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ASSESSMENT OF THE MEDICINAL PROPERTIES AND COMPONENTS OF THE BLEND OF THREE INDIGENOUS ESSENTIAL OILS (*Syzygium aromaticum*, *Monodora myristica*, AND *Xylopia aethiopica*) FROM AFRICA

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ABSTRACT

The essential oils of *Syzygium aromaticum, Monodora myristica, and Xylopia aethiopica* have been used widely in Africa for medicinal purposes. This work is aimed at finding out the combined medicinal efficacy of the three essential oils. The crude EOs were extracted by the hydrodistillation method. The chemical components were determined by GC-MS analysis. The phytochemicals, antidiabetic, anti-inflammatory, and antioxidant activities were determined by standard analytical methods. The GC-MS analysis indicated eugenol (75.08%) as the major component in the EO of *S. aromaticum*, isocaryophyllene (29.36%) in the EO of *M. myristica*, isospathulenol (8.67%) in the EO of *X. aethiopica*, and eugenol (34.25%) in the blend of the EOs. The phytochemicals in all the EOs and the blend were at varying values. α-amylase and α-glucosidase inhibition showed that the EO blend with an IC_{50} value of 1250.69 µg/mL and 1080.56 µg/mL, respectively, had the highest inhibition compared with other EOs. *S. aromaticum* had the highest activity against the anti-inflammatory indicators. The least inhibitory activity for DPPH was recorded with *M. myristica* EO. *S. aromaticum* recorded the highest inhibitory efficacy against ABTS and nitric oxide assays, respectively. The blend recorded the highest inhibitory activity against lipid peroxidation, with an IC_{50} value of 827.22 µg/mL. The findings demonstrated that the crude EOs and the blend exhibited medicinal activities. However, the EO blend had higher potency.

Keywords: Essential oil, Blend, Antioxidant, Anti-inflammatory, Anti-diabetic.

INTRODUCTION

Naturally occurring substances that have a wide range of pharmacological effects can be found abundantly in plants (Samtiya et al., 2021). Products from plants are good sources of novel medications because of this attribute, as well as their exceptional chemical variety. Plant components and secondary metabolic profiles can also help with biological grouping, especially for plants that adjust morphologically to varying regions or settings (Samtiya et al., 2021).

Essential oils (EOs) are taken from the bark, leaves, roots, and stems of plants. It's also become more popular recently to employ natural or plant-based medicinal remedies. Essential oils are among the most abundant natural product families and possible sources of physiologically active compounds. They are also among the most promising and extensively studied plant secondary metabolites (Samtiya et al., 2021). When comparing to various natural medications, the percentage of essential oils used in these natural treatments is seventy percent (Yeshi et al., 2022). It has been possible to extract around 3000 essential oils thus far, most of them from the Rutaceae, Myrtaceae, Zingiberaceae, and Asteraceae families. Of these, over 300 essential oils are marketed in the culinary and perfume industries, and a value of over $15 billion is predicted by 2025 (Yeshi et al., 2022).

The naturally occurring substances included in essential oils provide them with a variety of advantageous qualities. In addition to their excellent tolerance and efficacy when tested in human and livestock trials for both the avoidance and management of ailments, such as tumours and the syndrome of obesity, essential oils and their byproducts have recently attracted interest (Ly et al., 2021). Furthermore, these naturally occurring compounds possess potent disinfectant, anti-allergic, antibacterial, anti-oxidant, and immune-stimulating qualities (Dawood et al., 2022).

Clove (*Syzygium aromaticum*) is a member of the family of Myrtaceae and has been demonstrated to have a variety of medicinal functions, such as antioxidant, anti-inflammatory, and anti-diabetic (Alghazzaly et al., 2022). Biologically active compounds abound in *S. aromaticum*, which also possesses anti-inflammatory effects and can control several medical

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conditions such as drowsiness, coughing, constipation, indigestion, diarrhoea, and stomach dilation. It can also relieve the pain associated with gastrointestinal spasms and ease cramps in the uterus (Foda et al., 2022). The primary component of the extract from *Syzygium aromaticum* is eugenol, a phenolic chemical (Batiha et al., 2020). It contains a monoamine oxidation inhibitor, is neuroprotective, and has antioxidant properties (Batiha et al., 2020).

African nutmeg, or *Monodora myristica*, is a member of the Anonaceae family and is an aromatic plant that is underappreciated in Asia and Africa, yet is full of medicinal properties (Afolabi et al., 2021; Ekeanyanwu et al., 2021). It grows best in the West African evergreen forest and is native to West Africa. The flavorful powdered form of *M. myristica* seeds is used as a spice to enhance the sweetness of baked goods, stews, soups, and pastries (Irondi et al., 2023). Additionally, the powdered seed can be used to pepper soup as a stimulant to help ladies with constipation and postpartum passive uterine haemorrhage (Irondi et al., 2023). Anti-depressant, anti-inflammation, and anti-nociceptive are a few of the biological properties of *M. myristica* that have been scientifically documented (Ekeanyanwu et al., 2021).

Tall, slender, fragrant, evergreen tree of *Xylopia aethiopica* of the family of Annonaceae, can reach heights of 15 to 30 m and diameters of 60 to 70 cm (Yin et al., 2019). It is believed to originate natively in the African Savanna, especially in Senegal, Ghana, Nigeria, Cameroon, and Ethiopia, to mention a few countries (Yin et al., 2019).

