Chemical composition, antibacterial efficacy, and antioxidant capacity of essential oil and oleoresin from *Monodora myristica* and *Tetrapleura tetraptera* in Southeast Nigeria

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Specific to the West African sub-region, previous studies involving fruit, stem, and bark of *Tetrapleura tetraptera* as well as seeds of *Monodora myristica* have largely focused on phytochemical properties of aqueous and methanolic and ethanolic extracts. To supplement existing information, the chemical composition, antibacterial efficacy (tested against *Escherichia coli* and *Staphylococcus aureus*), and antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl (DPPH∙) radical scavenging, ferric reducing power, and total antioxidant capacity) of essential oil and oleoresin extracted from *T. tetraptera* fruit and *M. myristica* seeds cultivated in Southeast Nigeria, were studied. Essential oil and oleoresin were respectively extracted by steam distillation and aqueous maceration. By way of gas chromatograph mass spectrometry (GC–MS) analysis, the chemical compounds from essential oil and oleoresin from *M. myristica* and *T. Tetraperta* samples totaled 6 and 5, as well as 27 and 16, respectively. Besides the oleoresin of *M. myristica* and the essential oil of *T. tetraptera* showing some resistance against *S. aureus*, the oleoresins seemed highly susceptible to *E. coli*—all of which demonstrated concentration-dependence to the antibacterial inhibition zone. Scavenging DPPH radical, reduction power activity, and total antioxidant capacity increased with essential oil and oleoresin extracts’ concentrations, which positions *M. myristica* and *T. tetraptera* spices as very promising for food preservation, especially against autoxidation and microbial spoilage.

Globally, edible plants continue to be of research interest given their natural compounds and medicinal properties that are capable of improving health and preventing/combating diseases. Spices are among such particularly large groups of edible plants with beneficial natural ingredients usually added to foods. Furthermore, the capacity to influence both aroma and taste of foods is largely owed to the presence of essential/volatile oils that comprise terpenes and terpenoids along with various aliphatic hydrocarbons, acids, alcohols, etc., able to control food spoilage and prolong shelf life. In addition to essential oils, spices show potential health benefits through antimicrobial, antioxidant, antidiabetic, anti-inflammatory, anti-viral, and antiprotozoal capacities. Additionally,
the chemical composition and medicinal properties could be influenced by genetics and the type of extraction method\(^1\). In addition to their abundance from rural to urban areas, spices from African flora have helped in the discovery of novel drugs useful in biomedicine, which have helped to promote health and tackle diseases such as cancer, tumors, etc. Examples of indigenous spices in Africa include black pepper (*Xylopia aethiopsica*), West African pepper (*Piper guineense*), *Mentha piperita*, *Ocimum gratissimum*, *Tetrapleura tetraptera* and *Monodora myristica*. Unlike the exotic types, most of the above-named spices are wholly used and continue to receive research attention given the reported phytochemical constituents, antioxidant, and antimicrobial capacities\(^3,3-7\).

On one hand, the *T. tetraptera* is a deciduous perennial flowering medicinal plant of West African origin belonging to the pea family (Fabaceae), and commonly known as *aridan* and *yanayan* amongst the Yoruba and Urhobos respectively in Nigeria and *prekese* in Ghana\(^6,9\). The essential oil present in *T. tetraptera* provides a distinctive pleasant fragrance and aroma, making its dry fruit a popular seasoning spice in South-eastern Nigeria\(^5\). Ghanaians believe that *T. tetraptera* fruits contain multivitamins, useful in the management of jaundice, inflammation, convulsion, fever, and leprosy\(^4,8\). The phytochemical constituents of *T. tetraptera* fruits provide medicinal attributes demonstrated by molluscsidical, antimicrobial, antioxidant, anticonvulsant, anti-inflammatory, antimalarial, antidiabetic, and anticancer properties\(^8-11\). Microbiological studies show the extract of *T. tetraptera* exhibits excellent antimicrobial activity against Gram-positive and Gram-negative bacteria\(^4,2\). On the other hand, the *M. myristica*, also known as African/cabasha nutmeg, is a perennial plant of the family Annonaceae, which thrives in the tropics of West Africa and the Caribbean. Common names of this plant in Nigeria include churu, arwto, ehiri, airama, and akerewa, lubushi\(^2\). The seed is rich in oil and is of great value due to its medicinal and nutritional qualities\(^1\). The fruits and seeds are used as stimulants, stomachic, against headaches, and sores, and as a natural insect repellent. The essential oil contains compounds like α-phellandrene, α-pinene, myrcene, limonene, and pinene. The pleasant aroma, like nutmeg, makes this spice useful in the preparation of traditional dishes in Nigerian communities\(^6\). Spice seed extracts are believed to possess both antioxidant \(^2\) and antimicrobial properties\(^3\). *M. myristica* could be effective in the treatment of stomach aches, febrile pains, eye diseases, and hemorrhoids\(^3\), while *Tetrapleura tetraptera* helps to tackle diabetes mellitus, arthritis, hypertension, epilepsy, and asthma\(^2\).

Both *T. tetraptera* and *M. myristica* are among such common indigenous spices in Nigeria that are underutilized/valued, despite their sharp aroma and flavor that is highly perceptible by the sense of smell\(^4,4\). However, specific to West Africa sub-region, previous studies involving fruit, stem, and bark of *T. tetraptera* as well as seeds of *M. myristica* have largely focused on phytochemical properties of aqueous and methanolic and ethanolic extracts. There is a need, therefore, for further investigations into the essential oil and oleoresin of this *T. tetraptera* and *M. myristica*, specifically their chemical composition, antioxidant, and antibacterial properties and such studies would require respective activities of steam distillation maceration technique, which would help unravel their potential/relevance in the various communities in southeast Nigeria where they serve as a natural preservative to prevent food spoilage. To supplement existing information, this current work investigated the chemical composition, antibacterial efficacy, and antioxidant capacity of essential oil and oleoresin extracted from *T. tetraptera* fruit and *M. myristica* seeds cultivated in Southeast Nigeria. Specifically, the essential oil and oleoresin respectively extracted by steam distillation and aqueous maceration were subsequently subject to analytical tests in adherence to the relevant institutional guidelines. Furthering the knowledge and understanding underpinning the capacities of these extracted essential oil and oleoresin to tackle food spoilage challenges is warranted to help consolidate the product development potential of both *T. tetraptera* fruit and *M. myristica* seeds.

