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Synergistic and antagonistic medicinal activities of essential oil of *Monodora myristica*

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ABSTRACT

Synergetic and antagonistic medicinal activities of crude and fractionated essential oil from the seed of Monodora myristica were investigated. The crude essential oil was extracted by steam distillation and then subjected to column chromatography; three fractions (N1, N2, and N3) were obtained. To identify the components, gas chromatography and mass spectrometry (GC-MS) investigation was utilized. The phytochemical parameters were determined by standard analytical methods. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used to evaluate the antioxidative capacity. and, the anti-diabetic activity was performed using α-amylase and α-glucosidase assay. The Agar diffusion method was used to determine the antibacterial activities. The GC-MS analysis indicated p-cymene (21.12%) as the major component of the crude essential oil. The antioxidant activity using DPPH varied from 614 to 704 µg/mL, while the ABTS activity ranged between 281-342 µg/mL. The α-amylase assay showed fraction N1 with IC_{50} value of 3.21 µg/mL, while for α-glucosidase assay, fraction N2 with IC_{50} 2.15 µg/mL recorded the least value. The minimum inhibitory concentration values for the crude extract and all the fractions against the strains of bacteria ranged from 6 to 12.5 mg/mL Both crude extract and fractionated essential oil showed antioxidant, anti-diabetic and antibacterial activities.

1. INTRODUCTION

The main controversy that humans have encountered over time is related to the use of synthetic chemical products, such as food preservatives, pesticides, and pharmaceuticals that people ingest or use in their daily lives (Hayes et al., 2006) it impacts our health and the environment. Natural products are good lead molecules that can be further modified during the course of drug development and are essential sources for novel medications. The varied architectures and complex carbon skeletons of natural products have led to the significant use of natural products in medication discovery. Drugs obtained from natural sources are preferable to chemical products, making them good entities for further drug development. (Chin et al., 2006). It is believed that Africa is blessed with many plant species, many of which have a high potential as medicine used by local healers (Manach et al., 2004).

Natural products and their constituents have recently become the most successful source for the development of novel drugs (Rocha et al., 2005). Volatile oils, also known as essential oils, are

distilled from plants' fruits, seeds, leaves, and other scent-producing organs. The presence of oil glands or oil sacs in some of the plant's mentioned parts contains this oil (Mahato et al., 2019). There are several medicinal and biological uses for essential oils, including their antimicrobial, anticancer, anti-mutagenic, and antioxidant capabilities (Raut and Karuppayil, 2014). The Africa Nutmeg (*Monodora myristica*) is a woody blooming perennial member of the Annonaceae plant family that is edible (Hemingway, 2004). It is typically found in many West African countries like Nigeria, Cameroon, Liberia, Uganda, Angola, and Kenya (Burabai et al., 2009). The seeds of *M. myristica* are valued for their therapeutic and economic potential (Omobuwajo et al., 2003). It is successfully employed as a spice in Nigeria and because of its aromatic flavor (Raphael et al., 2010). Aside from that, *M. myristica* has been found useful in the treatment of headaches and hypertension (Owokotomo and Ekundayo, 2012). Several reports had identified the medicinal use of the stem and bark to cure stomach ache, fever pain, hemorrhoids, and eye disorders (Uwakwe and Nwaoguikpe, 2008; Ogu et al., 2011). The essential oil has consisted of several components which literature had revealed are responsible for the various activities they exhibit (Ogu et al., 2011). Medicine is mostly prepared in a condition in which it contains an active ingredient in most local remedies used as folkloric medicine, the mixture comprises several compounds (Uwakwe and Nwaoguikpe, 2008). The shortfall of this is that most of the compounds are not known since some of the compounds can be toxic. The vast array of these compounds in a mixture can synergize their action (Iwu et al., 1987).

While several studies have been conducted on the medicinal activities of the essential oil of *M. myristica*, we have some school of thought who believes that the several compounds present in plant extract could be responsible for the synergic activity, while some also believe that some compound could produce anti-synergic effects. This research compares the medicinal activities of the crude essential oil of *M. myristica* and the fractions obtained via column chromatography to determine if the fractions obtained will show if the components observed in them exhibit synergic or anti-synergic activities.

2. MATERIALS AND METHOD

2.1. Materials

African nutmeg seeds (*M. myristica)* were collected from a local store (Oja-Oba) in Oshogbo Osun State, Nigeria 7.7654°N, 4.554°E. The seed was identified with a herbarium specimen, IFE-17945, at the Botany Department of Obafemi Awolowo University. The seeds were air-dried and cut into smaller bits.

