Nig. J. Biotech. Vol. 35 (2018) 1-7
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v35i1.1

Analysis of Selected Medicinal Plants used in the Treatment of Malaria and Typhoid Fever in Ebonyi State, Nigeria

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ABSTRACT

The study was aimed at evaluating selected medicinal plants used in the treatment of malaria and typhoid fever. Materials used include medicinal plants used locally in treating malaria and typhoid fever sourced from different villages in Ebonyi State. The analysis of the medicinal plants was conducted using random amplified polymorphic DNA (RAPD) markers and NTSYSpc software version 2.02. Different RAPD markers including OPB-1, OPB-2, OPB-3, OPB-5, OPB-12 and OPH-12 were used to amplify the DNA of these plants. These markers were found to be polymorphic except OPB-3 which did not produce any band. It was observed that RAPD markers can effectively amplify DNA sequences of different medicinal plants. The data matrix of RAPD profiles obtained from fragments of each amplicon were scored as 1 (presence of alleles) or 0 (absence of alleles). A dendrogram of the plants using unweighted pair group mean (UPGMA) clustered the genotypes into groups. The dissimilarity values were 0.26 and 1 as minimum and maximum with an edge length of 1.32. Principal component analysis of the generated amplicons resulted to clusters with unique genetic identity. The polymorphism detected among the plants genotypes will be useful in selecting genetically diverse species in future breeding programme.

Key Words: Medicinal plants, Malaria/Typhoid, RAPD, Ebonyi State, Nigeria

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Introduction

Malaria is today a disease of the poor and underdeveloped countries, but remains an important health problem globally. In Africa, the occurrence of malaria has been rising at a shocking proportion in the last era with a record of 300 to 500 million cases each year causing 1.5 to 2.7 million deaths with more than 90% in children less than 5 years of age (W. H. O. 1996).

Medicinal plants have been used in virtually all cultures as a source of medicine (El-Beyrouthy *et al.*, 2013). During the last few decades, there has been an increase in the study of medicinal plants and their traditional usage in different parts of the world (Vogel, 2008). Also, over the years, there has been steady increase in the dependence on the use of plants and herbs as medicine in health sector especially their usage in the treatment of malaria and typhoid fever (Soforowa, 2014).

Apart from malaria, typhoid fever was discovered to be more prevalent contagious disease in Bambontos region (Zofou *et al*. 2009). The worldwide assessment of the incidence of typhoid fever is assumed to be 16 million to 33 million cases with 500,000 to 700,000 deaths per year (W. H. O. 2001).

Herbal medication remains the major sources of healing for most persons infected with malaria and typhoid fever. Because of its broad knowledge structure, herbal medication involves the utilization of substances, dosage and practices due to social and cultural norms and beliefs and also showcases the know-hows and thought of some of people. In order to determine, avoid and cure societal and spiritual disparity this idea is spread across generations (Diallo and Paulsen, 2000).

About 80% of the global population depends on herbal treatment for their health care delivery, and most of these people use plants or their ethics (Gupta *et al.* 2005). The curing of chronic and acute infectious diseases is based on a large quantity of ingredients obtained from plants in folk medicine (Okafor 2001). As a result of unavailability of health clinics and social services within the rural communities and under developed nations most dwellers depends on these plant natural resources for their health care. However, because of high price in availability, cultural mismatch and self-sufficiency several persons are presently resorting to folk practice for primary health treatment. (Kamatenesi-Mugisha *et al.*2005). Based on ethic favorites and apparent efficacy some people also adopt traditional medicines (WHO, 2002).

The physiological changes that occurs in the human body is usually caused by the substances available in medicinal plants (Nwachukwu *et al.*, 2010), and the fresh or dried parts of plants can be used to inhibit and cure different types of sicknesses (Joharchi and Amiri, 2012). Generally, the utilization of medicinal plants differs from species to species, as illnesses changes from one pattern to another in different sites (Nwachukwu *et al*., 2010). Nevertheless, at the market level medicinal plants and their parts are usually contaminated or changed by other plants thereby causing loss of value and hazard due to physical relatedness of plant part that causes misidentification by users and absence of standard identification system (Joharchi and Amiri, 2012).

Random amplified polymorphic DNA marker system is simple, fast, cheap and widely used for genetic diversity studies and marker assisted selection. Considering the poverty and unavailability of health post in some rural areas of the state and the cost of treatment, it is imperative to identify, authenticate and ensure the availability of plant species with the potential of curing. This study analyzed selected medicinal plants in Ebonyi State used in the treatment of malaria and typhoid fever using RAPD markers.