X. aethiopica fruit is commonly called "Guinea pepper" or "Negro pepper," has numerous recognised applications in traditional medicine in several countries in West Africa. It has historically been employed in the treatment of numerous ailments, such as coughing, syphilis, insulin resistance, gastroenteritis, haemorrhoids, uterine fibroids, malaria, and amenorrhoea in females (Yin et al., 2019). Upon scientific examination, several of the alleged traditional applications were validated. These encompass, among other things, painkillers, anti-inflammatories, insulin-like substances, antiplasmodial, and antibacterial properties. In addition to the previously mentioned health advantages, *X. aethiopica* fruit is a well-known spice that is utilised because of its high nutritional content (Melo et al., 2021).

Despite the increased focus and encouragement, the majority of published literature on essential oils is centered on determining the therapeutic value of an individual oil (Leigh-de et al., 2021). In contrast, as the foundation of aromatic treatment is the procedure of blending various essential oils to generate an improved medicinal impact, essential oils are generally employed in numerous mixtures when used for aromatic medicine (Bunse et al., 2022). Increased antibacterial, antioxidant, anti-inflammatory and antihistaminic benefits can be achieved by blending essential oils, as the scientific evidence on aromatic treatments thoroughly indicates (Leigh-de et al., 2020). Prior research has indicated that certain manufactured and native essential oils provide medicinal properties when blended (Orchard et al., 2019).

People have endured years of suffering from a wide range of ailments and pains. One of the various methods for battling illnesses is the use of natural medicines to treat a range of ailments (Kruk et al., 2019). The trend towards natural pharmaceuticals is acquiring popularity in the wake of the launch of multiple important medications due to growing concerns about the increasing toxicities associated with primary pharmaceuticals (Akram et al., 2021). A further trend that is gaining more and more recognition is the propensity of essential oil molecules to demonstrate both synergistic and antagonistic behaviour. According to a recent investigation on cell behaviour, an oil's functionality may be influenced by the component proportions as much as by the components' identities (Cimino et al., 2021). To create an oil blend with unique benefits, there has been curiosity about blending, or mixing, different essential oils due to the potential for both synergistic and adverse effects. Future studies on the synergy, antagonistic effects, and complementary impact of essential oil blends will benefit from the present results, which will make it possible to compare the function of this mix with that of the individual essential oils and maybe other blends.

This blend of Eos is a mixture of essential oils from *S. aromaticum* bud, *M. myristica* seed, and *X. aethiopica* seed. While many of these distinct varieties of EOs and their active components are well-known to offer different medicinal benefits, we looked to investigate the effect of essential oil blends on indicators associated with diabetes, inflammatory conditions, and oxidative damage.

EXPERIMENTAL

Materials and methods

Plant Materials

African Nutmeg (*Monodora myristica*) seeds, Clove bud *(S. aromaticum*), *and* Negro pepper pod (*X. aethiopica)* were picked from an orchard at Ijebu-Ode, Ogun State, after their identification at the Department of Plant Biology, Osun State University, Osogbo, Osun State, Nigeria.

Sample Pretreatment

Extraction of Essential Oils

The powdered seeds (10 g) were extracted using the hydro-distillation process. The seed was pulverized into smaller particles to enhance its surface area, and thereafter put into a 1000 mL round-bottom flask using a funnel. Following that, 200 mL of distillate water was introduced. Subsequently, the solution was agitated using a glass stirring rod. The heating mantle was linked to an electrical outlet and activated. The water began to flow through the condensation chamber of the

Clevenger apparatus, which was then attached to a flask. The flask was thereafter put on the heating mantle. The mixture underwent heating for 5 hours, following which the resulting extract was obtained. The yield was then estimated.

GC-MS Analysis

A flame ionisation detector (FID) and an HP-5MS (30m × 0.25 mm x 0.25 μm) capillary column were fitted to an Agilent 6890N apparatus for gas chromatography and mass spectrometry analysis. An Agilent Technologies 5973N mass spectrometer was used to identify the constituents of the essential oil. The oven's thermostat was set at the beginning at 60°C for one minute, followed by a ramp of 10°C min−1 to 180°C for one minute, and a ramp of 20°C min−1 to 280°C for fifteen minutes. These were the GC configurations. At 270°C, the injector temperature was kept constant. The samples (1 μL) were split 1:10 and injected neat. Helium served as the carrier gas, flowing at a rate of 1.0 mL min1. At two scans per second, spectra were scanned from 20 to 550 m/z. Most of the substances were identified using gas chromatography via comparison of their retention indexes to those reported in the scientific literature or to real chemicals that were kept in our testing facilities. The retention indices were calculated using the same operating parameters and compared to a homologous sequence of n-alkanes (C_8–C_{24}). To confirm the identity, their mass spectra on both columns were compared to mass spectra from published works or those kept in the NIST 05 and Wiley 275 libraries. After calculating constituent proportions using GC peak areas, adjustment variables were not used (Wesołowska et al., 2019).

Preparation of the Blend

The blend was prepared by mixing equal masses of the three essential oils in a ratio of 1:1:1.

Quantitative Phytochemical Analysis
Total phenolic content (TPC)

The quantification of the phenolic content was performed with a spectrophotometer (Kim et al., 2022). The essential oil (0.1 mL) was combined with 1 mL of Phenol Folin–Ciocalteu combination, followed by the addition of 9 mL of pure distilled water. Following agitation, 10 mL of 7% Na_2CO_3 was introduced, followed by rapid dilution with fresh distilled water, generating 25 mL total volume. The entire mixture was kept at 23°C for 90 min, and the absorbance was measured at a wavelength of 750 nm. The complete experiment was done thrice to guarantee precision with the standard gallic acid. TPC was quantified as mg GAE (gallic acid equivalents)/gram dry weight extract/fraction.