2.2. Methods

2.2.1. Extraction of the essential oil

The seed of *M. myristica* was ground to Mesh size of 0.5 mm and was extracted by the method of hydrodistillation for a period of 6 hours. The essential oil was dried with sodium sulfate (anhydrous). It was stored in an airtight container at 4°C in a refrigerator.

2.2.2. Fractionation

Crude extract (15 g of M. myristica) essential oil was put through column chromatography with a 3 ×100 cm column size to separate the crude extract into various fractions. Silica gel of particle size 0.063-0.200 was used as the stationary phase. A polarity gradient of the hexane/ethyl acetate system (100/00, 80/20, 60/40, 40/60, 20/80) was used to carry out the elution, then ethyl acetate/methanol (100/0, 80/20, and 100/0) mixture system. Using a separating funnel, a measured volume (100 mL) of each solvent combination was continuously added to the column. Test tubes were filled with 10-milliliter aliquots of the eluted fractions. Fractionation monitoring was done by thin layer chromatographic (TLC) aluminum plate. The fractions were spotted on pre-coated silica gel (F_{254}) and developed using the solvent ratio it was eluted with. The plate was taken out and hot air was used to dry it. The TLC plate was visualized by spraying with sulphuric acid reagent and heating the plate in a hot oven at 110°C for 5 min. Three fractions, N1, N2, and N3, were obtained after the solvent evaporated on a rotary evaporator at 40°C.

2.2.3. GC-MS analysis

The Agilent 6890N instrument, which has a flame ionization detector and a capillary column HP-5MS (30.0 m × 0.250 mm x 0.250 µm), was used to analyze the GC-MS data. With the use of an Agilent Technologies 5973N mass spectrometer, the constituent parts of the essential oil were determined. For one minute, the GC was at 60°C. It

then climbed at 10°C per 60 seconds to 180°C for one minute, and then at 20°C per minute to 280°C for fifteen minutes. The injector's temperature was kept at 270°C, and a 0.001 mL sample was injected neatly with a split ratio of 1:10. The carrier gas was helium, flowing at 1.0 mL min−1. At two scans per second, the spectrums scanning ranged from 20- 550 m/z. The compounds were identified by mass spectrometry (MS), and the identity of their constituents was verified by comparing their mass spectrum with sample references or with data that existed in the NIST 2008, mass spectral data both in the library and in the literature, and their Kovat's the retention index in comparison with hydrocarbons with respect to C8–C32 n-alkanes.

2.2.4. Determination of total phenolic acids

The Folin-Ciocalteu method (Folin and Ciocalteu, 1927) was utilized to determine this. A 125 µL volume of crude essential oil and fractions were mixed with distilled water and Folin-Ciocalteu's reagent. The Na_2CO_3 (7%) solution was added after the mixture had stood for six minutes. A SpectrumLab70 spectrophotometer was used to evaluate the absorbance of the mixture after it had stood for a period of 90 minutes. The result was stated in gallic acid equivalents (GAE).

2.2.5. Determination of saponin and tannin

Spectrophotometric analysis, as reported by Folin and Ciocalteu (1927), was used to calculate the saponin content. About 2 g of the sample was measured into a beaker and 2-methylpropan-1-ol was included. The mixture was transferred into a beaker containing a 40% magnesium carbonate ($MgCO_3$) solution after being filtered through No. 1 Whatman filter paper. After transferring around a milliliter of the solution into a volumetric flask, 2 milliliters of iron (III) chloride ($FeCl_3$) solution were introduced, and the quantity was adjusted with distilled water. This was left to stand for 30 minutes to develop the color, and a SpectrumLab70 spectrophotometer was employed to determine the absorbance at 380 nm. With very minor adjustments, the following standard Folin and Ciocalteu (1927) technique was used to determine the total tannin content: Using 80% ethanol, 0.5 mL of the sample extract was diluted. To two milliliters of Folin-Ciocalteu reagent, 0.1 milliliter was added from the diluted sample. A 7% sodium carbonate solution was added after 8 minutes, and the mixture was incubated for two hours. Tannic acid curve was used as the standard to assess the tannin content after the absorbance was measured at 760 nm.

