	1.3224
R(2,8)	1.06 26	1.0639	1.0637	1.0757	1.077	1.0769
R(3,6)	1.37 11	1.3715	1.3742	1.3981	1.3981	1.4004
R(3,7)	1.35 48	1.3605	1.3597	1.3587	1.3649	1.3649
R(5,6)	1.39 19	1.3972	1.387	1.3987	1.404	1.3969
R(6,9)	1.42 48	1.4212	1.4254	1.4317	1.4276	1.4326

Table 6: Optimized Bond Length (Å) of Thymine Molecule

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3738	1.3696	1.3686	1.3991	1.3944	1.393
R(1,3)	1.3815	1.3881	1.3829	1.3847	1.3924	1.3873
R(1,4)	0.9976	0.9988	0.9924	1.0143	1.0157	1.0091
R(2,5)	1.212	1.2271	1.223	1.2326	1.2483	1.2433
R(2,6)	1.3741	1.3732	1.3715	1.3929	1.3916	1.39
R(3,7)	1.3257	1.3304	1.3346	1.3485	1.3537	1.3569
R(3,8)	1.0698	1.0711	1.07	1.0828	1.0842	1.0831
R(6,9)	1.3897	1.39	1.3875	1.4133	1.4136	1.4102
R(6,11)	1.0008	1.0026	0.996	1.0175	1.0195	1.0126

It can also be seen that as for Adenine, the bonds R(1,7):N1-H7,R(2,14):C2-H14,R(5,15):C5-H15,R(9,11):N9-H11 and R(9,12):N9-H12 between nitrogen and hydrogen and between carbon and hydrogen atoms at the various positions possess the lowest values of bond lengths. These are the strongest bonds in adenine molecule meaning they cannot be easily broken. On the other hand, bonds R(1,2):N1-C2,R(1,6):N1-C6,R(3,4):N3-C4,R(4,6):C4-C6 and R(4,8):C4-C8 between carbon-carbon atoms and carbon-nitrogen atoms at the specified positions have the highest values of bond lengths. These are the weakest bonds.

Similar results were also obtained for the other molecules. It was therefore concluded that at all levels of theory, the bonds between nitrogen and carbon and between carbon and carbon atoms at

the various positions possess the highest values of bond lengths. On the other hand, bonds between nitrogen and hydrogen atoms and carbon-hydrogen atoms at the specified positions have the lowest values of bond lengths. This is an indication that the bonds involving hydrogen atoms are the strongest.

It can also be stated, from the results that on an average basis the bonds N-H and C-H are basically of the same strength and N-C and C-C are also of the same strength.

The results from the table show that at all levels of theory, there is a strong agreement among the bond lengths obtained by the different methods.

OPTIMIZED BOND ANGLES

Table 7: Optimized Bond Angles of Adenine

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,6)	106.8441	106.933	106.9907	106.9772	107.0713	107.0353
A(2,1,7)	127.6483	127.5265	127.3686	127.7674	127.6484	127.5482
A(6,1,7)	125.5075	125.5405	125.6407	125.2554	125.2803	125.4165
A(1,2,3)	112.187	112.0817	112.2797	112.4611	112.2087	112.5762
A(1,2,14)	121.8943	122.2484	122.243	121.9453	122.3718	122.2024
A(3,2,14)	125.9187	125.6699	125.4773	125.5936	125.4196	125.2214
A(2,3,4)	105.3612	105.3	105.2197	104.561	104.706	104.5209
A(3,4,6)	110.1155	110.0733	110.1507	111.0176	110.771	110.8548
A(3,4,8)	132.6599	132.4343	132.6584	132.2169	132.0249	132.3465
A(6,4,8)	117.2247	117.4924	117.1909	116.7655	117.204	116.7988
A(10,5,13)	126.4473	126.2086	126.7007	127.5888	127.045	127.6224

Table 8: Optimized Bond Angle (°) of Cytosine Molecule

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,4)	124.7092	124.6837	124.5331	124.7105	124.6213	124.4866
A(2,1,10)	121.0508	121.2152	121.2376	121.386	121.4693	121.5534
A(4,1,10)	114.24	114.1011	114.2293	113.9036	113.9093	113.96
A(1,2,6)	117.592	117.5996	117.5107	118.5766	118.3732	118.2114
A(1,2,8)	117.848	117.7027	117.7431	117.4017	117.3017	117.4317
A(6,2,8)	124.56	124.6977	124.7462	124.0217	124.3251	124.3569
A(4,3,9)	119.7181	119.4776	119.3418	118.9569	118.7688	118.7889
A(1,4,3)	115.2864	115.7962	116.1621	115.3658	115.9696	116.165
A(1,4,5)	118.1921	117.7388	117.9869	117.7426	117.4351	117.7849

Table 9: Optimized Bond Angle (°) of Guanine Molecule

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,3)	106.8717	106.8445	106.9553	106.9842	106.9483	106.9518
A(2,1,4)	127.8567	127.7202	127.5296	128.0053	127.8296	127.7323
A(3,1,4)	125.2716	125.4353	125.5152	125.0106	125.2221	125.3159
A(1,2,5)	111.303	111.3332	111.552	111.7852	111.6843	112.0369
A(1,2,8)	122.0868	122.3867	122.4276	122.0661	122.435	122.3245
A(5,2,8)	126.6101	126.2801	126.0204	126.1487	125.8808	125.6386

Table 10: Optimized Bond Angle (°) of Thymine Molecule

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,3)	123.2323	123.3435	123.3451	123.6028	123.6647	123.7163
A(2,1,4)	116.0362	116.0254	115.9101	115.4864	115.6158	115.3827
A(3,1,4)	120.7315	120.6311	120.7448	120.9107	120.7195	120.9009
A(1,2,5)	123.2293	123.2202	123.0562	123.2368	123.2086	123.0644
A(1,2,6)	113.1562	113.5251	113.7297	112.2729	112.7339	112.8873
A(5,2,6)	123.6144	123.2548	123.214	124.4903	124.0575	124.0482
A(1,3,7)	122.7811	122.5007	122.5477	122.6131	122.407	122.3857
A(1,3,8)	115.1221	114.8972	115.1228	115.6125	115.2731	115.3995
A(7,3,8)	122.0969	122.6021	122.3295	121.7744	122.32	122.2148
A(2,6,9)	127.862	127.3639	127.1482	128.4327	127.7665	127.6599

As for the cytosine molecule, it was observed that angles A(2,1,4):C-N-C, A(6,2,8):N-C-C, A(3,4,5):N-C-C and A(3,9,8):N-C-C have the highest bond angles. On the other hand, A(4,1,10):C-N-H and A(3,9,12):N-C-H bonds have the lowest bond angles. The observed variation in bond angles within the cytosine molecule suggests that the geometry around specific bonds differs. The bonds A(2,1,4):C-N-C, A(6,2,8):N-C-C, A(3,4,5):N-C-C, and A(3,9,8):N-C-C, characterized by higher bond angles, indicates a relatively open and less constrained geometry around these bonds.

Conversely, the bonds A(4,1,10):C-N-H and A(3,9,12):N-C-H, containing hydrogen atoms, which exhibit the lowest bond angles, suggest a more closed or constrained geometry. The lower bond angles in these bonds may be indicative of increased repulsion or crowding around the involved atoms, leading to a more compact arrangement.

In summary, the observed variations in bond angles in the cytosine molecule imply distinct geometric characteristics in different parts of the molecule, possibly influencing its structural and functional properties.

Similar observations, were also made with other molecules.

It can also be seen that there is a strong agreement among the bond angles at all levels of theory.

SUMMARY AND CONCLUSION

In this work, we unravelled the intricacies of DNA nucleobases - Adenine (A), Guanine (G), Cytosine (C), and Thymine (T). Employing the sophisticated Gaussian 03 package, we employed quantum chemical methods to delve into the very essence of these nucleobases.

Our research scrutinized the optimized geometrical structures, focusing on bond lengths and angles, while probing the thermodynamic

parameters including entropy, enthalpy, and heat capacity. The molecular dance further unfolded as dipole moments were meticulously calculated using both Restricted Hartree–Fock (RHF) and Density Functional Theory (DFT) methods, with 3-21G, 3-21+G, 6-31G, and 6-31+G basis sets.

What emerged was not just data but a symphony of agreement. The optimized geometrical parameters, irrespective of the method employed, harmonized seamlessly. Thermodynamic properties resonated in unison across different calculation methods, affirming the robustness of our findings.

As we compare the duet of DFT and RHF methods, their harmonious collaboration throughout our exploration leaves us with a melodic confirmation of the molecular landscape.

RECOMMENDATIONS

It is recommended that other levels of theory should be used to carry out the computation.

The research may also be carried out using another software like General Atomic and Molecular Electronic Structure System..

In order to witness a tremendous progress in this area of study because of its function in life, Universities and other institutions of higher learning should shoulder the responsibility of purchasing the softwares required for the research because of their high cost.

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DISPERSION OF HUMAN INTESTINAL GEOHELMINTH OVA IN SELECTED REFUSE DUMPSITES IN LOKOJA LOCAL GOVERNMENT AREA OF KOGI STATE

Egbunu, Abiodun Abosedede (1)

Amos, O.(2)

Alabi, Precious Bukola(1)

Abdullahi, Danladi Mohammed(1)

1. Science Laboratory Technology Department, School of Applied Sciences, Kogi State Polytechnic, Lokoja

2. Federal University Lokoja

E-mail: sisterb.ae@gmail.com

ABSTRACT

Geohelminths pose a major public health concern especially among children whose play habits involve sand play, particularly in the tropical and sub-tropical regions of the world where sanitary conditions are poor. A study to determine the prevalence of geohelminths found in different dumpsites in Lokoja Local Government Area of Kogi State was carried out. Soil samples were collected from four dumpsites in Crusher, Old market, Cantonment, Okadigbo and Kogi State Polytechnic areas of Lokoja Local Government area. Ova and larvae of the geohelminths were recovered using zinc sulphate flotation technique. The overall prevalence of geohelminths recorded in Crusher dumpsite were as follows; Trichiuris trichiura (21.8%), Ascaris lumbricoides (33.50%), Ancylostoma duodenale (20.4%) and Entamoeba histolytica (24.8%). In Old Market dumpsite; Trichiuris trichiura (35.2%), Ascaris lumbricoides (20.1%), Ancylostoma duodenale (27.9%) and Entamoeba histolytica (16.9%). In Cantonment dumpsite were as follows; Trichiuris trichiura (29.6%), Ascaris lumbricoides (28.4%), Ancylostoma duodenale (20.9%) and Entamoeba histolytica (20.9%). In Okadigbo; Trichiuris trichiura (27.5%), Ascaris lumbricoides (23.2%), Ancylostoma duodenale (37.7%) and Entamoeba histolytica (11.6%). In Kogi State Polytechnic dumpsite; Trichiuris trichiura (11.1%), Ascaris lumbricoides (12.7%), Ancylostoma duodenale (6.3%) and Entamoeba histolytica (15.9%). The overall prevalence of geohelminths in the various dumpsites shows highest prevalence of Ascaris in Crusher, of Trichuris in Old Market and Cantonment, Of Ancylostoma duodenale in Okadigbo. Entamoeba recorded the lowest prevalence in all the dumpsites in this study. This study adds its voice to the cry for a maintenance of hygiene, basic environmental and sanitation, adequate waste management services and health education on personal hygiene for environmental development and sustainability in Kogi state and in the country in general.

Keywords:

Geohelminths, ova, dumpsites, Prevalence

INTRODUCTION

Soil-transmitted helminths (STH) are among the leading causes of global health problems especially among the poorest and deprived communities where implementation of control measures is difficult.

They are considered among the neglected tropical diseases (NTD). The most widely distributed helminth infections include cestode infections (e.g. taeniasis and echinococcosis); The main species that infect humans in order of prevalence are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), hookworms (*Necator americanus* and *Ancylostoma duodenale*) and tapeworm (*Taenia solium*) Approximately 1.5 billion people are infected with STHs worldwide. (WHO 2023)

Human infection is influenced by poverty, poor personal hygiene, inadequate sanitation and overcrowding. Infections may result in anaemia, retarded growth, and impaired development.

Geohelminth is the second leading cause of death among children below 6 years in Nigeria (Eke et al 2015) Regardless of WASH programming status, nutritional outcomes were significantly influenced by age of the children. Children are more likely to have lower z-scores as they grow older. Mogaji et al 2016)

Most of these STH have also been classified as water borne diseases. According to different WHO reports, waterborne diseases cause about 1.7 million human deaths annually, and 88% is attributed to unsafe water supply, poor sanitation, and lack of hygiene. It is described that approximately, 10% of the population in the developing countries are infected with various intestinal worms. The use of faecal contaminated water is the main source of intestinal parasites. Disease may be acquired through accidental ingestion of these STHs by eggs contaminated water, food, or soil.

AIM OF STUDY

The aim of this study is to determine the prevalence of soil transmitted helminth infection and compare the difference prevalence in different community in Lokoja, Kogi state, Nigeria.

STUDY AREA

The study areas; Crusher, Cantonment, Old market, Okadigbo and Kogi State Polytechnic of Lokoja local government area chosen for this study are densely populated and are poorly planned and poorly developed area.

Lokoja lies about 7.8023 degrees North of the Equator and 6.7333 degrees East of the Meridian. It is about 165km Southwest of Abuja, Federal Capital Territory of Nigeria, as the crow flies, and 390km Northeast of Lagos by same measure. It is located on the confluence of River Niger and River Benue, with a total land coverage of about 63.82 sq. km

METHODOLOGY

Soil samples were collected from five locations of each dumpsite. Soil samples were collected using the method of Stojcevic et al., (2010). Soil was taken from at least 2cm deep into the soil using a hand trowel sterilized by cleaning with 95% ethanol. Soil samples were placed into labelled screw capped bottles and transported to the biology laboratory of Kogi State Polytechnic Lokoja. The soil samples were then analysed for the presence of human intestinal geohelminth ova and larvae.

All the samples were analysed using zinc sulphate flotation technique of Ogbolu et al., (2011). Each soil sample was sieved to remove debris and coarse particles. About 5gm of the soil was mixed thoroughly with 10mls of distilled water, strained into test tube and centrifuged at 2500rpm for 3mins.

The supernatant was decanted and the sediment was mixed with 10mls zinc sulphate solution of 33% weight per volume(1.18-1.20specific gravity). This was filled to the brim of the test-tube with a cover slip placed on the test-tube, to collect the ova and allowed to stand for a few

minutes. The cover slip was carefully removed and placed on a clean glass slide and examined under the light microscope at x10 and x40 magnifications for the presence of ova or cyst of parasites.

RESULTS AND DISCUSSIONS

Table. 1: Prevalence (%)of Geohelminth ova/cyst found in different dumpsites in study area

Study Area dumpsites	A.lumbricoides	T. trichura	<i>Ancylostoma duodenale</i>	E. histolytica	Total
Crusher	77 (33.4%)	49 (21.3%)	47 (20.4%)	57 (24.8%)	230
Old Market	44 (20.1%)	77 (35.2%)	61(27.9%)	37(16.9%)	219
Cantonment	23(28.4%)	24(29.6%)	17(21%)	17(21%)	81
Okadigbo(Felele)	16(23.2%)	19(27.5%)	26(37.7%)	8(11.6%)	69
Kogi Poly	3(5.2%)	7(12.1%)	38(65.5%)	10(17.2%)	58
Total(%)	163(24.8%)	176(26.8%)	143(21.8%)	129(19.6%)	657

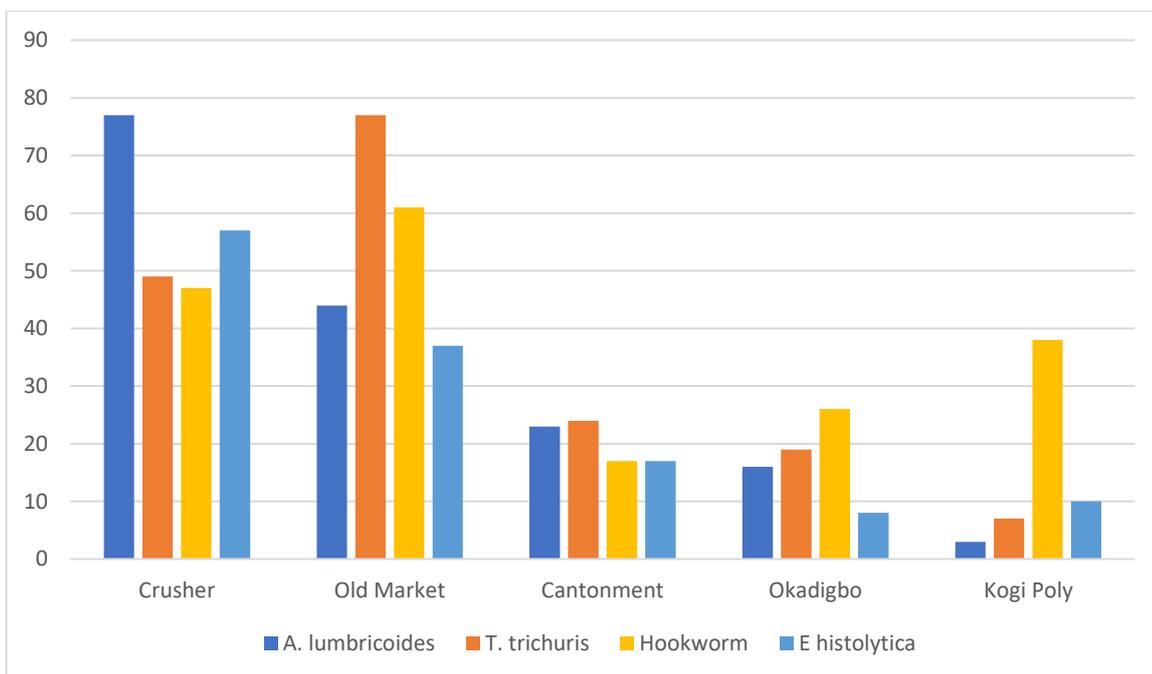


Figure 1. Prevalence (%)of Geohelminth ova/cyst found in different dumpsites in study area

In Crusher *A. lumbricoides* has highest prevalence of 33.4% this agrees with the study by Oluwole et al., (2015)while Hookworm (*Ancylostoma duodenale*) having the lowest prevalence of 20.4%. Studies carried out by Ojurongbe et al,

(2014) shows a higher intensity of Trichuris than Hookworm as recorded in this study.

In Old Market *T. trichiura* has highest prevalence while *E. histolytica* has lowest prevalence rate. This confirmed the findings of Yahaya, O., et al.(2023) Working with vegetables sold in Egah market, Kogi State,

Nigeria. which revealed 9.5% *T. trichiura* prevalence while *E. histolytica* had 3.8% . which happened to be the lowest in the study

In Cantonment highest prevalence rate was also recorded for *T. trichiura* while the lowest prevalence was found in both and *E. histolytica*

Ancylostoma duodenale prevalence rate was highest at the Okadigbo dumpsite while prevalence of *E. histolytica* was also low at this dumpsite. These results agree with studies by Oyebamiji et al., (2018) which showed that the highest prevalence was observed in the refuse dump (74.2%) followed by toilet area (36.5%) while the lowest was at house vicinities (1.6%). Fifty-seven percent of the respondents use pit latrine while 20.6% still practice open defecation. A high transmission risk was observed as large percentage (66.8%) of the respondents showed inadequate knowledge of how to avoid STH infections. Moreover,

64.0% and 25.2% reported that they often walk barefooted and suck fingers respectively. As recorded at the Old market dumpsite, *T. trichiura* has highest prevalence while *E. histolytica* has lowest prevalence rate at the Kogi State Polytechnic dumpsite.

TABLE 2.
Distribution And Prevalence (%) of Geohelminth ova/cyst found in different dumpsites in study area.

STUDY DUMPSITES	NUMBER OF OVA	PREVALENCE %
Crusher	230	35%
Old Market	219	33.3%
Cantonment	81	12.3%
Okadigbo(Felele)	69	10.5%
Kogi Poly	58	8.8%
Total(%)	657	

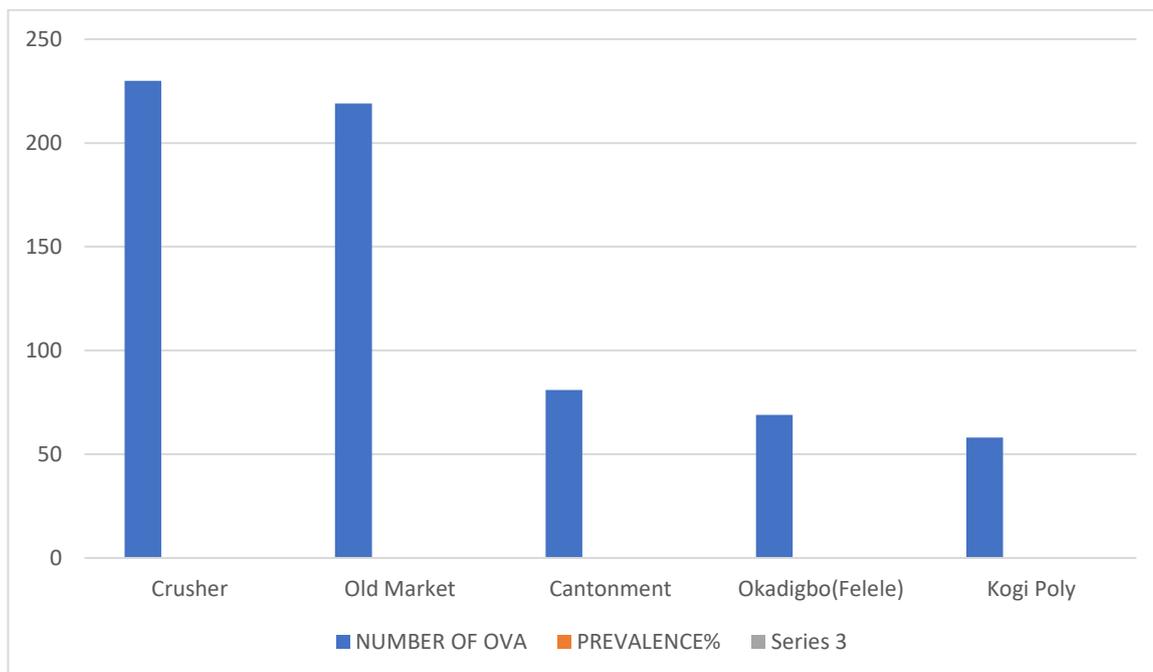


Figure 2: Distribution And Prevalence (%) of Geohelminth ova/cyst found in different dumpsites in study area

The Crusher dumpsite high ova prevalence can be attributed to the concentration of refuse at this particular dumpsite. Old market dumpsite ova prevalence ranks 2nd. The low ova prevalence in Cantoment, Okadigbo and Kogi State Polytechnic could be attributed to the fact that it is less accessible to human defecation activities. Result of Babamale et al (2015) had also revealed significantly higher prevalence and intensity of hookworm infection in individuals from poorer households than those from better-off households. Asaolu et al. (2003) reviewed the various sanitation interventions around the world, and it affirmed from the review, that the use and availability of latrines enhanced the reduction of parasitic ova and larvae, while settings where defecation prevails enhanced these. (Ugbomoiko et al 2012). The high prevalence of parasitic contamination of soil observed in the study may be due to inadequate knowledge of how to prevent transmission of STH which may pose a high risk of re-infections in the study area even after treatment.