Total flavonoid contents (TFC)

The spectrophotometric approach is the most straightforward and economical way to determine a plant's flavonoid content (Park et al., 2008). The reaction combination was generated in an experiment tube by the sequential mixture of 0.30 mL essential oil, 0.15 mL of $NaNO_2$ (0.5 mol/L) coupled with 0.30 M $AlCl_3{\cdot}6H_2O$ and 3.40 mL methanol (30%). The mixture was held for 5 min, after which 1 mL of 1 M NaOH was blended into it. At 506 nm wavelength, the transparency of the reaction mixture was monitored.

Total tannin content (TTC)

With a few minor adjustments, the Ejikeme et al. (2014) procedure was used to quantify the tannin content. This method involved soaking 500 mg of EO in 60 mL of distilled water. After being shaken by a machine for one hour, the sample was screened. In a volumetric flask with a capacity of 500 mL, the filtrate was adjusted to the appropriate level. 5 mL of the aforementioned filtrate was combined with 2 mL of 0.1 M $FeCl_3$ and potassium ferricyanide (0.006 M) prepared in 0.1 M HCl. Using a wavelength spectrophotometer, the intensity of absorption was measured at 200 nm with a standard gallic acid curve. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry plant material.

Determination of total proanthocyanin

Proanthocyanin was performed with the technique outlined by Chen et al. (2016). The combination was left to stand for 15 minutes after being blended with 0.5 mL of the 0.1 mg/mL extract solution, 3 mL of the 4% vanillin-methanol solution, and 1.5 mL of HCl. A 500 nm measurement was made of the absorbance. The final concentration of 0.1 mg/mL was used to analyze the extract samples in terms of catechin acid equivalent.

Determination of total Alkaloids

The EO (0.1 mL) was mixed with 200 mL of 10% acetic acid in ethanol. The mixture was allowed to sit for 4 minutes before being filtered. After concentrating the resulting filtrate in a water bath, NH_4OH was introduced drop by drop until the precipitation process was finished. The residue was washed using diluted NH_4OH. When it had dried, the residue was measured (Elshafie et al., 2017).

Determination of Saponin

Using the spectrophotometric approach outlined by Elshafie (2017), the saponin content was ascertained. Isobutyl alcohol, also known as but-2-ol, was poured into a beaker containing around 2 g of EO. To the mixture, 40% magnesium carbonate ($MgCO_3$) solution was added after filtration. To 1 mL of the solution, 2 mL of $FeCl_3$ solution was added, and the remaining volume was adjusted with distilled water. The absorbance was measured at 380 nm using a SpectrumLab70 spectrophotometer after this had been permitted to remain for 30 minutes to generate the colour.

Quantitative Test for Terpenoids

For 24 hours, 9 mL of ethanol and 100 mg of the extract were combined (Elshafie et al., 2017). The filtrate formed by adding 10 mL of petroleum ether was extracted using a separating funnel. After being divided into glass vials and carefully weighed, the ether extract was left to dry entirely at room temperature for 10 minutes (T_W). The total terpenoids present were then estimated:

$$T_G - T_W / T_G \times 100. \quad (1)$$

where T_G = Initial weight, T_W = Final weight.

Anti-Oxidant Analysis
DPPH radical scavenging assay

According to Kim et al. (2022), the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (DRSA) was used to examine the extracts' capacity to scavenge free radicals. A 0.1 mM solution of DPPH in methanol was made, and 1.6 mL of EO in methanol was combined with 2.4 mL of this solution. The mixture was kept for 30 minutes in the dark. Using spectrophotometry, the absorbance was obtained at 517 nm. The % of DPPH radical scavenging activity (%*K*) was determined:

$$(\%K) = \{(J_0 - J_1)/J_0\} \times 100. \quad (2)$$

J_1= absorbance of the essential oil/standard, and J_0= absorbance of the control.

ABTS radical scavenging activity

According to Schaich et al. (2015), the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay was carried out. $K_2S_2O_8$ (2.45 mM) solution and ABTS solution (7 mM) were reacted in a ratio of 1:1 and left in the dark for a night to produce a dark liquid comprising ABTS radical cations. Before being used in the experiment, the thermostat was adjusted to 30°C, and the ABTS radical cation was diluted with methanol (50%) to get absorbance at 745 nm. Calculation of the % inhibition was done using:

$$\text{Inhibition effect}(\%) = [(\text{control absorbance} - \text{sample absorbance})/(\text{control asbsorbance})] \times 100. \quad (3)$$

Lipid peroxidation inhibition assay

The procedure outlined by Ayala et al. (2014) was followed to determine the lipid peroxidation inhibition assay (*LPI*). Mixtures containing 100 µL, 10 mM FeSO4, 100 µL, 0.1 mM Ascorbic acid, and 0.3 mL of EO were mixed and left for twenty minutes. The mixture was incubated at 37 °C, after which 1.5 mL of (1%) thiobarbituric acid and 1 mL of (28%) trichloroacetic acid were added. After a final 15 minutes of heating at 100 °C, the solution was left to cool at 25 °C, and absorbance was taken at 532 nm. Using the following formula, the percentage inhibition of lipid peroxidation (% *LPI*) was determined:

$$\%LPI = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}. \quad (4)$$

Nitric oxide scavenging assay

The Griess reagent approach developed by Bhaskar and Balakrishnan (2009) was employed to analyse the extracts' ability to scavenge nitric oxide and their antioxidant activity. The Griess reagent was created by adding a comparable quantity of $C_6H_8N_2O_2S$ (1%) in H_3PO_4 (5%) and naphthylenediamine (0.1%) in distilled water. A batch of 10 mM $Na_2[Fe(CN)_5NO]$, prepared in saline buffered with phosphate, weighing 50–800 µg/ml, was given to each EO. Following treatment with 1 mL of Griess reagent, the mixture was left for three hours and subjected to spectrophotometric testing at 546 nm.

$$\text{Nitric oxidescavenging activity } (\%) = = \frac{(CTR \text{ absorbance} - SPL \text{ absorbance}) \times 100}{\text{Control absorbance}}. \quad (5)$$

CTR= control, *SPL*= sample.