Materials and Methods

Plant samples were collected from villages in Ebonyi State and identified by a qualified taxonomist in the Department of Applied Biology, Ebonyi State University Abakaliki, Ebonyi State.

DNA Extraction using CTAB Method

Fresh young leaf tissue (100 g) was weighed and grinded with 1000 µl of extraction buffer, vortexed and poured into sterile tube of 1.5 ml. It was incubated in water bath at 60°C for 10 min, and equal volume of phenol, chloroform, and iso-amylacohol at 25: 24: 1 was added. It was vortexed and centrifuged at 1, 3000 g for 10 min and 450 µl of the supernatant was removed and poured into 1.5 ml sterile tube and 400 µl of cold isopropanol was added into the supernatant. It was mixed and incubated for 3 h at 20°C in freezer and centrifuge at 1,3000 g for 10 min to sediment the DNA. The supernatant was decanted to ensure that the pellets was not distributed and 500 µl of 70% ethanol was added to the pellets and centrifuged again at 1,3000 g for 5 min to wash the pellet, then the ethanol was decanted and the DNA air-dried at room temperature. The pellets was suspended in 100-200 µl of nuclease free water for storage or used.

Table 1: Studied Plants Used in the Treatment of Malaria

Botanical Name	Common/Local Name	Part Used
Chromolena ordorata	Nwogbalike	Leaf
Ficus vogelii	Ogbu mbeke	Leaf
Psidium quajava	Guava	Leaf
Morinda lucida	Nvuishhi	Leaf

Table 2: Plants Used in the Treatment of Typhoid Fever

Botanical Name	Common/Local Name	Part Used
Swietenia macrophylla	Inyima mmanu	Leaf
Melostoma	Ikpisunku	Leaf
Spoudias mumbin	Utzu Enyi	Leaf

DNA Extraction using CTAB Method

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Electrophoresis of the Extracted Genomic DNA

The extracted DNA was separated in 1.5% agarose gel. 2 µl of the 6X DNA loading dye was mixed with 5 µl of the extracted DNA and the mixture was loaded into the gel wells. 10 µl of 100 base pair DNA ladder was used as molecular size marker and was loaded into the first well and ran alongside with the samples at 100 volts for 1 h in the electrophoresis tank. The gel was viewed under UV trans-illuminator and the image captured using gel documentation system BR Biochem. Life Science LTD, Tilak Nagar, New Delhi. The bands were scored on the basis of presence (1) or absence (0).

Polymerase Chain Reaction (PCR) Analysis

PCR amplification was performed in volume of 25 µL which consisted of 20 ng of genomic DNA, 2.5 µl of 10 x buffer Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa, 1.5 µl of 50 mM $MgCl_2$ (Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa) 2.0 µl of 2.5 mM dNTPs (Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa), and 0.2 µl of 500U Taq DNA polymerase (Inqaba Biotechnical Industries (Pty) Ltd. Pretoria, South Africa), 1.0 µl of 10 µM of each primer and 15.05 µl DEPC-treated water (invitrogen corporation). The PCR cycling profile used was an initial step at 94°C for 5 min., 35 cycles at 94°C for 20 sec, 60°C for 30 sec, 72°C for 1 min, and a 10 min final extension at 72°C. Amplified products were resolved on 2% agarose gel, visualized under UV light and photographed.

Results and Discussion

Banding Profiles Obtained using RAPD Markers

The data obtained from the scoring of the amplicons were used for phylogenetic reconstruction using unweighted pair group mean arithmetic (UPGMA) and dissimilarity index in Jaccard's option (Jaccard, 1908) were used for the analysis. The analysis was conducted using NTSYSpc software version 2.02.