CONCLUSION

This study provides evidence of high load of geohelminths in study areas with high human activities such as Old Market and the crusher dumpsite which needs proper waste management through recycling and compost processing. Poor sanitation characterised by open defecation and non-treatment of excreta before entering the environment facilitates STH transmission (WHO 2017). The worms' high reproductive capability produces thousands of eggs, and therefore, are easily dispersed. Poor sanitation could stall a viable STH PC programme as this could promote reinfection and reversion of endemicity level to the pre-control status. (Oyetunde et al 2023). For the control of these infections, the World Health Organization (WHO) has advocated an integrated approach, which includes access to appropriate sanitation, hygiene education, and

preventive chemotherapy (i.e., large-scale, periodic distribution of anthelmintic drugs). It is therefore recommended that sanitation, proper waste disposal and Health education on parasite transmission from primary school as well as grassroots hygiene should be given adequate attention and screening of human activities from dumpsites should be enforced in order to ensure environmental development and sustainability in our nation and the world at large. This study thus adds its voice to the cry for a maintenance of hygiene, basic environmental and sanitation, adequate waste management services and health education on personal hygiene for environmental development and sustainability in Kogi state and in the country in general.

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APPLICATION OF MULTI-BILINEAR BINOMIAL REGRESSION MODEL TO MODELLING
HETEROGENOUS MORTALITY VALUE

By

Lawal F. K

Department of Statistics

Kogi State Polytechnic, Lokoja

Kogi State

lawalfkray@yahoo.com

08030826962

And

Prof. G.M Oyeyemi

Department of Statistics

University of Ilorin, Ilorin

Kwara State

Abstract

A crucial component of mortality modeling is mortality forecasting, which aids in population projections and the calculation of longevity risk for the purpose of actuarial bond pricing. This study examines the Lee-Carter model in the context of diverse populations and suggests a multi-bilinear binomial regression model as an extension of the original model. The models were applied to death rates with a variety of features, and the outcomes were contrasted. The National Bureau of Statistics provided data on male mortality in Nigeria for the age group of five class intervals, spanning 19 years, which the article used for the analysis. Using Aikake Information Criterion (AIC) and Bayesian Information Criterion (BIC) as measures, the suggested model outperforms the Lee-Carter model in terms of fit to the male mortality rates in Nigeria.

Keywords: Mortality, Lee-Carter, Bilinear, Binomial, AIC

1.0 Introduction

Mortality refers to the pernicious course by which living members of the population gradationally die out (Oeppen and Vaupel, 2002). The future of human survival and mortality risk has attracted renewed intrigue in modern time. The historic increase in life expectancy shows little sign of slowing and increased survival which is an important contributor to population senescent. The field of mortality and longevity risks and generally, the accurate forecasting and financial control of such risks has become a topic of great interest to academics activities and financial professionals (Edwards & Munhenga, 2011). Over the last centuries significant increases in life expectancy have been observed for the developed countries (Oeppen and Vaupel, 2002). However, this observed decline in mortality rates is not a general pattern, as periods with gender-specific divergence between countries have emerged. In particular in the Scandinavian countries we have observed a decrease in the mortality rates for Norwegian and Swedish females, but a stable or in some cases even increasing mortality rate for

Danish females in the period from 1980-1995, see e.g. Jacobsen et al. (2004). A similar divergence has not been observed for Scandinavian males. This is an interesting puzzle: why have women in some developed countries had an increasing longevity whereas women in other developed countries have had a stable or decreasing longevity? This phenomena is not unique to the Scandinavian countries. Meslé and Vallin (2006) showed that the USA and the Netherlands exhibit a similar slowdown in life expectancy as the Danish women over this period whereas e.g. France and Japan displayed stable positive improvements in life expectancy. The older generation consumes a growing share of their wealth as they age in decades ahead (Marleen, et al, 2014). It is believed that this will put pressure on the company's balance sheets and government pension scheme. It is however observed in Taruvinga & Gatawa, (2010) that these arrangements of underestimating the population life expectancy, has led to forecasts that are not accurate. The future of mortality is of interest not only for its own usefulness but paramount in the

area of population forecasting which is cardinal to economic, social and health planning. The future requirement of health and social security for ageing population is now a considerable assignment for countries to battle with (Alders and De Beer 2005).

Therefore, the pattern of mortality depends on the distribution of age and sex of each population and mortality rate were thus measured separately for male and female. By mortality rates, this referred to the ratio of deaths to mid-year population size for a given interval of age and time. Mortality rates are among the most important parameters used in evaluating the population health and social levels. In addition to their importance in determining the level of the population's natural growth, Population's growth rate, calculating mortality prospects, creating life tables, Life Insurance and Annuities are products designed to manage financial uncertainty related to how long an individual will survive. Hence, the life time random variable X and its associated mortality models are the basic components in Actuarial Mathematics. In the last 50 years, human mortality has been seen to

follow a ceaseless though constantly irregular declined drift, Lee-carter (1992). The prospects of longer life are viewed as a positive change for individual and a substantial social achievement but have led to concern over their implications regarding the public spending on old age support and needs.

This research work proposed an extension of Lee-Carter mortality model to modelling heterogeneous mortality data using Nigeria male mortality data obtained from Nigeria National Bureau of Statistics.

2.0 Literature Reviewed

To fit and forecast death rates in the US, Lee and Carter introduced a stochastic model in 1992 that was based on a factor analytic method. Numerous individuals have put forth different changes to the LC technique. These include the Tuljapurkar et al. (2000), Lee & Miller (2001), and Booth et al. (2002) methods as well as the LC method without adjustment. Booth et al. (2005) assessed the forecast accuracy of the LC approach and its variations for the first time, and Booth et al. (2006) conducted additional research. The LC method has also undergone a number of

extensions. Among them, the extension put forth by Hyndman & Ullah (2007) has drawn more and more interest among statisticians and demographers.

To predict mortality and fertility rates, their approach incorporated the concepts of functional principal component regression, nonparametric smoothing, and functional data analysis. Erbas et al. (2007) used this method to predict Australia's breast cancer mortality rates. Hyndman & Booth (2008) have also expanded this approach to incorporate the forecasting of migration rates in Australia and to enhance the variance estimation. It was recently expanded by Hyndman & Shang (2009) to enable the weighting of more recent data. In numerous nations, the Lee-Carter (LC) model has been extensively employed for actuarial and demographic purposes due to its relatively strong performance and ease of use. To predict mortality and fertility rates, their approach incorporated the concepts of functional principal component regression, nonparametric smoothing, and functional data analysis. Erbas et al. (2007) used this method to predict Australia's breast cancer mortality rates. Hyndman & Booth

(2008) have also expanded this approach to incorporate the forecasting of migration rates in Australia and to enhance the variance estimation. It was recently expanded by Hyndman & Shang (2009) to enable the weighting of more recent data. In numerous nations, the Lee-Carter (LC) model has been extensively employed for actuarial and demographic purposes due to its relatively strong performance and ease of use.

Lee-Carter (LC) model structure is given as in equation 2.1

$$\log m_{x,t} = \alpha_x + b_x k_t + \varepsilon_{xt}, \sum_x b_x = 1, \sum_t k_t = 0 \tag{2.1}$$

Where, m_{xt} is the matrix of the central death rates at age $X = (x_1, \dots, x_n)$ in year t , ($t = t_1, \dots, t_{n-1}$). The term, ε_{xt} , represents the deviation of the model from the observed log-central death rates and it is expected to be Gaussian Normal i.e $\varepsilon_{xt} \sim N(0, \delta^2)$.

The method assumes a pattern of change in the age distribution of mortality, such that mortality rates decline at different ages maintaining the same time. However, in practice, the general speed of decline at different ages may vary. Wilmoth (1995) opined that the mortality rates at

old ages were observed in Sweden to decline more gradually than at other ages 5 to 50 relative to the older and younger ages.

According to Cains et al. (2009), the LC model is unable to produce a non-trivial correlation structure between the year-on-year changes in mortality rates at different ages. The LC model demonstrate mortality development over time for all ages to one single trend, implies perfect correlation between changes which does not seems biologically reasonable.

The LC model does not incorporate cohort effects in the forecasted mortality rates. The incorporation of cohort effect is a desirable property of any stochastic mortality model (Renshaw and Haberman, 2006).

3.0 Methodology

We propose a modified Lee-Carter model along Renshaw and Haberman model (2006), by including another factor age-Period cohort that will equally preserve the cohort effect and the heterogeneous characteristics of mortality experience in countries with heterogeneous mortality data such as Nigeria by adding an extra

bilinear term to Lee-Carter model. This will take care of the effect of age group cohort in the model and the heterogeneous effect not captured in LC model. Hence, the proposed model is given in equation 3.1

$$\text{Log} (m_{x,t}) = \alpha_x + b_x^{(1)}k_t^{(1)} + b_x^{(2)}k_t^{(2)} + b_x^{(0)}\gamma_{t-x} + E_{xt} \tag{3.1}$$

Where x is the age variable and t the year under investigation and E_{xt} is the random error.

In order to estimate the model, following Renshaw and Haberman (2006), binomial distribution of deaths was assumed and then used log link function targeting the force of mortality μ_{xt} .

$$L(\alpha, b_i, k_i) = \ln \left(\prod_t \prod_x w_{xt} \left(\frac{E_{xt}^0}{\hat{d}_{xt}} \right) \left(1 - \frac{\hat{d}_{xt}}{E_{xt}^0} \right)^{E_{xt}^0 - d_{xt}} \left(\frac{\hat{d}_{xt}}{E_{xt}^0} \right)^{d_{xt}} \right) \tag{3.2}$$

$$= \sum_t \sum_x w_{xt} \left(\ln \left(\frac{E_{xt}^0}{\hat{d}_{xt}} \right) + (E_{xt}^0 - d_{xt}) \ln \left(1 - \frac{\hat{d}_{xt}}{E_{xt}^0} \right) + \left(d_{xt} \ln \left(\frac{\hat{d}_{xt}}{E_{xt}^0} \right) \right) \right) \tag{3.3}$$

In the model w_{xt} are weights taking the value 0 if a particular (x, t) data cell is omitted or 1 if the cell is present and equation 3.4

$$\hat{d}_{xt} = E_{xt} g^{-1} \sum_t \sum_x \left\{ \left(\alpha_x + b_x^{(1)} k_t^{(1)} + b_x^{(2)} k_t^{(2)} + b_x^0 \gamma_{t-x} \right) \right\} \quad (3.4)$$

Is the expected number of deaths predicted by the model with g^{-1} denoting the inverse of the link function of g. The log likelihood was maximized using Newton Raphson method and because of the presence of the bilinear terms, $b_x^{(1)} k_t^{(1)}, b_x^{(2)} k_t^{(2)}$, the uni-dimensional N Newton method proposed by Goodman (1979) for estimating log-linear models with bilinear terms was implemented. In iteration v+1, a single set of parameters is updated fixing the other parameters at their current estimate using the following updating scheme

$$\hat{\theta}^{(v+1)} = \hat{\theta}^{(v)} - \frac{\frac{dL(V)}{d\theta}}{\frac{d^2L(V)}{d\theta^2}} \quad (3.5)$$

Where $L^{(v)} = L(\theta^{(v)})$

$$(3.6)$$

Using the iterative procedure by setting the starting values for $\hat{a}_x, \hat{b}_x^i, \hat{k}_t^i$ and $\hat{\gamma}_{t-x}$

In our models, there are five sets of parameters, these are: $a_x, b_x^{(1)}, k_t^{(1)}, b_x^{(2)}$ and $k_{t-x}^{(2)}$ terms.

The updating scheme is as follows, starting with $a_x^0 = 0, b_x^{(0)} = 1, k_t^{(0)} = 0,$

Compute

$$\hat{a}_x^{(v+1)} = \hat{a}_x^{(v)} - \frac{\sum_t (D_{xt} - D_{xt}^{(v)})}{\sum_t D_{xt}^{(v)}} \quad (3.7)$$

$$\hat{k}_t^{1(v+1)} = \hat{k}_t^{1(v)} - \frac{\sum_t (D_{xt} - D_{xt}^{(v+1)}) \hat{b}_x^{1(v)}}{\sum_t D_{xt}^{(v+1)} (\hat{b}_x^{1(v)})^2} \quad (3.8)$$

$$\hat{b}_t^{1(v+1)} = \hat{b}_t^{1(v)} - \frac{\sum_t (D_{xt} - D_{xt}^{(v+1)}) \hat{k}_t^{1(v)}}{\sum_t D_{xt}^{(v+1)} (\hat{k}_t^{1(v)})^2} \quad (3.9)$$

$$\hat{b}_x^{2(v+1)} = \hat{b}_x^{2(v)} - \frac{\sum_t (D_{xt} - D_{xt}^{(v+1)}) \hat{k}_t^{2(v+1)}}{\sum_t D_{xt}^{(v+1)} (\hat{k}_t^{2(v+1)})^2} \quad (3.10)$$

$$\hat{k}_t^{2(v+1)} = \hat{k}_t^{2(v)} - \frac{\sum_t (D_{xt} - D_{xt}^{(v+1)}) \hat{b}_x^{2(v)}}{\sum_t D_{xt}^{(v+1)} (\hat{b}_x^{2(v)})^2} \quad (3.11)$$

$$\hat{\gamma}_{t-x}^{1(v+1)} = \hat{\gamma}_{t-x}^{(v)} - \frac{\sum_t (D_{xt} - D_{xt}^{(v+1)}) \hat{b}_x^{0(v+1)}}{\sum_t D_{xt}^{(v+1)} (\hat{b}_x^{0(v)})^2} \quad (3.12)$$

$$\widehat{D}_x^{(va_x, vb_x, vk_t, vb_x, vk_t, b_x^0, \gamma_{t-x}^{(v)})} = E_{xt}$$

$$\exp^{(\widehat{a}_x^{va} + \widehat{b}_x^{1(vb_1)} \widehat{k}_t^{(1)vk_t} + \widehat{b}_x^{2(vb_2)} \widehat{k}_t^{(2)vk_t} + \widehat{b}_x^{(0)} \gamma_{t-x}^{(v)})}$$

(3.13)

Is the estimated number of deaths after va updating the parameter a_x, vb_x , update the parameters, $b_x^{(1)}$, vk_t . Updates the parameter $k_t^{(1)}$, $vb_x^{(2)}$, updates the parameter $b_x^{(2)}$ and vk_t updates the parameter $k_t^{(2)}$ and γ_{t-x} . This is made possible to optimize the binomial likelihood by monitoring the deviance as described in Renshaw and Haberman (2003).

From this updating, we arrived at the mortality model estimate as given in equation 3.14

$$\widehat{\eta}_{xt} = \widehat{a}_x + \widehat{b}_x^{(1)} \widehat{k}_t^{(1)} + \widehat{b}_x^{(2)} \widehat{k}_t^{(2)} + b_x^0 \gamma_{t-x}$$

(3.14)

The models were used to analyse the Nigeria male mortality from 1998-2010 as the available mortality data at the time of the research. Hence, The two models to be consider in this paper are Lee-Carter model and Multi bilinear binomial

regression mode as presented in equation 3.15 and 3.16 respectively.

$$LC: \quad \log m_{x,t} = \alpha_x + b_x k_t + \varepsilon_{xt} \quad ,$$

(3.15)

$$PM; \quad \text{Log}(m_{x,t}) = \alpha_x + b_x^{(1)} k_t^{(1)} + b_x^{(2)} k_t^{(2)} + b_x^{(0)} \gamma_{t-x} + E_{xt}$$

(3.16)

4. Analysis

In this unit, we apply the two models to life mortality data obtained from National Bureau of Statistics covering 19 years in age group of five interval. The year under study is 1998 to 2010 as earlier stated. The two models used for the analysis are stated in equation 3.15 and 3.16. The two models were applied to Nigeria male mortality and the discussion of the results and presentations of figures below.

4.1 Discussion of the result

Figures 1, is the Lee-Carter parameters for male Nigeria mortality rates. From the graphs, it was observed that α_x slope downward while the coefficient of age $\beta_{x,t}$ slope upward with sig-sag shape. The k_t parameter slope in reverse of the, $\beta_{x,t}$, this is equally visible from tables 1 and 2 of the parameters. This show that the model produces poor curves and fit for the

heterogeneous mortality data. Considering the parameters of the proposed model (Multi-bilinear Binomial Regression model) given in figures 2, α_x parameter produce a normal curve shape while the cohort parameter portrait a sine shape. k_t^1 and, k_t^2 produce a smoother curves better than the curve produce by LC model for Nigeria male mortality, it was also observed that the curves Produce by the parameter β_x^2 is smoother than LC model given in figure 2.

4.2 Comparison of accuracy of the models

It is commonly expected that models with more parameters will fit the data better when comparing the goodness-of-fit of various models.

It is now common in the mortality literature to use information criteria that modify the maximum likelihood criterion by penalizing models with more parameters, thereby ruling out the possibility that the better fit observed in a model is the result of over-parametrization and allowing comparison of the relative performance of several models. The Bayesian Information Criteria (BIC) and the Akaike Information Criteria (AIC) are two of these criteria. The parameters of the two models fitted to the male mortality data from Nigeria are shown in Table 3.

We saw that both factors result in the same ranking, with the model with the lowest AIC and highest BIC being regarded as the best model. As can be seen from Table 3, the multi-bilinear binomial regression model (PM1) outperforms the LC model in terms of AIC and BIC. This is particularly evident when contrasting Figure 2 of the proposed model with Figure 1 of the LC parameters, since the curves generated by the latter are less angular than those by the former. Thus, the suggested model works better than the LC model.

