*FUDMA Journal of Sciences (FJS)*
*ISSN online: 2616-1370*
*ISSN print: 2645 - 2944*
*Vol. 10 No. 23, December (Special Issue), 2026, pp 46 – 54*
*DOI: https://doi.org/10.33003/fjs-2026-1023-6294*

# Evaluation of Antioxidant Activities and Antimicrobial Potentials of *Monodoramyristica* (African Nutmeg)

***[1]Ikuesan Felix Adeleke, [1]Alaka Saudat Oyedunmade and [2]Karigidi Kayode Olayele**

[1]Department of Microbiology, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria
[2]Department of Biochemistry, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria

*Corresponding authors' email: fa.ikuesan@oaustech.edu.ng
ORCID iD: https://orcid.org/0000-0003-2000-3463

**Received:** 21 Oct. 2026
**Accepted:** 22 Nov. 2026
**Published:** 27 Nov. 2026

**ABSTRACT**
The increased development of resistant microbial strains to synthetic and semi-synthetic drugs spurred the search for plant-based antimicrobial with therapeutic potentials. *Monodoramyristica* is a prominent spice in the folk medicine as a part of polyherbal formulation for disease management. This study was conducted to investigate the antioxidant and antimicrobial potentials of ethanol extract of *Monodoramyristica* **(**MM). Standard analytical techniques were used to for qualitative and quantitative assessment of phytochemicals and antioxidants. The antimicrobial potentials of the *Monodoramyristica* ethanol extract was assayed by the agar- well diffusion technique against selected bacterial and fungal isolates from poultry wastewater. The quantitative phytochemical screening of the extract revealed the varying amount of alkaloid (12.75%), saponin (1%), tannin (1.95%), total phenol (0.6%) and total flavonoid (15%). The antimicrobial potentials showed minimum inhibitory concentration (MIC) against the tested bacteria; *Klebsiellapneumoniae, Pseudomonas aeruginosa* and *Bacillus subtilis* respectively at 62.5 (µg/mL), 125.0 (µg/mL) and 1000 (µg/ml)without effect on *Pseudomonas fluorescens at the* 31.25 – 1000 (µg/mL) while the minimum fungicidal concentration (MFC) was 6.25 (µg/mL)for *Aspergillus niger*. The MFC of the extract was 50.00 (µg/mL) for both *Penicillium* and *Aspergillus flavus* but exhibited no effect on *Rhizopus* sp.at the assayed concentrations of 3.125 – 100 (µg/mL).In conclusion ethanol extract of *Monodoramyristica* possessed phytochemicals, antioxidant and antimicrobial properties which might justify its usage as antimicrobial agent in traditional medicine.

**Keywords:** Antimicrobial, Antioxidant, Microorganisms, *Monodoramyristica*, Phytochemicals

## INTRODUCTION

Microbial susceptibility to synthetic and semi-synthetic drugs are on the decline because of the increased development of resistant microbial strains (Seukepet al., 2023). The use of medicinal plant to complement or provide alternative to conventional drugs is evolving and have significant role in the global health system for curing different diseases and ailments (Singh et al.,2022). There have been several reports globally on the antimicrobial activities of medicinal plants to mitigate the global emergence of multi-drug -resistant strains of diseases – causing microbes such as *Escherichia coli*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*(Tavareset al, 2013; Kebede et al., 2021;Dubaleet al., 2023). Different plants and their parts (root, stem and leaves) have been reported to contain diverse phytochemicals like phenols, quinones, flavonoids, tannins and coumarins (Pancheet al, 2016; Ojatula & Ikuesan, 2022) with antibacterial and antifungal effects (Daset al, 2016). Medicinal plants from the tropical and subtropical climates have many medicinal properties against diseases caused by microorganisms. One of the prominent medicinal plants of tropics is *Monodoramyristica.*

*Monodoramyristica* popularly known as African nutmeg is a member of family Annonaceae. The calabash nutmeg is native to the West, Central and East African forest belts. Its seeds are used as condiment in the preparation of soup, and as stimulant and aroma addition to other medicines (Uwakwe & Nwaoguikpe, 2008). In the traditional medicine, it is used in the management and treatment of diabetes mellitus and hypertension (Agiriga & Siwela, 2017). The seed has been reported to possess bioactive compounds and exhibited *in-vitro* antioxidant activities and inhibition of lipid peroxidation in rats (Karigidiet al.,2022). The seeds have been reported to contain phytochemicals such as steroids, saponins and terpenoids. Also, proximate analysis has showed that its seed contains 15% moisture, 19% crude protein, 36% carbohydrate, and 12% crude lipid contents (Nkwochaet al., 2018). Also, the antibacterial potential of its seed protein hydrolysates was reported by Osukoyaet al.(2021).

Due to emergence and recurring cases of microbial resistance and reduced antibiotic susceptibility, there is search for botanicals with antimicrobial potential. Therefore, this study is designed to evaluate the phytochemical content, antioxidant activities and antimicrobial potentials of ethanol extract of *Monodoramyristica*in order to justify its usage as antimicrobial agent in traditional medicine.

## MATERIALS AND METHODS

### Collection and authentication of plant material

Seeds of *Monodoramyristica* used in the study were purchased from the local market, in Okitipupa, Ondo State, Nigeria. The plant material was authenticated by the Taxonomist, Mr. O. T. Aturu of the Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria and a voucher specimen number OAUSTECH/H-00660 was assigned.



Figure 1: Mature seeds of *Monodoramyristica*

**Preparation of seed extract of *Monodoramyristica***

The method of Azwanida (2015) was adopted for preparation of extract. Fresh seeds of *Monodoramyristica* were de-hulled and sorted to get rid of dirt and bad seeds. The seeds were air-dried for 3 days to remove the water contents. Thereafter, the seeds were milled to fine powder by using manual engine grinder (Model Corene, A.5 Lander YCIA S.A).The seed powder was sieved with mesh to remove coarse particles (Odeje & Rabia, 2026).One hundred gram of powdered *Monodoramyristica* was soaked in 2 L of ethanol for 72 hours with frequent agitation. Thereafter, the extract was filtered using clean cheese cloth and concentrated with rotary evaporator (Cole Parmer IKA RV 10 digital) at 50$^{o}$C. The concentrate was lyophilized using a freeze-drier and stored at 40$^{o}$C until needed.