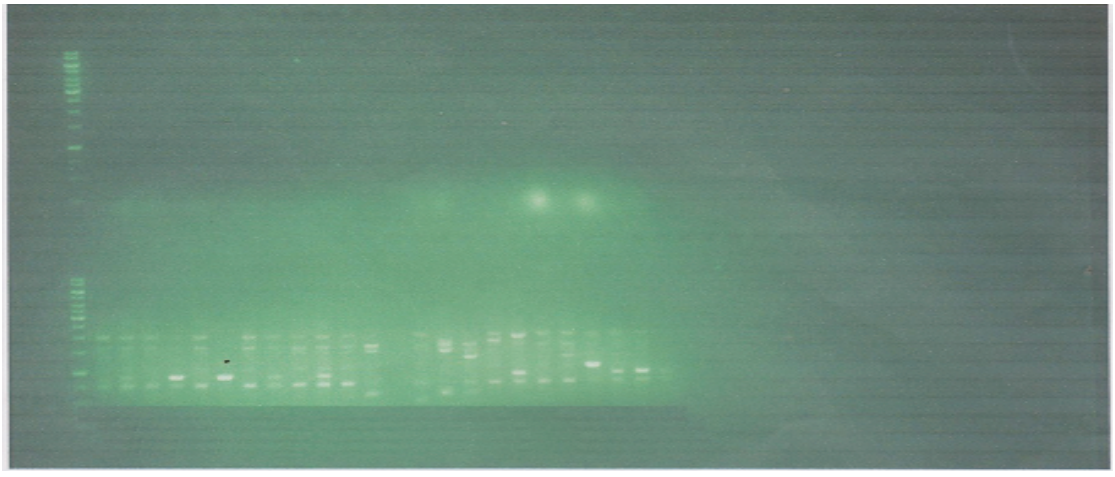

Figure 1: DNA Samples Amplified with OPB-1 Marker

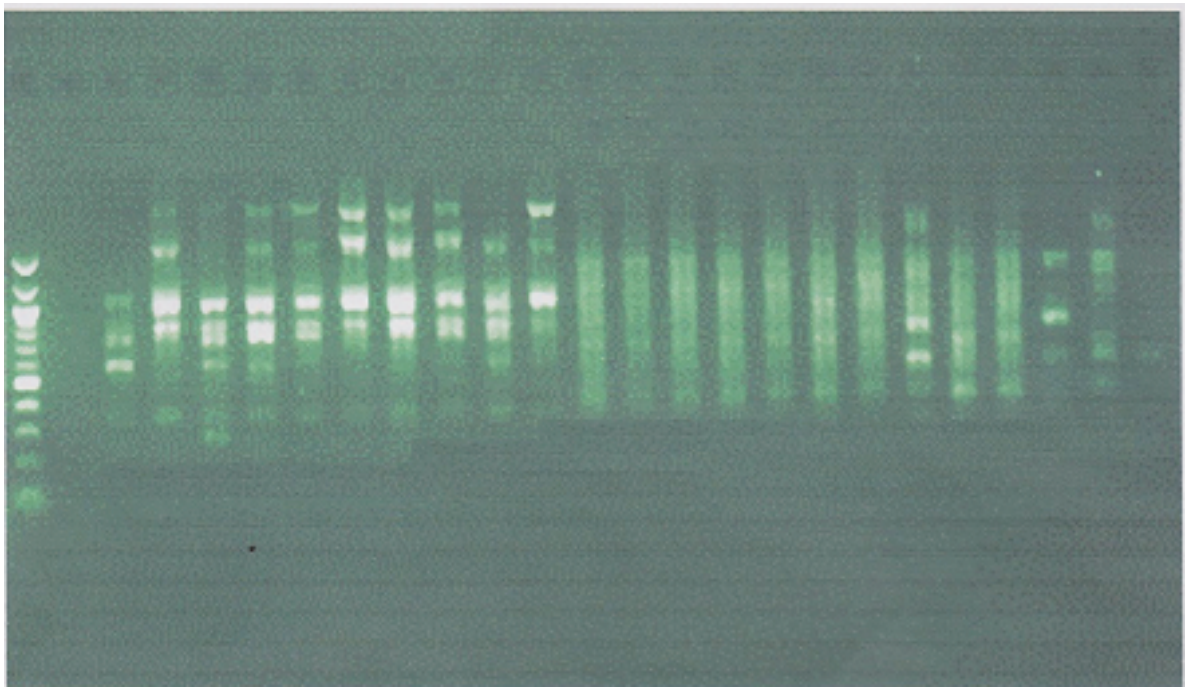

Figure 2: DNA Samples Amplified with OPB-5 Marker