**Materials and methods**

**Schematic overview of the experimental program.** Figure 1 shows the schematic overview of the experimental program of this current study, which depicts the major stages from procurement of plant materials, and processing of plant parts extraction processes, before the subsequent analytical measurements. For emphasis, this conducted research was directed to understand how the extracted essential oil and oleoresin from *T. tetraptera* fruit and *M. myristica* seeds would thrive specific to the context of chemical composition, antibacterial efficacy (tested against *Escherichia coli* and *Staphylococcus aureus*), and antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl (DPPH)- radical scavenging, ferric reducing power (FRAP), and total antioxidant capacity (TAC). Additionally, the analytical measurements were carried out independently using the different essential oil and oleoresin samples obtained from individual batches of *T. tetraptera* and *M. myristica* seeds. Importantly, the analytical measurements conducted were in adherence to the relevant guidelines prescribed by the Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Furthermore, all chemicals and reagents used in this work, which were procured from reputable registered chemical retailers, were of analytical grade standard.

**Procurement and processing of plant materials.** Fresh *T. tetraptera* fruit (approx. 1000 g) and *M. myristica* seeds (approx. 800 g) were procured as two separate batches from a local market in Owerri Imo State, in the Southeast of Nigeria. Furthermore, Fig. 2 shows the photo images of *M. myristica* seeds (Fig. 2a) and *T. tetraptera* fruits (Fig. 2b). To further confirm the samples, the samples were identified at the Department of Plant Biotechnology of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The processing of fresh *T. tetraptera* fruit and *M. myristica* seeds was carried out following the method described by\(^4\) with modifications. Specifically, the spices were washed with clean running water, sun-dried (72 h), and then oven dried using a Memmert UN30 (Memmert GmbH + Co. KG, Schwabach, Germany) oven at 55 °C for 72 h. The *M. myristica* seeds were cracked manually to recover the nibs, whereas the fruits of *T. tetraptera* were cut into small pieces, and both were kept in ambient conditions until required for further processing.
Figure 1. The schematic overview of the experimental program of this current study, which depicts the major stages from procurement of plant materials, processing of plant parts extraction processes, prior to the subsequent analytical measurements.

Figure 2. Photo images of *Monodra myristica* (African nutmeg) seeds (a) and *Tetrapleura tetraptera* fruits (b).
Extraction processes of essential oil and oleoresin. Already cracked *M. myristica* seeds, and small pieced cut *T. tetraptera* fruit samples were individually ground into coarse and fine particles using an attrition type mill K-Tron type T-35 volumetric feeder (K-Tron Corp. Pitman NJ USA). The coarse particles were kept for essential oil extraction, whereas those of fine powder was for oleoresin extraction. On one hand, essential oil extraction involved the steam distillation method as described by Chemat et al. 15 with slight modification. Briefly, the conventional type steam distillation apparatus with a cylindrical Pyrex body supported by Teflon grid at its lower end, with a rastostat-controlled heating facility, was used. The essential oil was dried over anhydrous sodium sulfate (Na$_2$SO$_4$) and stored in airtight amber bottles at 4 °C until used. On the other hand, the oleoresin extraction involved the maceration technique modified from Fernández-Ronco et al. 16. For each spice, 200 g of finely ground spice was macerated in 400 mL of distilled water in a 1000 mL conical flask and tightly corked. This was shaken vigorously at 30-min intervals for 3 h, after which the supernatant was decanted and the residue was filtered out. After extraction, the supernatant from each spice was collected and evaporated at 60 °C in a stifling air oven (Memmert UN30, Memmert GmbH + Co. KG, Schwabach, Germany) for 8 h. The oleoresins were stored in airtight amber bottles and kept in the refrigerator until used.

Analytical measurements. Determination of chemical composition. The chemical composition of the crude essential oil and oleoresin samples was determined following the routine method employed in the laboratory of the Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. This involved the gas chromatograph mass spectrometry (GC–MS) analysis, specifically the Agilent 6890 gas chromatograph (Supelco, Bellefonte, PA, USA) equipped with an on-column automatic injector, flame ionization detector, and HP 88 capillary column (100 m x 0.25 μm film thickness). The carrier gas was helium at a constant flow rate of 1.0 mL/min. The detector was maintained at 250 °C and both injectors at 220 °C with integrator chart speed at 2 cm/min. The initial column temperature was set at 100 °C (hold time = ~ 2 min) to the final temperature of 180 °C at a rate of 50 °C/min, the volume injected was 1.0 μL and the split ratio was 50:1. Total chromatogram was auto-integrated by Shem-Station, and emergent chemical constituents were identified by comparison with both published mass spectral database (NIST02.L) and available literature data.

Determination of antioxidant capacities. Free radical scavenging activity. Free radical scavenging activity of the already extracted essential oil and oleoresin were measured using the radical 1,1-diphenyl-2-Picrylhydrazyl (DPPH) as described by Shah et al. 18 with slight modifications. This involved dissolving ~ 25 mg of DPPH in 100 mL methanol, and thereafter storing at 20 °C till needed. From the working solution obtained from the DPPH stock solution by methanol, 1 mL was added to 100 μL of various concentrations of test samples, incubated for 45 min, thoroughly shaken, and then left in the dark under ambient conditions. Ascorbic acid served as the standard, scavenging effect was calculated by Eq. (1):

\[
\text{% Scavenging Effect} = \left( \frac{A_{DPPH} - A_{EOIL}}{A_{DPPH}} \right) \times 100
\]

where $A_{DPPH}$ = absorbance of DPPH at 517 nm and $A_{EOIL}$ = absorbance of oils/resins at 517 nm.

Iron (III) to iron (II)-reducing power assay (FRAP). The ability of essential oil and oleoresin to reduce Fe$^{3+}$ to Fe$^{2+}$ was assessed by the method of Hinneburg, Dorman, and Hiltunen 19 with slight modifications. A 2-mL aliquot of sample was added to 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6, 2.5 mL) containing potassium ferricyanide [K$_3$Fe (CN)$_6$] (1%, 2.5 mL). After the reaction mixture had incubated at 50 °C for 25 min, then 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. Subsequently, the supernatant (5 mL) was mixed with 2.5 mL of water, and 0.5 mL of 0.1% aqueous FeCl$_3$, followed by an absorbance reading at 700 nm. Gallic acid was used as a standard reference compound.