**Phytochemical analysis of the extract**

A small portion of the dry extract was used for phytochemical screening tests following the methods of Trease & Evans (1989) and Harborne (1973).

**The Qualitative Phytochemical Screening of seed extract of *Monodoramyristica***

The phytochemical screening of crude extract of *Monodoramyristica* was carried out by the method described by(Ogunmefunet al., 2017).

***Screening for phenols and tannins***

The extract was screened for the presence of phenols and tannins was screened using the standard method described by Trease& Evans (1989). Two milli-litres (2 mL) of 2% solution of Ferrous chloride ($FeCl_2$) was thoroughly mixed with the crude extract of *Monodoramyristica* and then observed for black or blue green colour indicating the presence of tannins and phenols.

***Screening for flavonoids***

To test the crude extract of the assayed sample for flavonoids, the method described by Trease& Evans (1989) was used. Fragments of magnesium ribbon and concentrated hydrogen chloride (HCl) was mixed with the crude extract of *Monodoramyristica* seed and then observed after few minutes for the appearance of a pink colour scarlet to indicate the presence of flavonoids.

***Test for saponins***

This test was carried out using the method described by Ogunmefunet al., (2017).Exactly 5 mL of distilled water was added onto the crude extract of sample in a test tube and then shaken vigorously. Foam formation indicated the presence of saponins.

***Test for alkaloids***

The screening for alkaloids was carried out by the method described by Harborne (1973). The powder of the *Monodoramyristica* extract was mix with few drops of 2N HCl. The aqueous layer which developed was decanted and then two drops of Mayer's reagent was added. Formation of white turbidity or precipitate indicated the presence of alkaloids.

**Test for terpenoids (Salkowski test)**

The test was performed by using dried extract (50mg) and soaked in 5 mL of ethanol. Extract was mixed in 2 mL of chloroform. It was slightly warmed and then cooled. Exactly3 mL of concentrated $H_2SO_4$ was added slowly along the sides of test tubes. A radish brown colored precipitation was formed at the interface indicating the presence of terpenoids (Indumathiet al.,2014).

**Quantitative determination of phytochemical constituents of seed extract of *Monodoramyristica***

***Determination of Alkaloid***

The method of Harborne (1973) was used for the quantitative determination of alkaloid on sample of *Monodoramyristica* seed extracts. Fivegrams sample was weighed into a 500 mL beaker and 200 mL of 10% acetic acid in ethanol was added. This was allowed to stand for 4 minutes, filtered and then concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered with 12.5 cm Gem filter paper. The residue was then oven-dried and considered as alkaloid expressed as percentage of alkaloid using the formula

% Alkaloid = (weight of alkaloid)/(weight of sample) ×100

***Determination of Flavonoid***

The method reported by Ejikemeet al. (2014) was used for the quantification of flavonoid extract. Exactly 50 $cm^3$ of 80% aqueous methanol was added to 2.50 g of crude *Monodoramyristica* seed extract sample in a 250 $cm^3$ Erlenmeyer flask, covered, and allowed to stand for 24 hours at room temperature. The resulting supernatant was discarded and the residue was re-extracted (three times) with the same

volume of methanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of sample. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible of predetermined weight was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as:

$$\%\, Flavonoid = (weight\ of\ Flavonoid)/(weight\ of\ sample) \times 100$$

***Determination of Saponin***

The methods of Obadoni & Ochuko, (2002) was used for the quantification of saponin in crude extract of sample. Exactly 5 grams of powder sample was dissolved in 100 $cm^3$ of 20% aqueous methanol in a 250 $cm^3$ Erlenmeyer flask and then heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 $cm^3$ of 20% aqueous methanol, after filtration, it was heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 $cm^3$ over water bath at 90°C, 20 $cm^3$ of diethyl ether was added to the concentrate in a 250 $cm^3$ separator funnel, and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice and 60 $cm^3$ of n-butanol was added and extracted twice with 10 $cm^3$ of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated in percentage:

% Saponin = (weight of saponin)/(weight of sample) ×100

***Determination of Tannins***

The quantitative determination of tannin was conducted using the analytical method of Ogunmefunet al. (2016). Exactly 0.2 g of finely ground sample was transferred into a 500 ml sample bottle and 100 ml of 70% aqueous acetone was added and properly covered. The bottles were kept in shaker water bath for 2 h and then maintained at 30°C. The solution was thereafter centrifuged and the sediment was stored in ice. Exactly 0.2 ml of solution was pipetted into test tubes and 0.8 ml of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water and 0.5 ml of Folin-ciocateau reagent was added to both sample and standard followed by 2.5 ml of 20% $Na_2CO_3$ the solutions were then shaken vigorously and allowed to incubate for 40 minutes at room temperature; its absorbance was then read at 725 nm against a reagent blank concentration of the same solution from a prepare standard tannic acid curve.

***Determination of Phenols***

Exactly 0.50 g sample was boiled for 15 minutes with 50 $cm^3$ of ether for the extraction of the phenolic components. Exactly 10 $cm^3$ of distilled water, 2 $cm^3$ of 0.1 N ammonium hydroxide solutions, and 5 $cm^3$ of concentrated amyl alcohol were also added to 5 $cm^3$ of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm, 0.20 g of tannic acid was dissolved in distilled water and diluted to 200 mL mark (1 mg/$cm^3$) in preparation for phenol standard curve. Varying concentrations (0.2 1.0 mg/$cm^3$) of the standard tannic acid solution was pipetted into five different test tubes to which 2 $cm^3$ of $NH_4OH$, 5 $cm^3$ of amyl alcohol, and 10 $cm^3$ of water were added. The solution was made up to 100 $cm^3$ volume and left to react for 30 minutes for colour development. The optical density was determined at 505 nm Keay et al. (1964).