2.2.6. Determination of flavonoid and alkaloid

One milliliter of distilled water was used to dissolve about 0.25 g of the extract. Next, 1 milliliter of 1M NaOH solution, 0.150 milliliters of freshly made aluminum chloride ($AlCl_3$), and $NaNO_2$ solution (5%) were added. After letting the mixture remain for five minutes, the absorbance at 510 nm was determined. on a SpectrumLab70 spectrophotometer. The outcome was given as equivalents of quercetin (QE).

After weighing the sample (0.3 g) into a beaker, 200 mL of ethanol containing CH_3COOH (10%) was introduced, and the mixture was permitted to stand for four minutes. After filtering, the extract was concentrated to a quarter of its initial size in a water bath. The extract was slowly mixed with ammonium hydroxide solution until the precipitation was achieved. After gathering the precipitate, it was filtered and cleaned with diluted ammonium hydroxide. After drying, the residue was weighed. (Folin and Ciocalteu, 1927).

2.2.7. Determination of cardiac glycosides

A conical flask filled with chloroform was filled with around 2 mL of the sample. The resultant mixture was then filtered, while 2 mL of pyridine and 29% sodium nitroprusside were added, and shaken vigorously for ten minutes. After that, 20% NaOH was applied to develop the color, and the absorbance was measured at 510 nm. on a SpectrumLab70 spectrophotometer.

2.2.8. Quantitative test for terpenoids

A 100 mg extract was soaked for 24 hours in 9 mL of ethanol (Folin & Ciocalteu, 1927). After filtering, the extract was extracted using a separating funnel and 10 mL of petroleum ether. Glass vials with pre-weighed contents were used to separate the ether extract, which was then allowed to fully dry (X). After ether was evaporated, the formula below was used to determine the yield (%) of total terpenoids present: $(X_i - X / X_i \times 100)$

Where X_i =initial weight and X_f = final weight.

2.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

The DPPH free radical scavenging activity of the tested crude essential oil and fractions was determined by the method outlined by Goncalves et al. (2011). The reaction mixture's color shift was observed at 517 nm. A 1 mL aliquot of 0.30 mM DPPH ethanolic solution was combined with 2.5 mL

of the essential oil at different concentrations, along with standard ascorbic acid (50.0, 100.0, 150.0, 200.0, and 250.0 µg/mL). The mixture was left to incubate in the dark at ambient temperature for half an hour, at which point the absorbance at 517 nm was measured. A blank made of ethanol was used. A negative control was provided by 1 mL of DPPH solution (0.3 mM) with 2.5 mL of ethanol. The studied extract's scavenging effectiveness was demonstrated by the decolorization of DPPH from purple to yellow. Each test was conducted trice. The percentage inhibition of DPPH was computed with the formula below:

$$DPPH\ activity\ (\%) = [(BC\ CTR - BCSPL)] /(BC\ control) \times 100$$

Where $BC\ CTR$ is the absorbance of $DPPH\ radicals + methanol$; $BC\ SPL$ is the absorbance of $DPPH\ radical + sample\ or\ standard$.

2.4. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity

The essential oil ability to scavenge ABTS radicals was ascertained in accordance with Goncalves et al. (2011). After 16 hours of dark storage at room temperature, 5.0 mL of a 0.014 M ABTS solution and 5.0 mL of a 0.0049 M $K_2S_2O_8$ solution reacted to form the ABTS cation radical. Before usage, this solution's absorbance at 734 nm was modify to 0.700 ± 0.020 by diluting it with ethanol. After homogenizing the crude essential oil and fractions at different concentrations with 1 mL of ABTS solution, the absorbance at 734 nm was measured. Every experiment included ethanol blanks, and all measurements were completed at least six minutes later. Likewise, the standard group's reaction mixture was created by combining 50 µL of BHT with 950 µL of ABTS solution. IC50 µg/ml was used to express the ABTS scavenging ability in relation to the antiradical activity. The formula below was used to get the inhibition percentage of the ABTS radical:

$$ABTS\ scavenging\ activity\ (\%) = \left\{\frac{(B_0 - B_1)}{B_0}\right\} X100$$

Where B_0 = absorbance of the control B_1 = absorbance of the sample.