Phosphomolybdate assay (total antioxidant capacity). The total antioxidant capacity (TAC) of the essential oil and oleoresin was determined by the phosphomolybdate method of Jan et al. 20 with slight modifications. An aliquot (~0.1-mL) of the sample was mixed with 1 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), thereafter covered and incubated in a water bath at 85 °C for...
Table 1. Major chemical compounds of essential oil and oleoresin found in *Monodora myristica*.

| S/N | Chemical compounds                  | *Monodora essential oil (%) | *Monodora oleoresin (%) | Retention time (min) |
|-----|-------------------------------------|----------------------------|-------------------------|----------------------|
| 1   | 1-α-Pinene                          | –                          | 2.60                    | 6.594                |
| 2   | α-Phellandrene                      | –                          | 6.40                    | 9.547                |
| 3   | α-Pinene                            | 8.71                       | –                       | 9.972                |
| 4   | β-Myrcene                           | 3.17                       | –                       | 12.556               |
| 5   | α-Phellandrene                      | 32.09                      | –                       | 12.969               |
| 6   | 2-Carene                            | 3.90                       | –                       | 13.907               |
| 7   | p-Cymene                            | 50.58                      | –                       | 13.832               |
| 8   | 1,2-(Methylene cyclopropyl) Cyclopendene | –                        | 2.65                    | 17.347               |
| 9   | 2,4-Dimethyl-1,3-Cyclopentadiene    | –                          | 2.93                    | 21.094               |
| 10  | 2-Acetyl-cyclopentanone             | –                          | 85.42                   | 21.401               |
| 11  | δ-Cadinene                          | 1.56                       | –                       | 31.259               |

75 min. When samples had been cooled, the absorbance was measured at 765 nm. The ascorbic acid was used as a standard reference compound. The TAC was estimated using the Eq. (2) below:

\[
TAC \, (\%) = \frac{(\text{Abs. of control} - \text{Abs. of sample})}{\text{Abs. of control}} \times 100 \tag{2}
\]

**Statistical analysis.** The data of three replicates from different samples were subjected to factorial approach analysis of variance (ANOVA). Results were represented as means ± standard error, with mean separation conducted using Fisher’s least significant difference (LSD) per measured variable. The probability level was statistically significant at p < 0.05 (95% confidence). Minitab 2018 software (Minitab, LLC, USA) was used to run the data.

**Results and discussion**

**Chemical composition of essential oil and oleoresin.** The main chemical compounds of essential oil and oleoresin of *M. myristica* and *T. tetraptera* are shown in Tables 1 and 2, with their respective GC–MS chromatograms shown in Figs. 3, 4, 5 and 6. Specific to *M. myristica*, the identified chemical compounds predominantly monoterpenes respectively totalled 6 and 5 in essential oil and oleoresin. Chemical compounds in essential oil would include p-cymene at 50.58% as peak, followed by α-phellandrene at 32.09%, with α-pinene at 8.71% as least, whereas in oleoresin would include, 2-acetyl cyclopentanone (85.42%) as predominant, with α-phellandrene (6.40%) as least. Only δ-cadinene (1.56%) appeared in the sesquiterpene but very little. Previous reports about different extracts of *M. myristica* specific to Nigeria obtained variations in chemical constituents. For example, a report of seed oil of *M. myristica* from southwest Nigeria obtained major constituents like α-phellandrene epoxide (3.02%), carvacrol (2.09%), and δ-cadinene (2.21%)21, as well as p-cymene (31.5%), α-phellandrene (18.1%), α-pinene (6.1%), β-pinene (5.1%)22. Elsewhere also, essential oil of *M. myristica* obtained by steam distillation showed chemical constituents like α-phellandrene (50.4%), α-pinene (5.5%), myrcene (4.35%), and germacrene-D-4-ol (9.0%)23, whereas another seed oil reported elsewhere showed chemical constituents like germacrene-D-4-ol (25.48%), tricyclo[5.2.1(1,5)] dec-2-ene (13.35%), δ-cadinene (11.09%) and linalool (15.10%), and from their stem barks with chemical constituents like γ-cadinene (31.31%), α-elemene (17.98)23. In a neighbouring country of Chad and Cameroon, the essential oils extracted by hydrodistillation from fruits of *Xylopia aethiopica* (Dunal) A. Rich, *Xylopia parviflora* (A. Rich) Benth. and *M. myristica* (Gaertn) showed α-phellandrene (Chad = 52.7%; Cameroon = 67.1%) as a major constituent24. Specific to *T. Tetraptera*, the chemical compounds of essential oil and oleoresin respectively totalled 27 and 16 (Tables 2 and 3, as well as Figs. 2, 3, 4 and 5). On one hand, the essential oil comprised carboxylic acids, phenolic acids, and esters, for example, 1-naphthalencarbonitrile, 2-methoxy- (8.26%), dodecanedioic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (7.90%), benzenamine, 4-methoxy-N-(triphenylphosphoranylidene)- (7.78%), 4-dibenzo furanamine (7.08), etc. On the other hand, the oleoresin comprised terpenes, for example, γ-terpinene (25.63%), linalool (20.74%), caryophyllene (9.68%), β-pinene (9.38%), 2-carene (6.98), γ-cadinene (5.47%), etc. Terpenes were not found in the essential oil of *T. tetraptera*. The essential oil extracted from *T. tetraptera* fruits via hydro-distillation by Erukainure et al.4 largely showed acetate and carboxylic acid, with little traces of terpenes. Probably, the presence of linalool might account for the plant’s peppery nature, whereas both γ-terpinene and α-thujene might account for the flavor/fragrance. Elsewhere, the root and stem ethanol extract of *T. tetraptera* spicce respectively obtained 70.14%25 and 32.01%26. Polyphenols like eugenol, quercetin, rutin, and tyrosol, according to Moukette et al.1, were considered 5.88–10.79-fold more concentrated in the water and ethanolic extracts of the fruits compared to the barks. Furthermore, α-phellandrene and α-pinene has been identified as a major chemotype for *M. myristica* seeds, whereas cirtal and linalool as major chemotypes of *T. tetraptera*4,21,22. Such factors as environmental influences, experimental conditions, place of origin, post-harvest handling of the fruits, adaptive metabolism of plants, and
plant part analyzed might be responsible for the variations in the chemical compounds that have been detected in *M. myristica* and *T. tetraptera* samples at this current work.