Anti-inflammatory Activity
Lipoxygenase enzyme inhibition assay

The pH borate buffer solution, stop solution, enzyme concentration, and substrate concentration were all optimised for the lipoxygenase inhibition experiment. Following optimisation, lipoxygenase activity was measured using a UV-Vis spectrophotometer device (Alzarea et al., 2021). The parameters included methanol as the solution stop, linoleic acid as the substrate of choice (900 µM), lipoxygenase (5,000 units/mL), and borate buffer (0.2 M, pH 8.5). This equation was used to determine the lipoxygenase inhibition value's inhibition percentage:

$$\%\text{Lipoxygenase inhibition} = \frac{(E-F)-(G-H) \times 100\%}{(E-F)}. \quad (6)$$

The variables *E*, *F*, *G*, and *H* in this equation represent the absorbance of the blank solution (containing the enzyme), the control blank solution (containing the enzyme but lacking the enzyme), and the standard/sample solution (containing the enzyme but lacking the enzyme).

Xanthine oxidase (XO) inhibition assay

Using a spectrophotometer, the xanthine oxidase inhibitory activity was measured as stated by Alzarea et al. (2021). The assay combination was made up of 50 µL of essential oil (1 mg/mL in phosphate buffer), 150 µL of phosphate buffer (0.066M; pH, 7.5), and 50 µL of enzyme solution (0.28U/mL). Following a three-minute pre-incubation period at room temperature (25°C), 250 µL of the substrate

solution (xanthine, 0.15 M in the same buffer) was added to start the reaction. Additionally, an unaltered solution devoid of enzymes was made. At 295 nm, the response was observed for three minutes, during which time the absorbance (V_0) was noted. As a negative control, phosphate buffer was utilized (activity of the enzyme without essential oil). ibuprofen served as the positive control.

Inhibition (%) = [(V_0 ctl- V_0 spl) X 100]/ V_0 ctl.

V_0 ctl = enzyme activity in the absence of essential oil.

V_0 spl = enzyme activity when there is essential oil or ibuprofen.

Membrane Stabilizing Assay

The methodology of Oyedapo et al. (2010) was adjusted to determine the red blood cell membrane stability capability. The mixture was made of 1.0 mL of EOs, 1.0 mL of hyposaline, and 0.4 mL of 0.14 M phosphate buffer at pH 7.6. To the mixture, 0.5 mL of 2.5% (v/v) erythrocyte was included. Thirty minutes were spent incubating the reaction mixture at 56 ℃, after which it was cooled and then centrifuged for ten minutes at 704 rpm. The tube containing no essential oil at all served as the control. At 560 nm, the absorbance was measured while the control lacked erythrocytes.

$$\%\text{Membrane Stability} = \frac{\{\text{abs test} - \text{abs drug control}\} \times 100}{\text{Abs drug} - \text{Abs drug control}}. (7)$$

Anti-diabetes Activity

α-Amylase inhibition assay

The assay used to assess the α-amylase inhibitory activity was modified from McCue and Shetty (2005) approach. Porcine pancreatic α-amylase solution (PPA) containing 1.3 U/mL and 200 µL of 0.02 M sodium phosphate buffer (pH 6.9; 6.7 mM NaCl) are both included in the reaction mixture. After pre-incubating the reaction medium for five minutes at 37°C, 200 µL of the 0.4% starch solution in the buffer mentioned above was added, and the mixture was incubated for ten minutes at 37°C. After adding 600 µL of $C_7H_4N_2O_7$ solution to the reaction, it was heated to boiling for seven minutes and cooled in cold water. After adding diluting with 1 mL of distilled water, the absorbance was taken at 540 nm. As a positive control, acarbose, a commercial inhibitor, was utilized at concentrations between 50 and 800 µg/mL. Instead of using a substrate, a blank buffer solution was employed. The control tube with total enzyme activity was the one containing the enzyme solution without any essential oil or aspartame. This equation was used to determine the percentage of inhibition of α-amylase:

$$\text{Inhibition of } \alpha - \text{amylase activity}(\%) = \left((AD - AE)/AD\right) \times 100. (8)$$

Here, *AE* represents the tested sample's absorbance, and *AD* (100% enzyme activity) represents the absorbance of the control (acarbose or essential oil).

α-glucosidase inhibitory assay

A modified version of Tao et al. (2013) approach was used to test inhibitory activity on α-glucosidase. 200 µL of a crude rat intestinal α-glucosidase enzyme solution (adjusted to 0.2 U/mL as initial concentration in phosphate buffer 67 mM, pH 6.8) was combined with 200 µL of the sample, which included solutions of essential oil (50-800 µg/mL) or acarbose (50-800 µg/mL), a positive control. Additionally, 1 mL of phosphate buffer was added. After pre-incubating the mixture for 10 minutes at 37°C, 300 µL of p-nitrophenyl-α-D-glucopyranoside solution (10 mM) was included, and the mixture was kept for 40 minutes at 37°C. In addition, 3 mL of sodium carbonate Na_2CO_3 (100 mM) was added to the mixture, and the reaction was brought to an end. The released p-nitrophenol's absorbance was determined at 405 nm. Substrate was replaced with buffer solution as a blank. The control tube with total enzyme activity was the one containing the enzyme solution without any essential oil or aspartame.

Inhibition of *α*-glucosidase activity (%) = ((*AD*-*AE*)/*AD*) *100

In this instance, *AE* = absorbance (sample), *AD* = absorbance of the control (100% enzyme activity) (essential oil or acarbose).