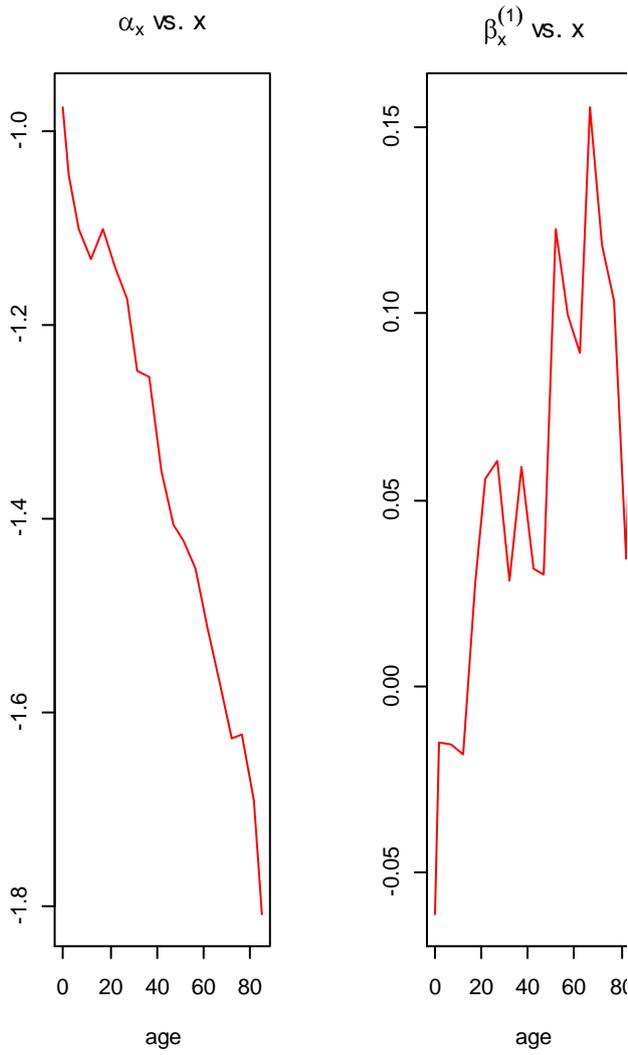


FIGURE 1: LC PARAMETERS FOR MALE MORTALITY IN NIGERIA

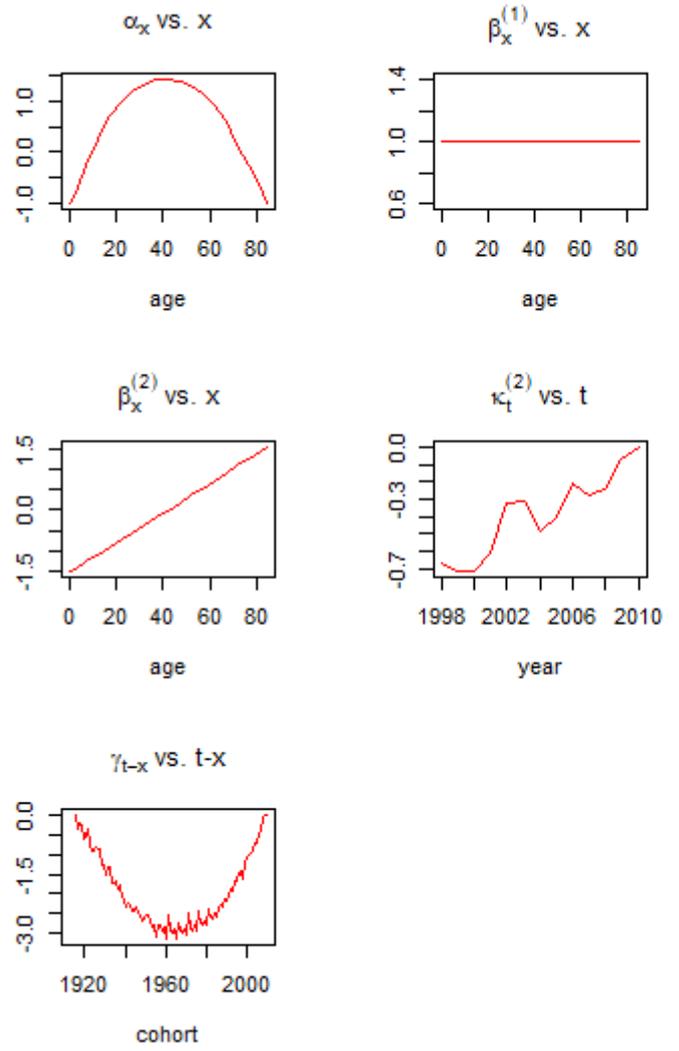


FIGURE 2: PM PARAMETERS FOR MALE MORTALITY IN NIGERIA

Table 1: Showing the parameters of Lee-Carter and Proposed mortality model

AGE	α_x LC	PM	LC	β_{2x} PM
0	- 4.04912	- 2.97029	-0.70858	-1.51805
2	- 4.09545	- 3.83091	-0.49857	-1.44585
7	- 4.11037	- 6.06391	-1.19791	-1.26536
12	- 4.12178	- 8.11861	-1.04172	-1.08486
17	-4.159	- 9.89117	-0.26485	-0.90437
22	- 4.20955	- 11.4479	0.338962	-0.72388
27	- 3.97416	- 12.7028	2.547099	-0.54338
32	-4.1961	- 13.6878	-0.1057	-0.36289
37	- 4.12784	- 14.4124	0.472427	-0.18239
42	- 4.01221	- 14.8779	-0.14034	-0.0019
47	- 4.15991	- 15.0977	0.215226	0.178594
52	- 4.18654	- 15.0584	-0.0042	0.359088
57	- 4.25865	- 14.7157	0.437976	0.539582
62	- 4.15507	- 14.1449	0.720789	0.720077
67	- 4.24685	- 13.3517	0.271844	0.900571
72	- 4.16945	- 12.2491	-0.36423	1.081065
77	- 4.18678	- 10.8645	0.206455	1.261559
82	- 4.20621	- 9.16668	0.450291	1.442053
85	- 4.26364	- 8.08904	-0.33497	1.55035

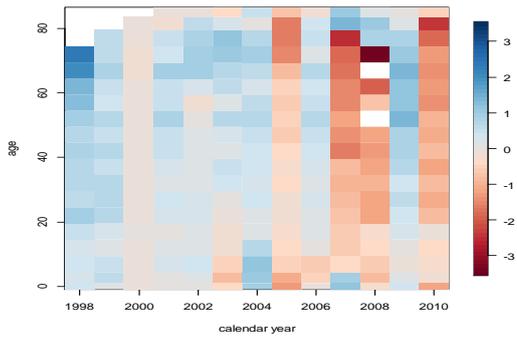


FIGURE 3: HEAT MAPS OF DEVIANCE RESIDUAL FOR LC MALE MODEL

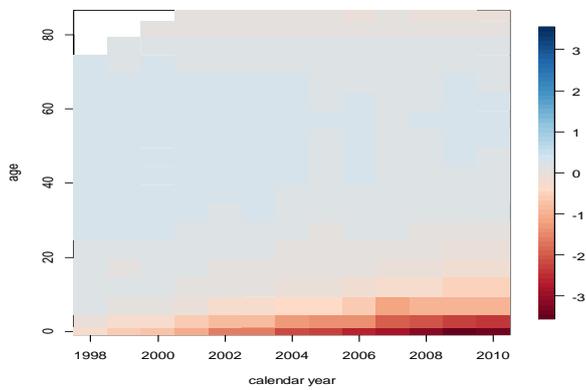


Figure 4: Heat maps of deviance residual for PM Male model

Table 2: The k_t parameters of the LC and the Proposed model

YEAR	LC		PM	
	MALE	MALE		
	K_t	k_{2t}	k_{1t}	
1998	-0.4810	1.5865	0.0000	
1999	0.0015	-0.4993	0.0140	
2000	0.1611	0.1827	0.0359	
2001	0.0412	0.1084	0.0279	
2002	0.1599	0.4063	0.0060	
2003	-0.0067	0.0600	0.0743	
2004	0.0456	0.4467	0.0204	
2005	0.4167	1.3735	0.0180	
2006	-0.0930	1.1633	0.0693	
2007	0.0575	0.7472	0.0655	
2008	0.0927	0.5315	0.1089	
2009	-0.0544	0.1855	0.0431	
2010	-0.3411	0.0000	0.0154	

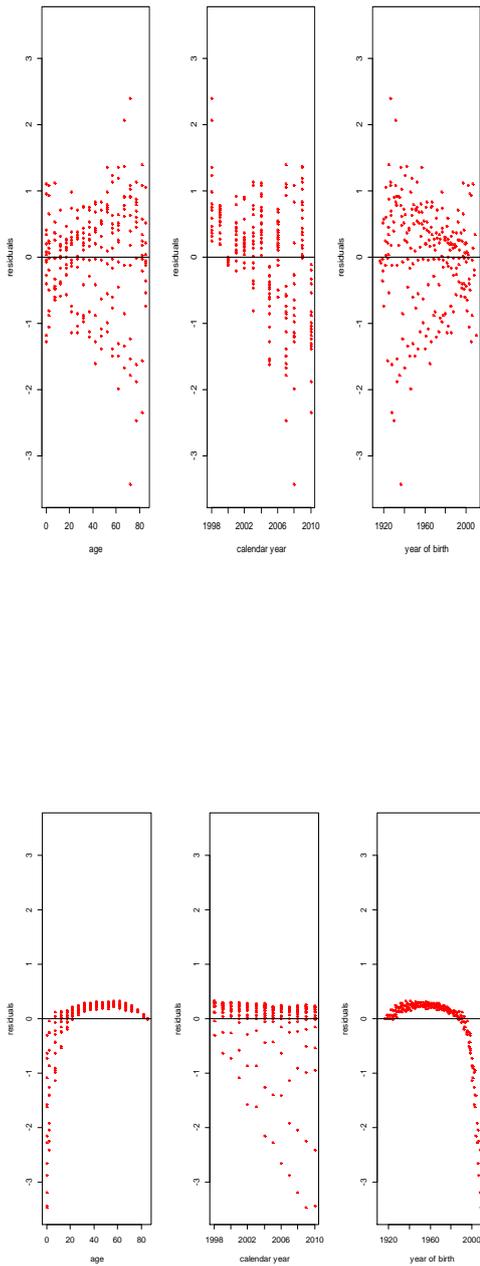


Figure 6: Scattered residuals of PM1 Model for Male

Table 3: Comparison of Parameters

Models/Parameters		AIC	BIC	DEV	LOG	NO.P ARA
LC	MA	252	269	225	-	49
	LE	6.58	7.34	6.35	121 4.29	
PM 1	MA	202	223	105	-	175
	LE	6.40	6,24	9.16	838. 20	

5 Conclusion

The significance and practicality of mortality modeling were highlighted in the study's introduction in Section 1. The study examined the Lee-Carter model and its extensions in Section 2. The analysis of the literature revealed that the majority of the LC model was created in a county with historical data and a constant death rate. As a result, the application of these models to model mortality in developing nations with inconsistent mortality data has not been completed.

The Lee-Carter model and the mathematical analysis of the suggested models are briefly

described in Section 3. The analysis's findings, their interpretation, discussion, and some consequences were covered in Section 4. When it came to modeling male mortality in Nigeria, the suggested model outperformed the LC model, as evidenced by the fact that it provided the lowest AIC and BIC. Furthermore, it is clear that the suggested model's curves are smoother than the Lee-Carter model's. It is advised that population statistics be projected for nations with varied death rates, like Nigeria, using the proposed model, based on the study's suggestion and the results obtained.

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REVIEW ON GENE MUTATION: NEW PROCEDURE TO FIGHT DIABETES, OBESITY AND HEART DISEASES.

Esugah-Mopah, N. M.,¹ Imaji, M.,^{1*} and Balogun, S. G.^{1**}

¹Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic Lokoja.

ABSTRACT

Gene mutation is a permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most people. The occurrence of mutation causes a change or alteration in genetic material; a random change in a gene (basic unit of hereditary) on chromosome resulting in a new trait or characteristics that can be inherited. In other words, mutation can be a source of beneficial genetic variation, or it can be neutral or harmful in effect. Hereditary and acquired (or somatic) mutations are the two major classes of gene mutation. Diabetes is a metabolic condition in which the body does not produce sufficient insulin to regulate blood glucose levels or where the insulin produced is unable to work effectively. There are two types of diabetes namely diabetes insipidus (type 1) and diabetes mellitus (type 2). The International Diabetes Federation (IDF) in 2017 reported that there are about 422 million people with diabetes in the world and this figure is expected to rise 629 million in 2045 with about 70% of this number living in middle- or low-income countries. This thereby makes the need for an effective, affordable, and accessible management/treatment of diabetes a global problem. Diabetes and obesity share some common susceptibility genes (FTO and GNDPA 2), because obesity is a common factor in the etiology of diabetes and hypertension, they share common pathways such as SNS, RAAS, oxidative stress, adipokines, insulin resistance, and PPARs common susceptibility genes. Recent study on gene mutation points to new way to fight diabetes, obesity and heart disease reveals that genetic mutations associated with loss of SGLT1 function are associated with lower post-prandial glucose levels and avoidance of obesity. SGLT2 inhibitors increase urinary glucose excretion, reduce body weight, and lower mortality and hospitalization for heart failure.

Key words: Gene, Mutation, Heart disease, insulin, Diabetes, Body weight.

1.0 INTRODUCTION

Functionally damaging missense variants in SGLT1 protects from diet-induced hyperglycemia in multiple populations (Song *et al.*, 2016; Sara *et al.*, 2018). Reduced intestinal glucose uptake may protect from long-term cardiometabolic outcomes, providing support for therapies that target SGLT1 function to prevent and treat metabolic conditions (Sara *et al.*, 2018). After ingestion, complex carbohydrates are enzymatically broken down to produce monosaccharides (glucose, galactose, and fructose), which are absorbed in the small intestine and used as substrate for the body's metabolically active tissues. The sodium/glucose co-transporter (SGLT)-1 protein is a rate-limiting factor for absorption of glucose and galactose in the small intestine and it uses transmembrane sodium gradients to drive the cellular uptake of these molecules (Abdullah *et al.*, 2011). Loss-of-function mutations, including missense, nonsense, and frameshift mutations, of the SGLT1 gene result in impaired cellular glucose transport and cause glucose-galactose malabsorption (GGM). However, in the process of identifying causal mutations for GGM, SGLT1 gene variants that are associated with subtle abnormalities of glucose absorption *in vivo* have been identified; the importance of these variants, which do not result in GGM, is unknown (Martin *et al.*, 2006).

1.1. Definition of Terms:

1.1.1 Gene: Gene is the basic unit of heredity; basic unit capable of transmitting characteristics from one generation to the next. It consists of a specific sequence of DNA or RNA that occupies a fixed position locus on a chromosome (Song *et al.*, 2010).

1.1.2 Mutation: Mutation is a change or alteration in genetic material; a random change in a gene on chromosome resulting in a new trait or characteristics that can be inherited. Mutation can be a source of beneficial genetic variation, or it can be neutral or harmful in effect (Song *et al.*, 2010).

1.1.3 Gene Mutation

A gene mutation is a permanent alteration in the DNA sequence that makes up a gene, such that the sequence differs from what is found in most people. Mutations range in size; they can affect anywhere from a single DNA building block (base pair) to a large segment of a chromosome that includes multiple genes (Cheng, 2011).

1.2 Classification of Gene Mutations

1.2.1 Hereditary Mutations: These are inherited from a parent and are present throughout a person's life in virtually every cell in the body. These mutations are also called germ line mutations because they are present in the parent's egg or sperm cells, which are also called germ cells (Sara *et al.*, 2018). When an egg and a sperm cell unite, the resulting fertilized egg cell receives DNA from both parents. If this DNA has a mutation, the child that grows from the

fertilized egg will have the mutation in each of his or her cells (Sara *et al.*, 2018).

1.2.2 Acquired (or Somatic) Mutations: These occur at some time during a person's life and are present only in certain cells, not in every cell in the body. These changes can be caused by environmental factors such as ultraviolet radiation from the sun, or can occur if an error is made as DNA copies itself during cell division. Acquired mutations in somatic cells other than sperm and egg cells) cannot be passed to the next generation (Cheng, 2011). Genetic changes that are described as *de novo* (new) mutations can be either hereditary or somatic. In some cases, the mutation occurs in a person's egg or sperm cell but is not present in any of the person's other cells. In other cases, the mutation occurs in the fertilized egg shortly after the egg and sperm cells unite (Sara *et al.*, 2018). (It is often impossible to tell exactly when a *de novo* mutation happened.) As the fertilized egg divides, each resulting cell in the growing embryo will have the mutation. *De novo* mutations may explain genetic disorders in which an affected child has a mutation in every cell in the body but the parents do not, and there is no family history of the disorder. (Sara *et al.*, 2018). Somatic mutations that happen in a single cell early in embryonic development can lead to a situation called mosaicism. These genetic changes are not present in a parent's egg or sperm cells, or in the fertilized egg, but happen a bit later when the embryo includes several cells. As

all the cells divide during growth and development, cells that arise from the cell with the altered gene will have the mutation, while other cells will not. Depending on the mutation and how many cells are affected, mosaicism may or may not cause health problems. Most disease-causing gene mutations are uncommon in the general population. However, other genetic changes occur more frequently (Martins *et al.*, 2006). Genetic alterations that occur in more than one (1) percent of the population are called polymorphisms. They are common enough to be considered a normal variation in the DNA. Polymorphisms are responsible for many of the normal differences between people such as eye color, hair color, and blood type. Although many polymorphisms have no negative effects on a person's health, some of these variations may influence the risk of developing certain disorders (Mugo *et al.*, 2007).

1.3 Diabetes

Diabetes is a metabolic condition in which the body does not produce sufficient insulin to regulate blood glucose levels or where the insulin produced is unable to work effectively. The International Diabetes Federation (IDF) in 2017 reported that there are about 422 million people with diabetes in the world and this figure is expected to rise 629 million in 2045 with about 70% of this number living in middle- or low-income countries.

Types of diabetes:

1.3.1 Type 1 diabetes: This an auto-immune condition in which the cells that produce insulin are destroyed, so lifelong treatment with insulin is required to prevent death. About 10% of people with diagnosed diabetes have type 1 diabetes (Song *et al.*, 2010).

1.3.2 Type 2 diabetes: This accounts for at least 90% of all cases of diabetes. Diabetes mellitus is a group of metabolic diseases characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Ogurtsova *et al.*, 2017). It occurs when the body either stops producing enough insulin for its needs or becomes resistant to the effect of insulin produced. The condition is progressive requiring lifestyle management (diet and exercise) at all stages. Over time most people with type 2 diabetes will require oral drugs and or insulin and type 2 diabetes may remain undetected for many years (IDF, 2017).

1.3.2.1.2 Risk Factors for Type 2 Diabetes

i. Obesity and overweight (Body Mass Index (BMI) and Waist Circumference)

Being overweight or obese especially for longer periods of time (a BMI of 25kg/m² or more) is the main modifiable risk factor for type 2 diabetes (Gatineau *et al.*, 2014), especially in early adulthood (Kodama *et al.*, 2014). A recent study found that severely obese people (BMI ≥ 40) were at an even greater risk of type 2 diabetes, when compared to obese people with a lower BMI (BMI 30 - 39.9) (Vinciguerra *et al.*, 2013). A large waist circumference is associated with increased likelihood of developing type 2 diabetes. Men are at higher risk of type 2 diabetes if they have a waist circumference of 94 - 102 cm and are at very high risk if it is more than 102 cm (Leeds, 2014). Women are at higher risk if they have a waist circumference of 80 – 88 cm and at very high risk if it is more than 88 cm.

ii. Genetic Determinant and Molecular Pathway

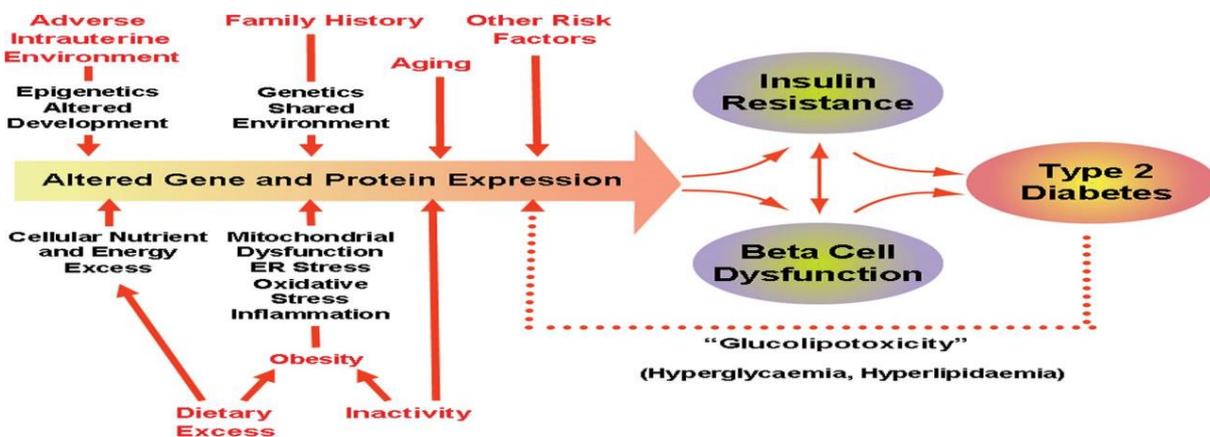


Figure 1: Genetic determinant and molecular pathway

Source: (Gatineau *et al.*, 2014)

1.3.2.2. Linkage Mechanisms of these Metabolic Syndromes

i.Theories of why obesity may lead to type 2 diabetes include:

Abdominal obesity may cause fat cells to release pro-inflammatory chemicals (Gatineau *et al.*, 2014). These chemicals can make the body less sensitive to the insulin it produces by disrupting the function of insulin responsive cells and their ability to respond to insulin (Freemantle *et al.*,

2008; Despres, 2014), obesity may trigger changes to the body's metabolism that cause adipose (fat) tissue to release increased amounts of fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors that are involved in the development of insulin resistance. When insulin resistance is accompanied by dysfunction of pancreatic islet beta-cells (the cells that release insulin) it leads to failure to control blood glucose levels (Kahn *et al.*, 2006).

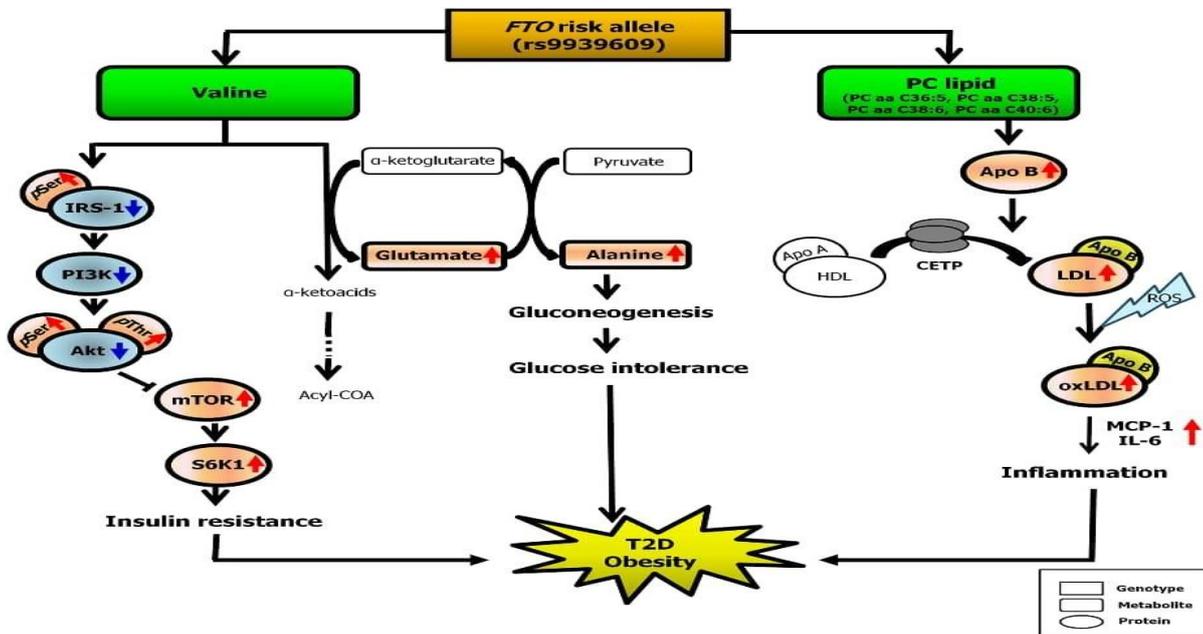


Figure 2: The FTO gene, promoting obesity and overeating, a linkage mechanism to type 2 diabetes.