***Quantitative test for terpenoids***

Dried plant extract 100 mg (wi) was taken and soaked in 9 mL of ethanol for 24 hours (Indumathiet al., 2014). The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the percentage (%) yield of total terpenoids contents was measured by the formula.

(wi-wf/wi×100)

**In vitro Antioxidant Assay**

***Determination of Total Phenolic content (TPC)***

Total phenolic contents of the extract were determined using the method of Kim et al. (2003) as modified by Karigidiet al. (2018). Exactly 0.5 mL of the sample was mixed with 0.5 mL (10 %) of Folin-Ciocalteu phenol reagent. After 5 mins, 2.5 mL of 7 % $Na_2CO_3$ was added and then followed immediately by addition of 2.5 mL of distilled water and shaken thoroughly. The mixture was kept in the dark for 90 minutes at room temperature. TPC was evaluated from Gallic acid standard curve and expressed as Gallic acid equivalent (mg GAE/100 g).

***Determination of Total flavonoid content (TFC)***

The total flavonoid contents of the extract were determined using the procedure described by Park et al. (2008). Exactly 0.3mL of the sample was mixed with 3.4 mL (30 %) of methanol, 0.15 mL (0.5 M) of $NaNO_2$ and 0.15 mL (0.3 M) of $AlCl_36H_20$ successively. After 5 minutes, 1 mL of 1 M NaOH was added and mixed well. TFC was expressed as Quercetin equivalent (mg QUE/100g).

***Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity***

DPPH radical scavenging activity of the extract was done using the method of (Omiyale and Karigidi2023). Exactly 1.0 mL of sample was added to 4 mL of DPPH solution (40 mg/L) prepared in methanol. The mixture were mixed thoroughly and left in the dark for 30 minutes. The absorbance was read at 520 nm. The inhibition percentage was calculated

Percentage inhibition of DPPH = $\{(A_{Crl} - A_{Spl})/ (A_{Crl})\} \times 100$

Where, $A_{Crl}$ = Absorbance of control and $A_{Spl}$ = Absorbance of the sample

***Total antioxidant capacity Phosphomolybdate assay (TAC)***

TAC of the extract was determined using the method described by(Omiyale & Karigidi 2023). Sample (0.4 mL) and 4.0 mL of phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was thoroughly mixed together. The mixture was capped and incubated in a boiling water bath at 95°C for 90 minutes. The mixture was cooled to room temperature and the absorbance of the mixture was measured at 695 nm. The total antioxidant capacity was calculated from Ascorbic acid calibration curve and expressed as ascorbic acid equivalent (mg AAE/100g).

***Determination of Ferric reducing antioxidant potential (FRAP)***

FRAP of the extract was done using the method of Benzie and Strain (1996). The FRAP working reagent consisting of25 mL acetate buffer, 2.5 mL TPTZ solution, and ferric chloride in ratio 10:1:1 was freshly prepared and warmed at 37 °C for 2 mins before use. 0.2 mL of the sample and 2.80 mL of the FRAP reagent were added together and incubated for 30 min

at room temperature. FRAP was evaluated from ferrous sulphate standard curve and expressed as (mg $Fe^{2+}E/100g$)

***$H_2O_2$ scavenging activity***

The ability of plant extract to scavenge hydrogen peroxide can be estimated according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (50mM pH 7.4). Extract (20–60g/mL) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide.

Percentage inhibition of $H_2O_2$ = $\{(A_{Crl}- A_{Spl})/ (A_{Crl})\} \times 100$

Where, $A_{Crl}$ = Absorbance of control and $A_{Spl}$ = Absorbance of the sample

**Microbiological analysis**

***Media preparation***

All media used in this study were prepared according to the manufacturer's specification. These media include; nutrient agar, Nutrient broth, Sabouraud dextrose agar, Potato Dextrose broth and Mueller Hinton Agar (MHA). The prepared media were covered with cotton wool and wrapped with aluminum foil, the content was stirred with a magnetic stirrer and heated to dissolve properly and then, sterilized by autoclaving at 121° C for 15 minutes and was maintained at about 45° C for use.

***Microorganisms used in bioassay***

The bacterial species used in the study were *Bacillus subtilis, Klebsiellapneumoniae, Pseudomonas fluorescens,* and *Pseudomonas aeruginosa*. The fungal isolates were *Aspergillusniger, Aspergillusflavus, Rhizopus*sp., *Penicillium*sp. These organisms were isolates from poultry wastewater collected from the stock at the Microbiology Laboratory of Microbiology Department, University of Ibadan, Ibadan, Nigeria. Purity and identity of isolates were confirmed using standard microbiological procedures (Ikuesan et al, 2020; Akter et al,2022).The pure isolates of each of the test organisms were inoculated in sterile slants containing Nutrient agar and potato dextrose agar for bacteria and fungi respectively and refrigerated at 4°C (Whiliki et al., 2023).

***Assessment of antimicrobial activities of Monodoramyristica***

The agar-well diffusion method described by Ansari et al.(2021);Bhandari et al., (2024)was used to determine the antimicrobial activity of ethanol extract of *Monodoramyristica* against test bacterial and fungal isolates. Overnight broth cultures of each pure isolate from poultry wastewater were obtained, diluted with sterile distilled water and standardized by comparing the turbidity with 0.5 McFarland turbidity standard (Amarillas et al., 2024).Exactly 0.1 mLaliq out of 24 hr old broth culture of each test organism was introduced to 9.9 ml distilled water to prepare a 1: 100 dilution of the organism (Ogunmefunet al., 2016). Twenty milliliter (20 mL) of sterile molten MHA maintained at about 45 $^0$C in a McCartney bottle was then inoculated with an aliquot (0.1) mL from the dilution and then rocked gently to allow proper mixing of inoculum with the medium. The medium (MHA) - culture mixture was poured into a well-labelled sterile Petri dish and allowed to solidify. Wells of 4 mm in diameter each were bored evenly onto the uniformly inoculated Mueller Hinton agar/Sabouraud dextrose agar plates so that they are no closer than 20 mm from each other, center to center. Each well was filled with 100 µl ml of the extract at varying concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL and 31.25 µg/mL) reconstituted with 10% Dimethyl sulphoxide (DMSO) and sterilized by 0.45 µm membrane filter. Each well was allowed to dry for 40 min(Nwiyi et al., 2022) to ensure complete diffusion of the extract with the agar surface and the agar plates were then incubated at 37ºC. The DMSO served as a negative control while streptomycin (25mg/ml) and fungi sol served as the positive control for bacterial and fungi respectively. The plates were incubated at 37°C for 24 hours and then observed for zone of inhibition. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured using Vernier caliper, including the diameter of the Streptomycin used as control.

