DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118

IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY
(A PUBLICATION OF FACULTY OF SCIENCE, FEDERAL UNIVERSITY GUSAU, NIGERIA)

Phytochemical Profiling, GC-MS Analysis, and Antiplasmodial Potentials of *Daniellia Oliveri* and *Morinda Lucida* Leaves

[1]Dibal, D.M., [1]Umar, Y.A.,[1]Vantsawa, P.A., and [1]*Muhammed, M.
[1]Depatrment of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria

***Corresponding Author's Email & Phone No.**: [1]muazmed@gmail.com 07033009793

Received on: January, 2025 **Revised and Accepted on:** February, 2025 **Published on:** March, 2025

ABSTRACT

The global demands for use of traditional herbal medicines obtained from medicinal plants for some aspect of primary health care needs is on the increase despite due to the rising spread of drug-resistant *Plasmodium falciparum* strains to artemisinin derivatives, because traditional herbal medicines have been the most accessible, available, affordable and cheap source of malaria treatments for most communities. This study aims to determine the phytochemical compounds and bioactive components present in aqueous crude extracts of *Daniellia oliveri*, *Morindalucida* and Combined recipe leaves. The phytochemical compounds and GC-MS identification of the bioactive components was determined. Results showed that Alkaloids, flavonoids, cardiac glycosides, tannin, steroid, saponins, terpenoids and phenol were present. Phenols had the highest mean concentration in *Danielliaoliveri* (74.80 mg/g), *Morindalucida* (97.78 mg/g) and Combined Recipe (80.85 mg/g) while Tannins had the lowest mean concentration in *D. oliveri* (3.10 mg/g), *M. lucida* (3.91 mg/g) and Combined Recipe (3.32 mg/g). GC-MS identification of the bioactive compounds revealed a total of 25 and 49 compounds in *Danielliaoliveri* and *Morindalucida* leaves respectively. It could be concluded that the aqueous crude extracts of *Danielliaoliveri* and *Morindalucida* leaves potentially contained phytochemicals and bioactive compounds reported to be active against malaria parasites.

Keywords: Phytochemical, GC-MS Analysis, Antiplasmodial, *Danielliaoliveri*, *Morindalucida*

1.0 INTRODUCTION

The global demand for herbal remedies is on the rise, WHO estimated that 80 % of the populations of some Asian and African countries presently use traditional herbal medicine for some aspect of primary health care needs despite the sophistication in the medical and technological ecosystem (Shadia *et al*., 2016).

The spread of drug-resistant *Plasmodium falciparum* strains to ACTs have increasingly been reported, resulting in reduced parasite susceptibility to artemisinin derivatives, leading to the development of new antimalarial drugs from traditional herbal medicines (Apinjoh *etal*., 2019). Traditional herbal medicines have been the most accessible, available, affordable and cheap source of malaria treatments for most communities (Tizazu *et al*., 2020). The recognition and validation of traditional medicine is important and could lead to the discovery of new plant-derived drugs following the reported cases of drugs-resistance developed by *Plasmodiumfalciparum* strains to quinine isolated from Cinchona species and artemisinin isolated from *Artemisiaannua* L., (Cimanga, 2019), which are potent sources of bioactive natural metabolites (Samin *etal*., 2020).

The approval of artemisinin-based combination therapies (ACTs) by World Health Organization (WHO) as the first-line treatment for malaria was a breakthrough in the study of the synergy of antimalarial drugs affirming drug combination as one of the potent strategies for a sustained fight against malaria (Ochara, 2022).

Danielliaoliveri (*D. oliveri*) (Rolfe) (syn. Paradaniellia oliveri Rolfe) was first named after William Freeman Daniell (Choo *etal*., 2020), a medicinal plant of the genus Daniellia that belongs to the family Fabaceae, sub-family Caesalpinioideae and commonly known as African copoiba balsam (Lawal *etal*., 2022). *Danielliaoliveri* tree is commonly found in Amazonia, some part of America, and Africa (Galba *etal*., 2020). *D.oliveri* plant is native to tropical West and Central Africa, in Nigeria it is traditionally known as 'Maje' in Hausa, 'Emi iya' in Yoruba, 'iya /ozabwa /agba' in Igbo (Lawal *etal*., 2022), 'Agba' in Igala, 'Ukpilla' in Igede (Muazu *etal*., 2022) and 'Aha' in Idoma languages (Shauibu *etal*., 2018).

In addition to *D.oliveri*, four other notable species in the genus *Daniellia* includes; *Danielliathurifera*, *Danielliaogea*, *Danielliapynaertii* and *Danielliaoblonga* (Keay, 1958).

*M*orinda *lucida* Benth is a member of the genus Morinda, a flowering medicinal plant that belongs to the family Rubiaceae, the genus, comprises about 80 species

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

that occur throughout the tropics. In Africa, the 5 species that are predominantly found are *Morindacitrifolia*, *Morindageminata*, *Morindalongiflora*, *Morindamorindoides* and *Morindalucida* (*M. lucida*) Benth (Zimudzi and Cardon, 2005). M. lucida Benth is one of the medicinal plants commonly found in Nigeria (Nwanna, 2021), and is called ''Oruwo'' in Yoruba Language, ''Eze-ogu'' in the Igbo language, ''Njisi'' in Hausa language, ''Ufu ogile'' in Igede language and ''Ogele'' in Igala language. The therapeutic/folkloric use of the leaves, stem bark and roots of *M. lucida* plant by the natives of different communities across the geopolitical zones of Nigeria includes; fever, malaria, diabetes, heart diseases among others (Amodu *etal*., 2020; Ojewumi and Dedeke, 2020).

Quantitative phytochemical analysis revealed the presence of tannins; alkaloids flavonoids; terpenoids; saponins; phenolic acid; glycoside in various concentrations and the variation in the concentrations of these bioactive ingredients in the different parts of *D. oliveri* and *M. lucida* plants explains their diverse use in the treatment of different health challenges (Sodimu *etal*., 2021).

Nwanna, (2021), further discovered that the alkaloids in *Morindalucida* plant have anti-oxidative, anti-nociceptive, anti-inflammatory, and anti-cholinergic properties with a total of (1.55/100mg) distinct plant-derived alkaloids such as dicentrine, atropine, and aporphine, detected by Gas Chromatography-Mass Spectroscopy (GC-MS) analytical techniques.

1.1 Preparation of Traditional Herbal Medicines

Over the decades, various methodologies have been adopted by the traditional healers, herbs traders, people knowledgeable about the traditional herbal medicines for the preparation of roots, barks, fruit, seed, leaf, stems of medicinal plants, useful in the prevention and curing of various diseases. These methods includes infusion, maceration, crushing and grinding (Arslan *etal*., 2021). However, the most commonly adopted method is infusion, practiced by an estimated 43.3% of the traditional healers in Kaduna State, northwestern Nigeria (Sodimu *etal*., 2021).