Source: (Gatineau *et al.*, 2014)

ii. Putative Pathophysiologic Mechanisms in the Development of Hypertension in Diabetes Mellitus

In Hong Kong studies of single nucleotide polymorphisms (SNPs), SNPs that predict the development of diabetes were found also to

predict the development of hypertension (Bernard and Cheung, 2012). Obese persons have a significantly higher risk of hypertension and type 2 diabetes (Bernard and Cheung, 2012). The obese (ob) gene was the first gene identified to be related to the onset of obesity (Ingalls *et al.*,

2004). Genome-wide association studies have revealed a number of genes influencing the susceptibility to obesity (Wang *et al.*, 2010). The FTO gene, promoting obesity and overeating, was one of the key obesity susceptibility genes. Together with the GNDPA 2 gene, they predict persistent central obesity in the Chinese population (Wang *et al.*, 2010). It is not

surprising to find that diabetes and obesity share some common susceptibility genes, because obesity is a common factor in the etiology of diabetes and hypertension, they share common pathways such as SNS, RAAS, oxidative stress, adipokines, insulin resistance, and PPARs common susceptibility genes (Mugo *et al.*, 2007; Bernard and Cheung, 2012).

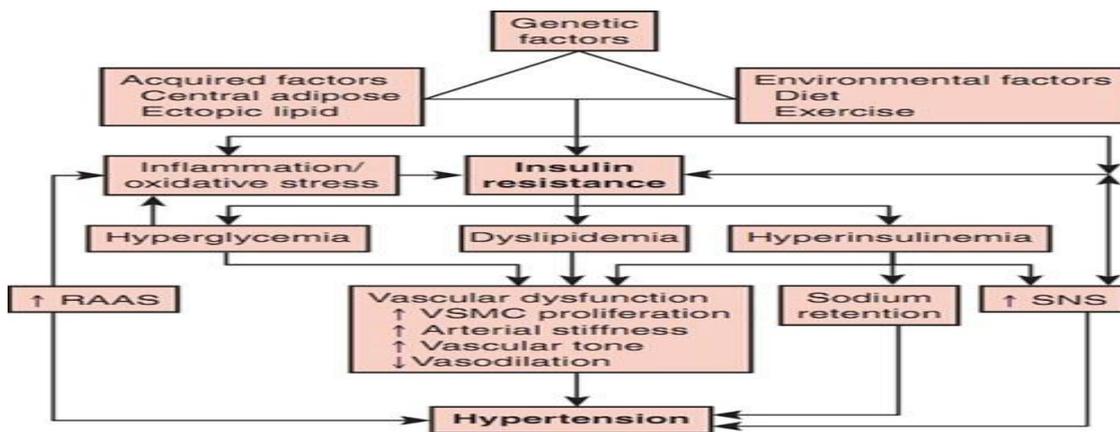


Figure 3: Summary of putative pathophysiologic mechanisms in the development of hypertension in diabetes mellitus. RAAS - renin-angiotensin-aldosterone system; SNS - sympathetic nervous system; VSMC - vascular smooth muscle cell. (Mugo *et al.*, 2007).

Source: (Mugo *et al.*, 2007).

4. Recent Study on Gene Mutation Points to New Way to Fight Diabetes, Obesity and Heart Disease

The sodium glucose cotransporter SGLT1 is strongly expressed in the apical brush border of the small intestine and the late proximal tubule of the kidney, where it is critical for absorption/reabsorption of glucose into the blood stream. Inhibition of SGLT1 and combined inhibition of SGLT1/SGLT2 is an interesting new anti-hyperglycemic concept (Cheng, 2011).

SGLT1 inhibition improves glucose homeostasis in diabetic patients by reducing dietary glucose absorption in the intestine and by increasing the release of gastrointestinal incretins like glucagon-like peptide-1. SGLT1 inhibition has a small glucosuric effect in the normal kidney and this effect is increased when more glucose is delivered to SGLT1 in the late proximal tubule, as shown during inhibition of SGLT2 and as can occur in diabetes (Martins *et al.*, 2006).

In short-term studies, inhibition of SGLT1 and combined SGLT1/SGLT2 inhibition appeared to

be safe and not associated with increased rates of hypoglycemia or clinical relevant gastrointestinal side effects. Summarily, SGLT1 absorbs glucose from the intestine, while both SGLT2 and SGLT1 are involved in renal reabsorption. Genetic mutations associated with loss of SGLT1 function are associated with lower post-prandial glucose levels and avoidance of obesity. SGLT2 inhibitors increase urinary glucose excretion, reduce body weight, and lower mortality and hospitalization for heart failure. Potential adverse effects include urogenital infections and euglycemic ketoacidosis. Loss-of-function mutations in the SGLT1 (sodium/glucose co-transporter-1) gene result in a rare glucose/galactose malabsorption

disorder and neonatal death if untreated (Mugo *et al.*, 2007).

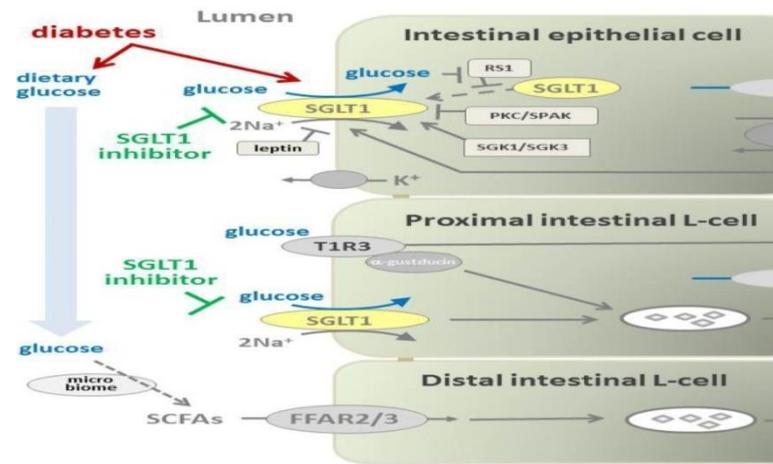


Figure 4 : SGLT1 inhibition reduces intestinal glucose absorption and enhances the sustained GLP-1 release from the distal intestine

Source: (Song *et al.*, 2016).

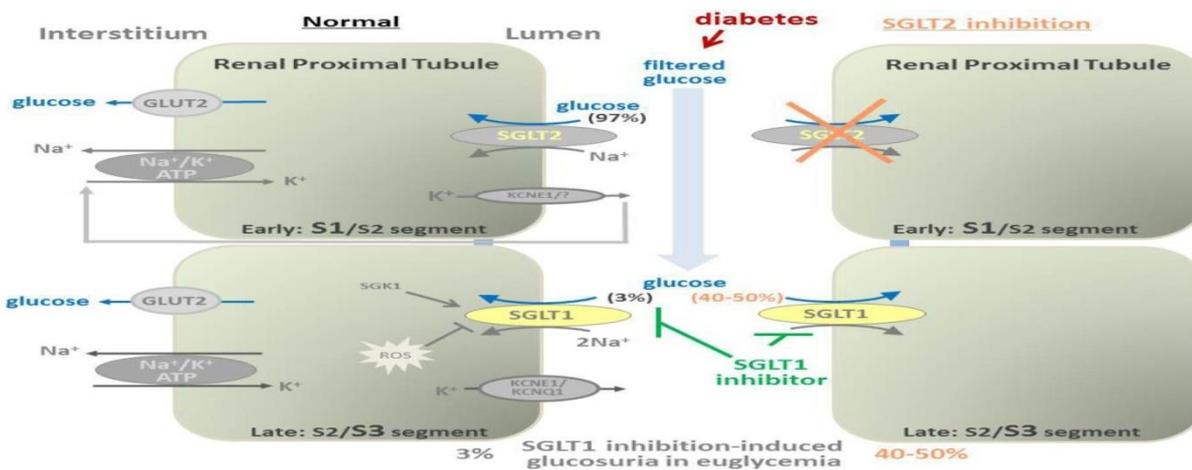


Figure 5: SGLT1 inhibition enhances glucosuria, particularly when upstream SGLT2 is overwhelmed by hyperglycemia or when SGLT2 is inhibited.

Source: (Song *et al.*, 2016).

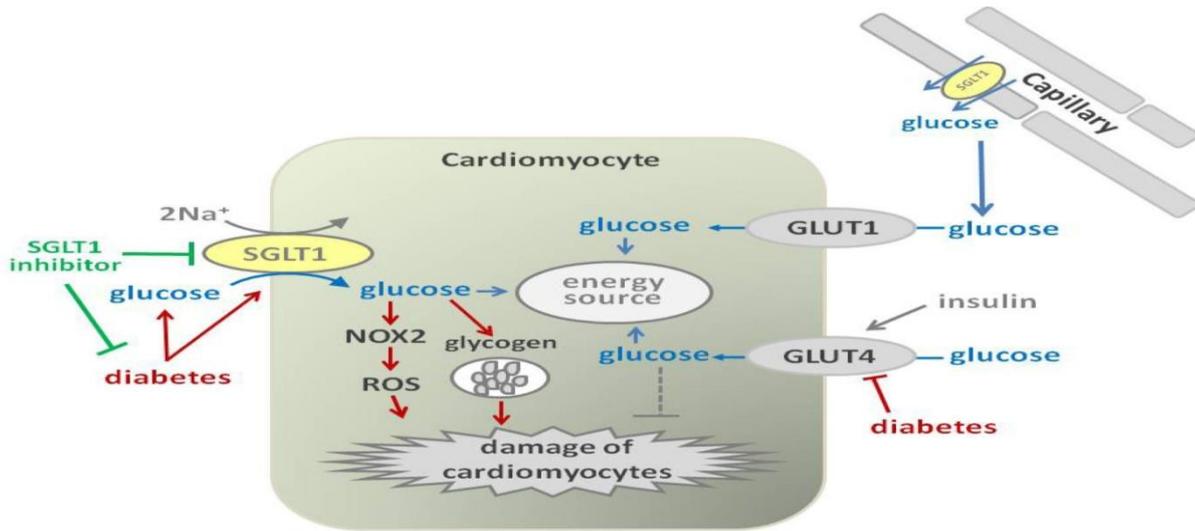


Figure 6: Potential effects of SGLT1 inhibition on the heart – a two-edged sword? In addition to fatty acids, cardiomyocytes use glucose as an energy source for glycolysis and oxidative phosphorylation. GLUT1 is responsible for basal glucose uptake and GLUT4 mediates insulin-induced glucose uptake.

Source: (Song *et al.*, 2016).

5 Conclusion

The SGLT1 N51S/A411T/H615Q haplotype was associated with protection from postprandial hyperglycemia and obesity in European- and African American subjects from 4 U.S. communities and 2 European cohorts from Finland. These data provide support that reduction of postprandial glucose levels via SGLT1 inhibition may be associated with reduced incidence of diabetes mellitus, heart failure, and/or mortality in certain populations at risk.

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DENSITY FUNCTIONAL THEORY(DFT) AND RESTRICTED HARTREE FORK (RHF) BASED
STUDY OF THE STRUCTURES AND THERMODYNAMIC PROPERTIES OF THE MOLECULAR
COMPONENTS OF DEOXYRIBONUCLEIC ACID(DNA)

BY

SALIHU ABUBAKAR, salbak2000@gmail.com,

ESUGA-MOPAH NINMA MARY

OLATUNDE OPEYEMI STEPHEN

Department of Science Laboratory Technology,

Kogi State Polytechnic, Lokoja

ABSTRACT

Deoxyribonucleic acid (DNA) is a molecule that contains the instruction an organism needs to develop, live and reproduce. These instructions are found inside every cell and are passed down from parents to their children. In this work, the DNA nucleobases i.e. Adenine (A), Guanine (G), Cytosine(C) and Thymine (T) were investigated using Gaussian 03 package which employs quantum chemical methods calculation. The optimized geometrical structures calculated are the bond lengths and bond angles while the thermodynamic parameters are the entropy, enthalpy, and heat capacity of the molecules were also calculated using Restricted Hartree–Fock (RHF) and Density Functional Theory (DFT) methods with 3-21G, 3-21+G, 6-31G and 6-31+G basis sets. The values of optimized geometrical parameters of each of the molecules obtained by the different methods were compared with one another. It was found that there is a good agreement between the calculated values. The values of the thermodynamic properties of each of the molecules calculated by the different methods were in agreement with one another.

Keywords: Nucleobases, thermodynamics, quantum, optimised.

INTRODUCTION

Biophysics, as an interdisciplinary science, employs principles from physics to examine intricate macro-molecular systems within living organisms (Alberts et al., 2014). Living organisms operate as self-regulated chemical entities, guided by molecular signals, receptors, and transducers (Nelson et al., 2020). Nucleic acids, biopolymers, proteins, and enzymes constitute essential subsystems in this context (Phillips et al., 2012). Biophysicists delve into the investigation of intramolecular and intermolecular interactions, exploring electronic and structural alterations, as well as the transfer of electrons, protons, ions, and energy within biological systems (Nelson et al., 2020). Theoretical challenges arise from the inherent openness and far-from-equilibrium nature of living systems.

Historically, studies on biological systems were dominated by disciplines such as biochemistry, molecular biology, structural biology, and genetics. These fields have yielded precise information concerning the chemical compositions of organisms, reaction pathways, and the elucidation of genetic codes (Alberts et al., 2014). A potential shift towards a new era suggests an integral role for physics and mathematics in advancing biophysics (Nelson et al., 2020). Biochemistry, focusing primarily on direct atomic contact, encounters challenges in addressing subtle, weak forces contributing to various biological phenomena, including the solvation problem.

Biological function emanates from specific chemical reactions and cascades, wherein molecules rely on either quantum mechanical or classical interactions. The primary task for theoretical biophysicists revolves around scrutinizing the physical characteristics of biological molecules and simple systems, all while considering the openness of biological systems (Phillips et al., 2012). Routine exchanges of energy and matter transpire between biological systems and their

environment, with components like proteins undergoing continuous restructuring, showcasing stability timescales longer than their biological functions.

Modeling assumes a critical role in scientific progress. A biological model serves to illustrate interrelationships, a biochemical model portrays complex chemical reactions, and a computational model simulates processes. In contrast, physical models encompass theoretical descriptions with equations of motion, enabling quantitative predictions and emphasizing the necessity to move beyond mere reproduction of known results (Alberts et al., 2014).

NUCLEIC ACIDS

Nucleic acids, including DNA and RNA, play a crucial role in living organisms by preserving and transmitting genetic information. (Shen, 2019) Despite their one-dimensional nature, understanding the three-dimensional structure of nucleic acids is essential for governing their organization, functions, and interactions with proteins.

Each nucleotide, the basic unit of nucleic acids, comprises a phosphate group, a sugar group, and a nitrogen base. The specificity of nucleic acid units is determined by the organic base, related to either the purine or pyrimidine ring system. DNA contains four bases: Adenine (A), Guanine (G), Cytosine (C), and Thymine (T), while RNA replaces Thymine with Uracil (U) (Troop, 2012)

James Watson and Francis Crick proposed a DNA structure model, emphasizing the anti-parallel arrangement of two strands held together by hydrogen bonds between nitrogenous bases. Hydrogen bonds that hold together the two strands of nucleotides in DNA have been the main scope of many experimental and theoretical investigations for three decades. The importance of this molecular interaction is due to its role in DNA replication and complementarity of nucleic acid bases which is the cornerstone of the genetic code (Espejo and Gonzalez, 2007).

Watson-Crick base pairing ensures complementary interactions, with Adenine pairing with Thymine (or Uracil in RNA) and Guanine pairing with Cytosine (Hartl and Ruvolo, 2012). This precise base pairing is crucial for DNA replication, preventing detrimental gene mutations (Friedberg et al, 2005).

The aim of this research is to study the molecular structure and thermodynamic properties of Guanine, Cytosine, Adenine and Thymine.

There have been exceptional developments of experimental methods to investigate the structure of nucleic acids at the atomic level. However there are many instances in which some information are not obtainable. As a result of the limitations of experimental techniques and recent development of computer hardware and simulation algorithms, we have witnessed an expansion of computational studies of nucleic acids in recent times. The current computational methods provide us with information complementary to the experimental techniques. A deeper investigation of the molecular structures and properties of the molecular components of the DNA is required because of their significance in cell life.

THEORETICAL BACKGROUND

Geometry optimization: Geometry optimization is a name for the procedure that attempts to find the configuration of minimum energy of a molecule. The procedure calculates the wave function and the energy at a starting geometry and then proceeds to search a new geometry of lower energy. This is repeated until the lowest energy geometry is found. The procedure calculates the force on each atom by evaluating the gradient (first derivative) of the energy with respect to atomic position. Geometrical parameters are: bond lengths, bond angles and dihedral angles. (Riahi et. al, 2010).

Bond length is the distance between the nuclei of two atoms joined by a covalent bond. Bond length depends on the particular atoms in the bond and on the bond order. The shorter the bond length, the larger is the value of bond energy. It is expressed in Angstrom units (Å) or picometers (pm). $1\text{Å} = 10^{-10}\text{m}$ and $1\text{pm} = 10^{-12}\text{m}$.

Bond angles are the angles made by the lines joining the nuclei of the atoms in a molecule. The bond angles

of a molecule, together with the bond lengths define the shape and size of the molecule.

Restricted Hartree-Fork (RHF):In computational Physics and Chemistry, the Restricted Hartree-Fork (RHF) method is a method of approximation for the determination of the wavefunction and the energy of a quantum many-body system in a stationary state. It is used for closed shell atoms or molecules such as He, H₂O, NH₃, CH₃COOH etc. Most molecules fall in this group.

The most common type of *ab initio* calculations is performed in the framework of the Hartree-Fock (HF) method, which is based on the central field approximation. This means that the Coulombic electron-electron repulsion is taken into account by integrating the repulsion term. This gives the average effect of the repulsion, but not the explicit repulsion interaction. This is a variational calculation, meaning that the approximate energies calculated are all equal to or greater than the exact energy. One of the advantages of this method is that it breaks the many-electron Schrodinger equation into many simpler one-electron equations (below is an example for an electron “1”):

$$\hat{F}(1) \varphi_i(1) = \varepsilon_i \varphi_i(1)$$

Each one-electron equation is solved to yield a single-electron wave function- $\varphi_i(1)$, called an orbital, and an energy - ε_i , called an orbital energy. The orbital describes the behavior of an electron in the average Coulomb-exchange field of all the other electrons expressed in the Fock operator, \hat{F} . The Fock operator also contains the kinetic energy operator and the Coulomb interaction with nuclei.

Density Functional Theory (DFT)

The density functional theory is a computational quantum mechanical modeling method used in Physics, Chemistry and Material science to investigate the electronic structure (principally the ground state) of many-body systems, in particular atoms, molecules and the condensed phases (Robert et. al, 2002). It is presently the most successful (and also the most promising) approach to compute the electronic structure of matter. Its applicability ranges from atoms, molecules and solids to nuclei and quantum and classical fluids. DFT predicts a great variety of molecular properties: molecular structures, vibrational frequencies, atomization energies,

ionization energies, electric and magnetic properties, reaction paths etc.

Beyond Hartree-Fock approximation, the great advantage of density functional theory stems from the inference of correlation effects. More exactly, the density functional approach is based on a strategy of modelling the electron correlation via general functionals of the electron density. Following the work by Kohn and Sham, the approximate functionals employed by current DFT methods separate the electronic energy into several terms:

$$E = E_T + E_V + E_J + E_{XC}$$

Where E_T is the kinetic energy term, E_V includes terms describing the potential energy of the nuclear-electron attraction and of the repulsion between pairs of nuclei, E_J is the electron-electron repulsion term, and E_{XC} is the exchange-correlation term and includes the remaining part of the electron-electron interactions.

The energy sum $E_T + E_V + E_J$ corresponds to the classical energy of the charge distribution ρ . The exchange-correlation term E_{XC} accounts for the exchange energy arising from the antisymmetry of the quantum wavefunctions and for the dynamic correlation in the motions of individual electrons.

Hohenberg and Kohn demonstrated that E_{XC} is entirely determined by the electron density:

$$E_{XC}(\rho) = \int f(\rho_\alpha(r), \rho_\beta(r), \nabla\rho_\alpha(r), \nabla\rho_\beta(r)) d^3(r)$$

Where ρ_α , ρ_β are referring to the corresponding α , β spin densities.