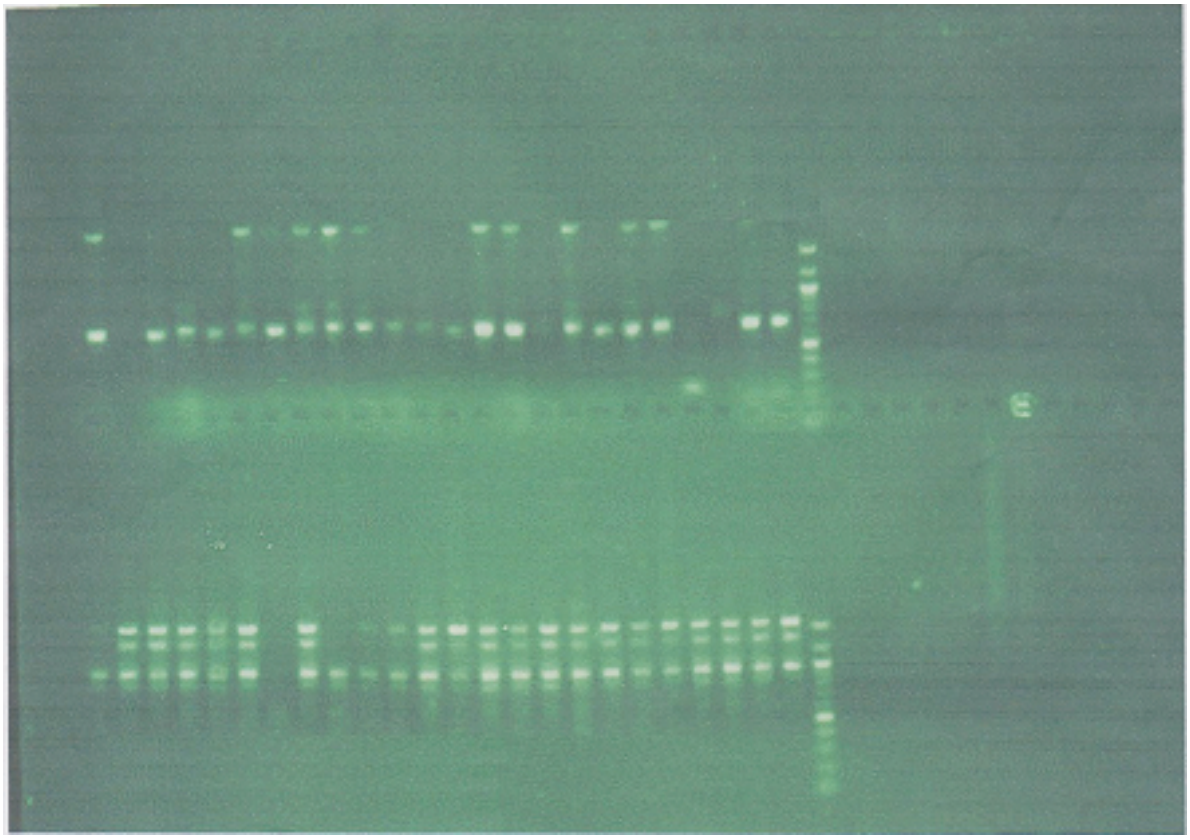

Figure 3: Samples Amplified with OPB-12 and OPH-12 Markers

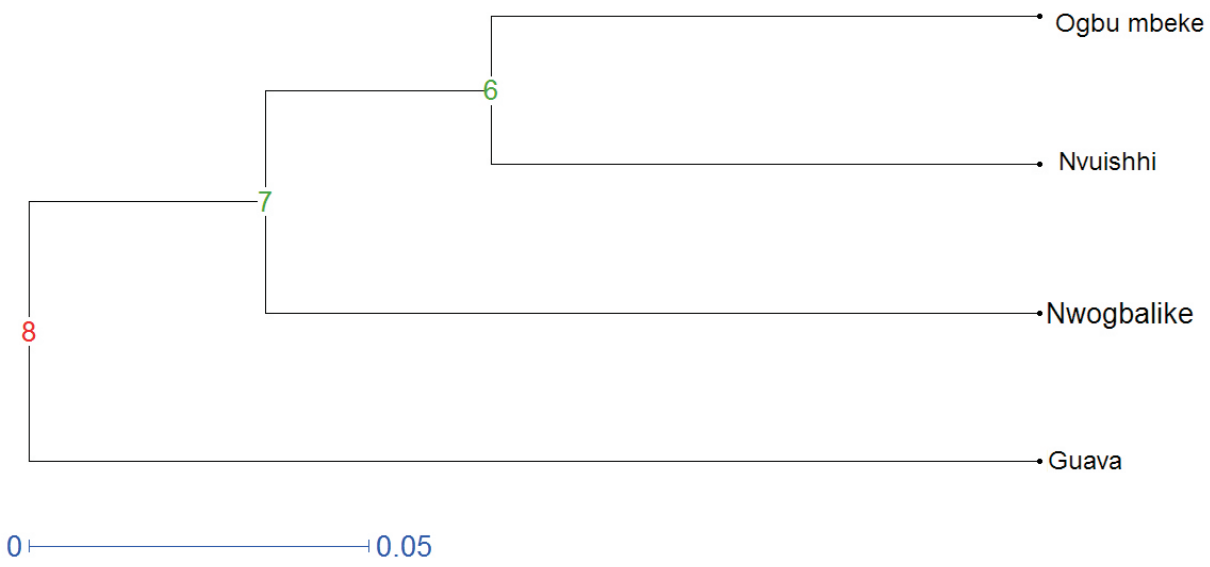

Figure 4: Dendogram (unrooted tree) of Selected Medicinal Plants for Malaria.