1.2 Phytochemical Compounds in Medicinal Plants

Phytochemicals are bioactive compounds found in plants, responsible for their medicinal properties. These compounds which have been classified into several categories are alkaloids, saponins, cardiac glycosides, flavonoids, terpenoids, anthraquinones, tannin, phlobatannin, reducing sugar, phenol and steroid (Sodimu *etal*., 2021).

1.3 Phytochemical Compounds in *Daniellia oliveri* and *Morinda lucida* Leaves

Quantitative phytochemical analysis of *D. oliveri* and *M. lucida* leaves revealed the presence of tannins; alkaloids; flavonoids; terpenoids; saponins; phenolic acid; glycoside in various concentrations and variation in the concentrations of these bioactive ingredients in the different parts of *D. oliveri* and *M. lucida* plants explains their diverse use in the treatment of different health challenges (Sodimu *et* al., 2021). Nwanna, (2021), further discovered that the alkaloids in *Morinda lucida* plant have anti-oxidative, antimalarial, anti-nociceptive, anti-inflammatory, and anti-cholinergic properties.

2.0 MATERIALS AND METHODS

2.1 Plant Leaf Collection

Danielliaoliveri and *Morindalucida* leaves were collected in March, 2024, from the Plant Science and Technology Garden, Prince Abubakar Audu University, Anyigba, Kogi State, North-Central Nigeria. Anyigba is situated between Latitude 7°27' and 7°31' North of the equator, between Longitude 7°09' and 7°12' East of the Greenwich meridian (Figure 1).

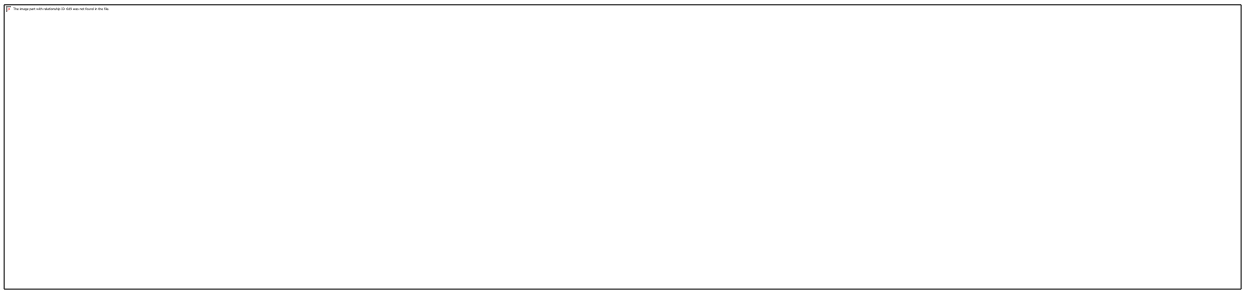

Figure 1: Map of Anyigba, Kogi state
Source: Ifatimehin *et-al*., (2014).

2.2 Plant Leaf Authentication

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

Danielliaoliveri and *Morindalucida* leaves were recognized and verified by an expert in the Department of Biological Sciences, Nigerian Defence Academy, Kaduna State, North-Western Nigeria. Voucher specimens were deposited while voucher numbers were assigned as follows: NDA/BIOH/202424 for *Danielliaoliveri* leaves and NDA/BIOH/202425 for *Morinda lucida* leaves.

2.3. Techniques for Extraction of Medicinal Plants

Extraction is important in medicinal plant studies for phytochemical analysis (Arslan et *al*., 2021). The various extraction techniques are decoction, maceration, digestion, infusion, percolation, superficial extraction, Soxhlet extraction, ultrasound-assisted, and microwave-assisted extractions (Abubakar and Haque, 2020).

2.4. Solvents for Extraction and Polarity

Solvents commonly used in extraction of medicinal plants are polar solvent (water, alcohols), intermediate polar (acetone, dichloromethane), and nonpolar (n-hexane, ether, chloroform) (Abubakar and Haque, 2020). Commonly used solvents and polarity includes Water (1.000), Methanol (0.762), Ethanol (0.654), n-Butanol (0.586), Acetone (0.355), Dichloromethane (0.309), Chloroform (0.259), Ethyl acetate (0.228), Diethyl ether (0.117), Petroleum ether (0.117) and n-Hexane (0.009) (Abubakar and Haque, 2020).

2.4.1 Water-Based Extraction

Infusion: Involves steeping plant material in hot water to extract bioactive compounds.

Decoction: Involves boiling plant material in water to extract bioactive compounds.

Maceration: Involves soaking plant material in cold water to extract bioactive compounds.

Percolation: Involves passing water through a bed of plant material to extract bioactive compounds.

Expression: Involves using pressure or heat to extract bioactive compounds from plant material (Cao, *et al*., 2020).

2.5 Preparation of Leaves and Aqueous Crude Extract

The method of Chaniad *etal*., 2022 was adopted in the preparation of leaves and aqueous crude extract. *D. oliveri* and *M. lucida* leaves were screened by the removal of pigmented and dried leaves. Leaves were cleaned, allowed to air-dry for three weeks at room temperature, pulverized with mortar and pestle to obtain the plant powdered samples. Decoction method of extraction was used to prepare aqueous extracts by dissolving 100g of the pulverized *D. oliveri* and *M. lucida* leaves each in 4000 mL of distilled water and allowed to boil for 3 hours respectively. Similarly, a 100g combination of both pulverized *D. oliveri* and *M. lucida* leaves comprising 50g of the pulverized *D. oliveri* and 50g of the pulverized *M. lucida* leaves (Combined Recipe) was prepared by dissolving in 4000 mL of distilled water and allowed to boil for 3 hours Chaniad *et al*., 2022. Subsequently, the liquid portion was separated from the residue using Whatman filter paper (Whatman, Buckinghamshire, England) (Chaniad *etal*., 2022; Afolabo and Abejide, 2020). The filtrates were combined and concentrated to dryness using a rotary evaporator (Stuart, SO1, UK) set at 40^{o}C (Kalay *etal*., 2020).

The percentage (%) yield was calculated using the formula adopted by Adam *etal*, (2019) as follows:

$$Extract\ yield\ (\%) = \frac{Weight\ of\ dried\ extract}{Weight\ of\ dried\ plant\ sample}\ X\ 100$$

Finally, the aqueous crude leaf extracts of *D. oliveri*, *M. lucida* and Combined Recipe were refrigerated at 4^{o}C.

2.6 Fractionation of Crude Extracts

Fractionation and purification of phytochemical substances were achieved through the application of various chromatographic techniques such as paper chromatography, thin-layer chromatography, gas chromatography, and high-performance liquid chromatography. Compounds obtained were characterized using diverse identification techniques such as mass spectroscopy, infrared spectroscopy, ultraviolet spectroscopy, and nuclear magnetic resonance spectroscopy (Abubakar and Haque, 2020).

Several methods including the detection of functional group, presence of multiple bonds and rings, hydrogen and carbon arrangement as well as full structural elucidation are applied in the identification of compounds from medicinal plant extracts. Mass spectroscopy (MS) method of identification of compounds provides abundant information on organic molecules from medicinal plant extracts based on chemical structure and molecular weight through sequencing and identifying the unknown compound in a mixture (Abubakar and Haque, 2020).