E_{XC} is usually divided into components, referred to as the *exchange* and *correlation* parts, but actually corresponding to the same-spin and mixed-spin interactions, respectively:

$$E_{XC}(\rho) = E_X(\rho) + E_C(\rho)$$

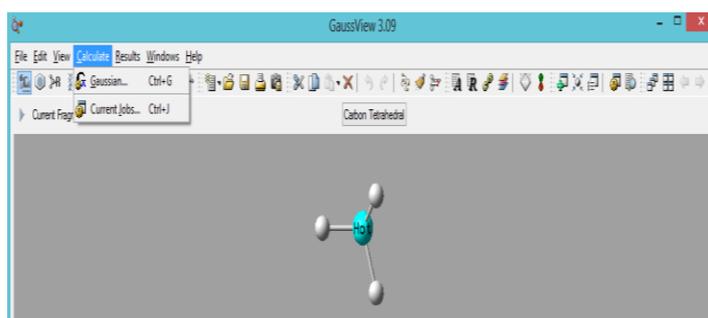
Pure DFT methods are defined by pairing an exchange functional with a correlation functional. For example, the well-known BLYP functional pairs Becke's gradient-corrected exchange functional with the gradient-corrected correlation functional of Lee, Yang and Parr. (Gang and Charles, 2007)

3.0 METHODOLOGY

Materials Used

Some of the materials used include a 64-bit operating system HP laptop, Gaussian 03 application software and Open Babel software. The molecular structures of Guanine, Cytosine, Adenine and Thymine bases were obtained from Ligand expo database. Ligand expo (formerly Ligand Depot) is an online database which provides chemical and structural information about small molecules (so-called ligands) within the structure entries of the Protein Data Bank (PDB)

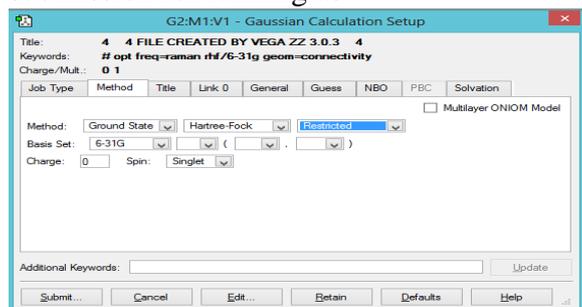
Computational Procedure: This work employed computational methods to carry out all the computations. All calculations were performed using Windows Version Gaussian 03 Package. The Gaussian input file was obtained by using Open Babel Package to convert the file from PDB file format. Once this was completed, the Gaussian menu path was used to open the Gaussian Calculation Setup dialog. As illustrated in the figure below.



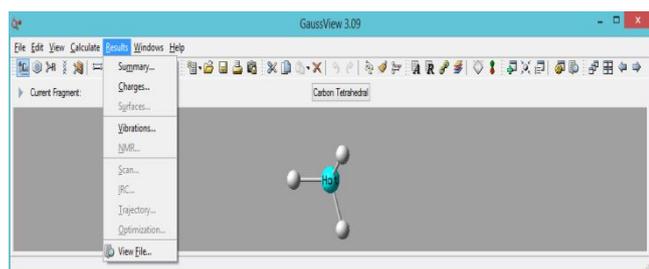
The Gaussian Calculation Setup Window

This dialog allows you to set up virtually all types of Gaussian calculations and to submit them from GaussView. Clicking on calculate menu displayed another dialogue box where the job type, in this case, optimization was chosen and then another dialog where the different types of calculations were set up before

submission for running as



The results of the calculations were obtained by clicking on results menu as shown below.



The molecular structures of Adenine, Cytosine, Guanine and Thymine were optimized using RHF and DFT methods with the Gaussian 03 package program. Different basis sets such as 3-21G, 3-21+G, 6-31G and 6-31+G were used. Geometric parameters such as the optimized bond lengths and bond angles were obtained. Some thermodynamics properties of each of the molecules such as enthalpy, heat capacity, and entropy.

4.0 RESULTS AND DISCUSSION

THERMODYNAMIC PROPERTIES OF THE NUCLEOBASES

Some of the thermodynamic properties of adenine, cytosine, guanine and thymine calculated in this work includes enthalpies, heat capacities and entropies. The properties as shown in Tables 1 and 2 were calculated at different levels of theory (RHF and DFT).

Enthalpy

It can be seen from Tables 1 and 2 that at each level of theory with the different basis sets, that

guanine has the highest values of enthalpies while cytosine has the lowest values. The order is indicated as follows: $G > T > A > C$. This indicates that guanine is the most energetic of the molecules. There was also a very strong agreement between the values of enthalpies obtained by the different methods together with the basis sets used.

Heat Capacity

Guanine is also observed to have the highest values of heat capacities at each level of theory with all the basis sets used while cytosine has the lowest values. The order is as follows: $G > A > T > C$. This means that guanine is the largest of the molecules. Therefore, it is the most stable.

Entropy(S)

The molecule with the highest entropy is guanine while cytosine has the lowest entropy. There is also a strong agreement between the values of entropies obtained by the different methods.

Table 1 The Thermodynamic Properties Obtained By RHF Method

Molecules	E(kCal/Mol)			CV(Cal/Mol-kelvin)			S(Cal/Mol-kelvin)		
	3-21G	3-21G +	6-31G	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
Adenine	77.498	80.786	78.602	26.176	26.207	25.436	80.786	80.928	79.789
Cytosine	68.322	68.160	69.689	21.470	21.566	22.802	76.257	76.439	77.641
Guanine	83.844	83.783	84.761	27.305	27.313	27.081	84.341	84.662	84.437
Thymine	81.512	81.325	81.978	25.003	24.984	24.856	81.640	81.637	81.624

Table 2 The Thermodynamic Properties Obtained By DFT Method

Molecules	E(kCal/Mol)	CV(Cal/Mol-kelvin)	S(Cal/Mol-kelvin)
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Molecules	3-21 G	3-21G +	6-31G	3-21G	3-21G +	6-31G	3-21G	3-21G+	6-31G
		Adenine	71.245	71.290	72.013	27.372	27.408	26.871	83.991
Cytosine	63.078	63.004	64.228	24.167	24.202	25.596	81.370	80.880	82.168
Guanine	77.740	77.768	78.437	30.291	30.266	30.067	87.756	88.129	86.898
Thymine	75.545	75.460	75.926	27.230	27.167	27.000	83.710	83.288	83.383

OPTIMIZED BOND LENGTHS

The optimized bond lengths obtained by different methods for the molecules are listed in Tables 3,4,5 and 6.

Table 3: Optimized Bond lengths of Adenine.

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3892	1.3899	1.3817	1.3979	1.3996	1.3926
R(1,6)	1.3673	1.3706	1.3655	1.3852	1.388	1.3841
R(1,7)	0.9951	0.9965	0.9902	1.0124	1.0138	1.0073
R(2,3)	1.2926	1.2979	1.2953	1.3252	1.3299	1.3259
R(2,14)	1.0634	1.0647	1.0645	1.0762	1.0775	1.0774
R(3,4)	1.3956	1.4015	1.3915	1.4002	1.407	1.3999
R(4,6)	1.3812	1.38	1.3824	1.4035	1.4019	1.4046
R(4,8)	1.3951	1.3944	1.3987	1.4075	1.4059	1.4105

Table 4: Optimized Bond lengths of Cytosine

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3528	1.3563	1.3569	1.3628	1.3682	1.3686
R(1,4)	1.4226	1.417	1.4117	1.458	1.448	1.4433
R(1,10)	1.0001	1.0015	0.9951	1.0178	1.0192	1.0126
R(2,6)	1.3489	1.355	1.3518	1.3611	1.3689	1.3659
R(2,8)	1.37	1.3729	1.3739	1.3893	1.3901	1.3914
R(3,4)	1.3744	1.3715	1.3722	1.3242	1.3848	1.3857

Table 5; Optimized Bond Length (Å) of Guanine Molecule

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.3956	1.3973	1.3875	1.4036	1.4066	1.3981
R(1,3)	1.3553	1.3589	1.3549	1.3766	1.3792	1.3762

R(1,4)	0.99 52	0.9966	0.9902	1.0125	1.014	1.0074
R(2,5)	1.29 03	1.2946	1.2928	1.3211	1.3253	1.3224
R(2,8)	1.06 26	1.0639	1.0637	1.0757	1.077	1.0769
R(3,6)	1.37 11	1.3715	1.3742	1.3981	1.3981	1.4004
R(3,7)	1.35 48	1.3605	1.3597	1.3587	1.3649	1.3649
R(5,6)	1.39 19	1.3972	1.387	1.3987	1.404	1.3969
R(6,9)	1.42 48	1.4212	1.4254	1.4317	1.4276	1.4326

Table 6: Optimized Bond Length (Å) of Thymine Molecule

Bond Length	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
R(1,2)	1.37 38	1.3696	1.3686	1.3991	1.3944	1.393
R(1,3)	1.38 15	1.3881	1.3829	1.3847	1.3924	1.3873
R(1,4)	0.99 76	0.9988	0.9924	1.0143	1.0157	1.0091
R(2,5)	1.21 2	1.2271	1.223	1.2326	1.2483	1.2433
R(2,6)	1.37 41	1.3732	1.3715	1.3929	1.3916	1.39
R(3,7)	1.32 57	1.3304	1.3346	1.3485	1.3537	1.3569
R(3,8)	1.06 98	1.0711	1.07	1.0828	1.0842	1.0831
R(6,9)	1.38 97	1.39	1.3875	1.4133	1.4136	1.4102
R(6,11)	1.00 08	1.0026	0.996	1.0175	1.0195	1.0126

It can also be seen that as for Adenine, the bonds R(1,7):N1-H7,R(2,14):C2-H14,R(5,15):C5-H15,R(9,11):N9-H11 and R(9,12):N9-H12 between nitrogen and hydrogen and between carbon and hydrogen atoms at the various positions possess the lowest values of bond lengths. These are the strongest bonds in adenine molecule meaning they cannot be easily broken. On the other hand, bonds R(1,2):N1-C2,R(1,6):N1-C6,R(3,4):N3-C4,R(4,6):C4-C6 and R(4,8):C4-C8 between carbon-carbon atoms and carbon-nitrogen atoms at the

specified positions have the highest values of bond lengths. These are the weakest bonds.

Similar results were also obtained for the other molecules. It was therefore concluded that at all levels of theory, the bonds between nitrogen and carbon and between carbon and carbon atoms at the various positions possess the highest values of bond lengths. On the other hand, bonds between nitrogen and hydrogen atoms and carbon-hydrogen atoms at the specified positions have the lowest values of bond

lengths. This is an indication that the bonds involving hydrogen atoms are the strongest.

It can also be stated, from the results that on an average basis the bonds N-H and C-H are

basically of the same strength and N-C and C-C are also of the same strength.

The results from the table show that at all levels of theory, there is a strong agreement among the bond lengths obtained by the different methods.

OPTIMIZED BOND ANGLES

Table 7: Optimized Bond Angles of Adenine

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,6)	106.844 1	106.933	106.990 7	106.977 2	107.071 3	107.035 3
A(2,1,7)	127.648 3	127.526 5	127.368 6	127.767 4	127.648 4	127.548 2
A(6,1,7)	125.507 5	125.540 5	125.640 7	125.255 4	125.280 3	125.416 5
A(1,2,3)	112.187	112.081 7	112.279 7	112.461 1	112.208 7	112.576 2
A(1,2,14)	121.894 3	122.248 4	122.243	121.945 3	122.371 8	122.202 4
A(3,2,14)	125.918 7	125.669 9	125.477 3	125.593 6	125.419 6	125.221 4
A(2,3,4)	105.361 2	105.3	105.219 7	104.561	104.706	104.520 9
A(3,4,6)	110.115 5	110.073 3	110.150 7	111.017 6	110.771	110.854 8
A(3,4,8)	132.659 9	132.434 3	132.658 4	132.216 9	132.024 9	132.346 5
A(6,4,8)	117.224 7	117.492 4	117.190 9	116.765 5	117.204	116.798 8
A(10,5,13)	126.447 3	126.208 6	126.700 7	127.588 8	127.045	127.622 4

Table 8: Optimized Bond Angle (°) of Cytosine Molecule

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,4)	124.709 2	124.683 7	124.533 1	124.710 5	124.621 3	124.486 6
A(2,1,10)	121.050 8	121.215 2	121.237 6	121.386	121.469 3	121.553 4
A(4,1,10)	114.24	114.101 1	114.229 3	113.903 6	113.909 3	113.96
A(1,2,6)	117.592	117.599 6	117.510 7	118.576 6	118.373 2	118.211 4
A(1,2,8)	117.848	117.702 7	117.743 1	117.401 7	117.301 7	117.431 7
A(6,2,8)	124.56	124.697 7	124.746 2	124.021 7	124.325 1	124.356 9

A(4,3,9)	119.718 1	119.477 6	119.341 8	118.956 9	118.768 8	118.788 9
A(1,4,3)	115.286 4	115.796 2	116.162 1	115.365 8	115.969 6	116.165
A(1,4,5)	118.192 1	117.738 8	117.986 9	117.742 6	117.435 1	117.784 9

Table 9: Optimized Bond Angle (°) of Guanine Molecule

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,3)	106.871 7	106.844 5	106.955 3	106.984 2	106.948 3	106.951 8
A(2,1,4)	127.856 7	127.720 2	127.529 6	128.005 3	127.829 6	127.732 3
A(3,1,4)	125.271 6	125.435 3	125.515 2	125.010 6	125.222 1	125.315 9
A(1,2,5)	111.303)	111.333 2	111.552	111.785 2	111.684 3	112.036 9
A(1,2,8)	122.086 8	122.386 7	122.427 6	122.066 1	122.435	122.324 5
A(5,2,8)	126.610 1	126.280 1	126.020 4	126.148 7	125.880 8	125.638 6

Table 10: Optimized Bond Angle (°) of Thymine Molecule

Bond Angles	RHF			DFT		
	3-21G	3-21G+	6-31G	3-21G	3-21G+	6-31G
A(2,1,3)	123.232 3	123.343 5	123.345 1	123.602 8	123.664 7	123.716 3
A(2,1,4)	116.036 2	116.025 4	115.910 1	115.486 4	115.615 8	115.382 7
A(3,1,4)	120.731 5	120.631 1	120.744 8	120.910 7	120.719 5	120.900 9
A(1,2,5)	123.229 3	123.220 2	123.056 2	123.236 8	123.208 6	123.064 4
A(1,2,6)	113.156 2	113.525 1	113.729 7	112.272 9	112.733 9	112.887 3
A(5,2,6)	123.614 4	123.254 8	123.214	124.490 3	124.057 5	124.048 2
A(1,3,7)	122.781 1	122.500 7	122.547 7	122.613 1	122.407	122.385 7
A(1,3,8)	115.122 1	114.897 2	115.122 8	115.612 5	115.273 1	115.399 5
A(7,3,8)	122.096 9	122.602 1	122.329 5	121.774 4	122.32	122.214 8
A(2,6,9)	127.862)	127.363 9	127.148 2	128.432 7	127.766 5	127.659 9

As for the cytosine molecule, it was observed that angles A(2,1,4):C-N-C, A(6,2,8):N-C-

C, A(3,4,5):N-C-C and A(3,9,8):N-C-C have the highest bond angles. On the other hand,

A(4,1,10):C-N-H and A(3,9,12):N-C-H bonds have the lowest bond angles. The observed variation in bond angles within the cytosine molecule suggests that the geometry around specific bonds differs. The bonds A(2,1,4):C-N-C, A(6,2,8):N-C-C, A(3,4,5):N-C-C, and A(3,9,8):N-C-C, characterized by higher bond angles, indicates a relatively open and less constrained geometry around these bonds.

Conversely, the bonds A(4,1,10):C-N-H and A(3,9,12):N-C-H, containing hydrogen atoms, which exhibit the lowest bond angles, suggest a more closed or constrained geometry. The lower bond angles in these bonds may be indicative of increased repulsion or crowding around the involved atoms, leading to a more compact arrangement.

In summary, the observed variations in bond angles in the cytosine molecule imply distinct geometric characteristics in different parts of the molecule, possibly influencing its structural and functional properties.

Similar observations were also made with other molecules.

It can also be seen that there is a strong agreement among the bond angles at all levels of theory.

SUMMARY AND CONCLUSION

In this work, we unravelled the intricacies of DNA nucleobases - Adenine (A), Guanine (G), Cytosine (C), and Thymine (T). Employing the sophisticated Gaussian 03 package, we employed quantum chemical methods to delve into the very essence of these nucleobases.

Our research scrutinized the optimized geometrical structures, focusing on bond lengths and angles, while probing the thermodynamic parameters including entropy, enthalpy, and heat capacity. The molecular dance further unfolded as dipole moments were meticulously calculated using both Restricted Hartree-Fock (RHF) and Density Functional Theory (DFT)

methods, with 3-21G, 3-21+G, 6-31G, and 6-31+G basis sets.

What emerged was not just data but a symphony of agreement. The optimized geometrical parameters, irrespective of the method employed, harmonized seamlessly. Thermodynamic properties resonated in unison across different calculation methods, affirming the robustness of our findings.

As we compare the duet of DFT and RHF methods, their harmonious collaboration throughout our exploration leaves us with a melodic confirmation of the molecular landscape.

RECOMMENDATIONS

It is recommended that other levels of theory should be used to carry out the computation.

The research may also be carried out using another software like General Atomic and Molecular Electronic Structure System..

In order to witness a tremendous progress in this area of study because of its function in life, Universities and other institutions of higher learning should shoulder the responsibility of purchasing the softwares required for the research because of their high cost.

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POTENTIAL SYNERGY BETWEEN *Moringa oleifera* SEED POWDER AND ALUM IN WATER TREATMENT

¹Mohammed Suleiman, ²Yusuf Campus Abdulsamad & ³Mohammed Bala Nasir

^{1&2}Department of Science Laboratory Technology, School of Applied Sciences, Kogi State Polytechnic, Lokoja.

³Department of Urban and Regional Planning, School of Environmental Technology, Kogi State Polytechnic, Lokoja.

Corresponding E-mail: mohammedsuleimanozas@gmail.com

Abstract

Treatment of water with either alum or Moringa seed has being in existence for a long time. Coagulation and settling experiment using jar test were performed on locally available Moringa oleifera solution to determine the actual dosage that would yield optimum removal of turbidity; microbial load, alkalinity and pH. The pH and alkalinity of all were within the acceptable standard for drinking water which are 6.5 - 8.5 world health organization (WHO) and the optimum dose of blend found to be 60mg/l of alum and 40 mg/l of Moringa seed which have a residual turbidity of 3.20 nephelometric turbidity unit (NTU) from the initial turbidity of 129 nephelometric turbidity unit (NTU) and a total microbial count in 30ml of water sample. This also is in agreement with WHO standard of less than 5 NTU turbidity and less than 100 microbial counts per ml of sample. While alum alone has dosage of 100mg/l gave a residual turbidity of 2.61 nephelometric turbidity units (NTU) and a microbial counts of 280ml-1 sample. Moringa seed alone with dosage of 100mg/l gave a residual turbidity of 68 nephelometric turbidity units and total microbial count of 50ml-1. Thus, the used of Moringa oleifera seed and alum blend reduces alum requirement by about 40 - 60% and gave the best water quality at low cost and from the results indicated that Moringa oleifera has a double advantage compared to commercial alum because of the presence of Phytochemicals which have been reported to poses anti-microbial properties with potentials for conjunctive use with alum for water purification in rural communities.

Keywords: *Moringa, Nephelometric turbidity, Microbial counts, Coagulation, Optimum, alkalinity*

1. Introduction

Moringa oleifera is a deciduous plant native of northern India, but introduced into the tropic African generally. The tree is fast growing and has been planted in many tropical countries. Its common names are horse - reddish tree, drum stick (English), Zogulle (Hausa), Ewe lle (Yoruba), and OkweOyibo (Igbo) (Abba, *et al.*, 2019).

The capsule of the fruit is about 20 - 45 cm long, and is pendulous, linear, acuminate and ribbed. It contains numerous globular seeds, about 1cm wide with three membranous wings at the base and apex. Scattered African named have, in the past used the paste of the seeds as a natural coagulant for water purification in Sudan and other parts of African. The plant is also known to have a wide application in therapy ranging from its use as an anti scorbutic and anti irritant in Nigeria, for the treatment of diarrhoea, rheumatism and goitre in Mauritius to its use for treating nervous debility and leprosy in India (Amagloh and Benany, 2017).

The seeds can as well be consumed after drying and used as a condiment and garnish. Moreover, they are not toxic. As a multi - purpose plant, it is also of great environmental interest for plantations, in compounds, around fields and on nursery bed (WHO, 2018).

Of the large number of plant materials that have been used over the years, the seeds form *Moringa oleifera* have been shown to be one of the most effective primary coagulants for water treatment especially in rural communities, reported that while aerating well water in rural areas of Sudan for the reduction of carbon dioxide prior to softening, numerous complaints of red in hot water systems were received even when aeration was continued and carbon dioxide neutralized with lime in the regular plant treatment process (Crapparet *al.*, 2017). This complains ceased and did not reoccur as *Moringa* seeds were used (Wrightet *al.*, 2018).

Alum is a double sulphate of potash and aluminium, and in this case breaks into potassium sulphate which remains in solution and a basic aluminium sulphate. This basic sulphate of aluminium is the composition of which is undetermined, precipitates as a more or less gelatinous and flocculent mass and carries down with it the foreign matters and human bodies (Wanger and Lanoix, 2019).

The sulphuric acid set free in the formation of the basic aluminium sulphate attacks the earthy and alkaline carbonates, which are always present, and forms with the sulphates, setting carbonic acid free (Muyibi and Okuofu, 2015).

Aluminum sulphate acts like alum, Aluminum acetate and ferric acetate do not give such good

result. In later years an extensive use of alum has been made in many processes of purifying water.

By the small amount of alum to water, it can be filtered through ordinary paper without difficulty and yield a brilliantly clear nitrate in which there is no trace of suspended particles (Craun and McCabe, 2017).

Generally, alum (aluminum sulphate), an organic coagulant and its synthetic polymeric derivatives are widely used in water treatment and it has been used for drinking water treatment since 500 BC (Doer Berth, 2019).

It is not only settles certain hazardous chemical and suspended solids but also bacterial colonies. The use of alum to remove suspended solids was first occurred in Egypt during 13th to 15th centuries BC (Egesiet *al.*, 2019).

Water is a transparent fluid which forms the world's stream, lakes, Oceans and rains; it is the major constituent of the fluids of living things. The health of a community depends to a large extent on the ample provision of whole some water supply (Folkardet *al.*, 2016).