2.5. Antidiabetic activity assay

2.5.1. Alpha-amylase inhibitory assay

The antidiabetic activity of *M. myristica* crude essential oil and fractions was determined by α-amylase and α-glucosidase assay as described previously by Ali et al. (2006). Porcine pancreatic (EC 3.2.1.1) α-amylase (0.50 mg/mL) was added to 500 µL of the essential oil and 0.020 mol/L Na_3PO_4 buffer (pH 6.9 with 0.0060 mol·L−1 NaCl), which were then incubated for 10 minutes at 25°C. Subsequently, the reaction mixture was supplemented with 500 µL of starch solution (1%). After that, the reaction was halted using 1.0 mL of $C_7H_4N_2O_7$ color reagent after 10 minutes of incubation at 25°C. After five minutes of incubation in a water bath (boiling), the mixture was given time to recede to ambient temperature. The UV-Visible spectrophotometer (Spectrumlab 752S) was used to measure the absorbance at 540 nm. Using the identical process, a reference was created by substituting distilled water for the essential oils. The % inhibition of α-amylase was evaluated:

$$\%\ \text{inhibition} = \frac{[Abs_{ref} - Abs_{sam}]\ X\ 100}{Abs_{sam}}$$

Where Abs_{ref}=absorbance of reference Abs_{sam} =absorbance of the sample

2.5.2. Alpha-glucose inhibitory assay

Ten minutes were spent at 25°C incubating 50.0 µL of the sample and 0.1 mL of α-glucosidase solution in 0.10 mol/L phosphate buffer (pH 6.9). Next, 0.05 mL of 0.10 mol/L phosphate buffer (pH 6.9) containing 5.0 mmol/L p-nitrophenyl-α-DD-glucopyranoside [p-nitrophenyl-α] solutions were added. After five minutes of incubation at 25°C, the reaction mixture's absorbance was measured at 405 nm using a UV-visible spectrophotometer. (Spectrumlab 752S). The% inhibition of the α-glucosidase inhibitory action was reported (Apostolidis et al., 2007). Percentages of the blank control were used to express the results.

Percentage inhibition is calculated as:

$$\%\ \text{inhibition} = \frac{\left[Abs_{ref} - Abs_{sam}\right] X\ 100}{Abs_{sam}}$$

Where Abs_{ref}=absorbance of reference Abs_{sam}= absorbance of the sample

2.6. Antibacterial screening

The three types of bacteria were used in this investigation were *Escherichia coli* (NCIB 1161], *Klebsiella pneumonia* (NCIB 1161] and *Bacillus cereus* (NCIB 1161] which were obtained from the Central Research Laboratories, University of Ilorin. Isolates were added to nutritional broth and cultured for 24 hours at 37°C to create the bacteria used in the study.

2.7. Antimicrobial sensitivity profiling

The zone of inhibition was ascertained using the Agar well diffusion method. Agar Mueller-Hinton was employed. After cooling to 45°C, 20–25 mL of the molten medium was applied to 150 mm-sized, pre-sterilized plates. Following this, sterile cotton swabs were used to equally distribute 16–24 hour-old bacterial species cultures over the whole surface of an agar plate, resulting in an even plate surface growth. The Petri plates were allowed to air dry. Using a sterile borer, about three to four wells of 6 mm in diameter and 5 mm in depth were perforated into the agar surface to hold the extracted samples. Dmethyl sulfoxide (DMSO) was used as the negative control and 10 µg of gentamicin was utilized as a positive control after about 50 µL of the undiluted extract was poured into each well. Following a half-hour at room temperature, the plates were incubated for a full day at 37°C to encourage the development of bacteria. A ruler was used to measure the zones of inhibition following incubation, and the results were reported in millimeters. (Sadeghi-Nejad *et al*., 2010)

2.8. Determination of minimum inhibition concentration (MIC)

Using the agar dilution procedure, the minimum inhibitory concentration was found. The MIC was assessed for extracts that demonstrated antibacterial activity against any pathogen in the agar well diffusion assay. Each extract was used in this test at various concentrations. An overnight suspension of every organism in nutrient broth was made, and 50µL was added to each test tube. The preparations were then incubated for 24 hours at 37°C. Each tube was injected on nutrient agar with a sterile cotton swab after incubation to determine whether or not bacterial growth was inhibited. Bacterial growth on solid media suggested that an extract concentration was not effective in suppressing the germs. According to Andualem et al. (2014), the minimum inhibitory concentration (MIC) is the minimal concentration of an antibiotic that prevents a microbe from growing visibly after being incubated for a night. Figure 1 shown the scheme of the process involved in the extraction of the crude and fractionated essential oil from the seed of *Monodora myristica*.