Statistical Analysis

The result was presented as the mean of three readings. The data obtained were subjected to Analysis of variance (ANOVA). The values were significantly different when $P<0.05$.

NUMERICAL RESULTS

Yield

S. aromaticum, *M. myristica*, and *X. aethiopica* essential oils were extracted with a yield of 11.34%, 2.74% and 1.44% respectively.

GC-MS Analysis

The analysis of the EO of *S. aromaticum* through GC-MS is shown in Table 1. Twenty-nine compounds were observed from the essential oil of *S. aromaticum*, of which the major components are eugenol (75.08%) and caryophyllene Oxide (4.83%). The GC-MS analysis of *M. myristica* obtained in Table 2. revealed the major constituents to be isocaryophyllene (29.36%), germacrene D-4-ol (20.31%), germacrene D (9.72%), and oplopanone (6.681%). Thirty-one compounds were obtained from the analysis of *X. aethiopica* essential oil, and the main compounds were isospathulenol (8.67%), chavibetol (6.99%), β-elemene (6.76%), and calamenene (5.23%) as revealed in Table 3. The GC-MS result of the blend

of essential oils is obtained in Table 4, which revealed the main components as eugenol (34.25%) and chavibetol (16.57%). This showed a decrease in the value of eugenol in the blend compared to what was obtainable in *S. aromaticum*, but an increase in the value of chavibetol in the blend compared to what was found in the *X. aethiopica*.

Table 1. Components from GC-MS Analysis of *S. aromaticum*.

Name of Compounds	% Composition	Retention Time
Eugenol	75.08	3.408
Caryophyllene Oxide	4.83	4.947
Vanillin	2.30	3.946
Trans-Anethole	1.79	9.902
Homoranillyl Alcohol	1.67	5.857
Vanilla Ethyl ether	1.51	11.419
Sinapaleolehyde	1.45	5.817
1,3-Thiazolidin-4-ones-	1.44	11.213
Garyophyilene	0.94	5.336
Beta-caryophyllene Oxide	1.24	5.410
Pulegone	0.74	5.073
1-(2-(2-(tert-Butyl)phenoxyl)ethyl)-1-3-dihydro-2H-benzo[d]imidazole-2-thione	0.51	11.304
2-[3-hydroxy-4-methoxyphenyl)]-hydrazine-boxamide	0.46	5.748
α-Acoreno	0.41	6.618
Nor Camphorone	0.31	4.054
Tricyclo[4.1.0.0(3,7)] heptanes	0.27	11.476
2-methoxy-4-(2-propenyl) phenol	0.26	3.534
Benzoyl benzonate	0.24	5.960
4-hydroxy quinoline	0.22	5.697
Epianastrophin	0.22	6.229
Barpisoflavone A, 3Me derivatives	0.21	10.938
(1R,7S,E)-7-Isopropyl-4-, 10-dimethylenecyclodec-5-enol	0.20	6.068
Indole	0.15	5.021
Phenyl 4-methoxy benzoyl ether	0.15	11.636
Thiazolidin-4-one,3-(2-furfury)-5-(2,3-dihydro-2-oxo-3-indoly)-2-Thioxo	0.13	11.522

Table 2. Components from GC-MS analysis of *M. myristica*.

Name of Compounds	% Composition	Retention Time
Isocaryophyllene	29.36	3.677
Germacrene D-4-ol	20.31	4.729
Germacrene D	9.71	4.077
Oplopanone	6.68	5.782
Farnesoic acid	4.66	6.297
Camphor	3.19	3.516
Valerenol	2.89	5.971
Cuparene	2.42	5.073
Eugenol	2.16	3.242
2-methyladamantane	2.15	3.459
Farnesol	2.13	4.174
Farnesol	2.08	4.317
Isospathulenol	1.81	6.223
Muurola-4 (14),5-diene, cis-	2.51	5.565
2-caren-4-ol	1.53	3.711
Isoniazid pyruvate	1.48	14.119
n-Hexadecanoic acid	1.45	7.087
Geraniol	1.14	7.121
2-pentadecanone	1.12	5.645
Docosatetraenoic acid	1.11	8.540
Geranic acid or Chrysanthemic acid	1.10	3.745
Alpha-cadinene T1n3	1.05	4.289
2-(Propoxycarbonyl)benzoic acid	0.94	10.011

Table 3. Components from GC-MS analysis of the *X. aethiopica*.

Name of Compounds	% Composition	Retention Time
Isospathulenol	8.67	5.147
Chavibetol	6.99	5.010
β-Elemene	6.76	3.488
Calamenene	5.23	4.615
Muurolol	4.62	5.296
Germacrena D	4.53	4.106
Caryophyllene oxide	4.34	4.809
γ-Curcumene	3.86	6.566
Oxepine, 2,7-dimethyl-	3.72	6.354
Aromadendrene	3.07	6.435
Spathulenol	2.98	4.787
16.beta, H-kauran-16-ol	2.85	8.575
α-Farnesene	2.81	7.533
Cic-2-,alpha,-Bisabolene epoxide	2.64	5.691
2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	2.62	7.402
Alloaromadendrene epoxide	2.61	5.828
α-CadineneT1n3	4.07	4.060
3-Oxatricyclo[5.1.0.$0^{2,4}$]octane, 4,6,6-trimethyl-2-[(1E)-3-methyl-1,3-butadien-1-yl	2.54	6.721
(4*Z*,8*E*)-*N*-(3-chlorophenyl)bicyclo[10.1.0]trideca-4,8-diene-13-carboxamide	2.31	6.188
Aromandendrene	2.21	5.239
FenchoneT1n3	2.21	6.835
Isoaromadrene epoxide	2.07	5.925
Caryophyllene	1.94	3.671
Salvial-4(14)-en-1-one	1.78	4.867
Humulene	1.64	3.900
Cadinol	1.52	5.193
Icosa-9, 11-diyne	1.47	6.892

Table 4. Components from GC-MS analysis of the Blend.