**Antibacterial efficacy of essential oil and oleoresin.** As mentioned earlier in this paper, there is evidence that the extract of *T. tetraptera* would exhibit very promising antimicrobial activity against Gram-positive/-negative bacteria\(^4,12\). Table 3 shows the antibacterial inhibition zone of essential oils and oleoresins of *M. myristica* and *T. tetraptera*, which appeared concentration-dependent to the bacterial pathogens. Whereas oleoresin obtained the highest susceptibility to *E. coli*, those of *M. myristica* together with the essential oil of *T. tetraptera* showed some resistance to *S. aureus*. Considering that the inhibition zone qualitatively deciphers the potentials of an antibacterial agent, the essential oil of *M. myristica* at concentration of 75 µg/mL obtained the highest zone (16 mm) against *E. coli* whereas the essential oil from *T. tetraptera* obtained the least activity.

**Table 2.** Major chemical compounds of essential oil and oleoresin found in *Tetraplura tetraptera*.

| S/N | Chemical compounds                                                                 | Retention time | Area (%) Essential oil | Area (%) Oleoresin |
|-----|-----------------------------------------------------------------------------------|----------------|------------------------|--------------------|
| 1   | 1-Oxa-4azaspiro [4.5] decan-4-oxyl,3,3-dimethyl-8-oxo-                            | 5.384          | 0.05                   |                    |
| 2   | α-Thujene                                                                       | 6.482          | 3.87                   |                    |
| 3   | 1-Octyn-3-ol                                                                     | 6.623          | 0.13                   |                    |
| 4   | 1,3-Cyclohexadiene, 5-ethyl-                                                     | 7.384          | 0.01                   |                    |
| 5   | 1,3-Cyclopentadiene, 5,5-dimethyl-                                               | 8.229          | 0.01                   |                    |
| 6   | β-Pinene                                                                        | 8.483          | 9.38                   |                    |
| 7   | Cyclopentyl acetylene                                                            | 8.567          | 0.01                   |                    |
| 8   | β-Phellandrene                                                                  | 9.272          | 2.47                   |                    |
| 9   | 1H-Imidazole-2,4(3H,5H)-dione, 5-phenyl-5-(2-pyridinyl)-                         | 9.750          | 0.05                   |                    |
| 10  | (±)-4-Carene                                                                    | 10.129         | 2.81                   |                    |
| 11  | Terpinolene                                                                     | 10.523         | 2.68                   |                    |
| 12  | γ-Terpinene                                                                     | 12.362         | 25.63                  |                    |
| 13  | Tetrasiloxane, decamethyl-                                                       | 12.820         | 3.69                   |                    |
| 14  | 3-Bromobenzoic acid, 10-undecenylester                                          | 13.384         | 5.85                   |                    |
| 15  | Cyclotrisiloxane, hexamethyl-                                                    | 13.835         | 5.90                   |                    |
| 16  | 2-Carene                                                                        | 13.388         | 6.98                   |                    |
| 17  | Benz(cd)indol-2-(1H)-one, 1-methyl-                                              | 14.032         | 4.68                   |                    |
| 18  | Carazolol                                                                       | 14.229         | 6.03                   |                    |
| 19  | Linalool                                                                         | 14.388         | 20.74                  |                    |
| 20  | 4-Dibenzo[1,5]furanamine                                                         | 14.454         | 7.08                   |                    |
| 21  | Dodecanic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester                          | 14.708         | 7.90                   |                    |
| 22  | Dodecanic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester                          | 14.933         | 6.29                   |                    |
| 23  | Dodecaneic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester                         | 15.187         | 6.97                   |                    |
| 24  | Benzenamine, 4-methoxy-N-(triphenylphosphoryliden)                               | 15.468         | 7.78                   |                    |
| 25  | Phenoxazine                                                                      | 15.694         | 5.40                   |                    |
| 26  | Phenoxazine                                                                      | 16.004         | 6.23                   |                    |
| 27  | 1-Naphthalene carbonitrile, 2-methoxy-                                           | 16.483         | 8.26                   |                    |
| 28  | Terpinen-4-ol                                                                   | 16.509         | 1.42                   |                    |
| 29  | 1,2-Cyclobexaneedicarboxylic acid, 4-bromophenyl ethyl ester                    | 16.736         | 5.03                   |                    |
| 30  | Benzenamine, 4-methoxy-N-(triphenylphosphoryliden)                               | 16.849         | 1.54                   |                    |
| 31  | 2,5-Cyclobexadien-1-one, 4-(phenylimino)-                                        | 17.018         | 5.98                   |                    |
| 32  | α-Terpinol                                                                       | 17.053         | 1.58                   |                    |
| 33  | Fumaric acid, 2,3-dichlorophenyl isoxenyl ester                                 | 17.299         | 4.84                   |                    |
| 34  | 2-Hydroxycarbazole                                                               | 17.638         | 2.74                   |                    |
| 35  | 4-Nitrophenyl laurate                                                            | 18.004         | 2.42                   |                    |
| 36  | Dodecanic acid, 2,4,5-trichlorophenyl ester                                     | 18.342         | 1.71                   |                    |
| 37  | Dodecanic acid, 1,2,3-propanetriyl ester                                         | 18.736         | 0.91                   |                    |
| 38  | Caryophyllene                                                                   | 24.597         | 9.68                   |                    |
| 39  | Humulene                                                                         | 25.467         | 1.30                   |                    |
| 40  | γ-elemene                                                                        | 26.887         | 5.47                   |                    |
| 41  | α-Farnesene                                                                     | 27.462         | 3.59                   |                    |
| 42  | δ-Cadinene                                                                      | 27.687         | 0.92                   |                    |
| 43  | β-Sinensal                                                                       | 34.312         | 1.48                   |                    |

Table 2. Major chemical compounds of essential oil and oleoresin found in *Tetraplura tetraptera*. 