2.7 Determination of Phytochemical Compounds

The qualitative and quantitative phytochemical analysis of the aqueous crude extracts of *D.oliveri*, *M.lucida* and Combined Recipe was carried out using the method as adopted by Huma *etal*., (2021) to examine the presence or absence of phytochemical compounds and to quantify the amount of the phytochemical compounds which includes; Alkaloids, Anthraquinones, Flavonoids, Cardiac glycosides, Tannin, Steroid, Saponins, Terpenoids and Phenol (Sodimu *etal*., 2021; Trease and Evans, 1978).

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

2.7.1 Qualitative Determination of Phytochemical Compounds

2.7.1.1 Alkaloids

2.7.1.1.1 Hager's test:

Into a 2 mL of the aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe respectively, few drops of Hager's reagent was dispensed. The appearance of yellow color precipitates was indicative of the presence of alkaloid in the aqueous crude extracts of *D.oliveri* and *M.lucida* leaves respectively.

2.7.1.2 Anthraquinones

To 1 mL of diluted aqueous crude extracts of *D. oliveri* leaves, *M. lucida* leaves and Combined Recipe respectively, 1 mL benzene was added in addition to 1mL of 10% ammonia solution. Appearance of red color on addition of ammonia solution indicated the presence of anthraquinones.

2.7.1.3 Flavonoids

2.7.1.3.1 Alkaline reagent test:

To the 1 mL aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe respectively in test tubes, 1 mL of 2N NaOH was added and a yellow color indicated the presence of Flavonoids.

2.7.1.4 Cardiac-Glycosides

2.7.1.4.1 Keller Killani test:

To 1µL of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe respectively, 1 mL glacial acetic acid was added, allowed to cool, and 2 drops of $FeCl_3$ was further added. A careful addition of 2 ml solution of conc. H_2SO4 through the wall of the test tube was finally made. The formation of brown ring at the interface showed the presence of de-oxy sugar characteristic of cardenolides.

2.7.1.5 Tannins

2.7.1.5.1 Alkaline Reagent test:

To 2 mL of NaOH in a test tube, 2 mL of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe were respectively dispensed. Change in color from yellow to red indicated the presence of tannins.

2.7.1.6 Steroids

2.7.1.6.1 Salkowski test:

To 1mL of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe in test tubes, 10 mL of chloroform was added into each extract respectively, followed by the addition of 10 mL concentrated sulphuric acid in the test tubes. Formation of two layers with the lower layer of yellow color along green fluorescence, and the upper layer of red colors was indicative of the presence of steroids.

2.7.1.7 Saponins

2.7.1.7.1 Froth formation with distil water:

Added to 2mL of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe respectively in graduated cylinders was 2 mL of distilled water. The mixture was shaken continuously for 15 min. Appearance of 1 cm layer of foam was indicative of the presence of saponins.

2.7.1.8 Terpenoids

To 2 mL of aqueous crude extracts of Daniellia oliveri leaves, *Morindalucida* leaves and Combined Recipe, 1 mL 1% HCl was added and kept for 5 hours. In addition, 1 mL of Trim-Hill reagent was added and placed in a water bath previously set to boiling temperature, heated for 5-10 minutes. A bluish green color appearance indicated the presence of terpenoids.

2.7.1.9 Phenols

2.7.1.9.1 Ellagic acid test:

To 1µL of aqueous crude extracts of *Danielliaoliveri* leaves, *Morinda lucida* leaves and Combined Recipe, a few drops of 5% Glacial acetic acid was added to each tube. In addition, 5% $NaNO_2$ was dropped into the mixture. The formation of muddy brown color was indicative of the presence of Phenols.

2.8 Quantitative Determination of Phytochemical Compounds

The method according to Huma *etal*., (2021) and Ezeonu and Ejikeme, (2016) was adopted for the quantitative determination of phytochemical compounds available in the aqueous crude extracts of *D.oliveri* leaves, *M. lucida* leaves and Combined Recipe respectively.

2.8.1 Quantitative Determination of Alkaloids

The methods of Harborne, 1973 was used for the quantitative determination of Alkaloids.

In a 250 cm^3 beaker containing 2.5 g of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe respectively, 200 cm^3 of 10% acetic acid in ethanol was dispensed and allowed to stand for 4 hours. In a water bath (DHG-9030A, China), the extracts was concentrated to one-quarter of the initial volume, gradually, 15 drops of concentrated ammonium hydroxide were added to each extract till precipitation emerged, followed immediately by filtration and allow to stand for 3 hours for sedimentation to occur. The supernatant was discarded. Using 20 cm^3 of 0.1M of

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

ammonium hydroxide, the precipitates were washed and filtered using Gem filter paper (12.5 cm). The residue was dried in the oven (DHG-9030A, China), weighed using electronic weighing balance Model (B- 218) and the concentration of alkaloid was calculated using the formula as presented below:

$$\%\ Alkaloid = \frac{Weight\ of\ Alkaloid}{Weight\ of\ sample}\ X\ 100$$

2.8.2 Quantitative Determination of Anthraquinones

Into a 100 mL volumetric flask, 10 mL of 1/10 dilution of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe was added respectively. Using UV-Vis (Ultra-Violet-Visible) spectrophotometer (Intec-290, India) at a wavelength of 325 nm, the concentration of anthraquinone was determined by checking the dilution factor of each sample compared to the standard anthraquinone solution (Khoomsab and Khoomsab, 2019).

2.8.3 Quantitative Determination of flavonoid

Repeatedly, 100 mL of 80% of aqueous methanol was added to 10 g of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe respectively. The mixture was filtered using Whatman filter paper (Whatman, Buckinghamshire, England) and the filtrates were respectively dried through vaporization in a rotary evaporator (Stuart, SO1, UK) and weighed till constant weight was achieved. The percentage concentration of flavonoid was calculated as follows:

$$\%\ Flavonoid = \frac{Weight\ of\ Flavonoid}{Weight\ of\ sample}\ X\ 100$$

2.8.4 Quantitative Determination of Cardiac-Glycosides

The amount of cardiac-glycosides was determined according to the methods adopted by Yadav *etal*., (2021).

Briefly, 50 µl of the aqueous crude extracts of *D. oliveri* leaves, *M.lucida* leaves and Combined Recipe was allowed to react with 150 µl of methanolic KOH for 2 hours at room temperature in test tubes. The UV (Ultra-Violet) absorption was measured at 355 nm. Scillaren A of different concentrations (20, 40, 60, 80, 100 µg) was used as a standard and results was expressed as mg SCE/g DW.

Where:

SCE (Scillaren A equivalent)

DW= (Dry weight).