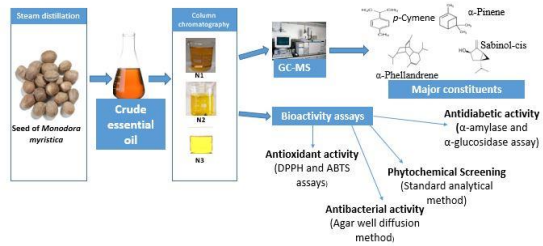

Figure 1. Scheme showing the summary of the chemicals extracted and fractionated from the seed of *Monodora myristica*

3. RESULTS AND DISCUSSION

Essential oils are composed of a vast array of components; the vast number of compounds may be culpable for the synergistic actions of the extracts. Sometimes, some components could have countered the desired effects of the extracts, which could cause an antagonistic effect on the extract. The crude essential oil of *M. myristica* extracted was separated into three parts utilizing column chromatography, N1, N2, and N3 with yield of 5.98, 2.20 and 4.43 g, respectively. The GC-MS analysis outcome of the crude essential oil and fractions N1, N2 and N3 of *M. myristica* appear in Tables 1, 2, 3 and 4, respectively. In the crude essential oil, it was observed that *p*-cymene had the highest composition (21.15%), followed by α-phellandrene (20.77%).

Table 1. Major constituents of the crude essential oil of *M. myristica*

Compounds	%	RI (cal)	RI (lit)
p-Cymene	21.15	1039	1041
α–Phellandrene dimer	20.77	993.2	999.1
Sabinol-cis	12.25	1125.4	1135.6
α-Pinene	5.8	930.1	934.5
λ-Muurolene	4.86	1465.2	1473
Limonene	3.78	1024.1	1029.5
β-Myrcene	3.21	976.4	983.1

RI (literature) represents the retention indices reported by Babushok et al., 2011. Retention indices of components for dimethyl silicone stationary phase

Table 2. Major constituents of the fraction N1 of the essential oil of *M. myristica*

Compounds	%	RI (cal)	RI (lit)
p-Cymene	48.89	1010.4	1015.1
λ-Muurolene	7.12	1506.5	1513.9
Thymol	5.63	1284.3	1290.1
Caryophyllene	4.08	1400.1	1406.8
β-Myrcene	3.09	980.3	989.2
γ–Terpinene	3.02	1047.1	1050.3

RI (literature) represents the retention indices reported by Babushok et al., 2011. Retention indices of components for dimethyl silicone stationary phase

Table 3. Major constituents of the fraction N2 of the essential oil of *M. myristica*

Compounds	%	RI (cal)	RI (lit)
Sabinol-cis	47.7	1130.1	1135.6
Linalool	13.64	1078.1	1086.3
α-Cadinol	9.81	1635.1	1640.2
γ–Muurolene	5.03	1489.1	1473
Germacrene	3.99		1480.6

RI (literature) represents the retention indices reported by Babushok et al., 2011. Retention indices of components for dimethyl silicone stationary phase

Table 4. Major constituents of the fraction N3 of the essential oil of *M. myristica*

Compounds	%	RI (cal)	RI (lit)
α-Pinene	51.33	1021.3	1025.4
α-Phellandrene	22.76	1161.3	1167.7

RI (literature) represents the retention indices reported by Babushok et al., 2011. Retention indices of components for dimethyl silicone stationary phase

The GC-MS analysis of fraction N1 revealed that *p*-cymene had the highest composition (48.89%) while γ-terpinene had the least value (3.02%). Also, thymol and terpinene, which were not listed as significant components in the crude essential oil, were observed in fraction N1. Owokotomo and Ekundayo (2012) also reported these compounds.

The highest constituent observed in fraction N2 showed that sabinol-cis had the highest composition of (47.70%) followed by linalool (13.64%) while germacrene (3.99%) was observed to have the lowest composition. Awojide et al. (2016) also reported sabinol as a major component in their work, while Sylvie et al. (2019) also found linalool present in the fraction obtained in their work. Fractions N3 had two major components, α-pinene (51.33%) and α-phellandrene (22.76%); both of these compounds were also reported elsewhere (Sylvie et al., 2019; Owokotomo & Ekudayo, 2012). The possible explanations for this variation include the fruits' origin, which was frequently unknown (Owolabi et al., 2009). Also, the kind of treatment the fruits underwent after the harvest may be another important reason.