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Elsewhere, inhibition zones of 8 mm have been reported and 13 mm for 2.5 mg/mL and 5.0 mg/mL, respectively, of essential oil *M. myristica* against *E. coli*\(^3\), whereas 11 mm for 2 mL also of *M. myristica* essential oil against *E. coli*\(^4\). Another study showed *T. tetraptera* stem extract against two *Salmonella* strains, with a remarkably high inhibition zone (> 20 mm diameter)\(^5\), besides the absence of carvone component believed as responsible for the weak activity of oleoresins\(^27,28\). Comparatively, the promising antibacterial activity of essential oil from *M. myristica* might be dependent on factors like chemical composition and its solubility\(^1\). The extraction process, cell wall composition of microbial entity, environmental conditions, chemotype of the plant parts used (which often influences biosynthetic pathways), as well as other physiological properties might also potential factors that contribute to the differences in the antibacterial activity.
Natural compounds (terpenes inclusive), which are secondary metabolites from plants, are responsible for the defense against pathogens and promising in controlling pathogenic/spoilage microbial entities in foods. The survival of the E. coli and S. aureus population involving different concentrations of Mondora myristica and Tetrapleura tetraptera essential oil and oleoresin can be seen in Table 4. Of both spices, the survival of E. coli and S. aureus population appeared significantly higher (p < 0.05) in the essential oil above those of oleoresin. Within three (3) days of storage, specific to concentrations 25 and 50 µg/mL of essential oil from T. tetraptera,
the pathogen population increased markedly \( (p < 0.05) \) at \( 2.15 \times 10^5 \) and \( 1.53 \times 10^5 \) CFU/mL (\( E. \) coli) and \( 2.5 \) and \( 2.03 \times 10^5 \) CFU/mL (\( S. \) aureus), respectively. Within 3 days of storage, however, no bacteria proliferated in \( T. \) tetraptera oleoresin as well as \( M. \) myristica essential oil and oleoresin. By day 5, the \( T. \) tetraptera essential oil respectively showed \( 7.05 \) and \( 6.10 \times 10^5 \) CFU/mL for \( E. \) coli and \( S. \) aureus (Table 5). However, \( S. \) aureus obtained \( 6.51 \) and \( 4 \times 10^7 \) CFU/mL at 25 and 50 µg/mL for \( M. \) myristica essential oil, significantly different \( (p < 0.05) \) from \( 1.5 \times 10^4 \) CFU/mL at 25 µg/mL for \( T. \) tetraptera oleoresin. Gram-positive bacteria are believed to reveal more susceptibility to antibacterial compounds compared to Gram-negative bacteria\(^{28,30}\), given the lipoproteins and

| Spices          | Concentration (µg/mL) | Escherichia coli | Staphylococcus aureus |
|-----------------|-----------------------|------------------|-----------------------|
| MM oleoresin    | 50                    | 12               | -                     |
|                 | 75                    | 13               | -                     |
| TT oleoresin    | 50                    | 13               | -                     |
|                 | 75                    | 15               | 8                     |
| MM essential oil| 50                    | 13               | 11                    |
|                 | 75                    | 16               | 13                    |
| TT essential oil| 50                    | -                | -                     |
|                 | 75                    | -                | -                     |

Table 3. Antibacterial zone of inhibition (mm) of \( M. \) myristica and \( T. \) tetraptera essential oils and oleoresins. M.M = \( M. \) myristica, T.T = \( T. \) tetraptera.

| Spices          | Storage period (day) | Escherichia coli | Staphylococcus aureus |
|-----------------|----------------------|------------------|-----------------------|
|                 |                      | Concentration (µg/mL) |                       |
|                 |                      | 25 | 50 | 25 | 50 |                      |
| M.M; essential oil | 1                   | \( 2 \times 10^5 \) ± 4 \( \times 10^5 \) ± 7 \( \times 10^5 \) ± 7 \( 6.45 \times 10^7 \) ± 707 |                       |
|                 | 3                   | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 |                       |
|                 | 5                   | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 6.51 \times 10^7 \) ± 212 | \( 4 \times 10^7 \) ± 0 |                       |
| M.M; oleoresin  | 1                   | \( 5 \times 10^5 \) ± 134 | \( 7.05 \times 10^7 \) ± 707 | \( 7 \times 10^7 \) ± 0 |                       |
|                 | 3                   | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 |                       |
|                 | 5                   | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 |                       |
| T.T; essential oil | 1                   | \( 6.65 \times 10^7 \) ± 212 | \( 4.90 \times 10^7 \) ± 134 | \( 9.05 \times 10^7 \) ± 707 | \( 7.01 \times 10^7 \) ± 141 |                       |
|                 | 3                   | \( 2.15 \times 10^7 \) ± 212 | \( 1.53 \times 10^7 \) ± 354 | \( 2.5 \times 10^7 \) ± 0 | \( 2.03 \times 10^7 \) ± 353 |                       |
|                 | 5                   | \( 7.05 \times 10^7 \) ± 707 | \( 7.05 \times 10^7 \) ± 707 | \( 6.10 \times 10^7 \) ± 141 | \( 6.10 \times 10^7 \) ± 141 |                       |
| T.T; oleoresin  | 1                   | \( 4 \times 10^7 \) ± 0 | \( 2 \times 10^7 \) ± 0 | \( 8 \times 10^7 \) ± 0 | \( 8 \times 10^7 \) ± 0 |                       |
|                 | 3                   | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 |                       |
|                 | 5                   | \( 0^\circ \) ± 0 | \( 1.5 \times 10^4 \) ± 707 | \( 0^\circ \) ± 0 | \( 0^\circ \) ± 0 |                       |

Table 4. Survival of \( E. \) coli and \( S. \) aureus population \( (10^7 \times \text{CFU/mL}) \) involving different \( M. \) myristica and \( T. \) tetraptera essential oils and oleoresin concentrations. Means with different superscript are significantly different. M.M = \( M. \) myristica, T.T = \( T. \) tetraptera.

| Extracts | Concentrations (µg/mL) | Ascorbic acid | M.M essential oil | M.M oleoresin | T.T essential oil | T.T oleoresin |
|----------|-------------------------|---------------|------------------|---------------|-----------------|--------------|
|          | 10                      | 92.60±1.78    | 62.4±17.54       | 66.47±1.63    | 59.37±3.62      | 68.38±3.21   |
|          | 20                      | 93.20±2.49    | 54.01±0.98       | 65.41±2.73    | 58.59±1.83      | 66.77±2.76   |
|          | 40                      | 95.82±2.33    | 53.76±1.87       | 64.85±2.21    | 61.34±1.83      | 70.54±0.91   |
|          | 80                      | 93.15±1.63    | 60.61±3.45       | 66.01±2.54    | 60.18±1.39      | 74.52±1.09   |
|          | 160                     | 92.43±3.68    | 64.70±1.50       | 70.80±1.61    | 61.05±1.43      | 83.89±0.91   |
|          | 320                     | 90.43±1.07    | 72.82±0.94       | 76.38±1.80    | 57.25±2.75      | 92.35±1.46   |
|          | 640                     | 87.73±3.67    | 79.3±0.17        | 77.09±2.97    | 56.75±1.38      | 93.4±0.92    |