**Determination of Minimum Inhibitory Concentration (MIC)**

The MIC of the extracts for each susceptible test organism was determined by a method described by Okeke et al. (2001) and Okoliet al. (2002). The minimum inhibitory concentration (MIC) in µg/mL was determined by comparing the different concentration of the extracts that have different zones of inhibition and then selecting the lowest concentration of each extract (Agatemor, 2009).

**Antibiotics sensitivity testing**

Antibiotic sensitivity test was carried out to ascertain the susceptibility of the test microbes to commercial antibiotics impregnated disc using the agar diffusion technique as described by (Lalitha, 2004). The bacterial colonies from the pure culture were transferred into the nutrient broth and incubated overnight. Thereafter, the test organisms were swabbed on separate Muller Hinton Agar (MHA) plates using a sterile cotton wool swab. The appropriate multi-disc depending on whether the test organism was a gram-negative organism or gram- positive organism was then placed gently and firmly onto the surface of the plates, using sterile forceps. The plates were left at room temperature for one hour to allow diffusion of the different antibiotics from the disc into the medium and incubated at 37ºC for 24 hours and then observed for zones of inhibition and sensitivity determined based on measured zone sizes. Zone of inhibition greater than 24 mm was considered sensitive, 14-16 mm moderate sensitive and less than 14 mm of inhibition was considered resistant. (National Committee for Clinical Laboratory Standard; NCCLS, 2006).

**Statistical analysis**

Results were presented as the mean ± SD of triplicate readings, analyzed using analysis of variance (ANOVA)

**RESULTS AND DISCUSSION**

The quantitative phytochemical screening of ethanol extract of *Monodoramyristica* presented in Table 1 revealed the presence of different phytochemicals which occur in varying amounts in the range 0.60 – 27.50% (w/w). Among these, terpenoid, followed by flavonoid was found to have the highest abundance of 27.50 % (w/w) and 15.0% (w/w) respectively while total phenols with 0.60% was the least. Phytochemicals such as Saponins, alkaloid, terpenoids and flavonoids are biologically active compounds found in plants. These compounds have been shown to exhibit many biological activities particularly antioxidant and antimicrobial (Nwozo et al., 2023). The secondary metabolites (flavonoids, alkaloids terpenoids and tannins) detected in this study were among those reported by several

authors in their studies of plant phytochemicals(Jimoh et al., 2020; Nwiyi et al., 2022; Ojatula & Ikuesan, 2022).Ogunmefunet al. (2017) reported that flavonoids, alkaloids and terpenoids are secondary metabolites produced by plants as a chemical defense against pests and diseases". Tannins are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Abdulhamid et al., 2023); they are referred to as natural antibiotics from plants. Therefore, the ample presence of the phytochemicals in ethanol extract of *Monodoramyristica* justifies its usage in folk medicine. For example, saponin has been shown to exert antioxidant and antimicrobial activities by El Idrissi et al.,(2023) while Kongkum et al. (2021) reported the antimicrobial and antioxidant activities of alkaloids isolated from twigs of *Uvariagr and iflora* Roxb. Also, Figure 1 showed the TPC, TFC, TAC and FRAP. They exhibited concentration dependent activities. Figure 2 presented the results of DPPH and $H_2O_2$ scavenging abilities. They also exhibited concentration dependent activities. Antioxidants are substances that scavenge or slow down the activities of free radicals thereby inhibiting oxidative mechanisms that lead to chronic and degenerative diseases in the body (Roy et al. 2023). Antioxidant ability assays can be divided into two; hydrogen atom transfer (HAT) and single electron transfer (SET) assays. Most of the HAT assays are kinetics based and involve a competitive reaction scheme while SET assays measure the ability of an antioxidant to reduce an oxidant with corresponding colour change when reduced (Karigidiet al. 2022). The antioxidant assays employed in the present study belong to SET (TAC, FRAP, DPPH and $H_2O_2$) category. The TAC and FRAP activities recorded for the ethanol extract of *Monodoramyristica*in the present study is lower than what was reported for methanol extract of *Monodoramyristica* by Karigidiet al.(2022).

**Table 1: Quantitative phytochemical constituent of *Monodoramyristica***

| **Phytochemical** | **(%) occurrence (w/w)** |
|---|---|
| Alkaloids | 12.75 |
| Flavonoids | 15.00 |
| Saponins | 1.00 |
| Tannins | 1.95 |
| Terpenoids | 27.80 |
| Total phenols | 0.60 |