Table 5 shows the results of the phytochemical analysis conducted quantitatively conducted on the fractions and crude essential oil of *M. myristica*. The research reported flavonoid (5.80 mg QE/g) and saponin (5.57 mg/g) as one of the major phytochemicals from the crude essential oil of *M. myristica;* this was comparable to what was found by Offor et al. (2018), who reported flavonoid (2.34 mg/100 g) and saponin (1.56 mg/100 g). Phenolic acid was observed in all except fraction N3; this corresponds to similar observations made by Adewole et al. (2013). The highest value of saponin was detected in fraction N2 (6.06 mg/g) which was also similar to what was observed in the crude essential oil but significantly different; the same phytochemical was reported by Offor et al. (2018). Tannin was only detected in the crude essential oil in a low quantity (0.338 mg/g); this was also reported by Adewole et al. (2013). Flavonoid was detected in all the fractions and crude essential oil of *M. myristica*, and it was highest in fraction N3 (6.86 mg QE/g); this was comparable to the work of Raphael et al. (2010). Alkaloid was not determined in the fractions and crude essential oil of *M. myristica*, which is similar to the work of Amabeoku (2009). The highest quantity of cardiac glycoside was recorded in fraction N1 (6.00 mg/g), similar to the work of Enabulele et al. (2014). Terpenoid had the highest quantity in fraction N3 (5.66 mg/g), similar to what was observed in fraction N1, but not significantly different in value. This phytochemical was also observed by Enabulele et al. (2014).

p-Cymene with the highest constituent of fraction N1 and crude essential oil, has reportedly shown anti-diabetic properties (Abbasi et al. 2018). This constituent may have been attributed to the phenolic component since phenolic acid possess high anti-diabetic property, as observed in fraction N1. Gowd et al. (2017) reported the anti-diabetes effects of phenolic acid through multiple mechanisms. Saponin, which was observed to have its highest constituent in fraction N2, was reported elsewhere (Oishi et al., 2007) to be an excellent anti-diabetic agent. The effect of saponin having an anti-diabetic property in this study may be because of the presence of linalool as a constituent of fraction N2. Tannin, which was only detected in the crude

essential oil of *M. myristica*, has been showed to possess antibacterial properties (Cotas et al., 2020). *p*-Cymene was found in abundance in the crude essential oil and might be responsible for the antibacterial activity of tannin in the crude essential oil. Several bacteria, including *E. coli*, can be inhibited by compounds derived from essential oils, such as *p*-cymene (Cotas et al., 2020).

α-Pinene has been reported to be the highest compound in the fraction N4. α-Pinene reportedly possesses antimicrobial and antioxidant properties (Duke, 1994). Flavonoid, found in abundance in fraction N3, might have contributed significantly to its antibacterial and antioxidant properties because of the presence of α-pinene. Although flavonoids have a wide range of biochemical activities, practically every category of flavonoids is best known for their ability to act as antioxidants (Heim et al., 2002). Mishra et al. (2013) revealed that plant extracts high in flavonoids from several species have antibacterial properties.

Cardiac glycoside has been observed to be the highest in fraction N1, this could have been attributed to the reason why it displayed a variety of antibacterial potentials (Arora & Sood, 2017). Thymol being present in fraction N1 might have contributed to its antimicrobial potential has it has been revealed elsewhere that it possesses antimicrobial activity (Karpanen et al., 2008).

Terpenoid, which was observed to be highest in fraction N3, was similar to what was observed in fraction N1 but significantly not different. Terpenoid has potent antibacterial properties (Shahbazi, 2015; Wang et al., 2016; Chan et al., 2016; Dogan et al., 2017). The antimicrobial property may be because of the presence of α-pinene (Duke, 1994).

The results of the free radical scavenging potentials of the crude essential oils and fractions are shown in Table 6. Two radical scavenging methods, namely DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azobis-2-aminopropane hydrochloride) assay were employed for the antioxidant activities. The values of inhibition concentration at 50% (IC_{50}) for DPPH, ABTS and those of some standards (Vitamin C and garlic acid) are shown in Table 7. The research recorded an IC_{50} of 716.00 µg/mL and 312.22 µg/mL for DPPH and ABTS free radical scavenging, respectively. This result was similar to what was reported by Noreen et al. (2017), who indicated an IC_{50} of 780.00 µg/mL for DPPH and 432.00 µg/mL for ABTS.

Free radicals are always fought against by antioxidants (Mollica et al., 2018., Zengin et al., 2017, Zengin et al., 2018; Sadeer et al., 2019). A prior investigation by Akinwunmi and Oluokun (2013) revealed that with a maximal DPPH radical scavenging activity of 41.20%, the flavonoid fraction demonstrated strong and noteworthy antioxidant potentials. The higher radical scavenging potentials exhibited by fraction N3, with IC_{50} of 614.17 µg/mL and 312.22 µg/mL for DPPH and ABTS, respectively, could cause a higher value of flavonoid presents in the fraction 6.86 mg QE/g. The higher antioxidant activity of fraction N3 could also be attributed to the higher value of α-pinene observed (Salehi et al., 2019).