In addition, to food, shelter and clothing, water is a major cause of death and diseases in our world. Water on or in and around the earth and this water there since existence of the earth and this water has been in use over and over again.

Water is the most commonly found natural compound on earth and lives depend on it generally (Jahnn, 2018).

To some extent, only a small proportion of this water is pure at any moment due to its various sources it could be river, rain underground channels, rock, streams etc (Leeet *al.*, 2015).

These listed sources of water contain dissolved chemicals and minerals which make the water obtained through these sources impure. The primary aim of this research is its application to third world countries in reference to the coagulants potential to prevent the spread of water borne disease. Further research into its efficacy in water purification is desirable so that it may be employed in providing easy and safe means of portable water for rural populations, since modern technology for portable is limited; it is very expensive and unavailable in those areas (Wrightet *al.*, 2018).

2 Methodology

***Moringa oleifera* Samples Collection and Analysis**

Moringa oleifera seed was collected from Okene Central North in Okene Local Government Area of Kogi State, Nigeria in August, 2021.

Sample Preparation

The seeds of *Moringa oleifera* were peeled to obtain the nuts and dried. Thereafter, the dried

seeds were pounded and sieved and also, commercially made alum was purchased from Old Market in Lokoja Kogi State, Nigeria and ground.

Processing of *Moringa oleifera* Seed Solution

The fully mature seed was collected and the outer coating was removed from the matured *Moringa oleifera* seeds and white kernel was air dried for three weeks before pounded in a mortar with pestle and then sieved with a sieve to obtain a very fine powder.

About 1g of the powdered Moringa seed was soaked in distilled water blended and the volume was made up to 100ml to make a bulk solution and the solution was filtered using filter paper to remove insoluble matter. By noting the various volumes used in treating water used in treating the various samples of the water were calculated.

Processing of Alum Solution

The commercial grade alum obtained from old market in Lokoja was pounded to powdered using mortar and pestle and 1g of the alum powdered was soaked in distilled water, blended and the volume was made up to 100ml.

Rain Water Samples Collection and Analysis

This particular sample of water was taken from Okehi Local government area Kogi state. The sample of water was collected in the 10 litre sterile plastic container and transported in a

carton to the laboratory for treatment and analysis.

The Jar Test

Reagents: alum solution and *Moringa oleifera* seed solution

Method: The jar test protocol was designed to stimulate coagulation, flocculation and settling at water treatment plants.

The jar test apparatus could accommodate four (4) 500ml jar at a time. 200ml of raw water were measured and introduced into each of the four beakers, labelled 1,2,3 and 4 on a flocculator with a measuring pipette, dosages of alum and Moringa solution were added into the beakers as rapidly as possible.

The sequence of addition was *Moringa oleifera* solution followed by alum solution. With stirring paddles lowered into the beakers, and the jar tests mixer turned on flask test mixing was done for 2 minutes at a speed of 100 resolution per minute (RPM), followed by slow mixing for 8 minutes at 25 RPM.

The beakers were observed and evaluated as to the specific dosages flocculation quality and the result was recorded immediately. The jar test mixer was turned off and the flocs were allowed to settle in the beakers for 30 minutes. The floc settling characteristic was observed, a lazy sample floc formation indicates poor coagulation, and properly coagulated water contains floc particles that are well formed and dense with the liquid between the particles clear.

Determination of the Actual Dosages Used in the Treatment of Water

Exactly 1g of the powder (solute) was dissolved in 100ml of distilled water to give the bulk solution. This implies that 100ml 1g of the solute. Therefore 1ml of the solution contains; This indicates that 1ml of the bulk solution contains 0.01g of the solute.

$$1 \times 1\text{g}/100 = 0.01\text{g of the solute}$$

Turbidity Test

Turbidity is an indication of clarity of water. Turbidity in water is caused by the presence of suspended matter, such as clay, silt, finely divided organic and inorganic matter, plankton and other microscopic organisms. The degree of turbidity depends upon the concentration, fineness or dispersion of suspended matter and light absorption property of the performance of treatment works processes such as coagulation, sedimentation and filtration.

The method used in this work was the nephelometric method using a turbid meter, this instrument was used to determine the turbidity of water by measuring the intensity of light scattered in one particular direction, predominantly at right angles to incident light and the unit is nephelometric turbidity units (NTU).

Determination of pH

The pH of the samples were determined using a pH meter (with glass electrode) standardized using pH buffer solutions and distilled water. pH meters are usually used to measure the pH of liquids through special probes and sometimes are used to measure the pH of semi - solid substances.

Determination of the Free Alkalinity

Exactly 50ml of the sample was added to two drops of methyl orange indicator and then titrated with 0.2m HCl to the pinkish end point. The titter value was then used to calculate the free alkalinity as described by (APHA - AWA - MPCP, 1975).

$$\text{Free alkalinity} = \frac{\text{titter value} \times 1000\text{cm}^3}{\text{Volume of sample measures}}$$

Microbial Test

The microbial test was carried out using the total plate count (TPC) media, which was aseptically prepared according to the manufacturer's instructions. The total plate count media was poured into the Petri dishes and labeled 1 to 7; inoculated with 1ml of the water sample, allowed to solidified and was incubated for 24 hours in an electro thermal incubator.

The growth of the microorganisms was then observed, counted and recorded per ml (i.e. number of microorganism per ml of water sample).

3. Result

Table 3.1: The Various Dosages of Each Coagulant Used i(Jar Test)

Jar No	1	2	3	4	5	6	7
	2ml of alum solution only added to 200ml water sample	0.4ml of <i>Moringa</i> solution + 1.6ml of alum solution added to 200ml water sample	0.8ml of <i>Moringa</i> solution + 1.2ml of alum solution added to 200ml water sample	1.2ml of <i>Moringa</i> solution + 0.4ml of alum added to 200ml water sample	1.6ml of <i>Moringa</i> solution + 0.4ml of alum added to 200ml water sample	2ml of <i>Moringa</i> solution added to 200ml of water sample	Coagulant free water sample added to 200ml of water.

Source: (Amagloh and Benang, 2017)

The jar test is a test designed to show the effectiveness of chemicals used in water treatment. Many of the chemicals used in water treatment can be evaluated on a small laboratory scale using the jar test. The most important of these chemicals are the ones used for coagulation such as alum and the polyelectrolyte e.g. sodium salt (NaCl). Using the jar test, it is possible to approximate the correct coagulation dosage for plant use with varying amount of turbidity, colour, microbial count and other factors that indicated raw water changes. The jar test is also a useful tool in evaluating new coagulant being considered for use.

Table 3.2: Physical and Chemical parameters of the jar test.

Jar No	1	2	3	4	5	6	7
Dosage of Alum alone 100 mg/l	Alum alone 100	80:20	60:40	40:60	20:80	<i>Moringa</i> alone100	Coagulant free water sample
Flock mark	5	6	5	4	3	1	0
Appearance time (minutes)	< 1	< 1	< 1	> 1	> 1	> 1	
Settling time (minutes)	< 15	< 15	< 15	>15	>15	>15	
Turbidity (NTU)	2.61	2.81	3.20	8.70	20.90	68.00	129.00
Clarity	Very clear	Very clear	Very clear	Clear	Cloudy	Cloudy	Very Cloudy
Odour	Odourless	Odourless	Odourless	Slight odour	Slight odour	Slight odour	Offensive odour
Colour	colourless	Colourless	Colourless	Slight colour	Slight colour	Slight colour	Dirty brown
pH	7.0	7.2	7.3	7.3	7.2	7.3	7.2
Alkalinity mg/l	8.0	8.8	12.0	16.0	22.0	30.0	8.9

Table 3.3:The result of microbial test

Jar no	1	2	3	4	5	6	7
Dosage of Alum coagulant ratio alone of alum to 100 <i>Moringa</i> mg/l		80:40	60:40	40:60	20:80	100	100
Total plate count (TPC)	280	100	30	41	49	50	100

4. Discussions

The table 3.2 and 3.3 represent the jar test result. The jar test number 1, 2,3,4,5, and 6 contains dosages. 100mg/l alum only 80mg/l ratio of alum to *Moringa* and 100mg/l of *Moringa* only respectively, the jar number 7 is a Coagulant free water sample used as a control. The flocs of jar number 1, 2, and 3 are better flocs as indicated by floc mark 5, 6 and 5 respectively as against the jar number 4,5 and 6 with floc mark 4,3 and 2 respectively, an indication that a higher alum ratio to *Moringa* formed a better or denser flocs while a higher ration of *Moringa* to alum form sluggish or lazy flocs. Again the jar number 1,2,3 and 4 forms flocs faster with the appearance time of the flocs less than 1 minute. The time required for the flocs to settle down at the bottom of the container is an important factor in ensuring a regular supply of treated water. It was also observed that the dosage of 100mg/l of alum alone 80mg/l: 20mg/, 60:40mg/l ratio of alum to *Moringa* powder settle in less than 15 minutes while that of 40:60mg/l, 20:80mg/l

ratio of alum to *Moringa* and 100mg/l of *Moringa* required more than 15 minutes to settle. The pH of all the dosages tested ranges from 7.0 to 7.3 which are within the permissible limits which is 6.5 to 8.5 of WHO, guideline for drinking water quality. It could be seen that the pH reduces as the concentrations of the dosing solution of alum were increased. This could be explained from the fact that the solutions then becoming more acidic. This was attributed to the fact that the alum produces sulphuric acid which lower the levels of pH. The increase in acidity could also due to trivalent cation aluminium which serves as a Lewis acid. Thus, it can accept a lone pair of electrons. The reverse was observed when *Moringa* concentration of the dosing solution was increased. The pH increases with increasing concentrations of the *Moringa* coagulant. Doer reported that the action of *Moringa oleifera* as a coagulant lies in the presence of water soluble cationic proteins in the seeds. These suggest that in water the basic amino acids present in protein of *Moringa* would accept a proton from water resulting in the hydroxyl group making the solution basic. This accounted for the basic pH values observed when the concentration of *Moringa* was increased compared to alum. The turbidity test shows that the dosage 100mg alum alone, 80mg: 20mg/l, 60:40mg/l, alum ratio to *Moringa* reduces the water turbidity form 129 nephelometric turbidity unit (NTU) to below 5 NTU which is the WHO's standard for drinking

water turbidity, the other dosages: 40:60mg/l, 20:80mg/l ratio of alum to Moringa and 100mg/l of Moringa had turbidity higher than 5NTU respectively.

Table 3.2 showed that the micro - organisms reduces as the dose of Moringa increases from 0 to 40mg/l and then increases as the dose of Moringa increases from 40 to 100mg/l. this is seen from the fact that the coagulant free sample containing over 300 count ml-1 with Moringa dose of 100mg/, this may be attributed to the enmeshment of the micro-organisms by the high concentration of particulate matters till present in the Moringa treated water as observed by Amagloh, Benang and Yazdani.

From the result above, the optimum dose for the coagulants blend from this work was 60:40mg/l to ratio of alum to Moringa. These alum requirements in the water treatment. This observation was in agreement with reported literature of Muyibi and Okuofu and Aghuwanshiet *al.*, that achieved a reduction of between 40:80% dosage requirements of alum. But the optimum dose in this work is quite different from that of Muyibi and Okuofu which was 20mg/l alum to 10mg/l Moringa dose with the water exhibits initial turbidity between 30 to 90 nephelometric turbidity units. This difference in the optimum dose may be due to the fact that the dose requirement is dependent on the initial turbidity of the water.

5. Conclusion

The purpose of this work was to verify the potential synergy between alum and *Moringa oleifera* seed powder blend in water treatment and its application to the third world countries in reference to the coagulants potentials to prevent the spread of water borne diseases.

The specific conclusions derived from these works are as follows:

- Blending alum and *Moringa oleifera* seed powder clarifies water often better than alum alone and also alum and Moringa seed powder reduced the microbial count in the water to within the permissible limit.
- The blending alum and *Moringa oleifera* seed in water treatment reduces alum requirement by 40 - 60% as well the cost of water treatment and the blending alum and *Moringa oleifera* seed powder in water treatment can effectively improve water sanitation in third world countries at low cost.
- The blending of alum and Moringa seed powder in water treatment has a major advantage because Moringa seed can be produced locally using the blend with alum rather than using alum and chloride to save foreign exchange and generate farm and employment income, therefore Moringa to create a new market for a community is there and studies projects are taking place examining this potential.

Recommendations

This method of water treatment can be used to provide portable drinking water in rural communities, for example the Eika in Okehi Local Government Area of Kogi State.

- Toxicological assessment of the seed of Moringa on various species of bacterial should be carried out to evaluate its anti-microbial property on different species of bacterial. This will enhance its application in water treatment as an anti-microbial agent and the jar tests should be carried out on more water source to determine the optimum dosage requirement before application.
- The effect of pH and turbidity of the water sample on the antimicrobial property should be studied on various levels of human life.

More study should be conducted on the usefulness of Moringa seed to know more of its economic importance to man.

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CHEMICAL EVALUATION OF BLACK CUMIN (*Nigela sativa*)

¹Mohammed Suleiman, ²Yusuf Campus Abdulsamad, ³Mohammed Bala Nazir

^{1&2}Department of Science Laboratory Technology, School of Applied Sciences,
Kogi State Polytechnic, Lokoja.

³Department of Urban and Regional Planning,
School of Environmental Technology,
Kogi State Polytechnic, Lokoja.

Corresponding E-mail: mohammedsuleimanozas@gmail.com

Abstract

The samples of black cumin seeds were purchased from International Market Lokoja, Kogi State, Nigeria and taken to the chemistry laboratory in Kogi State Polytechnic, Lokoja, Kogi State of Nigeria and analysed for the phytochemical composition using methanolic extract. The phytochemical analysis revealed the presence of bioactive compounds: Tannins, Saponins, Glycosides, Anthraquinones and Terpenoids and the absence of Steroids, Reducing sugar and Flavonoids. Their presence shows the medicinal properties of black cumin. Tannins for example are known to be made up of phenolic compounds Tannins possess homeostatic properties and therefore widely used as tropical application on superficial wounds and infections. The results of phytochemical analysis of the Black cumin shown that methanol seeds extract of Black cumin revealed the presence of tannins, terpenoids, Saponins, glycosides & Anthraquinones, but no steroids, reducing sugar & flavonoids. Black cumin seeds can be used for air freshener because the seed is made of eucalyptol which mostly contains terpene and cymene, which are organic aromatic compounds. Extract from the seeds of Black cumin seeds can be used in synergy with other compounds as food additive. Several studies report direct addition of aromatic plants essential oils and extracts to foodstuffs to exert an antimicrobial activity.

Keywords: Phytochemical, methanolic extract, Tannins, Saponins, Glycosides, Anthraquinones, Steroids, Reducing sugar and Flavonoids.

1. Introduction

There is an increasing awareness of the therapeutic potential of many natural products and medicinal plants that are frequently considered far less toxic and free from side effects than synthetic drugs (Newman and Cragg, 2017). A vivid example of that is the black cumin (*Nigella sativa*), which is probably the most extensively studied medicinal plant in recent literature. The medicinal plant *N. sativa* is an annual spicy herb belonging to the family Ranunculaceae. The plant has long been recognized in the Indian subcontinent as an important element in traditional popular medicine but now it is widely cultivated in many other parts of the world including the Mediterranean regions, the Middle East, Far East and several other countries in Asia as well as in Europe (Chevallier, 2019).

Historically, the black cumin has been considered a crop of a great medicinal importance in ancient Egyptian and Greek civilizations where it was used for treatment of various ailments such as headache, nasal congestion, toothache, and intestinal worms (Tembhurne et al., 2020).

The seed is traditionally known in the Middle East as habbat al-Baraka (habbat seed in Arabic; Al Baraka = blessing) due to its powerful healing qualities and remarkable therapeutic potentials. It became the most famous and popular medicinal plant among all Muslim communities because it was described by the Prophet Mohammed (PBUH) as a miraculous remedy. The most important active components of the volatile oils of the black seed are thymoquinone (TQ) and dithymoquinone (DTQ), and both were reported to have anti-inflammatory and antitumor properties (Ivankovic et al., 2016; Banerjee et al., 2020). Those two active ingredients (TQ and DTQ) were also reported to have remarkable immunomodulatory and immunotherapeutic potentials (Swamy and Tan, 2017, Salem, 2016).

In addition to the traditional use, it was also reported to have some beneficial effects in several aspects of poultry production such as improvement of body weight performance, food intake, feed conversion ratio, dressing percentage, carcass quality and visceral organ weight (Durrani et al., 2017; Abu-Dieyeh and Abu-Darwish 2018 and Miraghaee et al., 2019). Moreover, the dietary use of the black cumin was also reported to increase egg production, egg mass and egg shell thickness in laying hens (Nasir et al., 2016; Akhtar et al., 2019).

The black cumin has been regarded since ancient history as a miraculous remedy for various ailments and disease conditions in human patients (Ahmad et al., 2013; Tembhumet al., 2020). In fact, the seed has long been discovered in preserved crops found with Tutankhamun's tomb in Egypt and it was mentioned as a curative substance in the Holy Bible (Saleem, 2016). The Greek physician Dioskorides used black cumin to treat headache, nasal congestion, toothache and intestinal parasites. Hippocrates regarded the black cumin as a valuable remedy for hepatic and digestive disorders. It has been recorded that the black cumin was prescribed by ancient Egyptian physicians to treat the above mentioned ailments in addition to obesity, back pain, hypertension, gastrointestinal problems and bronchial asthma (Salem, 2016; Forouzanfar et al., 2020).

The black cumin was also used in folk medicine for hundreds of years in the Middle East and Far East as a traditional medicine for a wide range of illnesses. It was frequently used as a diuretic agent and to promote menstruation and increase milk production (Goreja, et al., 2019). It is worth mentioning that the black cumin has considerably received a special attention and interest in all Arab and other Muslim communities in which it was extensively used for treatment of various kinds of diseases.

The absolute confidence on the curative potential of the black cumin by Muslim communities was due to the fact that the Prophet Mohammed (PBUH) described the black seed as "a remedy for every illness except death" (Iavnkovicet al., 2016). Moreover, the famous Muslim philosopher and physician Avicenna (980-1037A.D.) stated in his famous legendary book "The Canon of Medicine" that the black cumin stimulates the body's energy and helps recovery from fatigue (Chevallier, 2017).

He further claimed that the black cumin is good for inner purification of the body by reducing mucous and strengthening lungs, treating fever, coughs, colds, toothache, headache, skin diseases, infected wounds and intestinal parasites in addition to its ability to protect against poisonous bites and stings (Ahmed et al, 2013; Hajhashemiet al., 2016; and Sayed, 2018).

2. Methodology

Samples Collection

The samples of black cumin seeds were purchased from International Market Lokoja, Kogi State, Nigeria and taken to the chemistry laboratory in Kogi State Polytechnic, Lokoja, Kogi State for analysis

Sample Preparation

The samples were washed with distilled water and thinly sliced, and then treated with chlorine concentrated solution. These samples were oven dried at temperature of 600C for 24 hours. The samples were ground with a blender (Model No. 205) and stored in air-tight container. Each sample (100g) was weighed and extracted with methanol which was used for the analysis.

Phytochemical Screening Methods

Phytochemical screening was performed using standard procedures described by Sofowora, (1993); Trease and Evans; (1989) and Ayoola et al.(2018).

Determination of Saponins in Black Cumin Seeds

About 0.4g of sample was added to 4ml of distilled water in test tubes and the solutions were shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Determination of Triterpenoids in Black Cumin Seeds

About 0.4g of the sample was dissolved in 1ml of chloroform. About 1ml of acetic anhydride was added, followed by the addition of 2ml of concentrated H₂SO₄. Formation of reddish violet colour indicates the presence of triterpenoids.

Determination of Tannins in Black Cumin Seeds

Two methods were used to test for tannins:

(a) To 10ml of freshly prepared 10% KOH in a beaker, 0.5g of extract was added and shaken to dissolve. A dirty precipitate observed indicated the presence of tannin.

(b) About 0.5g of each extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration.

Determination of Reducing Sugar (Fehling's test) in Black Cumin Seeds

About 0.5g of sample was dissolved in 5ml distilled water and filtered. The filtrate was hydrolysed with dilute HCl, neutralized with alkali (NaOH) and heated with Ferling's A and

B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Determination of Anthraquinones in Black Cumin Seeds

About 0.5g of sample was boiled with 10ml of H₂SO₄ and filtered while hot. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Determination of Steroids in Black Cumin Seeds

About 0.5g of extract was dissolved in 10ml of chloroform and equal volume of concentrated H₂SO₄ was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

Determination of Cardiac Glycosides (Keller-Killiani Test) in Black Cumin Seeds

About 0.5g of extract dissolved, 5ml of water was added and 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayered with 1ml of concentrated H₂SO₄. A brown ring at the interface indicated the presence of a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Determination of Flavonoids in Black Cumin Seeds

Two methods were used to test for flavonoids:

(a) A portion of the extracts were heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

(b) Dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids. Test for Alkaloids Extracts were dissolved individually in dilute HCl and filtered.

(a) Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

(b) Filtrate was treated with Dragendorff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloid. Filtrate was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloid is confirmed by the formation of yellow coloured precipitate.

Statistical Analysis

All determinations were carried out in triplicates. The results generated from the analysis were subjected to Statistical Package for Social Science (SPSS) version 16. Descriptive statistic was used to interpret the results. A P>value less than or equal to 0.05 was considered statistically significant.

3. Result

Table 1: Phytochemical Composition of Black Cumin

Phytochemical compound	Methanol extracts
Tannins	++
Saponins	+++
Anthraquinones	+++
Steroids	–
Glycosides	+++
Terpenoids	++

Reducing Sugar	–
Flavonoids	–

Key: +++ = Highly present, ++ = Moderately present, + = Slightly, - = Not detected

4. Discussion

The results of the phytochemical screening of black cumin revealed the presence of Tannins, Saponins, Glycosides, Anthraquinones and Terpenoids. Their presence shows the medicinal properties of black cumin. Tannins for example are known to be made up of phenolic compounds Tannins possess homeostatic properties and therefore widely used as tropical application on superficial wounds and infections.