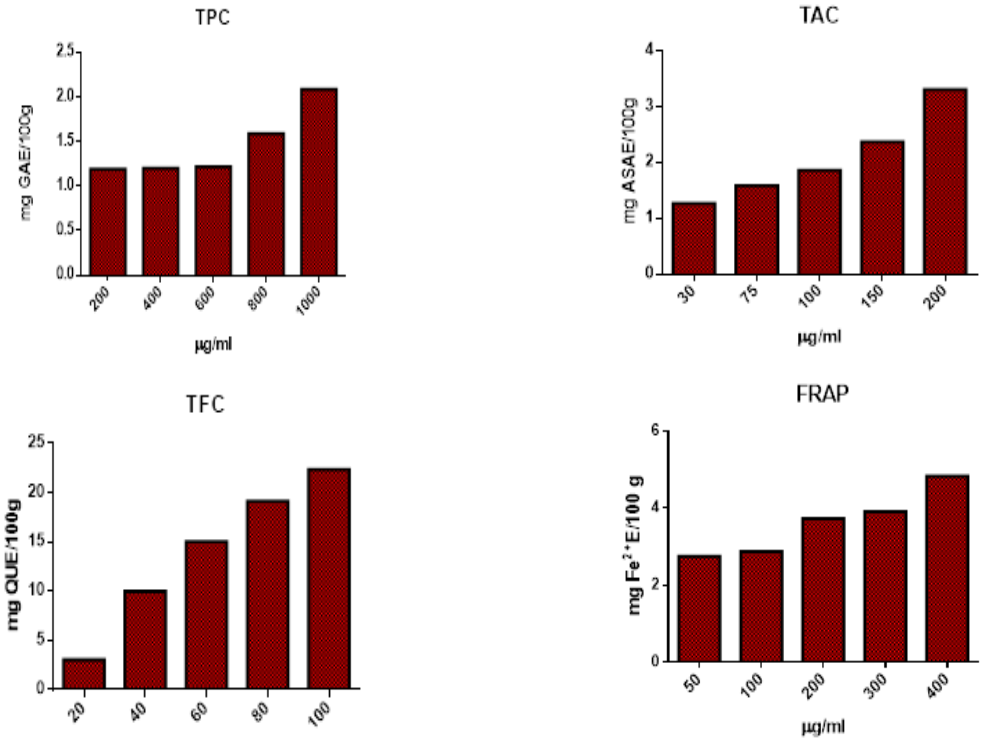


Figure 2: Bioactive and antioxidant components of ethanol extract of *Monodoramyristica*

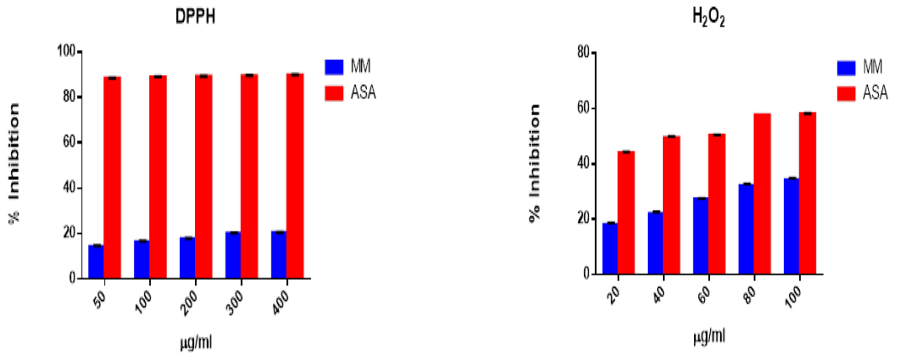


Figure 3: DPPH and $H_2O_2$ scavenging ability of ethanol extract of *Monodoramyristica*

The antibiotics sensitivity test with ciprofloxacin, gentamicin, erythromycin, trimethoprim and meropenem shown in Table 2 revealed that the all test bacterial isolates were sensitive to ciprofloxacin with varying zones of inhibition in the range of 26 – 34 mm. The isolates also exhibited absolute resistance (0 mm) to erythromycin, trimethoprim and meropenem and 8 - 10 mm to gentamicin. Table 3 also revealed that among the fungi, *Aspergillus niger* was the most sensitive (28 mm) to *Monodoramyristica* seed extract at 200µg/mL and 19 mm zone of inhibition against the positive control (fungi sol) while all the fungal test isolates were resistant to the negative control. The minimum inhibitory concentrations of *Monodoramyristica* on the test bacterial and fungal isolates are presented in Tables 4 and 5. Table2shows the minimum inhibitory concentration of *Monodoramyristica* ethanol extract against selected bacterial isolates at 62.5 (µg/mL), 125.0 (µg/mL) and 1000 (µg/mL) for *Klebsiellapneumoniae* (2 mm), *Pseudomonas aeruginosa* (4 mm)and *Bacillus subtilis*(6 mm) respectively while *Pseudomonas fluorescens* was profoundly resistant to the *Monodoramyristica* ethanol extract at the applied concentration of 31.25 – 1000 (µg/mL). Similarly, *Rhizopus*sp. demonstrated absolute resistance to the extract at the applied concentration of 3.125 -100 (µg/mL) while the extract showed minimum inhibitory concentration µg/mL of 6.25 on *A. niger* (2 mm), and 50 µg/mL for *A. flavus*(4 mm) and *Penicillium*sp. (6 mm).

**Table 2: Sensitivity Test against tested bacteria**

| **Antibiotics Disc** | **Zone of Inhibition (mm)** | | | |
|---|---|---|---|---|
| **µg** | ***Bacillus subtilis*** | ***Klebsiellapneumoniae*** | ***Pseudomonas aeruginosa*** | ***Pseudomonas fluorescens*** |
| Ciprofloxacin (5) | 30 | 26 | 30 | 34 |
| Gentamicin (10) | 8 | 8 | 10 | 8 |
| Erythromycin (15) | 0 | 0 | 0 | 0 |
| Trimethoprim (25) | 0 | 0 | 0 | 0 |
| Meropenem (10) | 0 | 0 | 0 | 0 |