2.8.5 Quantitative Determination of Tannins

The method as applied by Van Buren and Robinson, (1969) with little modification was used for the quantitative determination of tannin. Briefly, 500 mg of aqueous crude extracts of *D.oliveri* leaves, *M. lucida* leaves and Combined Recipe were measured in (50 mL) plastic bottles respectively. Following the addition of 50 mL distilled water in a mechanical shaker, the mixtures were shaken for one hour and later filtered. The volume of each was raised up to 50 mL in a volumetric flask. 5 mL of filtered solution was taken in test tube using a pipette, and 2 mL $FeCl_3$ (0.1 M) in HCl (0.1 N) and potassium ferrocyanide (0.008 M) was added. Absorbance was measured at 120 nm by the use of spectrophotometer in comparison to standard curve of gallic acid, results was depicted in the form of GAE (mg of Gallic Acid Equivalent) per gram of dried extracts.

$$Tannic\ acid\ (mg) = \frac{C\ X\ Extract\ Volume}{100\ g\ Aliquote\ Volume\ X\ Weight\ of\ Sample}\ X\ 100$$

2.8.6 Quantitative Determination of Steroids

To 1 mL of aqueous crude extracts of *D.oliveri* leaves, *M. lucida* leaves and Combined Recipe solution in 10 mL volumetric flask, 2mL of 4N Sulphuric acid and 2 mL of 0.5% w/v iron (III) chloride were added and gently mixed, followed by the addition of 5 mL of 0.5% w/v of potassium hexacyanoferrate (III) solution. The mixture was heated in a water bath (BIOBASE WT-60, China) maintained at 70±2°C for 30 minutes, occasionally, the mixture was shaken and diluted to the mark with distilled water. Compared with the reagent blank, the absorbance was measured at 780 nm and the concentration of steroids was obtained when compared with the standard curve (Panchal and Charuben, 2021).

2.8.7 Quantitative Determination of Saponin

Respectively, 10 g of aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe were obtained and dissolved in 50 mL of 20 % aqueous ethanol respectively. All samples were subjected to heat with a continuous stirring for four hours at 55°C in a water bath (BIOBASE WT-60, China). The solution was filtered. The same process was repeated in 100 mL of 20% ethanol. In the water bath (BIOBASE WT-60, China) at 90°C, the extracts was dried to 40 mL. In a separating funnel, 10 mL of diethyl ether was added and vigorously shaken, and two layers emerge. The aqueous layer was selected and purified repeatedly. Subsequently, 30 mL of n-butanol was added with further washing with the aid of 10 mL of 5% aqueous sodium chloride. Using a water bath for evaporation, the extracts were dried in oven till constant weight was achieved and the percentage amount of saponin was calculated (Huma *etal*., 2021) as follows:

$$\%\ \boldsymbol{Saponin} = \frac{\boldsymbol{Weight\ of\ Saponin}}{\boldsymbol{Weight\ of\ sample}}\ \boldsymbol{X\ 100}$$

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

2.8.8 Quantitative Determination of Terpenoids

At a room temperature, 10 g aqueous crude extracts of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe was soaked in 100 mL of alcohol for 24 hours, filtered and extracted using petroleum ether. The extract was dried and the quantity of terpenoids calculated using the formula of Ezeonu and Ejikeme (2016), as follows:

(wi-wf/wi×100).

Where:

wi= Initial weight

wf= Final weight

2.8.9 Quantitative Determination of Phenols

In 100 cm^3 of ether using a soxhlet apparatus, 2 g powder of *D.oliveri* leaves, *M.lucida* leaves and Combined Recipe were defatted respectively for 2 hours. With the aid of 50 cm^3 of ether, the phenolic components were extracted by boiling 0.50 g of each sample for 15 minutes. Using a measuring cylinder, 10 cm^3 of distilled water, 2 cm^3 of 0.1N ammonium hydroxide solution, and 5 cm^3 of concentrated amyl alcohol were dispensed into 5 cm^3 of each extract and left to react for 30 minutes for colour development. With the aid of spectrophotometer, the optical density was measured at 505 nm. Tannic acid (0.20 g) was dissolved in distilled water and diluted to 200 mL mark (1mg/cm^3) for the preparation for phenol standard curve. Varying concentrations (0.2–1.0mg/cm^3) of the standard tannic acid solution was pipetted into five different test tubes to which 2 cm^3 of NH_3OH, 5 cm^3 of amyl alcohol, and 10 cm^3 of water was added and the mixture was made up to 100 cm^3 volume, allowed for 30 minutes for colour development and at 505 nm, the optical density was determined (Keay *etal*., 1964).

2.9 GC-MS Determination of Phytochemical Components in the Aqueous Crude Extracts

The method of Olivia *et al*, (2021) was adopted for the Gas Chromatography-Mass Spectometry (GC-MS) determination of phytochemical components in crude extracts of *D. oliveri* leaves, *M. lucida* leaves and Combined Recipe. The GC-MS analysis was carried out in a combined 7890A Gas Chromatograph System (GCMS-QP2010 PLUS SHIMADZU, JAPAN) and Mass Spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD (Mass Selective Detector) with Triple-Axis detector. Each component's relative percentage amount was determined by comparing its average peak area to the sum of all areas. The system was controlled and data were collected using the supplier's MS solution software. Based on their retention indices, components was identified, and the National Institute of Standards and Technology's (NIST) database was used to interpret the mass spectrum. More than 62,000 patterns of well-known chemicals are included in the database. The resulting spectra of the crude extracts of *D. oliveri* and *M. lucida* leaves extracts' unidentified components was compared with the reference mass spectra of known components kept in the NIST library (NISTII).

2.10 Statistical Analysis

Data was subjected to analysis using Microsoft Excel, SPSS, Graph Pad and SAS 9.12 software. One – way analysis of variance (ANOVA) was used to determine the treatment effects. Pearson correlation test was used to evaluate relationship between measured parameters. Duncan multiple range test (DMRT) was used to separate the means. All data were analyzed at a 95% confidence interval and $P< 0.05$ was considered statistically significant.

3.0 RESULT AND DISCUSSION

3.1 Percentage Yield of *D. oliveri, M. lucida* and Combined Recipe

The Aqueous Crude Extracts of *D. oliveri* yielded 17.4 g indicating a percentage yield of 17.4%, *M. lucida* yielded 5.0 g; indicating a percentage yield of 5.0 %, and Combined Recipe yielded 8.4g indicating a percentage yield of 8.4% (Table 1).

Table 1: Percentage Yield of Aqueous Crude Extracts of *Daniellia oliveri*, *Morinda lucida* and Their Combined Recipe

Variables	*Daniellia oliveri*	*Morinda lucida*	*Combined Recipe*
Weight of Powder (g)	100	100	100
Vol. of Solvent (ml)	4000	4000	4000
Yield (g)	17.4	5	8.4
% Yield	17.4	5	8.4

Legend:
Vol. = Volume

3.2 Phytochemical Components Detected in *D. oliveri, M. lucida* and Combined Recipe

The Qualitative Phytochemical analysis of the Aqueous Crude Extracts of *D. oliveri*, *M. lucida* and Combined Recipe revealed the presence of Tannins, Phenols, Steroids, Flavonoids, Saponins, Glycosides and the absence of Anthraquinones. Alkaloids was present in *D. oliveri* but absent in *M. lucida* and Combined Recipe. Terpenoids was present in *M. lucida* and Combined Recipe but absent in *D. oliveri* crude extract (Table 2).