Black cumin is a widely observed plant which has a tremendous latent in terms of medicinal uses. Constantly, it contains different chemical constituents. This review comprises the pharmacological aspects of Black cumin as a rich source of phytonutrients. Numerous studies have shown that Black cumin exhibit numerous properties like Anti-inflammatory (Nagpal et al., 2020), Antiseptic (Kokateet al., 1999), Stimulant (Purohit et al., 2017), anti-malarial (Hardel Danendra Kumar et al., 2019).

In the present study which is found, one hand to be better for the extraction of tannins (Cork et Krockenberger 1991) Black cumin seeds extract is a natural food additive which is used as food in comparing the phytochemistry of the seeds to that of fruits for their possible use as natural food preservative in the food industries.

5. Conclusion

The results of phytochemical analysis of the Black cumin showed that methanol seeds extracts of Black cumin revealed the presence of tannins, terpenoids, Saponins, glycosides & Anthraquinones, but no steroids, reducing sugar & flavonoids.

Based on the findings in this study, Black cumin plant can be used for the production and development of new drugs for the treatment of variety of chronic diseases as this has shown that it contains bioactive constituents that have the properties. More research should be carried out to discover other metabolites substituent that may be contained by the plant apart from the one in this research work.

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MICROBIOLOGICAL ASSESSMENT OF BURUKUTU (BEER) DRINK SOLD IN EJULE, KOGI STATE, NIGERIA

¹Onoja, P. Okolo, ²Ichado, A. S. P. & ³Amodu Augustin Ojih

¹Department of Biology Education, Federal College of Education, Odugbo, Apa Local Government Area, Benue State, Nigeria

²Department of Science Laboratory Technology, Kogi State Polytechnic, Lokoja, Nigeria.

³Department of Mineral and Petroleum Resources Engineering, Kogi State Polytechnic, Lokoja, Nigeria.

Abstract

‘Microbiological Assessment of Burukutu (beer) sold in Ejule, Kogi State’ is work carried out to ascertain the microbial quality of burukutu beer produced and sold to people of Ejule town. Twenty samples of Burukutu (beer) were purchased from the drinking joints in Ejule market and transported to the laboratory in Alfa Laboratories Limited, Lagos, (A public Analyst and Consultancy Laboratory) for microbiological analysis. Plate count agar, MacConkey agar, Mannitol salt agar, Eosin methylene blue agar, Sabouroud Dextrose agar and Salmonella-Shigella agar were used following Standard Bacteriological procedures for the enumeration of total aerobic count, and isolation of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas spp*, *Lactobacillus spp* and *Streptococcus spp* respectively. Results obtained for the work indicates that the total aerobic counts ranged from 1.4×10^7 cfu/ml to 5.4×10^7 cfu/ml while the coliform counts ranged from 1.7×10^2 cfu/ml to 3.3×10^4 cfu/ml. The *E. coli* counts ranged from 1.8×10^2 cfu/ml to 3.4×10^4 cfu/ml *S. aureus* counts ranged from 1.9×10^3 to 1.7×10^4 cfu/ml and while the fungi counts ranged from 1.9×10^3 to 1.7×10^4 cfu/ml respectively. The microorganisms isolated with their percentage of occurrence were: *Enterobacter sp* (17%), *E. coli* (19.14%), *Lactobacillus sp* (12.8%), *S. aureus* (26.6%), *Pseudomonas spp* (15.95%) and *Streptococcus spp* (8.51%), respectively. *S. aureus* had the highest percentage of occurrence while, *Strep. sp* recorded the lowest. Among the fungal isolates *Aspergillus species* had the highest occurrence (28.6%) while *Candida species* had the least, occurrence (4.8%); *Mucor sp*, *Rhizopus sp*, *Penicillium sp* and *Geotrichum sp* recorded 15.2%, 26.7%, 19% and 5.7% of occurrence respectively. It was observed from this study that the Good manufacturing practices (GMP) and Good Hygienic Practices (GHP) of the processing and handling of burukutu beer needs to be improved upon to increase the microbiological qualities of the drinks in order to avert the potential public health threat due to microbial contamination.

Key words: Burukutu, fermentation, microbial contamination, Good Manufacturing Practices (GMPs), Good hygienic practices (GHPs).

Introduction

Burukutu (beer) is produced from millet, sorghum and maize in Nigeria. The choice of the type of cereals used for production depends on the availability and the preference of the people within the environment. Kolawole *et al.* (2007) affirms that Burukutu is an indigenous alcoholic beverage produced and consumed in Nigeria, Republic of Benin and Ghana. Burukutu is a beverage that has a Vinegar-like flavor, brownish in colour suspension, produced from grain of either sorghum, millets or maize.

Burukutu is traditionally brewed drinks that is popular and used widely in the middle and northern part of Nigeria as a prestigious drinks for common men, chiefs and elderly people. It is sometimes used as a ritual beverage for the worship of some deities and “Abegwu” (the gods of the dead) in Igala tradition (Kolawole *et al.* (2007))

The production of this beverage is carried out traditionally by malting, mashing, fermenting and maturing of the grains that were stepped in water overnight, the water is drained off and the grain spread in local basket or banana leaves and left to germinate. Grains are watered at interval during germination period of four to five days until the plumule attains a certain length. The sprouted grains can be ground into paste or allowed to dry in sun for one to two days before willing into powder (Achi, 2005).

Burukutu differ from European beers in opaque and having a thick consistency suspension because they are not filtered clear. The African beers are also sour because they are allowed to undergo an acidification step. Its fermentation is a continuous type because fermentation process are not arrested making the drinks consumed in an active state of fermentation. Procedure of fermentation vary from place to place and even from producer to producer (Holza *et al.*, 1991).

In some places, the souring process is done separately by producing a starter culture which serves as inoculum that is used in seeding the Burukutu worth and allowed to ferment (Wang *et al.*, 2015).The method and handling processes

of production encourages contamination with different types of microorganisms. Michodjehoun- Mestres *et al.* (2005) assert that the various production sites of burukutu has poor sanitation and hygienic condition which may be the reasons for high population of aerobic mesophiles recorded at the various stages of burukutu production.

It is worthy to note that the water used for steeping are of questionable quality, the grains are not sorted for gravel and other debus before sleeping. Waters used are gotten from tankers which has varying microbiological quantity. The presence of bacteria could have come from poor handling, poor handling, poor personal hygiene and lack of toilet facilities on the processing premises. The presence of *E. coli* and any enterobacteriaceae in burukutu can be attributed to improper sanitation condition during processing from water supply, un-sterilized utensils, exposure to open air and contamination by flies (Kolawole *et al.*,2007).

Microorganism associated with fermentation includes bacteria, yeast and occasionally molds (Muranja *et al.*, 2003). Different factors are used in the determination of the microorganisms present in any burukutu: the location of the grain, the composition of the grains; and the stages of fermentation, equipment, personnel and the handling processes are very important (Okafor, 1972).

The drink is a nutritious one that serves as source of energy and food hence all a sundry are fund of drinking Burukutu in the early hours in the morning because of this value. However it becomes an intoxicating drink in the afternoon or later in the day as fermentation progresses.

The quality of burukutu produced for people needs to be of high quality chemically and micro biologically to satisfy the people that patronize it with the nutrient and food value..Therefore the aim of this study was to assess the microbiological quality of burukutu beer produced in Ejule, Kogi State, Nigeria.

Methodology

Study Area

Ejule is a rural community situated in Ofu Local Government Area of Kogi State, Nigeria. The study area has an estimated population of 16,500 people consisting of 8,322 males and 8,178 females (NPC, 2006). It is located in the Middle Belt region of the country and covers a land mass area of 12sq.km. The study area lies on latitudes 60 57' 00" N and longitude 60 49' 60" E of the equator. Ejule is bordered to the north by Okele, to the south east by Ofakaga, to the south by Alloma and to the west by Alla. Hence, Ejule community is considered as secondary urbanized but suffers a serious setback in the area of water, health and infrastructure.

The study area lies within the humid semi-hot Savannah zone. The climate is dominated by two major air masses; the warm and the dry tropical continental wind from the Sahara Desert and the hot, humid tropical maritime wind from the Atlantic zone (the south West Monsoon wind). The wet/rainy season starts from middle of April to October while the dry/cool season runs from November to March. The study area has mean annual rainfall of about 1100mm while the mean temperature ranges between 28 0C to 34 0C. Relative humidity is about 74% to 80% for dry and wet seasons respectively. The vegetation of the area falls under tropical Guinean belt

Sample Collection

The burukutu beer samples were bought from Ejule market in Ejule, Kogi State, Nigeria. A total of twenty (20) samples of burukutu were purchased from various vendors in the market and were aseptically transported to the laboratory (Alfa Laboratories Limited, Lagos) in an ice packed cooler. They were analyzed on arrival to the laboratory.

Media Used

The microbiological media used were products of Difco Laboratories, England. They include plate count Agar used for the estimation of total aerobic count, Mac Conkey Agar for enumeration coliform count, Eosin Methylene

blue agar for the enumeration of *E.coli* and Sabouraud Dextrose agar used for the enumeration of fungal organisms, Salmonella Shigella agar for the detection of *Salmonella and Shigella species*. Mannitol Salt agar for the detection of *Staph. aureus*.

Enumeration Total Bacteria and Fungi

Total viable counts of the burukutu samples were determined by pour plate technique. The samples were diluted in seven (7) folds. Each of the appropriate dilution was pipetted into labeled sterile petri-dishes according to the different media used. Then the molten plate count Agar, MacConkey Agar, Mannitol Salt Agar, Eosin Methylene Blue Agar, Sabouraud Dextrose Agar, Salmonella- Shigella Agar at 45°C were poured into the petri-dishes containing 1ml of the dilution of samples. They were rock and swirled for proper mixing. The Sabouraud Dextrose plates were incubated at room temperature while other bacteriological plates were incubated at 37°C for 48hours in an incubator. The bacterial isolates were sub-cultured into agar slants and were used for biochemical analysis.

Identification and Characterization of Isolates

Bacterial Isolates were characterized and identified according to Cheesbrough, 2003, 2005 and 2006, Adeoye, 2007 and Bergeys Manual of Determinatives bacteriology (1994). Gram reaction, cell morphology, motility test, oxidase catalase production, starch hydrolysis, sugar fermentation, methyl red-voges proskaur reaction and urease production were carried out for the phenotypic bacterial identification and characterization.

Fungal isolates were examined microscopically and micro biologically using the needle mount technique according to Barnett and Hunter (1999)

Result

The mean value of counts of microorganisms isolated from the burukutu samples analyses from the study are is show in table 1 . The total aerobic counts ranged from 2.6×10^6 cfu/ml to

5.1 x 10⁷cfu/ml while the coliform counts ranges from 2.0 x 10³cfu/ml to 2.9 x 10⁵cfu/ml. The *E. coli* counts ranged from 1.2x10²cfu/ml to 2.4x10⁴cfu/ml. *S. aureus* counts ranged from 1.5 x 10³ to 2.4 x 10³ cfu/ml and while the fungi counts ranged from 1.63 x 10³ cfu/ml respectively.

The microorganisms isolated with their percentags of occurrence is depicted in Table 2. Briefly *Enterobacter sp*, *E. coli*, *Lactobacillus sp*, *S. aureus*, *Pseudomonas sp* and *Streptococcus spp* as 17% , 19.14%, 12.8%,

26.6%, 15.95%, and 8.51% respectively, with *S. aureus* having the highest percentage and *Strep. sp* with the lowest. The fungal species isolated were *Aspergillus species* with the highest occurrence of 28.6% while *Candida species* had the lowest occurrence of 4.8%.*Mucor sp*, *Rhizopus sp*, *Penicillium sp* and *Geotrichum sp* had 15.2%, 26.7%, 19% and 5.7% respectively.

Table 1: Means counts of the microorganisms isolated from the burukutu samples in cfu/ml

Shop	TBC	Coliform count	<i>E. coli</i> count	SSC	Fungi count	<i>Staph aureus</i>
A	3.4 x 10 ⁷	1.7 x 10 ²	1.8 x 10 ²	0	3.3 x 10 ³	2.5 x 10 ³
B	2.8 x 10 ⁶	2.8 x 10 ⁴	1.8 x 10 ³	0	3.1 x 10 ³	2.7 x 10 ³
C	1.4 x 10 ⁷	2.2 x 10 ³	2.2 x 10 ³	0	1.7 x 10 ⁴	1.9 x 10 ³
D	5.4 x 10 ⁷	3.3 x 10 ⁴	3.4 x 10 ⁴	0	2.4 x 10 ³	2.9 x 10 ³
E	4.6 x 10 ⁷	2.5 x 10 ⁵	2.1 x 10 ⁴	0	1.9 x 10 ³	1.9 x 10 ³

Table 2:Microorganisms isolated (Bacterial & Fungi)

Microorganism	No of isolates	% occurrence
Bacteria		
<i>Enterobacter species</i>	18	17.00
<i>Escherichia coli</i>	21	19.14
<i>Lactobacillus species</i>	17	12.80
<i>Staphylococcus aureus</i>	28	26.60
<i>Pseudomonas species</i>	19	15.95
<i>Streptococcus species</i>	14	8.51
Fungi		
<i>Aspergillus species</i>	31	28.6
<i>Mucor species</i>	16	15.2
<i>Rhizopus species</i>	26	26.7
<i>Perricillum species</i>	22	19.0
<i>Candida species</i>	6	4.8
<i>Geotrichumspeies</i>	8	5.7

Discussion

The high mean of microbial counts observed in the result may be as a result of handling process, the poor hygienic nature of the production area, the adherence to standard operating procedure for production and the type of water used in the processing. Exposure of burukutu during processing, storage and sales can lead to high

microbial load *Entnobacter species*, *Eschaichia coli*, *Lactobacillus species*, *Staphylococcus aureus*, *Pseudomonas species*, *Streptococcus species*, *Aspergillus species*, *Mucor species*, *Rhizopus species*, *Penicilium species*, *Candida species* and *Geotrichum species* were the organism encountered in the samples. This is in agreement with work of Kolawole *et al.*, 2007.

The presence of *Staphylococcus aureus* in the samples can be attributed to the handling processes, because the organism is normal flora of the mucous membrane and skin signifying that the handler is not maintaining body hand sanitation before engagement in the processing. This organism can be passed unto food during harvesting, processing and storage. *Staphylococcus aureus* are implicated in staphylococcal food poisoning which has symptoms such as diarrhea and vomiting (Eze *et al.*, 2008.).

Escherichia coli was encountered in the drink which might be as a result of the quality of water used. *E. coli* is a normal flora of the intestinal region of humans and vertebrates. The presence of these organisms in the sample is an indication of fecal contamination of the samples, which can be attributed to poor hygienic condition of burukutu processing previously reported by Kolawole *et al.*, 2007.

The presence of *Saccharomyces cerevisiae* (yeast) and lactic acid bacteria in the sample analyzed can be indicative of their role in the fermentation processes of burukutu beer. The process of cereal food fermentation has been a mutual process between yeasts and lactic acid bacteria in several cereals (Omemu *et al.*, 2007 and Omemu, 2011). Lactic acid bacteria which are responsible for production of lactic acid gives the burukutu its slight bitter/sour taste. Metabolites of yeasts such as acetate, carbon dioxide, propionate, succinate are responsible for the deterioration of the drinks after production (Eze *et al.*, 2011). The sample was observed to undergo continuous fermentation processes due the availability of nutrient in the burukutu for continuous growth of the yeast population which shows the non stabilization of the product.(Eze *et al.*, 2011).

Findings from the study has shown that burukutu beer contains some microorganisms that are pathogenic and harmful to man and can have a serious dissimulated public health risk to the consumers. As a result of these harmful pathogenic organisms presence, there is need for the creation of awareness to families, communities, producers, vendors and

consumers of burukutu on the possible health implications of consuming the product. Serious attention should be given to good manufacturing practice (GMPs) and Good Hygiene Practice (GHPs) in the production, handling and vending of this beverages so that the desired incentives value could be achieved. It is also necessary to find a solution to how the continuous fermentation could be curtailed to increase the freshness of the product after production.

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ANALYSIS OF TRACE METALS IN SOME SELECTED DRINKING WATER SOURCES IN ANKPA, KOGI STATE, NIGERIA

¹Onoja, P. Okolo and ²Ichado A. S. P. & ³Amodu Augustin Ojih

¹Department of Biology Education, Federal College of Education, Odugbo, Apa Local Government Area of Benue State,

²Department of Science Laboratory Technology, Kogi State Polytechnic, Lokoja, Kogi State.

³ Department of Mineral and Petroleum Resources Engineering. Kogi state polytechnic Lokoja.
Corresponding Author (aspichado@gmail.com)

Abstract

The inadequacy of quality water in Ankpa metropolis is almost endemic and this study investigated the trace metal level in some selected drinking water sources in Ankpa, Kogi State, Nigeria. Various drinking water sources were obtained and trace metal analysis was carried out using Atomic Absorption Spectrophotometric method of trace metal determination of Fe, Pb, Zn, Cu, Ni and Cd. The mean values of Fe, Pb, Zn, Cu, Ni and Cd obtained were 0.023-1.031mg/l; 0.18 -3.47mg/l; 0.11-0.12mg/l; 0.08-6.52mg/l; 0.02-1.04mg/l and 0.02-0.84mg/l respectively. These values are above World Health Organization (WHO) limits. Since all the water sources analyzed recorded high trace metal levels, it advisable for the enforcement of regulation guiding water quality, National Agency for Food and Drug Administration and Control (NAFDAC) to make sure that an ion exchange resin system be incorporated in the water treatment line to remove excess trace metals to make the water sources safe for drinking based on WHO standards for potable water.

Keywords: Trace metals, Quality, Drinking water, Potable water and WHO standards.

1.0 INTRODUCTION

The World Health Organization (WHO) reported that over two billion people lived in water-stressed countries in 2021, and at least 1.7 billion people consumed water from sources contaminated with feces in 2022 (WHO, 2023). The lack of access to clean water is often attributed to pollutants introduced into the environment. Freshwater sources can be contaminated by rainwater carrying dissolved airborne particulate matter from industrial and agricultural activities (Khan, 2014; Rueda-Holgado et al., 2014). Additionally, pollutants may originate from natural sources such as volcanic activities (George, 2014) or dissolved minerals from weathered rocks and soil. Several factors have been influencing global water supply, including climate change and global warming, which are altering evaporation patterns, precipitation, and stream flows, significantly impacting hydrological regimes (Molina, Luong, and Bernhofer, 2019). Global warming also affects evapotranspiration, stored water volumes, soil moisture, and water retention capacity (Lal, 2020). The ecological degradation of rivers, destruction of watersheds, and pollution of water sources have been exacerbated by climate change, increasing the demand for water-related services (Dunham et al., 2018; Jia et al., 2019). Water scarcity poses a threat to both urban and rural communities, leading to higher water supply costs, decreased reliability, prolonged droughts, floods, erosion,

and increased costs of irrigated food production (Schilling et al., 2020). Agricultural practices impact water quality, influencing flooding, precipitation, groundwater inflow, nutrient cycles, soil erosion, carbon sequestration, and other ecological processes (Kumar et al., 2021). Farming activities contribute to water pollution through the use of agrochemicals that enter surface and groundwater via erosion and leaching (Akhtar et al., 2021). The physicochemical properties of water bodies are altered by pollutants from agricultural activities, harming aquatic ecosystems and other organism's dependent on these polluted waters (Evans et al., 2019). Mine waste also poses a significant challenge to the sustainability of water bodies, contaminating both groundwater and surface water in disposal areas (Agboola et al., 2020). Numerous disasters have been linked to mine dumps in both active and abandoned mining sites. Adebayo et al. (2023) conducted a study on the determination of heavy metal contents in some drinking water sources in Osun State, Nigeria. Wastes containing hazardous heavy metals and radioactive substances pose significant threats to both human health and the ecosystem at large (Li, Wang, and Li, 2019). Although well water is generally expected to be pure and free from pollutants due to its isolation from the soil surface, this is not always the case. While groundwater quality remains high in some areas, it is increasingly under threat, particularly in developing countries. Factors

affecting groundwater quality include aquifer lithology, groundwater velocity, the quality of recharge waters, interactions with other water types or aquifers, as well as human activities and environmental conditions (Okeke et al., 2021).

Contaminated drinking water can lead to a rise in infectious diseases and noncommunicable diseases, with developing countries being more severely affected due to a lack of clean drinking water (Guarino, 2017). Issues related to sanitation and waste disposal in these countries further exacerbate water supply problems, resulting in the loss of productivity and lives due to communicable diseases (Muta'aHellandendu, 2012). Drinking water contaminated by heavy metals poses a serious risk to delicate biological systems, leading to various health issues (Apiamu et al., 2023). Some of the heavy metal pollutants of public health concern in drinking water include arsenic (As), cadmium (Cd), nickel (Ni), chromium (Cr), and lead (Pb). Both natural and human activities are major sources of heavy metal contamination in surface and groundwater (Yahaya et al., 2020). The increasing concentration of heavy metals in water bodies, regardless of the source, poses a significant threat to both human health and aquatic ecosystems (Yahaya et al., 2020).