Phytochemicals such as phenolic compounds have been shown to contribute immensely to the protective and antimicrobial effects of medicinal plants therefore boosting the tendency of these plants to mitigate pathogens and prevent microbial growth (Olaiya et al., 2016).Biological contaminants of bacterial and fungal origin signify a major cause of illnesses and disease burden. The bacterial and fungi used in this study have been isolated from the general environments of soil, dust, air and water and implicated in several human and poultry diseases. *Bacillus subtilis* is a known cause of food poisoning, *Klebsiella* spp. is frequently connected with poor sanitary practices and could indicate the potential danger of food borne infection. Also, the fungi are associated with production of different mycotoxins. These secondary metabolites; aflatoxins, patulin, ochratoxin, citrin etc which can damage human and poultry health. In this study, ethanol extract of *M. myristica* was able to inhibit some bacterial and fungal isolates. The bacterial isolates used in this study were predominantly gram negative. This investigation revealed that Gram positive *B. subtilis* was resistant to ethanol extract of *Monodoramyristica* at all concentrations less than 1000 µg/mL while Gram negative isolates except *Pseudomonas fluorescens* are susceptible at concentration of 62.5 µg/mL. The Minimum Inhibitory Concentration (MIC) values for *Monodoramyristica* against the test isolates varied. The MIC values represent the lowest concentration of the extract at which no visible growth of the pathogen occurs. *Monodoramyristica* demonstrated varying MIC values against *Klebsiellapneumoniae, Bacillus subtilis,* and *Pseudomonas aeruginosa,* ranging from 125 µg/mL to 1000 µg/mL. Notably, *Pseudomonas fluorescens* did not exhibit any growth inhibition even at the highest concentration tested. These results suggest that *Monodoramyristica* has varying degrees of effectiveness against different bacterial strains, with MIC values providing insights into its potential as a therapeutic agent for specific infections (Oyebode et al., 2021). The zones of inhibition of less than 14 mm due to ethanol extract of *Monodoramyristica* on the tested bacteria species suggested that the organisms are resistant to the extracts at the applied maximum concentration of *Monodoramyristica*. This assertion is drawn from the recommendation of National Committee for Clinical Laboratory Standards (2006) that zone of inhibition greater than 24 mm was considered sensitive, 14-16 mm moderate sensitive and less than 14 mm of inhibition was considered resistant. The antibacterial activity of ethanol extract of *Monodoramyristica* is concentration dependent. This assertion agrees with the finding of Whiliki et al., (2023) and Ojatula & Ikuesan (2022) report on the methanol extract of *M. myristica* and *Cymbopogoncitratus***.** The results of this study revealed that all the test bacteria were resistant to gentamicin, erythromycin trimethoprim and meropenem.

This implies these commercial antibiotics are not choice drugs for infections caused by *B. subtilis*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Among the fungi used to assay the antifungal activities of *Monodoramyristica* ethanol seed extract at 200 µg/mL, only *A. niger* showed sensitivity while, *A. flavus* and *Rhizopus*sp. were sensitive to the positive control with zone of inhibition of 19-24 mm. This result suggested that *Monodoramyristica* seed extract can be used for the treatment of Aspergillosis caused by *A. niger*.

**Table 3: Antifungal activities (mm) of *M.myristica* against selected fungi**

| **Fungal Isolate** | **Zone of Inhibition (mm)** | | |
|---|---|---|---|
| | ***Monodora* 200µl/ml** | **Negative** | **Positive** |
| *Penicillium*sp. | 13 | R | R |
| *Aspergillusflavus* | 11 | R | 19 |
| *Aspergillusniger* | 28 | R | 19 |
| *Rhizopus*sp. | 0 | R | 24 |

*Legend: Positive control: Fungi sol, Negative control: Dimethysulfoxide, R: Resistant*

**Table4: Minimum inhibitory concentration (mm) of ethanol extract of *Monodoramyristica* against selected bacterial isolates**

| **Extract (µg/mL)** | **Zone of Inhibition (mm)** | | | |
|---|---|---|---|---|
| | ***Bacillus subtilis*** | ***Klebsiellapneumoniae*** | ***Pseudomonas aeruginosa*** | ***Pseudomonas fluorescens*** |
| 1000 | 6 | 4 | 6 | R |
| 500 | R | 2 | 4 | R |
| 250 | R | 2 | 4 | R |
| 125 | R | 2 | 4 | R |
| 62.5 | R | 2 | R | R |
| 31.25 | R | R | R | R |

*Legend: R = Resistant*

**Table 5: Minimum inhibitory concentration (mm) of *Monodoramyristica* against selected fungi**

| **Extract (µg/mL)** | **Zone of Inhibition (mm)** | | | |
|---|---|---|---|---|
| | ***Penicillium*sp.** | ***Aspergillusflavus*** | ***Aspergillusniger*.** | ***Rhizopus*sp.** |
| 100 | 9 | 6 | 13 | R |
| 50 | 6 | 4 | 10 | R |
| 25 | R | R | 5 | R |
| 12.5 | R | R | 2 | R |
| 6.25 | R | R | 2 | R |
| 3.125 | R | R | R | R |

*Legend: R = Resistant*

MIC values against *Penicillum* sp., *Aspergillusflavus*, *Aspergillusniger*, and *Rhizopus* sp., ranging from 12.5 µg/mL to 100 µg/mL. However, *Rhizopus* sp. exhibited no growth inhibition even at the highest concentration tested. These results highlight the potential of *Monodoramyristica* as an antifungal agent, with MIC values providing insights into its efficacy against specific fungal pathogens or infections (Gbotosho et al., 2019). Dowe et al. (2016) asserted that the lower the MIC value, the more potent the antimicrobial agent, conversely, the higher the MIC value, the less potent the antimicrobial agent. The efficacy or effectiveness of antimicrobials is influenced by factors which include status off the microorganism, antimicrobial concentrations, inoculums size among others (Li et al., 2017)

## CONCLUSION

Based on this study, the ethanol extract of *M. myristica* possesses numerous phytochemicals and free radical scavenging activity which makes them potent natural source of antioxidants. The extract showed fungicidal and bactericidal properties at varying concentrations. Hence, the extract of *M. myristica*c can be used as potent natural antibacterial and antifungal agents against the investigated pathogens.

## REFERENCES

Abdulhamid, A., Fakai, I. M., Sani, I., Bagudo, A. I.,Hamisu, A. (2023). Screening for antibacterial properties of some traditional medicinal plants in Kebbi State, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, 31(1): 102-109.

Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Electronic Journal of Environment, Agriculture and Food Chemistry*, 8(3):195-200.