Name of Compounds	% Composition	Retention Time
Eugenol	34.25	3.345
Chavibetol	16.57	4.489
Germacrene D	4.62	4.112
δ-Cadinene	3.60	4.489
Caryophyllene	2.70	3.700
[1,1^1-Bipheny1]-2,2^1-diol,3,3^1-dimethoxy-5,5^1-di-2-propenyl-	2.45	10.726
.tau.Muurolol	2.36	5.256
4,8*a*-dimethyl-6-prop-1-en-2-yl-2,3,5,6,7,8-hexahydro-1*H*-naphthalen-2-ol	2.92	6.309
Anethole	2.21	9.885
2-Isopropyl-5-methyl-9-methylene[4.4.0]dec-1-ene	2.19	5.170
Di(2-ethylhexyl)phthalate	1.61	10.017
α-CadineneT1n3	1.56	4.318
α-Gurjunene	1.40	5.096
[4E,8E,13E]-1-(2-Hydroxyethyl)-1,5,9-trimethyl cyclotetra decatriene.	1.00	7.367
Kaur-16-en-18-al,(4.alpha)-	0.97	8.684
M-Camphorene	0.89	7.505
Sabinene hydrate, trans-	0.89	3.591

Phytochemical Analysis

The findings of the quantitative analysis of the phytochemical characteristics of crude essential oils and their blend are displayed in Table 5. All of the extracted crude essential oils of *S. aromaticum*, *M. myristica*, *X. aethiopica*, and the blend contained phenolic content. *S. aromaticum* had the highest phenolic value of 182.46 mg GAE/g, while *M. myristica* had the lowest phenolic content of 144.01 mg GAE/g. The blend had higher phenolic and flavonoid content than *M. myristica* EO. *X. aethiopica* had the highest flavonoid value of 43.48 mg CE/g, whereas *M. myristica* had the lowest flavonoid content of 24.34 mg CE/g. Tannin content of 17.41 mg GAE/g was highest in *S. aromaticum*, and it was the only EO with higher tannin than the blend, while the least tannin was found in *M. myristica* (9.85 mg GAE/g). The value of Proanthocyanin ranged between 8.15 to 9.91 mg CE/g, where *S. aromaticum* recorded the highest value, but the lowest in the blend. *M. myristica* had the highest alkaloid content of 8.70 mg/g, but *S. aromaticum* essential oil had the lowest alkaloid content of 6.30 mg/g; the blend recorded a higher alkaloid content than *S. aromaticum* and *X. aethiopica*. Saponin was highest in *M. myristica* (3.30 mg/g) and least in the blend (1.70 mg/g). The highest terpenoid was recorded in *X. aethiopica* with a value of 20.00 mg/g, while the lowest value was recorded in the essential oil of *M. myristica* (16.30 mg/g).

Table 5. Quantitative Phytochemical Components of the Essential Oils and the Blend.

Essential Oil Phytochemicals	*Syzygium aromaticum*	*Monodora myristica*	*Xylopia aethiopica*	Blend
Phenolic content (mg GAE/g Extract)	182.46 ± 1.23^{d}	144.01 ± 1.37^{a}	175.68 ± 2.21^{c}	161.02 ± 2.30^{b}
Flavonoid (mg CE/g Extract)	39.03 ± 0.72^{c}	24.34 ± 0.52^{a}	43.48 ± 0.35^{d}	30.02 ± 0.32^{b}
Tannin (mg GAE/g Extract)	17.41 ± 0.33^{d}	9.85 ± 0.42^{a}	11.32 ± 0.37^{b}	14.97 ± 0.35^{c}
Proanthocyanin (mg CE/g Extract)	9.91 ± 0.57^{c}	8.45 ± 0.18^{bc}	9.89 ± 0.36^{c}	8.15 ± 0.41^{a}
Alkaloid (mg/g)	6.30 ± 0.11^{a}	8.70 ± 0.07^{d}	6.70 ± 0.14^{b}	8.00 ± 0.37^{c}
Saponin (mg/g)	2.70 ± 0.01^{c}	3.30 ± 0.02^{d}	2.30 ± 0.01^{b}	1.70 ± 0.03^{a}
Terpenoid (mg/g)	18.70 ± 0.58^{b}	16.30 ± 0.23^{a}	20.00 ± 0.27^{c}	17.00 ± 0.32^{a}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Antioxidant Activities
DPPH

The percentage inhibitory activities of the crude essential oils, blend, and the standards (gallic acid and ascorbic acid) against DPPH were recorded in Table 6. The least percentage inhibitory activity with a concentration of 100 µg/mL was 7.73% observed in the crude essential oil of *M. myristica*, while the highest inhibitory activity value at 1600 µg/mL was recorded in the crude essential oil of *X. aethiopica* (71.75%). It was also observed that gallic acid, with an inhibition of 85.22% at a concentration of 1600 µg/mL, had a higher percentage inhibitory activity against DPPH than the crude essential oils and ascorbic acid. The blend showed a higher DPPH scavenging activity than *M. myristica* at all concentrations, while other crude EOs recorded a higher activity. Table 7 recorded the IC_{50} values of the crude essential oils, the blend, and the standards. It was observed that *X. aethiopica*, with the least IC_{50} value of 796.56 µg/mL, had a higher DPPH inhibition than other crude essential oils, the blend as well as ascorbic acid. *M. myristica*, with an IC_{50} of 1119.35 µg/mL, had the least DPPH inhibition, with only it having a higher IC_{50} than the blend.