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

Table 2: Qualitative Phytochemical Screening of*D. oliveri*, *M. lucida* and Combined Recipe

Phytochemical	*Daniellia oliveri*	*Morinda lucida*	Combined Recipe
Tannins	+VE	+VE	+VE
Phenols	+VE	+VE	+VE
Steroids	+VE	+VE	+VE
Anthraquinones	-VE	-VE	-VE
Flavonoids	+VE	+VE	+VE
Saponins	+VE	+VE	+VE
Terpenoids	-VE	+VE	+VE
Glycosides	+VE	+VE	+VE
Alkaloids	+VE	-VE	-VE

Legend: Present = +VE and absent = -VE

3.3. Mean Concentrations of Phytochemical Constituents in *D. oliveri*, *M. lucida* and Combined Recipe

The mean concentrations of various phytochemical constituents detected in Aqueous Crude Extracts of *D. oliveri*,*M. lucida* and Combined Recipe as presented in table 3 showed Phenols as the phytochemical with the highest mean concentration in *D. oliveri* (74.80 mg/g), *M. lucida* (97.78 mg/g) and Combined Recipe (80.85 mg/g).

Tannins had the lowest mean concentration in *D. oliveri* (3.10 mg/g), *M. lucida* (3.91 mg/g) and Combined Recipe (3.32 mg/g). Terpenoids was not applicable (NA) to *D. oliveri.*

However, low amount of Glycosides was detected in crude extract of *D. oliveri* (14.07 mg/g), *M. lucida* (39.04 mg/g) and Combined Recipe (18.62 mg/g).

The mean amount of Alkaloids in *D. oliveri* (10.20 mg/g), a lower amount in *M. lucida* (0.40 mg/g), and the lowest mean concentration (0.09 mg/g) contained in Combined Recipe.

There is significant difference ($p < 0.05$) between the mean concentration of alkaloid and other phytochemicals in *M. lucida* leaves. Also, there is significant difference ($p < 0.05$) between the mean concentration of alkaloid and other phytochemicals in *D. oliveri* leaves. In addition,

There is significant difference ($p < 0.05$) between the mean concentration of alkaloid in *M. lucida*, *D. oliveri* and the mean concentration of alkaloid in the Combined recipe of *D. oliveri* and *M. lucida* leaves.

Table 3: Mean Concentrations of Various Phytochemical Constituents Detected in Aqueous Crude Extracts

Phytochemical	***Morinda lucida***	***Daniellia oliveri***	***Combined Recipe***
Tannins (mg/g)	3.91 ± 0.04^{a}	3.10 ± 0.04^{c}	3.32 ± 0.00^{b}
Phenols (mg/g)	97.78 ± 0.98^{a}	74.80 ± 0.09^{c}	80.85 ± 0.10^{b}
Terpenoids (mg/g)	8.07 ± 0.14^{a}	NA	5.66 ± 0.05^{b}
Flavonoids (mg/g)	12.69 ± 0.18^{a}	5.42 ± 0.04^{c}	6.76 ± 0.08^{b}
Saponins (mg/g)	15.28 ± 0.00^{a}	4.42 ± 0.11^{c}	10.64 ± 0.31^{b}
Steroids (mg/g)	34.05 ± 0.04^{a}	19.78 ± 0.04^{b}	19.04 ± 0.02^{c}
Glycosides (mg/g)	39.04 ± 0.16^{a}	14.07 ± 0.03^{c}	18.62 ± 0.39^{b}
Alkaloids (mg/g)	0.40 ± 0.01^{b}	10.20 ± 0.02^{a}	0.09 ± 0.01^{c}

n = 2, values presented as mean ± sem. statistical analysis shows that the means with the same subscript letter on the same row are not significantly different at $p > 0.05$

Legend

NA: Not Applicable to the Sample

3.4 GCMS Identification of Compounds in *Daniellia oliveri* Leaves

Following the GCMS analysis, a total of 25 compounds were identified in *D. oliveri* leaves, in relation to the molecular weight and retention index, see the chromatogram of the GC-MS analysis (Figure 2).

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118

IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

Figure 2: Chromatogram of GCMS Identification of Compounds in *D. oliveri* Leaves

3.5 GCMS Identification of Compounds in *Morinda lucida* Leaves

Following the GCMS analysis, a total of 49 bioactive compounds were identified in *M. lucida leaves*, in relation to the molecular weight and retention index as shown by the chromatogram of the GC-MS analysis (Figure 3).

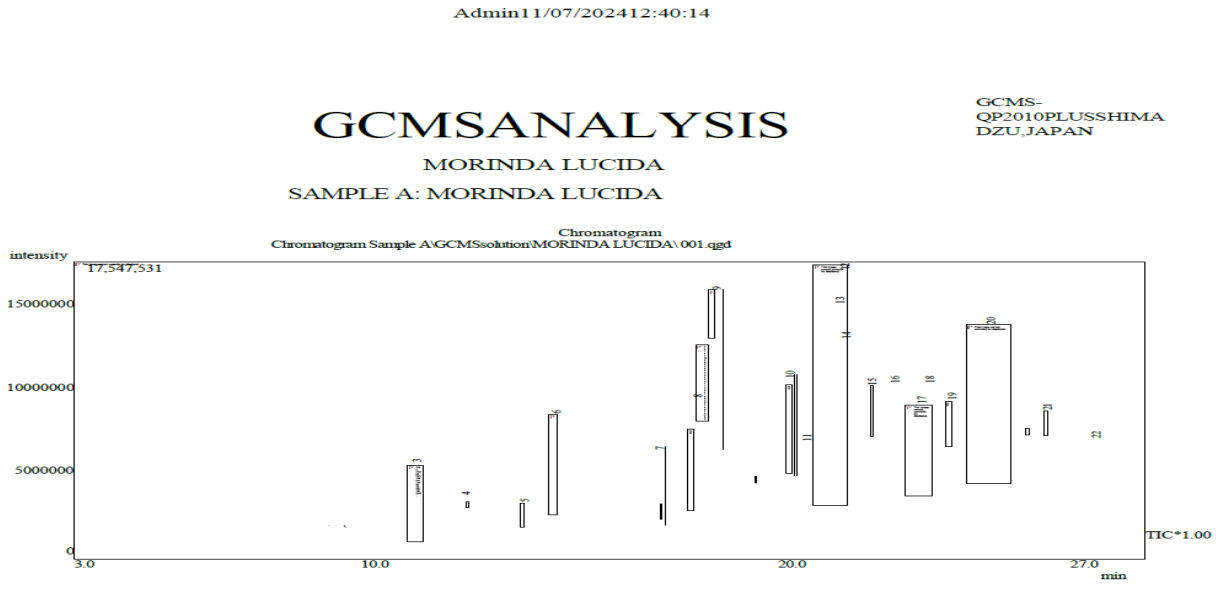

Figure 3: Chromatogram of GCMS Identification of Compounds in *M. lucida* Leaves

4.0 DISCUSSION

DOI:https://doi.org/10.57233/ijsgs.v11i1.794 ISSNp: 2488-9229; ISSNe: 3027-1118
IJSGS FUGUSAU VOL.11(1) WEBSITE: https://fugus-ijsgs.com.ng