Agiriga, A., Siwela, M. (2017).*Monodoramyristica* (Gaertn.) Dunal: A Plant with Multiple Food, Health and Medicinal Applications: A Review. *American Journal of Food Technology* 12 (4): 271-284

Akter, Y., Barua, R., Nasir Uddin, M., Muhammad Sanaullah, A. F., Marzan, L. W. (2022). Bioactive potentiality of secondary metabolites from endophytic bacteria against SARS-COV-2: An in-silico approach. *PLoS One*, 17(8), e0269962.

Amarillas, L., Padilla-Lafarga, F., León Chan, R. G., Padilla, J., Lugo-Melchor, Y., López Avendaño, J. E.,Estrada-Acosta, M. (2024). Isolation and characterization of a bacteriophage

with potential for the control of multidrug-resistant Salmonella strains encoding virulence factors associated with the promotion of precancerous lesions. *Viruses*, 16(11), 1711.

Ansari, J., Kushwaha, S. P., Ansari, V. A., Singh, K., Hasan, S. M. (2021). Agar well diffusion: A prominent method for in-vitro screening of antimicrobials. *International Journal of Botany Studies*, 6(5):836-839

Azwanida, N. N. (2015) A review on the extraction methods used in medicinal plants, principles, strength and limitation. *Medicinal and Aromatic Plants* 4(3): 1-6

Benzie, F., Strain, J. J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical of Biochemistry,* 239: 70–76.

Bhandari, A., Shrestha, K., Parajuli, P. (2024). A Comparative Study on the Phytochemical Profile, Antimicrobial Activity and Antioxidant Capacity of Flowers and Leaves from Selected Medicinal Plants. *Nepal Journal of Biotechnology*, 12(2), 101-108.

Das, Q., Islam, M. R., Marcone M. F., Warriner K., Diarra, M. S. (2016). Potential of berry extracts to control foodborne pathogens, *Food Control*: 1-13.

Dowe, E., Ahonkhai, I., Ayinde, B.A.. Uwumarongie, H.O. (2016). Phytochemical and antimicrobial evaluation of the methanol stem extract and fraction of *Massularia acuminate G. Don* (Rubiaceae) against isolated odontopathogens. *Ewemen Journal of Microbial Research*. 2(1): 13-21.

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology*, 51-62.

Ejikeme, M. C, Ezeonu, S. C., Eboatu, N.A. (2014) Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger-Delta area of Nigeria. *European Science Journal*. 10(18): 247-270.

El Idrissi Y., Elouafy Y., E. l., Moudden, H., Mghazli, N, El Guezzane, C., El Yadini, A., Harhar H., Zarrouk A., Goh, K. W., Ming L. C., Bouyahya, A (2023). Evaluation of Antioxidant and Antimicrobial Activity of Saponin Extracts from Different Parts of *Argania spinosa* L. Skeels. *Progress inMicrobes and Molecular Biology*, 6(1).

Gbotosho, O., Okuns, M., Adeyemi, O. S. (2019). Flavonoid content and antioxidant activity of the leaves of *Gouanialongipetala*. *Journal of Applied Pharmaceutical Science*, 9(7), 49-54.

Harborne J.B. (1973). Phytochemical Methods; A guide to modern techniques of plant Analysis.2nd Edition, London New York.

Ikuesan, F. A., Olukunle, O. F., Oyelakin, O. O., Boboye, B. E. (2020). Molecular characterization of crude oil degrading bacterial isolates from polluted soils and cow dung. *International Journal of Environment, Agriculture and Biotechnology*, 5(3): 533-541

Indumathi, C., Durgadevi, G., Nithyavani S., Gayathri, P. K. (2014). Estimation of terpenoid content and its antimicrobial property in *Enicostemmalitorrale*. *International Journal of Chem. Tech Research*. 6 (9):4264-4267

Jimoh, F. A., Ajao, A. T., Zakariyah, R. F., Odebisi-Omokanye, M. B., Abdulrahman, H. O. (2020).Phytochemical screening and antimicrobial effect of seeds of *Monodoramyristica*on selected microorganisms; *Nigeria Journal of Pure and Applied Sciences*, 33 (1): 3674-3686

Karigidi, K. O, Ojebode, M. E.,Olaiya, C. O (2018). Effect of Cooking on Antioxidant and Enzymes Activity Linked to Carbohydrate Metabolism and Lipid Peroxidation of Eggplant (*Solanum melongena*). *Pertanika Journal of Tropical Agricultural Science* 41 (4): 1717-1730

Karigidi, K. O., Akintimehin, E. S., Omoboyowa, D. A., Adetuyi, F. O. (2022). Phytochemical profiling and antioxidant activities of *Monodoramyristica* and *Dennettiatripetala* against lipid peroxidation in rat heart. *ACTA PharmaceuticaSciencia*, *60*(1), 49-63.

Keay, R. W., Onochie, C. F., Stanfield, D. P. (1964). Nigerian trees. Department of Forest Research Publishers, Ibadan; 1:103-265.

Kebede, T., Gadisa E., Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS one*, *16*(3), e0249253.

Kim, D. O., Jeong, S. W., Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of Plums. *Food Chemistry* 81: 321–326

Kongkum, N., Thanasansurapong, S., Surapanich, N., Kumutanat, W., Sukkum, C., Kuhakarn, C., Jaipetch, T., Reutrakul, V., Hongthong, S. (2021). Antioxidant and antimicrobial alkaloids isolated from twigs of *Uvariagrandiflora*Roxb. *ScienceAsia* 2;47.

Lalitha, M. K. (2004). Manual on antimicrobial susceptibility testing. *Performance standards for antimicrobial testing: Twelfth Informational Supplement*, *56238*, 454-456.

Li, J., Xie, S., Ahmed,S., Wang, F., Gu, Y., Zhang, C., Chai, X., Wu, Y., Cai, J., Cheng, G (2017). Antimicrobial Activity and Resistance: Influencing Factors. *Frontiers in Pharmacology*, 8: 364. https://doi.org/10.3389/fphar.2017.0036

NCCLS (2006). Performance Standards for Antimicrobial Disc Susceptibility Test, 4th Edition, Approved Standard M 2 – A 4, Villanova.