As reported in this work, flavonoids had the highest constituent in fraction N3, which might have contributed significantly to its antioxidant property because of their biological importance.

Table 5. Quantitative phytochemical parameters in *M. myristica*

Components	N1	N2	N3	Crude
Phenolic acid (mg GAE/g)	1.30±0.03^{b}	1.29±0.02^{b}	ND	1.10±0.02^{a}
Saponin (mg/g)	4.52±0.02^{a}	6.06±0.02^{b}	4.10±0.01^{a}	5.57±0.03^{b}
Tannin (mg/g)	ND	ND	ND	0.34±0.02^{a}
Flavonoid (mg QE/g)	4.16±0.02^{a}	4.20±0.01^{a}	6.86±0.02^{c}	5.80±0.01^{b}
Alkaloid (mg/g)	ND	ND	ND	ND
Cardiac glycoside (mg/g)	6.00±0.02^{b}	5.31±0.02^{a}	5.32±0.02^{a}	5.30±0.02^{a}
Terpenoid (mg/g)	5.64±0.02^{b}	5.48±0.02^{b}	5.66±0.02^{b}	4.60±0.01^{a}

The table displays each value as mean ± SD Significant differences are indicated by a different letter after values in the same column ($p < 0.05$).

Agati et al. (2012) reported that by chelating the trace elements involved in the production of free radicals or by inhibiting the enzyme, flavonoids limit the synthesis of reactive oxygen species (ROS). They also scavenge ROS and help regulate or maintain antioxidant defenses. Previous studies have showed that flavonoids and phenols protect

cells against oxidative stress-induced damage (Olamilosoye et al., 2018).

Table 6. Radical scavenging activities of the fractionated and crude essential oil of *M. myristica* against DPPH and ABTS

Sample	IC50 (µg/mL)	
	DPPH	ABTS
N1	734.60±20.80^{d}	331.11±22.30^{d}
N2	764.74±25.10^{e}	342.64±30.40^{e}
N3	614.17±30.20^{b}	281.48±24.60^{a}
Crude EO	716.00±24.20^{e}	312.22±28.10^{b}
Ascorbic	734.20±10.30^{d}	342.20±10.30^{e}
Gallic acid	482.86±10.20^{a}	321.10±10.20^{c}

The table presents each value as mean ± SD

The anti-diabetic activities of the fraction and crude essential oil of *M. myristica*, as evaluated by α-amylase and α-glucosidase assays are shown in Table 7. While all fractions and the crude essential oil of *M. myristica* showed activities against α-amylase and α-glucosidase, this could have been because of the existence of tannins, flavonoids, saponins, phenolic acid compounds and steroids detected in the sample.

Tafesse et al. (2017) stated that some of these bioactive constituents could control diabetes. The slight variation in the activities ranged from 3.21-3.90 mg/mL and 2.15-3.18 mg/mL for the α-amylase and α-glucosidase, respectively, cannot be far from the antagonistic and synergistic action of the various components present in the fractions and crude essential oil. The higher inhibition observed in fraction N1 (3.12 mg/mL) could be because of the fraction's higher value of phenolic acid (Tafesse et al., 2017; Gowd et al., 2017). A higher inhibition of α-amylase in fraction N1 may be attributed to the higher value of *p*-cymene found in the fractions. Abbasi et al. (2018) showed that *p*-cymene exhibits anti-diabetic activity. The higher α-glucosidase inhibition was observed with fraction N2 (2.15 mg/mL); this could result from the high saponin present in the fraction. Research has shown that extract with high saponin has anti-diabetic activities (Oishi et al., 2007). The high α-glucosidase inhibition could also result from the presence of linalool. Afifi et al. (1998) had once reported that linalool present in an extract produces anti-diabetic activities.

Table 7. In-vivo anti-diabetics activities of *M. myristica*

Sample	IC50 (mg/mL)	
	α-amylase	α-glucosidase
N1	3.21 + 0.01	2.46 + 0.01
N2	3.38 + 0.03	2.15 + 0.02
N3	3.90 + 0.01	3.18 + 0.02
Crude EO	3.65 + 0.02	3.10 + 0.01

Each value in the table is represented as mean ± SD (n = 3).