The study observed higher extraction yields from *D. oliveri* compared to *M. lucida* leaves, potentially due to the greater solubility of its phytochemicals in the selected solvent (Kumar *et al*., 2023). This aligns with previous research indicating that plant species with thinner cell walls and optimized extraction conditions tend to yield higher concentrations of bioactive compounds (Chibuye *et al*., 2023). Additionally, the combination of both plant extracts (Combined Recipe) resulted in a higher yield compared to *M. lucida* alone, likely due to synergistic effects that enhance solvent penetration and facilitate greater phytochemical extraction (Zhang *et al*., 2018).

The phytochemical analysis revealed the presence of tannins, phenols, steroids, flavonoids, saponins, and glycosides in all extracts, with variations in concentration. The complementary phytochemical profiles of *D. oliveri* and *M. lucida* may contribute to a more comprehensive extraction of bioactive compounds, enhancing the antioxidant potential of the extracts (Larissa *et al*., 2024). This agrees with findings that plant combinations often result in increased antioxidant activity and higher yields of valuable phytochemicals (Ndezo *et al*., 2022).

Interestingly, alkaloids were present in *D. oliveri* but either absent or in very low concentrations in *M. lucida* and the Combined Recipe. This could be attributed to polarity mismatches, as water, a polar solvent, may not efficiently extract nonpolar alkaloid compounds (Haq *et al*., 2020). Additionally, the lack of complementary phytochemical profiles in the Combined Recipe could contribute to the reduced alkaloid concentration (Zhang *et al*., 2018).

Phenols were the most abundant phytochemical in all extracts, with the highest concentration in *M. lucida*, followed by the Combined Recipe and *D. oliveri*. This distribution suggests that *M. lucida* contains more extractable phenolic compounds. In contrast, tannins had the lowest mean concentration in all samples, likely due to variations in phytochemical composition and solvent polarity (Haq *et al*., 2020). The mean concentrations of various phytochemical constituents detected may also be due to the presence of specific phytochemicals available in the respective extracts (Fotsing *et al*., 2022).

GC-MS analysis identified numerous bioactive compounds in both *D. oliveri* and *M. lucida* extracts, including fatty acids, methyl esters, and volatile organic substances. The presence of these compounds suggests significant pharmacological potential, particularly in terms of lipid-based bioactivity. The GC-MS technique was effective in separating and identifying compounds based on their mass-to-charge ratio (m/z), retention index, and molecular weight, confirming the presence of bioactive constituents with potential medicinal applications (Reineccius *et al*., 2024; Wakoli *et al*., 2024).

Daniellia oliveri was found to contain notable fatty acids and methyl esters such as methyl myristate, linoleic acid, oleic acid, and palmitoleic acid, all of which have pharmacological significance (Yakubu *et al*., 2017). Similarly, *M. lucida* contained bioactive compounds including phthalic acid, benzenedicarboxylic acid, myristic acid, and decanoic acid. These findings support existing research on the medicinal potential of these plants, particularly for their anti-inflammatory and antimicrobial properties (Adekunle *et al*., 2023).

Notably, the combination of plant extracts in the GC-MS analysis resulted in a greater diversity of bioactive compounds than when analyzed separately. This supports the hypothesis that synergy between plant constituents enhances the range and potency of bioactive compounds (Chaachouay, 2025). Reports indicate that synergistic effects among phytochemicals can significantly improve their biological activities (Nock and Amlabu, 2020).

The therapeutic potential of these extracts may largely be attributed to their phytochemical compositions. GC-MS profiling identified bioactive compounds known for their antimalarial properties, including fatty acid methyl esters and phenolic compounds. The high concentration of these compounds in *D. oliveri* is consistent with reports demonstrating its potential antiplasmodial activity (Mba *et al*., 2022). Similarly, *M. lucida*'s bioactive compounds have been linked to significant antiplasmodial effects (Oladeji *et al*., 2022).

Understanding the specific bioactive compounds responsible for antimalarial activity remains crucial for future research. Studies suggest that the synergy of phytochemicals in plant combinations could enhance antimicrobial efficacy (Vaou *et al*., 2022). This reinforces the potential of using combined plant extracts for optimal antiplasmodial activity, as observed with *M. lucida* and other medicinal plants (Adekunle *et al*., 2019). The findings indicate that the synergy between *D. oliveri* and *M. lucida* may result in enhanced therapeutic effects compared to their individual use (Afolabi and Abejide, 2020).

DOI:https://doi.org/10.57233/ijsgs.v11i1.794

ISSNp: 2488-9229; ISSNe: 3027-1118

IJSGS FUGUSAU VOL.11(1)

WEBSITE: https://fugus-ijsgs.com.ng

5.0 CONCLUSION

The qualitative, quantitative and GC-MS analysis of the aqueous crude extracts of *D. oliveri* and *M. lucida* leaves contains all the actual phytochemicals and bioactive compounds with antiplasmodial potentials as reported, and more studies are required to fully understand the synergistic antiplasmodial activities of these important medicinal plants.

Daniellia oliveri and *M. lucida* leaves possess potentials for antiplasmodial activities, singly or synergistically and therefore requires further research and possible drug development for the control of malaria in endemic communities.

Conflicts of interest

Authors declared no conflicts of interest.

Acknowledgments

Authors greatly acknowledge Dr Mrs K.B. Dikwa, entire staff of the Department of Biological Sciences, Nigerian Defence Academy, Kaduna State and, Dr. Babajide Bamidele Ajayi of, Federal University of Technology Akure, Nigeria.

REFERENCES

Abubakar, A.R., and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences. 2020(12):1-10.

Adam, O.A.O., Abadi, R.S.M. and Ayoub, S.M.H. (2019). The effect of extraction method and solvents on yield and antioxidant activity of certain Sudanese medicinal plant extracts. Journal of Phytopharmacology. 8(5):248-252.

Adekunle, D.O., Faboro, E.O., and Lajide, L. (2023). Identification and quantification of bioactive compounds in different extracts of *Morinda lucida* benth (rubiaceae) root using GC–MS analysis. Journal of the Nigerian Society of Physical Sciences. 5(4), 1534. https://doi.org/10.46481/jnsps.2023.1534.

Adekunle, O.A., Momoh, J.O., Damazio, O.A., Ajetunmobi, A.O., and Aiyegbeni, M.A. (2019). Phytochemical Analysis and Antiplasmodial (curative) Activities of Methanolic Leaf Extract of *Morinda lucida* (Ewe Oruwo) in Male Swiss Mice Infected with *Plasmodium berghei* NK65. International Journal of Tropical Disease & Health. DOI: 10.9734/ijtdh/2019/v37i130156.