ABTS

The percentage inhibitory activities of ABTS radical scavenging showed that *S. aromaticum* had a higher inhibition of 42.87% at a concentration of 1600 µg/mL, while the blend had the least inhibition of 34.90% (Table 8). *S. aromaticum* with an IC_{50} of 1746.96 µg/mL had a higher inhibition than other crude essential oils, while the blend of the essential oil with an IC_{50} of 2309.58 µg/mL showed the least ABTS radical scavenging activity (Table 9). Gallic acid, having an IC_{50} of 1471.11 µg/mL, showed a higher ABTS radical scavenging inhibition than all the essential oils and ascorbic acid.

Lipid Peroxidation

The percentage of lipid peroxidation ranged from 69.00 to 66.44% with a concentration of 1600 µg/mL. The blend recorded the highest inhibition of 76.80% compared to the crude EOs (Table 10). Table 11. displays the IC_{50} observed for the essential oils and standards. The values ranged from 604.39 to 922.00 µg/mL, where the blend of essential oils had the highest lipid peroxidation inhibition.

Table 6. Percentage Inhibitory Activity of DPPH by the essential oils, Blend and Standards.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Gallic Acid	Ascorbic Acid
100	9.95±1.52^{b}	7.73±0.11^{a}	14.09±0.65^{c}	10.32±0.65^{b}	13.48±0.23^{c}	9.42±0.11^{b}
200	24.53±0.22^{b}	24.42±0.32^{b}	31.93±0.76^{d}	23.05±0.11^{a}	30.02±0.22^{d}	26.34±0.65^{c}
400	38.82±0.32^{b}	33.08±0.87^{a}	49.16±0.87^{d}	34.07±0.97^{a}	48.47±1.19^{d}	45.33±0.87^{c}
800	54.36±0.65^{b}	47.78±1.52^{a}	57.43±0.87^{b}	48.09±3.90^{a}	66.08±0.98^{c}	56.66±0.22^{b}
1600	67.23±0.65^{b}	60.95±1.30^{a}	71.25±0.43^{c}	61.26±2.38^{a}	85.22±0.97^{d}	70.13±0.65^{c}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at p<0.05.

Table 7. IC_{50} for DPPH Radical Scavenging Potential of the Essential Oils, Blend, and Standards.

EO/Standards	Concentration IC_{50}(µg/mL)
S. aromaticum	957.35±5.32^{d}
M. myristica	1119.35±4.12^{e}
X. aethiopica	796.56±6.17^{b}
Blend	1092.58±7.32^{e}
Gallic Acid	662.56±8.21^{a}
Ascorbic Acid	871.71±4.81^{c}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at p < 0.05.

Table 8. Percentage Inhibitory Activity of ABTS by the Essential Oils, Blend, and Standards.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Gallic Acid	Ascorbic Acid
100	3.79±0.43^{c}	3.26±1.18^{c}	0.83±0.97^{a}	1.52±0.86^{a}	5.46±1.29^{d}	2.20±0.10^{b}
200	13.88±0.32^{bc}	12.37±0.5^{b}	9.48±1.39^{a}	11.46±1.29^{b}	14.11±0.21^{c}	11.31±0.97^{b}
400	26.40±1.07^{c}	24.28±1.0^{bc}	18.29±0.75^{a}	17.75±0.86^{a}	27.16±0.64^{c}	21.70±0.42^{b}
800	34.14±0.43^{c}	31.49±0.7^{b}	27.39±0.75^{a}	26.25±0.64^{a}	40.06±0.21^{d}	31.34±1.18^{b}
1600	42.87±0.11^{c}	41.40±2.3^{c}	37.71±0.75^{b}	34.90±1.72^{a}	49.01±0.86^{d}	41.65±0.32^{c}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at p < 0.05.

Table 9. IC_{50} Values for ABTS Radical Scavenging Potential of the Essential Oils, Blend, and Standards.

EO/Standards	Concentration IC_{50}(µg/mL)
S. aromaticum	1746.96±8.10^{b}
M. myristica	1891.36±5.20^{d}
X. aethiopica	2054.45±3.33^{e}
Blend	2309.58±6.12^{f}
Gallic Acid	1471.11±3.42^{a}
Ascorbic Acid	1803.04±6.21^{c}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at p < 0.05.

Table 10. Percentage Inhibitory Activity of Lipid Peroxidation by the Crude Essential Oils, Blend, and Standards.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Gallic Acid	Ascorbic Acid
100	8.26±1.20^{b}	5.40±0.75^{a}	8.40±0.60^{b}	7.90±0.75^{b}	9.22±0.15^{c}	8.47±1.20^{b}
200	21.61±1.20^{b}	17.70±1.05^{a}	26.40±0.15^{c}	23.90±1.20^{b}	35.80±0.00^{d}	26.59±0.75^{c}
400	42.70±0.75^{b}	37.30±3.90^{a}	47.00±2.10^{c}	43.54±0.75^{b}	59.00±0.75^{d}	48.00±1.05^{c}
800	59.90±1.35^{b}	57.20±0.60^{a}	60.50±1.65^{c}	61.90±1.80^{c}	65.25±0.35^{d}	64.40±0.30^{d}
1600	75.42±1.80^{c}	69.00±3.15^{a}	71.06±2.55^{b}	76.80±2.55^{c}	86.44±0.30^{e}	79.90±0.80^{d}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at p < 0.05.

Table 11. IC_{50} Values for Lipid Peroxidation Potential of the Essential Oils, Blend, and Standards.