Humans are exposed to heavy metals daily through activities such as mining, smelting, refining, fossil fuel combustion, and the use of various products (Khan et al., 2016). Due to their

chemistry and reactivity, these metals are highly mobile once they enter the ecosystem (Zhang et al., 2018). The kidneys, involved in filtration, are particularly vulnerable to damage when toxic environmental pollutants are ingested, with occupational exposure being a common cause of kidney disease (Xu et al., 2018). Arsenic in drinking water has been linked to vascular impairment in the lower limbs, leading to gangrene (Chang et al., 2018). Lead is toxic to the nervous system, potentially damaging the brain and other organs, causing stunted growth and hearing problems in children, and leading to anemia, hypertension, reduced sperm count, and an increased risk of miscarriages in adults (WHO, 2016). Lead can also interfere with blood formation, accumulate in the body, and cause death.

Chronic cadmium poisoning, known as itai-itai disease, causes renal tubular dysfunction, osteomalacia, and osteoporosis, and was first discovered in Japan in the early 20th century (Kubier, Wilkin, and Pichler, 2019). Cadmium exposure is also associated with glucose metabolism disorders, breast and lung cancer, cerebral infarction, and cardiac failure (Khan et al., 2017). Chromium poisoning has been linked to cancers in laboratory animals and workers exposed occupationally (Zhitkovich, 2011). Chromium can cause cardiovascular collapse due to severe hypovolemia, respiratory, liver, and kidney injury, and has been reported to cause death following ingestion of Cr (VI) in

several case studies involving children and adults (WHO, 2020). Nickel exposure in mammals increases the risk of liver cirrhosis and azotemia, marked by metabolic stress (Apiamu et al., 2023). Nickel poisoning may also affect cardiac function and cause acute hemorrhagic gastritis, with symptoms such as nausea, vomiting, diarrhea, dizziness, fatigue, headaches, and shortness of breath (WHO, 2021).

Nigeria, with an estimated population of 200 million, is the most populous black nation in the world, yet close to 70 million people lack access to safe drinking water (Ighalo and Adeniyi, 2020). The country's water and sanitation infrastructure are outdated and inadequate, leaving many areas without access to safe and clean drinking water (Ihieze and Obaniyi, 2023). Inadequate waste disposal and unregulated industrial practices contribute to the pollution of water sources, making clean and safe drinking water scarce in Nigeria, and putting the health of the population at risk. Despite abundant water resources, Nigeria faces significant water challenges due to poor water management, rapid population growth, urbanization, industrialization, and inadequate infrastructure, rendering many water sources unfit for human consumption (Ihieze and Obaniyi, 2023).

2.0 Methodology

Study Area

Ankpa is one of the Local Government Headquarters in the Confluence State (Kogi State). The state is located between Latitude 7.8° N and Longitude 6.7° E. It lies on the basement complex formation that comprise mainly of quartz, feldspar, granite and gneiss. Ankpa is a low moderate relief with few scattered laterites cap link where elevation rarely exceeds 400m above sea level. Annual average rainfall ranges between 100mm and 150mm with the annual humidity of 70%. The annual average temperature is about 27°C , with high temperature of 33°C in dry months of November, December, January and February. The average sunshine is 6.7 per day.

Sample collection

Six (6) brands of sachet water sold around Ankpa were sampled for six (6) weeks. That is a total of six (6) samples per weeks. A total of 36 samples of sachet water were sampled altogether. Also, two (2) well-waters from different locations within Ankpa were sampled once a week for six (6) weeks and borehole water sources were also sampled and transported to the laboratory in an ice cool pack for analysis.

Analysis of Water Samples

Water samples were analyzed using Atomic Absorption spectrophotometer (AAS). From each water sample collected, 150ml of water was taken, and was preserved by adding 1.0ml Conc. HNO₃ cooled and then stored in a refrigerator to stabilize the metals for up to 2 weeks, 100ml of the water sample was filtered and concentrated to about 60ml. using 100ml standard flask 5ml of HNO₃ was added and made up with De-ionized water, 1% of the HNO₃ was used as blank. The metal concentration of the water samples was read off from a standard curve.

$$\text{Metal concentration (mg/l)} = A \times B / C$$

Where

A = Concentration of metal in digested solution (mg/l)

B = Final Volume of digested solution(ml)

C = Sample size

The use of AAS was based on Beer – Lambert’s Law

$$\text{Log } I_0 / I_t = (a.b.c) \quad (1)$$

Where I₀ = Intensity of incident light

I_t = Intensity of transmitted light

a = Molar absorptivity

b = Thickness of medium

c = Concentration

Thus, the concentration and logarithmic of the transmittance are proportional. The procedures

employed are as indicated in the AAS manufacturer’s manual.

SPSS - Version 16

Reagent and glassware

- Reagents used were Analar grade
- Distilled water was used throughout the study Glassware / apparatus washed
- Glassware / apparatus washed thoroughly an oven dried
- 0.1m HNO₃ was used

This study sampled various drinking water sources in Ankpa, Kogi State with the following objectives: a) To determine the physicochemical parameters of selected rivers and well water, comparing the concentrations with World Health Organization (WHO) standards. b) To assess the concentrations of lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), and arsenic (As) in the water samples, comparing the results with WHO standards. The findings of this study will help evaluate the suitability of these water sources for human consumption by comparing contaminant levels with the WHO's recommended limits.

3.0 RESULTS AND DISCUSSION

3.1 Results

IRON (Fe)

Fig. 1 below shows the mean values of Iron plotted against the various water samples analyzed. The result revealed that SW1 table water and Ankpa public boreholes recorded iron

values of 0.33, 0.74 and 0.54mg/l respectively, which are higher than WHO standards of 0.3mg/l for potable water.

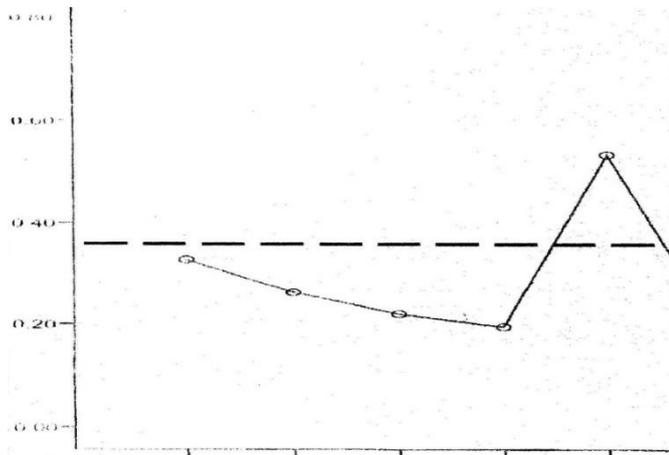
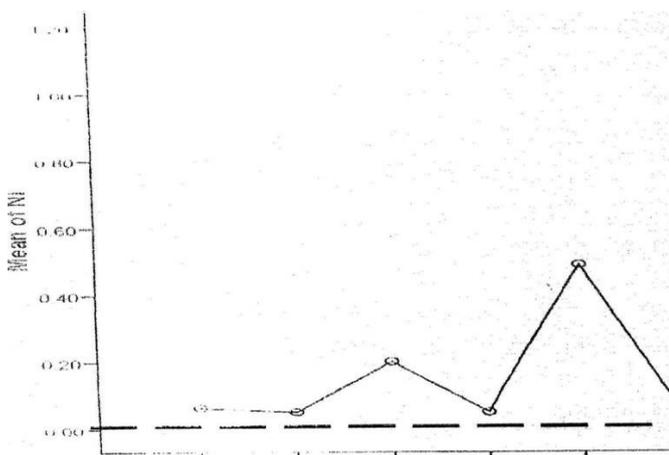


Fig. 1. Result of Iron Analysis

NIKEL (Ni)

Fig. 2 below shows the mean values of Nickel plotted against the various water samples analyzed. The result showed that SW1, SW2, SW3, SW4, SW5, SW6 table water, WWI well water, and BHW borehole water had Nickel (Ni) with the following value:0.07, 0.02 0.2, 0.05, 0.07, 0.035, 1.03, 0.75 and 1.03 mg/l respectively which are higher than WHO standards of 0.01 mg/l for potable water.



SW1 SW2 SW3 SW4 PBW
SW5 WW1 WW2 SW6 BHW

Fig. 2: Result of Nickel Analysis

Zinc (Zn)

Fig. 3 below showed the mean values of zinc (Zn) plotted against the various water samples analyzed. The result established that all were within the WHO Standards limits for potable water.

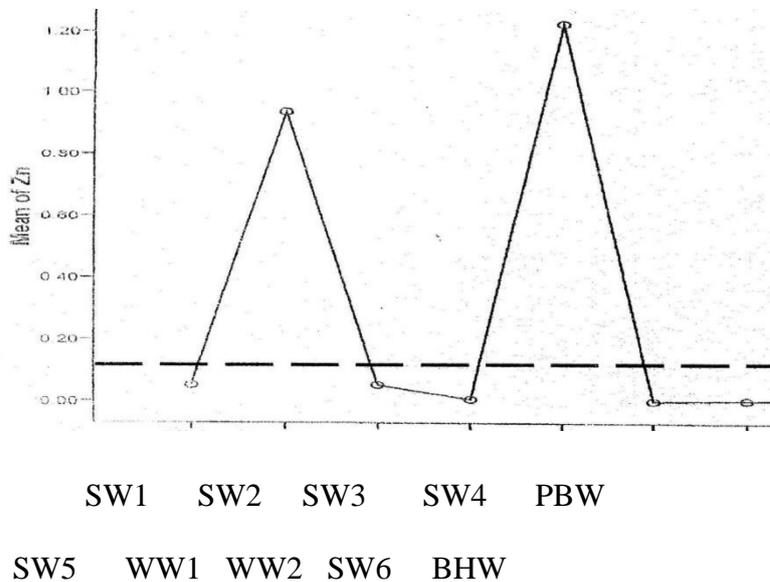


Fig. 3: Result of Zinc Analysis

Lead (Pb)

In Fig. 4 below, the mean values of lead (Pb) plotted against the various water samples analyzed shows that SW1, SW3, SW4, SW5, SW6 table water; PBW water works, WW1 and WW2 well waters; an BHW borehole water has lead (Pb) values of 0.9, 1.07, 0.02, 0.2, 2.87,

1.78, 3.47, 0.72 and 2.66mg/ l respectively, which are higher that WHO standards of 0.05 mg/l for potable water.

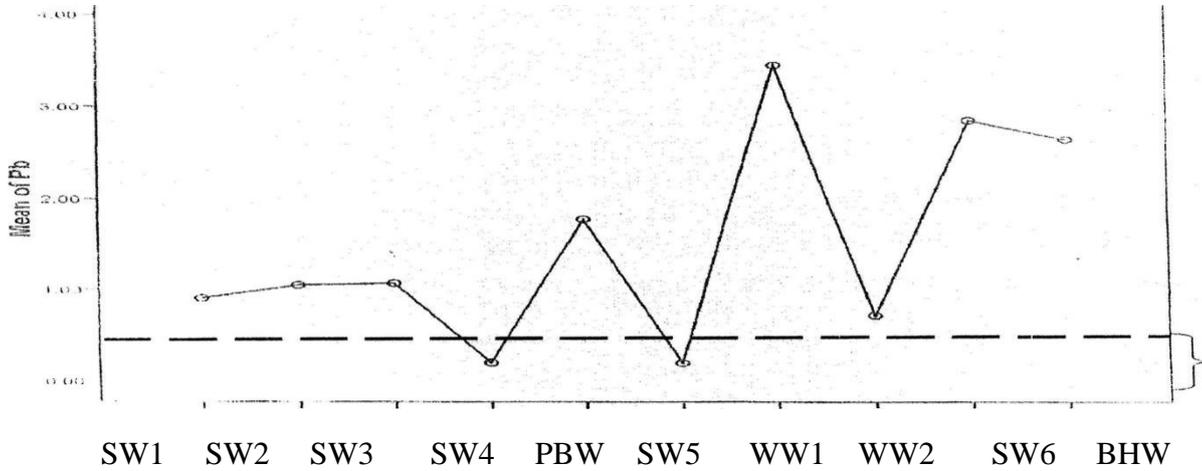


Fig. 4: Result of Lead Analysis

Cadmium (Cd)

In fig. 5 below, the means values of Cadmium (Cd) plotted against the various water samples analyzed. The result revealed that SW1, SW2, SW3, SW 4, SW 5, SW 6 table waters; PBW water works, WW2 well and BHW borehole waters has cadmium (Cd) values of 0.06, 0.06, 0.07, 0.02, 0.02, 0.05, 0.74, 0.04 and 0.05 mg/l respectively, which are higher than WHO standards of 0.01 mg/l for potable water. In fig. 5 below, the means values of Cadmium (Cd) plotted against the various water samples

analyzed. The result revealed that SW1, SW2, SW3, SW 4, SW 5, SW 6 table waters; PBW water works, WW2 well and BHW borehole waters has cadmium (Cd) values of 0.06, 0.06, 0.07, 0.02, 0.02, 0.05, 0.74, 0.04 and 0.05 mg/l respectively, which are higher than WHO standards of 0.01 mg/l for potable water.

Cadmium (Cd)

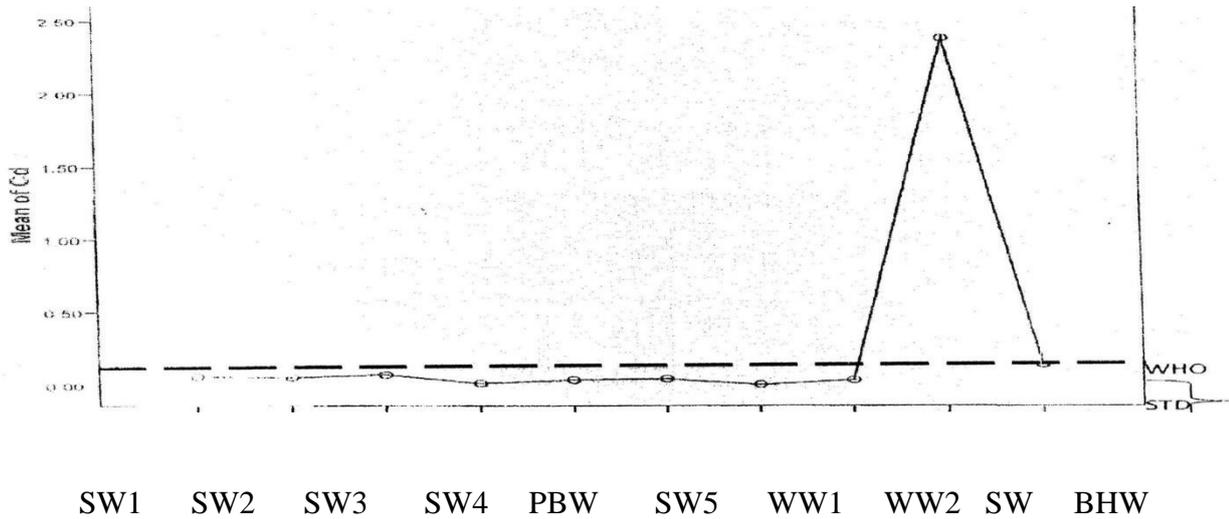


Fig. 5: Result of Cadmium Analysis

Copper (Cu) In Fig. 6 below, the mean values of Copper (Cu) plotted against the various water samples analyzed. The result showed that SW1, SW2, SW3, SW4, SW5, and SW6 table water, PBW water works, WW2 well water and

BHW borehole waters has copper (Cu) values of 2.19, 0.07, 1.95, 6.52, 6.13, 6.09, 2.88 and 4.6mg/l respectively, which are higher than WHO standards of 0.05 mg/l for potable water.

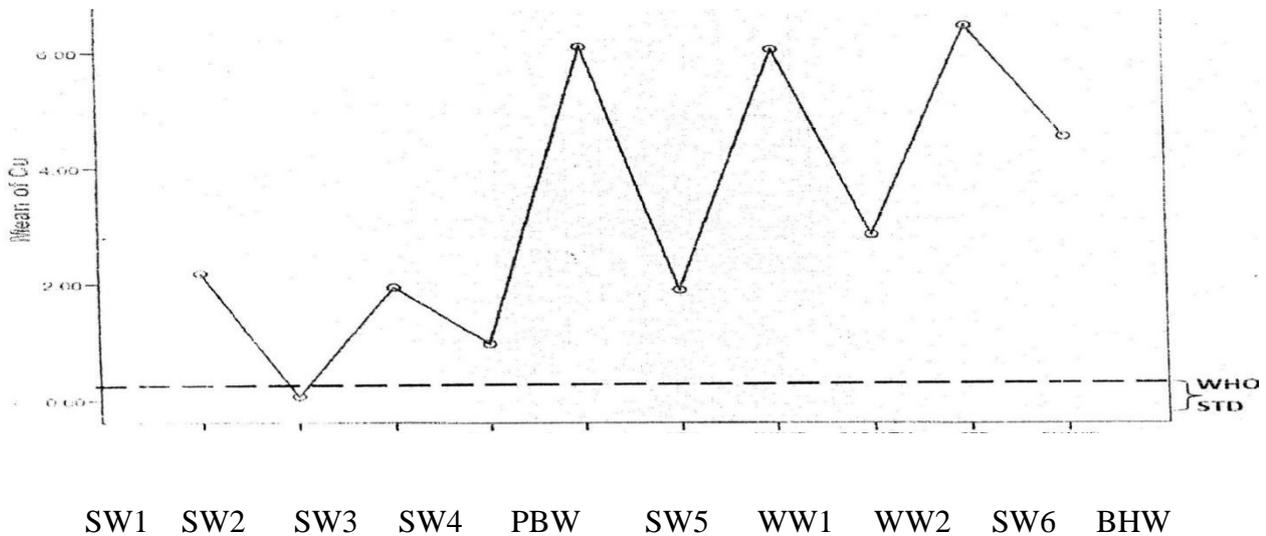


Fig. 6: Result of Copper Analysis

3.2 DISCUSSION

The mean values of Fe, Ni, Pb, Cd, and Cu observed in this study shows that they were higher than WHO standards for potable water with exception of Zn that was within the limits. This could be as a result of human activities such as open-air solid waste combustion, quarrying, road construction, dissociation of bedrock of the water course, gas station, saw mills, air borne particulates, industrial liquid waste, discharge of agricultural drainage, sewage effluents which might found their ways into the water body of river Maboro and its onward flow into River Niger. Also, the geological strata of the rock and the soil of the place where well and boreholes are dug and drilled might be a contributory factor to the high trace metal levels in the water.

The findings in this Fig. 1 shows the mean values of Iron plotted against the various water samples analyzed. The result revealed that SW1 table water and Ankpa public boreholes recorded iron values of 0.33, 0.74 and 0.54mg/l respectively, which are higher than WHO standards of 0.3mg/l for potable water. The work of Orewole *et al.*, (2007) that has 0.5mg/l as the mean values of iron in his water analyzed which shows an agreement with this work

The result from Fig 2.0 showed that SW1, SW2, SW3, SW4, SW5, SW6 table water, WW1 well water, and BHW borehole water had Nickel (Ni) with the following value:0.07, 0.02 0.2, 0.05, 0.07, 0.035, 1.03, 0.75 and 1.03 mg/l

respectively which are higher than WHO standards of 0.01 mg/l for potable water. This is in consonance with the works of Akpoborie *et al.*, (2011) which has means values of 0.07, 0.045 and 02 mg/l respectively for the various water analyzed,while

Fig. 3 showed the mean values of zinc (Zn) plotted against the various water samples analyzed. The result established that all were within the WHO Standards limits for potable water. This tally with the works of Akpoborie *et al.*, (2011) which has mean values of zinc as 0.11, 0.12 and 0.11 mg/l respectively for the various water analyzed, furthermore, Fig. 4.0 shows the mean values of lead (Pb) plotted against the various water samples analyzed shows that SW1, SW3, SW4, SW5, SW6 table water; PBW water works, WW1 and WW2 well waters; an BHW borehole water has lead (Pb) values of 0.9, 1.07, 0.02, 0.2, 2.87, 1.78, 3.47, 0.72 and 2.66mg/ l respectively, which are higher than WHO standards of 0.05 mg/l for potable water. This is in agreement with the work of Aderinola *et al.*, (2011) which has mean value of Pb as 0.14mg/l of the water analyzed.

The result obtained from fig. 5.0, shows the means values of Cadmium (Cd) plotted against the various water samples analyzed. The result revealed that SW1, SW2, SW3, SW 4, SW 5, SW 6 table waters; PBW water works, WW2 well and BHW borehole waters has cadmium

(Cd) values of 0.06, 0.06, 0.07, 0.02, 0.02, 0.05, 0.74, 0.04 and 0.05 mg/l respectively, which are higher than WHO standards of 0.01 mg/l for potable water. This is in agreement with the work of Yusuf et al (2006) with mean value of Cd as 0.05mg/l and in deviance with (Jarup *et al.*, 2003) with mean value for Cd as 0.01mg/l. In Fig. 6, the mean values of Copper (Cu) plotted against the various water samples analyzed. The result showed that SW1, SW2, SW3, SW4, SW5, and SW6 table water, PBW water works, WW2 well water and BHW borehole waters has copper (Cu) values of 2.19, 0.07, 1.95, 6.52, 6.13, 6.09, 2.88 and 4.6mg/l respectively, which are higher than WHO standards of 0.05 mg/l for potable water. This is in deviance with the work of Fortner *et al.*, (1981) which has mean valued Cu 0.06, 0.4 and 0.045mg/l. from the various water analyzed .

The assertion above is affirmed by Adekunle *et al.* (2004) that higher trace metals concentration in wet seasons implies increase in soil metal burden from atmospheric deposit of air particulates by wet precipitate and subsequent rain water run-offs of waste materials. Metal levels could also be traced to dissociation from bedrock through which the water flows. Copper enters water system through mineral dissolution and industrial effluents.

Jarup *et al.* (2003) also affirms that metal pollution of some rivers in Nigeria may be due to industrial discharges, corrosion of iron and

steel materials in building, leachates from dump sites and vehicles. High Pb concentration in some surface water samples have been attributed to use of leaded petrol in vehicles.

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