Afolabi, O.J., and Abejide, A.E., (2020). Antiplasmodial activities *of Morinda lucida* (Benth) and *Alstonia boonei* (De wild) in mice infected with *Plasmodium berghei*. National Research Centre. (2020) 44:85. https://doi.org/10.1186/s42269-020-00342-8.

Amodu, E.T.B. Momoh, S.O. Otoigiakhi, V.A. Iyeh, T.A. Owolabi, K.C. Ezenwa, E.O. Olayioye,

O.C. Iyoriobhe, O.F. and Aferuan (2020). Ethnobotany and ethnopharmacology of the Igala kingdom in Kogi east, Nigeria. Taiwania 65(2): 199-208. Doi: 10.6165/tai.2020.65.199.

Apinjoh, T.O., Ouattara, A., and Titanji, V.P.K. (2019). Genetic diversity and drug resistance surveillance of Plasmodium falciparum for malaria elimination: is there an ideal tool for resource-limited sub-Saharan Africa. Malaria Journal. 18, 217. https://doi.org/10.1186/s12936-019-2844-5.

Arslan, D., Aydin, M., and Turker, S. (2021). Extraction methods of medicinal and aromatic plants, its use in foods and evaluation in food supplement field. Turkish Journal of Agriculture-Food Science and Technology,.9(5):926-936.

Cao, P.N., Leclercq, L., Rossi, J.C., and Cottet, H. (2020). Water-based extraction of bioactive principles from Blackcurrant leaves and *Chrysanthellum americanum*: A comperative study. Food. 9(1478). DOI: 10.3390/foods9101478.

Chaachouay, N. (2025). Synergy, Additive Effects, and Antagonism of Drugs with Plant Bioactive Compounds. Drugs and Drug Candidates. 4(1), 4. https://doi.org/10.3390/ddc4010004.

Chaniad, P., Phuwajaroanpong, A., Techarang, T., Horata, N., Chukaew, A. and Punsawad, C. (2022). Evaluation of the antimalarial activity and toxicity of Mahanil-Tang-Thong formulation and its plant ingredients. BMC Complementary Medicine Therapeutics. 2022(22): 51. [Google Scholar] [CrossRef].

Chibuye, B., Singh, S. I., Chimuka, L., and Maseka, K. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Scientific African. 19, e01585. https://doi.org/10.1016/j.sciaf.2023.e01585.

Choo, L., Forest, F., Wieringa, J., Bruneau, A., and De la Estrella, M. (2020). "Phylogeny and biogeography of the Daniellia clade (Leguminosae: Detarioideae), a tropical tree lineage largely threatened in Africa and Madagascar". Molecular Phylogenetics and Evolution.146:106752.doi:10.1016/j.ympev.2020.106752.ISSN10557903. PMID 32028029. S2CID 211050305.

Cimanga, K., Nsaka, S. L., Tshodi, M. E., Mbamu, B. M., Kikweta, C. M., Makila, F. B., Cos, P., Maes, L., Vlietinck, A., Exarchou,. V., (2019). In vitro and in vivo antiplasmodial activity of extracts and isolated constituents of *Alstonia congensis* root bark. Journal of ethnopharmacology. ISSN 0378-8741 - 242(2019),

UNSP 111736. https://doi.org/10.1016/J.JEP.2019.02.019.

Ezeonu, C.S., and Ejikeme, C.M. (2016). Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian Softwoods. New Journal of Science. doi.org/10.1155/2016/5601327.

Fotsing, Y.S.F., Kezetas, J.J.B., El-Saber, B.G., Ali, I., and Ndjakou, B. L. (2022). Extraction of bioactive compounds from medicinal plants and herbs. IntechOpen. doi: 10.5772/intechopen.98602.

Galba, J.B., Lea, B.K.D., Eglantine, K.W., Herve, H.N.A., Bilbine, K.N., Jorelle, L.D.K., Roland,

R.N. and Harquin, S.F. (2020). Aqueous root bark extract of *Daniella oliveri* (Hutch and Datz) (Fabaceae) protects neurons against diazepam-induced amnesia in mice. Evidence-Based Complementary and Alternative Medicine. 2020(7815348): https://doi.org/10.1155/2020/7815348.

Haq, N., Muhammad, A. S., Najiha, R., and Najeeb, U. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. Brazilian Journal of Pharmaceutical Sciences. 56(3). DOI: 10.1590/s2175-97902019000417129.

Harborne, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London. p. 221.

Huma, F. H., Saira, B., Munazza, A. and Muhammad, R. K. (2021). Qualitative and quantitative analysis of phytochemicals in *Lepidium Pinnatifidum* Ledeb. Scholars International Journal of Traditional and Complementary Medicine. 4(5): 67-75.

Ifatimehin, O.O., Falola, O.O., and Odogbo, E.V. (2014). An Analysis of Spartial Distribution of Plasmodium Sporozoites and Effects of Climatic Correlates on Malaria infection in Anyigba Town, Nigeria. Global Journal of Health Science. 6(1):115-126.

Keay, R. W. J. (1958). Flora of west tropical Africa. Crown Agents for Oversea Governments and Administrations.

Keay, R. W. J., Onochie, C. F. A. and Stanfield, D. P. (1964). Nigerian Trees, Department of Forest Research Publishers, Ibadan, Nigeria.

Kalay, H., Gereziher, G. S., Aman, K., Gebretsadkan, H.T., Gomathi, P. and Mebrahtom, G.H. (2020). Antimalarial activity of *Meriandra dianthera* leaf extracts in *Plasmodium berghei*-infected Mice. Hindawi. Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2020/8980212.

Khoomsab, R. and Khoomsab, K. (2019). Extraction and determination of anthraquinone from herbal plant as bird repellent. Science & Technology Asia. 24(1):14-20.

Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K, S., and Oz, F. (2023). Major phytochemicals: Recent advances in health benefits and extraction method. Molecules. 28(2):887.https://doi.org/10.3390/molecules28020887.

Larissa, M.M., Dabole, B., Leutcha, P. and Emmanuel, T. (2024). Phytochemical constituents, antimicrobial activity of Daniellia oliveri, and X-ray crystal structure of polyalthic acid. Natural Product Communications. 19(9). Doi: 10.1177/1934578X241282853.

Lawal, H. Z., Olatunji, K., and Abdulazeez, A. (2022). Comparative study on the proximate, mineral, phytochemicals and antimicrobial analysis of *Daniellia oliveri*, *Leptadenia hastata* and *Vitex doniana* leaves. Bayero Journal of Pure and Applied Sciences. 13(1): 27 - 33 ISSN 2006 – 6996. http://dx.doi.org/10.4314/bajopas.v13i1.6S.

Mba, C.T., Chukwuma, M.O., and Chikeokwu, I. (2022). Antimalarial assay, isolation, and characterization of compounds responsible for antimalarial activity in *Daniellia oliveri* (Rolfe) Hutch. and Dalziel (Caesalpiniaceae). International Journal of Biochemistry Research & Review. DOI: 10.9734/ijbcrr/2022/v31i830342.