Table 5. DPPH radical scavenging activity of essential oils and oleoresins (%) at different concentrations in \( M. \) myristica and \( T. \) tetraptera spices. Means with the same superscript are not significantly different. M.M = \( M. \) myristica, T.T = \( T. \) tetraptera.
seeds could concur with maximum concentration of 120 µg/mL, contrasting 10–41% when concentration is enhanced antioxidant properties. Previous reports show roughly 29–37.7% scavenging powers of *M. myristica* (93.46%) that appeared significantly higher (p < 0.05) compared to the others, which specifically demonstrates *T. tetraptera* example, the highest concentration (640 µg/mL) of extracts1,4,36. Typically through the reduction of DPPH, the radical scavenging compounds reducing power4, although the higher reducing power for ethanolic and hydroethanolic extracts of fruit and bark fruit peel (at similar concentrations higher than 100 µg/mL) might possess the lesser extract of *T. tetraptera* hydrogel molecule37. Of all reactive oxygen species, the OH radical appears the stronger, and able to react with most biological molecules found in living cells. Among effective defences of living body against various diseases and hydroethanolic  extract of *M. myristica* oil could  resemble1. Acting as a defense mechanism against lipid peroxidation, the antioxidants in plant materials would bind to metal ions by reducing transition metals such as Fe2+ or Cu+33. Further, the reducing power of lipopolysaccharides present in the cellular wall/barrier-like structure that restricts the entry of hydrophobic compounds31. In essential oil, however, presence of terpenes should provide the major antibacterial capacity. More so, some simple phenols might appear in the form of phenylpropanoids32. Besides essential oils and oleoresins of this current work being concentration-dependent, their complex mix of several (minor) chemical components would assert multifunctional synergistic effects33,34.

### Antioxidant capacity of essential oil and oleoresin.

The DPPH radical scavenging activity of essential oils and oleoresins (%) at different concentrations in *M. myristica* and *T. tetraptera* spices can be seen in Table 5. In general, the extracts showed promising scavenging activity with a maximum of 93.46% and a minimum of 53.76%. DPPH radical scavenging activity increased with concentration except for *T. tetraptera* essential oil. The oleoresin of *T. tetraptera* showed the better DPPH radical scavenging activity across samples’ concentrations. For example, the highest concentration (640 µg/mL) of *T. tetraptera* oleoresin obtained maximum scavenging power (93.46%) that appeared significantly higher (p < 0.05) compared to the others, which specifically demonstrates enhanced antioxidant properties. Previous reports show roughly 29–37.7% scavenging powers of *M. myristica* seeds could concur with maximum concentration of 120 µg/mL, contrasting 10–41% when concentration is maximum at 400 µg/mL7. Lower DPPH scavenging has been recorded at 360 µg/mL (TAC value = 8.76). The TAC recorded in *M. myristica* oil obtained the peak TAC value (15.75) at 640 µg/mL concentration, significantly higher (p < 0.05) than that of 360 µg/mL (TAC value = 8.76). *T. tetraptera* oleoresin obtained the second-highest absorbance values, while *M. myristica* oleoresin had the least absorbance levels.

### Table 6. Reducing power activity of essential oils and oleoresins (%) at different concentrations in *M. myristica* and *T. tetraptera* spices. Means with different superscript are significantly different. M.M = Monodora myristica, T.T = Tetrapleura tetraptera.

| Samples         | Concentrations (µg/mL) | 10   | 20   | 40   | 80   | 160  | 320  | 640  |
|-----------------|------------------------|------|------|------|------|------|------|------|
| Ascorbic acid   | 0.08 ± 0.01            | 0.04± 0.01 | 0.03± 0.00 | 0.05± 0.00 | 0.06± 0.00 | 0.06± 0.00 | 0.07± 0.01 |
| M.M essential oil | 0.30 ± 0.30            | 0.15± 0.01 | 0.32± 0.02 | 0.72± 0.04 | 1.40± 0.10 | 3.23± 0.07 | 5.81± 2.45 |
| M.M oleoresin   | 0.11 ± 0.02            | 0.29± 0.01 | 0.40± 0.05 | 2.05± 0.34 | 4.32± 1.63 | 8.76± 3.14 | 15.75± 5.51 |
| T.T essential oil | 0.07± 0.00             | 0.14± 0.01 | 0.25± 0.05 | 0.48± 0.21 | 1.12± 0.09 | 2.87± 0.12 | 6.10± 0.56 |
| T.T oleoresin   | 0.08± 0.00             | 0.14± 0.02 | 0.32± 0.02 | 0.67± 0.06 | 1.42± 0.01 | 2.93± 0.29 | 6.97± 2.96 |

### Table 7. Total antioxidant capacity of essential oils and oleoresins (%) at different concentrations in *M. myristica* and *T. tetraptera* spices. Means with different superscript are significantly different. M.M = Monodora myristica, T.T = Tetrapleura tetraptera.

| Samples         | Concentrations (µg/mL) | 10   | 20   | 40   | 80   | 160  | 320  | 640  |
|-----------------|------------------------|------|------|------|------|------|------|------|
| Ascorbic acid   | 0.04± 0.01             | 0.04± 0.01 | 0.03± 0.00 | 0.05± 0.00 | 0.06± 0.00 | 0.06± 0.00 | 0.07± 0.01 |
| M.M essential oil | 0.30± 0.30             | 0.15± 0.01 | 0.32± 0.02 | 0.72± 0.04 | 1.40± 0.10 | 3.23± 0.07 | 5.81± 2.45 |
| M.M oleoresin   | 0.11± 0.02             | 0.29± 0.01 | 0.40± 0.05 | 2.05± 0.34 | 4.32± 1.63 | 8.76± 3.14 | 15.75± 5.51 |
| T.T essential oil | 0.07± 0.00             | 0.14± 0.01 | 0.25± 0.05 | 0.48± 0.21 | 1.12± 0.09 | 2.87± 0.12 | 6.10± 0.56 |
| T.T oleoresin   | 0.08± 0.00             | 0.14± 0.02 | 0.32± 0.02 | 0.67± 0.06 | 1.42± 0.01 | 2.93± 0.29 | 6.97± 2.96 |
this current work seemed higher than those of *M. myristica*\(^9\), including those of aqueous and ethanolic extracts of fruits, pulps, and seeds of *T. tetraptera*. Nonetheless and for emphasis, the TAC assay is based on the ability of a reducing agent (antioxidant) to reduce molybdenum (VI) ions to molybdenum (V) giving a green phosphomolybdate (V) complex at 765 nm.\(^{20,37,38}\)