The results of the antibacterial screening of the samples showed that the crude essential oil and the fractions of *M. myristica* showed activities against all strains of the bacteria (Tables 8, 9 and 10). At a concentration of 6.25 mg/mL, none of the samples had any activity of *E. coli*. The highest activity was observed with a concentration of 50 mg/mL. The crude essential oil had the highest zone of inhibition of 5.20 mm with the minimum inhibitory concentration (MIC) value of 6.00 mg/mL, followed by fraction N2 with a zone of inhibition of 3.80 mm and MIC value of 10.00 mg/mL against *E. coli.*

The Agar diffusion method applied to determine the antimicrobial activities of the fractions (using different solvents and oil). All tested organisms were sensitive against the bacterial strains, indicative of different zone of inhibition observed. The crude extract with a minimum inhibitory concentration (MIC) of 6.00 mg/mL had the highest antibacterial activity against *E. coli*; this was also reported by Adewole et al. (2013). Fraction N3 with least MIC value of 5.00 mg/mL recorded highest antibacterial activity against *K. pneumonia* and the MIC value of 6.00 mg/mL was reported in fraction N3 as having the highest antibacterial activity against *B. cereus.*

Table 8. Anti-bacteria activities of fractionated and crude essential oil of *M. Myristica* against *Escherichia coli*

Concentration/ MIC (mg/mL)	50	25	12.5	6.25	MIC
Sample	Diameter of zone inhibition (mm)				
N1	3.40± 0.10	3.10± 0.10	0.00± 0.00	0.00± 0.00	12.5
N2	3.80± 0.10	3.20± 0.10	1.10± 0.10	0.00± 0.00	10
N3	2.40± 0.10	1.60± 0.10	I.20± 0.10	0.00± 0.00	15
Crude EO	5.20± 0.10	3.20± 0.10	2.80± 0.10	0.00± 0.00	6

The table presents each value as mean ± SD

Table 9. Anti-bacteria activities of fractionated and pure oil of *M. myristica* against *Klebsiella pneumonia*

Concentration/ MIC (mg/mL)	**50**	**25**	**12.5**	**6.25**	**MIC**
Sample	Diameter of zone inhibition (mm)				
N1	4.00± 0.10	3.20± 0.10	1.00± 0.10	0.00± 0.00	9
N2	5.00± 0.10	4.00± 0.10	3.00± 0.10	0.00± 0.00	6
N3	6.00± 0.10	4.20± 0.10	3.00± 0.10	2.00± 0.10	5
Crude EO	3.00± 0.10	2.00± 0.10	0.00± 0.00	0.00 ± 0.00	12.5

The table presents each value as mean ± SD

Table 10. Anti-bacteria activities of fractionated and pure oil of *M. myristica* against *Bacillus cereus*

Concentration/ MIC (mg/mL)	**50**	**25**	**12.5**	**6.25**	**MIC**
Sample	Diameter of zone inhibition (mm)				
N1	4.40± 0.10	2.80± 0.10	2.00± 0.10	1.00± 0.10	10
N2	4.00± 0.10	2.00± 0.00	1.00± 0.10	1.00± 0.10	12.5
N3	6.00± 0.10	3.00± 0.10	2.00± 0.10	1.00± 0.10	6
Crude EO	4.00± 0.10	2.10± 0.10	1.00± 0.10	0.00± 0.10	12.5

The table presents each value as mean ± SD

Research has shown that extracts with high phenolic contents have antimicrobial activity (Obonga et al., 2019). It could also be the existence of terpenoids in the extracts (Dogan et al., 2017; Wang et al., 2016; Chan et al., 2016; Shahbazi, 2015). Terpenoids were present in all of the fractions and the crude essential oil but at a lower concentration (4.60 mg/g); this could be responsible for the activities in the samples. The different activities exhibited could also result from the different concentrations of phenolic and terpenoids.

4. CONCLUSION

Undoubtedly, the essential oil extracted from the seed of *M. myristica* contains several components. The fractionation of the crude essential oil showed the prominence of some components in some fractions more than others. It is clear from the result obtained that the crude essential oil exhibited synergistic activity on some biomarkers. In contrast, in some biomarkers, the antagonist effect of some components was clear in the lower activities recorded for the crude essential oil in some parameters determined. This study showed that synergistic activity was specific more to the antibacterial activity against *E. coli* while for other parameters observed, the antagonistic effect of the compound was recorded. This result is because of the varying degree of the components observed in the crude essential oil and the fractions observed.

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