Muazu, M., Abdulsalami, M.S., Dikwa, K. B., Dibal, D. M., Kokori, B.T., and Junaidu, Y. (2022). GC-MS analysis and antiplasmodial potentials of bioactive compounds present in methanolic and ethanolic leaf extracts of *Daniella oliveri*. Asian Journal of Biochemistry, Genetics and Molecular Biology. 12(1): 26-34; Article no.AJBGMB.83851 ISSN: 2582-3698.

Ndezo, B.B., Njikang, E.N.R., Tchuenguem, T.R., and Dzoyem, J.P. (2022). Phytochemical screening, antioxidant, and antimicrobial activities of seven underinvestigated medicinal plants against microbial pathogens. Advances in Pharmacological and Pharmaceutical Sciences. 10;2022:1998808. doi: 10.1155/2022/1998808. PMID: 36263083; PMCID: PMC9576442.

Nock, I., and Amlabu, W. (2020). Antimalarial potency and phytochemical profile of *Khaya senegalensis* Juss. (1830) (Maliaceae). Nigerian Journal of Scientific Research. 19(2):80–95.

Nwanna, E.E. (2021). Protective effect of alkaloid-rich extract of Brimstone tree (*Morinda lucida*) on Neurotoxicity in the Fruit-fly (*Drosophila melanogaster*) Model. African Journal of Biomedical Research. 24(2): 1119-5096.

Ochora, D.O., Esezah, K., K., Namukobe,J., Perpetua, I., Dancan, M., Wakoli, W. O., Edwin, W., Mwakio, R. A., Yeda, A. C., Cheruiyot, D. W., Juma, B. Andagalu, A.L., Roth, B.R., Ogutu, A.Y., and Hoseah, M.A., (2022). Synergism in Antiplasmodial Activities of Artemether and Lumefantrine in Combination with *Securidaca longipedunculata* Fresen (Polygalaceae). Plants 11(1): 47. https://doi.org/10.3390/plants11010047.

Ojewumi, A.W. and Dedeke, G.A. (2020). Evaluation of nutritional and phytochemical properties

of *Eucalyptus citriodora*, *Hibiscus sabdariffa* and *Morinda lucida* from Ogun State, Nigeria. Journal of stress physiology and Biochemistry. 16(2): 4-56.

Oladeji, O.S., Oluyori, A.P., and Dada, A.O. (2022). Antiplasmodial activity of *Morinda lucida* Benth. Leaf and bark extracts against *Plasmodium berghei* infected mice. Saudi Journal of Biological Sciences. 29(4):2475-2482.

Olivia, N.U., Goodness, U.C., and Obinna, O.M. (2021). Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Future Journal of Pharmaceutical Science. 7, 59. https://doi.org/10.1186/s43094-021-00208-4.

Panchal, M.D., and Charuben, V.J. (2021). Qualitative and quantitative phytochemical screening of three plants stem bark and leaves from Sapotaceae Family. International Journal of Multidisciplinary Educational Research. 6(8):1-6.

Reineccius, G.A., and Qian, M.C. (2024). Gas Chromatography In: Ismail, B.P., Nielsen, S.S. (eds)

Nielsen's Food Analysis. Food Science Text Series. Springer, Cham. https://doi.org/10.1007/978-3-031-50643-7_14.

Samin, M., Behzad, J., Parina, A., Miquel, M. and Javad, S.R. (2020). Medicinal plants used in the treatment of Malaria: A key emphasis to Artemisia, Cinchona, Cryptolepis, and Tabebuia genera. Phytotherapy Research. 34(7): 1556-1569.

Shadia, M. A., Abhinav, A., and Tarek, A. K. (2016). Health benefits and possible risks of herbal medicine. www.researchgate.net/publication/290430960.

Shauibu, G., Abu, A.H., Agwu, E.O., Ehile, V., Ezeudo, Z.E, Ogedemgbe, B., and Peter, S. (2018). Effects of *Daniella oliveri* aqueous leave extract on blood glucose, haematological and biochemical values in streptozotocin-induced type 2 diabetes in male albino rats. Vom Journal of Veterinary Science. 13(1): 81-87.

Sodimu, A. I., Olorukooba, M. M., Baba, G. O., Ademuwagun, A. A., Olaifa, R. K., Rasheed, M. F., Salau1, L. O., Musa, K., and Bello, M. I. (2021). Medicinal Utilization and phytochemical composition of Brimstone (Morinda lucida Benth) Tree: A case study of Igabi Local Government in Northern Guinea Savanna Area of Kaduna State, Nigeria. Australian Journal of Science and Technology. 5(2): 2208-6404.

Tizazu, D., Workineh, Y., and Ayalew, Y. (2020). Parental traditional medicine use for children and associated factors in North Mecha district, North West Ethiopia. Pediatric Health Medicine and Therapy. 11:505–512. doi:10.2147/PHMT.S275249.

Trease, E.G. and Evans, W.C. (1978) Pharmacognosy. 11th Edition, Balliere Tindall, London, 115-222.

Van Buren, J.P., and Robinson, W.B., (1969). Formation of complexes between protein and tannic acid. Journal of Agriculture and Food Chemistry. 17(4):0021-8561.

Vaou, N., Stavropoulou, E., Voidarou, C.C., Tsakris, Z., Rozos, G., Tsigalou, C., Bezirtzoglou, E., (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. Antibiotics (Basel). 11(8):1014. doi: 10.3390/antibiotics11081014. PMID: 36009883; PMCID: PMC9404952.

Wakoli, J., Anjum, A., Sajed, T., Oler, E., Wang, F., Gautam, V., LeVatte, M., and Wishart, D.S. (2024). GCMS-ID: a webserver for identifying compounds from gas chromatography mass spectrometry experiments. Nucleic Acids Research. 52(W1):W381-W389. doi: 10.1093/nar/gkae425. PMID: 38783107; PMCID: PMC11223868.

Yadav, P.B., Lekhak, U.M., Ghane, S.G., and Lekhak, M.M. (2021). Phytochemicals, antioxidants, estimation of cardiac glycoside (Scillaren A) and detection of major metabolites using LC-MS from Drimia species. South African Journal of Botany. 140(2021): 259-268.

Yakubu, E.O., Otitoju, O., and Onwuka, J. (2017) Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of aqueous extract of *Daniellia oliveri* Stem Bark. Pharmaceutica Analytica Acta. 8: 568. doi:10.4172/2153-2435.1000568.

Zhang, Q.W., Lin, L.G., and Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine. https://doi.org/10.1186/s13020-018-0177-x.

Zimudzi, C., and Cardon, D., (2005). *Morinda lucida* Benth. In: PROTA (Plant Resources of Tropical Africa/Ressources Vegetales de l'Afrique Tropicale), Jansen, P.C.M. and D. Cardon (Eds.). Wageningen, Netherlands.