**Conclusions**

In this study and from *M. myristica* and *T. tetraptera* spices found in the Southeast of Nigeria, both essential oil and oleoresin were secured, respectively, by steam distillation and maceration. Very useful compounds were obtained, through which some considerable antibacterial and antioxidant potentials were exhibited. Essential oil of *M. myristica* appeared to have enhanced antibacterial and antioxidant activity compared to the others. The *T. tetraptera*, however, seemed less effective to inhibit both *E. coli* and *S. aureus* populations. The extraction process, environment, and genetics would be crucial influences on the natural compounds found in each spice's essential oil and oleoresin, which would in turn strongly impact (both *M. myristica* and *T. tetraptera* spices’) antibacterial and antioxidant capabilities, and hold great promise in food preservation, especially against the autoxidation and microbial aspects of (food) spoilage. Considering the findings of this current work, the direction of future investigations should be to establish the purity of these chemical constituents of essential oil and oleoresin obtained from *M. myristica* and *T. tetraptera* spices, and their corresponding cytotoxic activities, which could possibly help better the understanding that underpin their pharmacological and other associated/related effects.

**Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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**References**

1. Moukette, B. M. et al. In vitro ion chelating, antioxidative mechanism of extracts from fruits and barks of *Tetrapleura tetraptera* and their protective effects against fenton mediated toxicity of metal ions on liver homogenates. *Evid.-based Complement. Altern. Med.* 2015, 423689. https://doi.org/10.1155/2015/423689 (2015).
2. Omar, J. et al. Quantitative analysis of bioactive compounds from aromatic plants by means of dynamic headspace extraction and multiple headspace extraction-gas chromatography-mass spectrometry: quantitative analysis of bioactive compound. *J. Food Sci.* 81, C867–C873. https://doi.org/10.1111/jfsc.13257 (2016).
3. David, O. M., Ojo, O. O., Olumekun, V. O. & Famurewa, O. Antimicrobial activities of essential oils from *Monodora myristica* (Gaertn Dunal) and *Xylopia aethiopica* (Dunal A. Rich) seeds. *Br. J. Appl. Sci. Technol.* 4, 3332–3341 (2014).
4. Erukainure, O. L. et al. Ethanol extract of *Tetrapleura tetraptera* fruit peels: Chemical characterization, and antioxidant potentials against free radicals and lipid peroxidation in hepatic tissues. *J. Taibah Univ. Sci.* 11, 861–867. https://doi.org/10.1016/j.jtusci.2017.03.007 (2017).
5. Udoh, G. A. & Etukudo, M. F. Essential oils and fatty acids composition of dry fruits of *Tetrapleura tetraptera*. *J. Appl. SCI. Environ. Manag.* 18, 419–424 (2014).
6. Ezeuko, A. S., Bamgboye, O. A., Jonathan, H. & Uchenna, D. Extraction, physicochemical, phytochemical analysis, and identification of some important compounds of *Monodora myristica* (African nutmeg) seed oil. *IIJRAS.* 4, 406–410 (2017).
7. Akinwunmi, K. F. & Oyedapo, O. O. Evaluation of antioxidant potentials of *Monodora myristica* (Gaertn) duneel seeds. *Afr. J. Food Sci.* 7, 317–324 (2013).
8. Adusei, S., Otchere, J. K., Oteng, P., Mensah, R. Q. & Tei-Mensah, E. Phytochemical analysis, antioxidant, and metal chelating capacity of *Tetrapleura tetraptera*. *Heliyon* 5, e02762. https://doi.org/10.1016/j.helijon.2019.e02762 (2019).
9. Ogbonugafor, H. A., Ugochukwu, C. G. & Kyrian-Oghonna, A. E. The role of spices in nutrition and health: A review of three popular spices used in Southern Nigeria. *Food Qual. Saf. J.* 1, 171–185. https://doi.org/10.1093/fqsj/fxy020 (2017).
10. Aygeman, K. et al. Antibacterial activity, and mechanism of *Tetrapleura tetraptera* stem extract against Salmonella strains and its application in raw chicken meat. *J. Food Process. Preserv.* 45, e14489 (2021).
11. Mbagang, A. T. et al. Cytotoxic phytochemicals from the crude extract of *Tetrapleura tetraptera* fruits towards multi-factorial drug resistant cancer cells. *J. Ethnopharmacol.* 267, 113632. https://doi.org/10.1016/j.jep.2020.113632 (2021).
12. Ekwenye, U. N. & Okorie, C. F. Antibacterial activity of *Tetrapleura tetraptera* Toub.pod extracts. *Int. J. Pharm. Biol. Sci.* 1, 731–741 (2010).
13. Owokotamo, I. & Ekundayo, O. Comparative study of the essential oils of *Monodora myristica* from Nigeria. *Eur. Chem. Bull.* 1, 263–265. https://doi.org/10.17628/ECB.2012.1.263 (2012).
14. Abayeh, O. J., Abdulrazaz, A. K. & Oluagbi, R. Quality characteristics of Canarium schweinfurthii Engl. oil. *Plant Foods Hum. Nutr.* 54, 43–48 (1999).
15. Chernet, F. et al. Microwave accelerated steam distillation of essential oil from lavender: A rapid, clean, and environmentally friendly approach. *Anal. Chim. Acta* 555, 157–160 (2006).
16. Fernández-Ronco, M. P., Gracia, L., de Lucas, A. & Rodríquez, J. F. Extraction of *Capicum annuum* oleoresin by maceration and ultrasound-assisted extraction: Influence of parameters and process modeling. *J. Food Process Eng.* 36, 343–352 (2013).
17. Balouti, M., Sadiki, M. & Issouadi, S. K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* 6, 71–79. https://doi.org/10.1016/j.jpha.2015.11.005 (2016).