Nkwocha, C. C., Nworah, F. N., Okagu, I. U., Nwagwe, O. R. (2018) Proximate and phytochemical analysis of *Monodoramyristica* (African Nutmeg) from Nsukka, Enugu State, Nigeria. *Journal of Food Nutrition Research*. 6(9): 597–601

Nwiyi, P. O., Nzubechi, A., Erumaka, I., Enwere S. (2022) In vitro antibacterial activities of *Vernoniaamygdalina* extract on microorganisms isolated from *Clariasgariepinus*. *Journal of Advances in Microbiology Research* 3(1): 15-21

Nwozo, O. S., Effiong, E. M., Aja, P. M., Awuchi, C. G. (2023). Antioxidant, phytochemical, and therapeutic properties of medicinal plants: A review. *International Journal of Food Properties*, 26(1), 359-388.

Obadoni, B. O., Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*. 86:2003-2008

Odeje, S. C., Rabia, H. (2026). Ethnobotanical potential of *Carica papaya*, *Psidium guajava* and *Azadirachta indica* leaf, bark extracts and their combination in inhibiting the growth of *Salmonella typhi*. *FUDMA Journal of Sciences* (FJS) 10 (3): 377 – 381

Ogunmefun, O. T., Ekundayo, E. A., Akharaiyi, F. C., Ewhenodere, D (2017). Phytochemical screening and antibacterial activities of *Tectonagrandis L. f.* (Teak) leaves on microorganisms isolated from decayed food samples. *Tropical Plants Research*, 4(3):376-382.

Ogunmefun, O. T., Saba, A. B., Fasola, T. R., Akharaiyi, F. C., Oridupa, A. O (2016). Phytochemistry and *in-vitro* Antimicrobial Evaluation of *Phragmantheraincana* (Schum.) Balle Extracts on Selected Clinical Microorganisms. *British Microbiology Research Journal* 14(3): 1-10

Ojatula A. O., Ikuesan F. A. (2022). Assessment of the biomolecules profiles and *in-vitro* biological activities of *Cymbopogoncitratus*. *Nigerian Journal of Pure and Applied Science*; 35(2): 4322 - 4332.

Okeke, M. I., Iroegbu, C. U.,Jideofor, C. O., Okoli, A. S., Esimone, C. O. (2001) Anti-microbial activity of ethanol extracts of two indigenous Nigerian spices. *Journal of Herbs and Spices Medicinal Plants*, 8(4):39-46.

Okoli, A. S., Okeke, M. I., Iroegbu, C. U., Ebo, P. U. (2002) Antibacterial activity of *Harunganamadagascariensis* leaf extracts. *Phytotherapy Resources*. 16:174-179.

Olaiya, O. O, Ojebode, M. E., Karigidi K. O. (2016). Antioxidant and Antibacterial Activities of the Essential Oils of *Cymbopogon citrates* and *Citrus sinensis*. *European Journal of Medicinal Plants*. 16(1):1-10.

Omiyale, B. O., Karigidi, K. O. (2023). Antioxidant activities and GC-MS profiling of fractions of methanol extract of *Andrographis paniculata*. *African Journal of Biotechnology*, *22*(8), 138-145.

Osukoya, O. A., Adewale, O. B., Falade, A. E., Afolabi, O. B., Awe, J. O., Obafemi, T. O., Kuku,A. (2021) Antioxidant and antibacterial propertiesof *Monodoramyristica* (Calabash nutmeg) seed protein hydrolysates. *Food Measure* **15**, 2854–2864 (2021). https://doi.org/10.1007/s11694-021-00871-4

Oyebode, O. T., Ojo, O. S., Adeyemi, O. S. (2021). Phenolic content and antioxidant activity of the methanol extract of *Eremomastaxspeciosa* leaves. *Free Radicals and Antioxidants*, 11(1), 53-58.

Panche, A. N., Diwan, A. D., Chandra S. R.(2016). Flavonoids: an overview. *Journal of Nutritional Science*, 5:47.

Park, Y. S, Jung S. T, Kang S. G, Heo B. K, Arancibia-Avila P, Toledo F., Gorinstein S, (2008). Antioxidants and proteins in ethylene-treated kiwifruits. *Food Chemistry* 107: 640–648

Roy, Z., Bansal R., Siddiqui, L., Chaudhary, N. (2023). Understanding the role of free radicals and antioxidant enzymes in human diseases. *Current Pharmaceutical Biotechnology.* 24(10):1265-76

Ruch, R. J., Cheng, S. J., Klaunig, J. E, (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen 10, 10031008.

Seukep, A. J, Nembu, N. E, Mbuntcha, H. G., Kuete V. (2023). Bacterial drug resistance towards natural products. In *Advances in Botanical Research* 106: 21-45). Academic Press.

Singh, D. B., Pathak, R. K., Rai D. (2022). From traditional herbal medicine to rational drug discovery: strategies, challenges, and future perspectives. *RevistaBrasileira de Farmacognosia*, 32(2): 147-159.

Tavares, L. S., Silva, C. S., F., de Souza, V. C., da Silva, V. L., Diniz, C. G., Santos, M. O. (2013). Strategies and molecular tools to fight antimicrobial resistance: *Frontiers in Microbiology*. 2013; 4:412.

Trease, G. E., Evans, W. C. (1989). Pharmacognosy. Eleventh Edition. BrilliarTrindel Can. Macmillian Publishers, London

Uwakwe, A. A., Nwaoguikpe R. N. (2008) *In-vitro* antisickling effects of *Xylopia aethiopica* and *Monodoramyristica*. *Medicinal Plant Research,*2(6): 119–124.

Whiliki, O. O., Dowe, E., Otue E. I. (2023). Phytochemical Screening and in –vitro Antimicrobial Activity of *Monodoramyristica* Seed Extract on Selected Human Pathogens *Nigerian Journal of Microbiology*, 37(1): 6599 – 6608

©2026 This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International license viewed via https://creativecommons.org/licenses/by/4.0/ which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is cited appropriately.