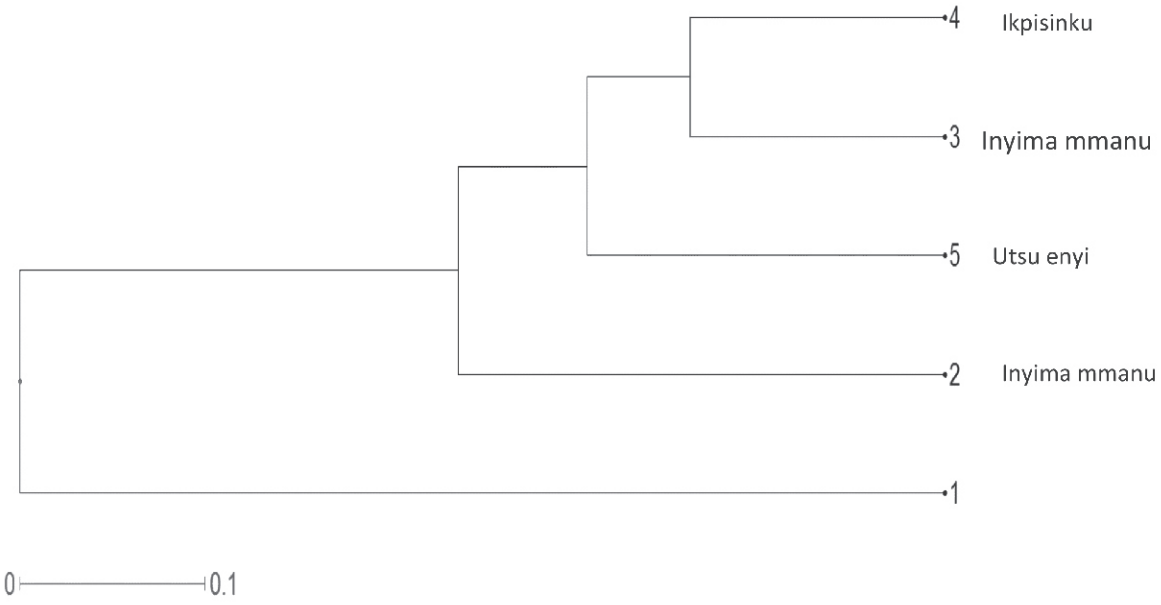

Figure 5: Dendogram (Unrooted trees) of Selected Medicinal Plants for Typhoid Fever

Africa is gifted by nature with a lot of herbal plant, which indigenous people acquire, preserve and pass to their next generation (Sabot *et al.,* 2010). The knowledge concerning traditional medicines and plants is presently transferred from generation to generation predominantly by word of mouth. This work identified different selected species of medicinal plants such as *Ficus vogelii* (Ogbu mbeke); *Morinda lucida* (Nvuishhi); inyima mmanu; utsu enyi; ikpisinku and opume using molecular markers.

A dendogram of the malaria medicinal plants accessions using unweighted pair group mean arithmetic (UPGMA) method separated the accessions with grouping level of 0.161, 0.227 and 0.293 (Figures 4). The dendogram of the accessions for the treatment of typhoid fever using unweighted pair group mean arithmetic (UPGMA) method separated the accessions with grouping level of 0.28, 0.39 and 0.48 with total edge length of 1.59 and dissimilarity minimum value of 0.3 and maximum value of 1.0 (Figures 5). The principal component analysis (PCA) of

the generated amplicons gave a representative of the accessions.However, this result is in accordance with the report of Yook *et al*. (2014) who observed that RAPD studies have been widely used for genetic variation of population. The results of the study showed that RAPD marker used established remarkable genetic difference among the medicinal plants examined.

Acknowledgements

This work was supported by a research grant from Tertiary Education Trust Fund (Tetfund) of the Federal Republic of Nigeria. The authors gratefully acknowledge the Federal University, Ndufu-Alike Ikwo (FUNAI), Ebonyi State, Nigeria for the research grant and facilities.

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