18. Shah, N. A. et al. Investigation on flavonoid composition and anti-free radical potential of *Sida cordata*. *BMC Complement. Altern. Med.* 13, 276 (2013).
19. Hinneburg, I., Dorman, H. J. D. & Hiltunen, R. Antibacterial activities of extracts from selected culinary herbs and spices. *Food Chem.* 97, 122–129 (2006).
20. Jan, S. Khan, M. R., Rashid, U. & Bokhari, J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of Monotheca buxifolia fruit. *Osong. Public Health Res. Perspect.* 4, 246–254 (2013).
21. Igwe, C. C., Yayi, E. & Moudachirou, M. Chemical constituents of the solvents extracted and hydrodistilled essential oils of African nutmeg (*Monodora myristica*) and turmeric (*Curcuma domestica*) in Southwest Nigeria. *Niger. Food J.* 1, 21–32 (2005).
22. Owolabi, O. S. et al. Bioactivity of three plant derived essential oils against the maize weevils *Sitophilus zeamais* (Motschulsky) and cowpea weevil *Callosobruchus maculatus* (Fabricius). *Elec. J. Environ. Agricul. Food Chem.* 8, 828–835 (2009).
23. Onyenekwe, P. C., Ogbadu, G., Deslaurier, H., Gagnon, M. & Collin, G. J. Volatile constituents of the essential oils of Mondora myristica (Gaertn.) dunal, J. Sci. Food Agric. 61, 379–381 (1993).
24. Bakarnga-Via, I. et al. Composition and cytotoxic activity of essential oils from Xylopia aethiopica (Dunal) A. Rich, Xylopia parviflora (A. Rich) Benth. and Mondora myristica (Gaertn) growing in Chad and Cameroon. BMC Complement. Altern. Med. 14, 1–8. https://doi.org/10.1186/1472-6882-14-125 (2014).
25. Lin, L., Agymang, K., Abdel-Samie, M. A. & Gui, H. Antibacterial mechanism of Tetrapleura tetraptera extract against Escherichia coli and Staphylococcus aureus and its application in pork. J. Food Saf. 39, e12693 (2019).
26. Adeyemo, E. et al. Phytochemical, antimicrobial, and Ge-Ms of African nutmeg (Mondora Myristica). Int. J. Pharm. Sci. Invent. 2, 25–32 (2013).
27. Martino, L. D., Feo, V. D., Formisano, C., Mignola, E. & Senatore, F. Chemical composition & antimicrobial activity of the essential oils from three chemotypes of origanum vulgare Isp. hirtum (link) letswaart growing wild in Campania (Southern Italy). Molecules 14, 2735–2746 (2009).
28. Singh, S. et al. Comparative studies of chemical composition, antioxidant and antimicrobial potentials of essential oils and oleoresins obtained from seeds and leaves of Anethum graveolens L. Toxicol. Open Access 3, 119. https://doi.org/10.4172/2476-2067.1000119 (2017).
29. Pichersky, E. Biosynthesis of plant volatiles: Nature’s diversity and ingenuity”. Science 311, 808–811. https://doi.org/10.1126/science.1118510 (2006).
30. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol. 94, 223–253 (2004).
31. Cox, S. D. & Markham, J. L. Susceptibility, and intrinsic tolerance of Pseudomonas aeruginosa to selected plant volatile compounds. J. Appl. Microbiol. 103, 930–936 (2007).
32. Aldred, E. Phenols. In Pharmacology: A Handbook for Complementary Healthcare Professionals (eds Aldred, E. et al.) 149–166 (Churchill Livingstone, 2009).
33. Koroch, A. R., Rodolfo Juliani, H. & Zygadlo, J. A. Bioactivity of essential oils and their components. In Flavours and Fragrances (ed. Berger, R. G.) 87–115 (Springer, 2007).
34. Singh, S. et al. Comparison of chemical composition, antioxidant and antimicrobial potentials of essential oils and oleoresins obtained from seeds of brassica juncea and sinapis Alba. MOJ Food Process. Technol. (MOJFPT) 4, 113–120. https://doi.org/10.15406/mojfpt.2017.04.00100 (2017).
35. Uyoh, E. A., Chukwurah, P., Akarika, C. R. & Antia, V. A. Potentials of two Nigerian spices—Piper nigrum and Mondora myristica as sources for cheap natural antioxidants. Am. J. Plant Sci. 4, 1105–1115. https://doi.org/10.4236/ajps.2013.45137 (2013).
36. Famobuwa, O., Lajide, L., Owolabi, B., Osuo, I. & Amuho, U. Antioxidant activity of the fruit and stem bark of Tetrapleura tetraptera Gaub (Mimosaceae). Br. J. Pharm. Res. 9, 1–4. https://doi.org/10.9734/bjprr/2016/21462 (2016).
37. Ahmed, D., Khan, M. M. & Saeed, R. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts of Adiantum caudatum leaves. Antioxidants 4, 394–409. https://doi.org/10.3390/antiox4020394 (2015).
38. Jee-Young, J., Pyo-jam, P. & Se-kwon, K. Antioxidant activity of a peptide isolated from Alaska Pollack (Theragra chalcogramma) frames protein hydrolysate. Food Res. Int. 38(1), 45–50. https://doi.org/10.1016/j.foodres.2004.07.005 (2005).
39. Mashiwani, Z., Khan, M. A., Irum, S. & Ahmad, M. Antioxidant potential of root bark of Berberis lycium Boyle, from Galliyat, western Himalaya, Pakistan. Pak. J. Bot. 231–234 (2013)

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Author contributions
Q.N.O., and E.U.U., were associated with conceptualization; data curation; formal analysis; investigation; methodology; and wrote the original draft; C.E.O. was associated with data curation; formal analysis; methodology; and reviewed and edited the final manuscript; S.J. was associated with funding acquisition, visualization, methodology, reviewed and edited the final manuscript; C.O.R.O. was associated with methodology, funding acquisition, project administration, validation, visualization, reviewed and edited the final manuscript. All authors agree to the last version submitted for publication.

Competing interests
The authors declare no competing interests.

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