EO/Standards	Concentration IC_{50}(µg/mL)
S. aromaticum	834.88±5.12^{e}
M. myristica	922.00±2.24^{d}
X. aethiopica	837.22±4.28^{c}
Blend	827.22±3.13^{c}
Gallic Acid	604.29±7.12^{a}
Ascorbic Acid	740.48±5.24^{b}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Nitric Oxide

The percentage inhibitory activities of each crude essential oil, the blend, and the standards against nitric oxide were recorded in Table 12. The percentage inhibition of nitric oxide at 1600 µg/mL ranges from 52.92 to 65.89% with the essential oil of *S. aromaticum* recording the highest inhibition of 57.65% of nitric oxide among the essential oils. The IC_{50} values obtained from the inhibition of nitric oxide by all of the essential oils, as well as the standards, are presented in Table 13. The crude essential oil of *S. aromaticum* (1104.62 µg/mL) exhibited the highest level of inhibitory action, whereas the essential oil of *M. myristica* (1335.00 µg/mL) had the lowest inhibitory action.

Table 12. Percentage Inhibitory Activity of Nitric Oxide by the Crude Essential Oils, Blend, and Standards.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Gallic Acid	Ascorbic Acid
100	13.92±0.42^{d}	9.97±0.74^{a}	10.94±0.42^{a}	11.39±2.12^{b}	13.87±0.53^{d}	12.44±0.64^{c}
200	26.37±0.85^{c}	18.22±1.38^{a}	22.11±1.80^{b}	19.72±1.59^{a}	28.71±0.11^{d}	23.61±0.53^{bc}
400	38.76±0.74^{c}	26.39±0.85^{a}	32.38±0.42^{b}	31.03±0.64^{ab}	39.51±0.53^{d}	32.68±0.85^{b}
800	50.37±1.06^{d}	42.20±2.65^{a}	45.37±0.11^{c}	44.45±1.17^{b}	50.30±0.53^{d}	44.98±0.00^{b}
1600	57.65±0.95^{c}	54.42±0.42^{b}	55.10±0.74^{bc}	54.95±0.74^{b}	65.89±0.74^{d}	52.92±0.21^{a}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Table 13. IC_{50} Values for Nitric Oxide Ion Scavenging Potential of the Crude Essential Oils, Blend, and Standards.

EO/Standards	Concentration IC_{50}(µg/mL)
S. aromaticum	1104.62±6.32^{b}
M. myristica	1335.00±4.18^{d}
X. aethiopica	1289.23±5.36^{c}
Blend	1285.19±7.46^{c}
Gallic Acid	979.00±6.12^{a}
Ascorbic Acid	1326.67±8.32^{d}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Anti-inflammatory Activities
Lipoxygenases Inhibition

The percentage inhibitory activities of lipoxygenases by the crude essential oils, the blend, and the standard were observed in Table 14. The result showed that *S. aromaticum*, with a percentage inhibition of 67.11% had the highest activity of all the essential oils at 1600 µg/mL. The essential oil blend exhibited the least inhibition of lipoxygenases at all concentrations. The IC_{50} values obtained for the lipoxygenase assay with all of the essential oils, as well as the standard, are presented in Table 15. *S. aromaticum* with an IC_{50} of 859.63 µg/mL showed a higher inhibition than the rest of the crude essential oils, while the blend with an IC_{50} of 3093.46 µg/mL recorded the least lipoxygenase inhibition. The standard ibuprofen had an IC_{50} of 767.67 µg/mL, which indicated a higher lipoxygenase inhibition than all the crude and the blend of the essential oils.

Membrane Stability Assay

At a concentration of 1600 µg/mL, the membrane stability inhibition ranged from 36.96 to 78.26%. The blend, with a percentage inhibition of 40.22%, showed a superior inhibition than *M. myristica* (36.96%). *S. aromaticum* recorded membrane stability inhibition of 45.65%, which was the highest percentage of inhibitory activity of all the crude essential oils and the blend. Table 16. It was also revealed that the standard (Ibuprofen) with a percentage inhibition of 78.26% had higher inhibition than all the crude essential oils and the blend at a concentration of 1600 µg/mL. Table 17. revealed the IC_{50} of the membrane stability assay of the

essential oils and ibuprofen. *S. aromaticum*, having the least IC_{50} of 1134.90 µg/mL, showed a higher membrane stability assay than the rest of the crude essential oils, as well as the blend.

Table 14. Percentage Inhibitory Activity of Lipoxygenases by the Crude Essential Oils, Blend, and Standard.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Ibuprofen
100	25.12±0.70^{d}	19.26±0.58^{b}	22.07±1.52^{c}	5.95±0.94^{a}	25.04±0.35^{d}
200	32.64±1.52^{b}	32.31±0.82^{b}	33.47±5.03^{b}	12.98±1.52^{a}	32.56±0.23^{b}
400	36.69±0.47^{b}	35.37±0.94^{b}	40.58±2.69^{c}	18.35±0.94^{a}	40.17±0.94^{c}
800	58.93±0.35^{d}	39.67±1.87^{b}	50.83±0.35^{c}	24.21±0.82^{a}	60.91±1.29^{d}
1600	67.11±0.94^{d}	43.31±1.40^{b}	58.60±1.29^{c}	28.51±0.82^{a}	71.98±1.99^{e}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Table 15. IC_{50} Values for Lipoxygenase Inhibition of the Crude Essential Oils, Blend, and Standard.

EO/Standard	Concentration IC_{50}(µg/mL)
S. aromaticum	859.63±2.12^{b}
M. myristica	1967.50±4.21^{d}
X. aethiopica	1060.95±3.33^{c}
Blend	3093.46±3.44^{e}
Ibuprofen	767.67±5.21^{a}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Table 16. Percentage Inhibitory Activity of Membrane by the Crude Essential Oils, Blend, and Standard.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Ibuprofen
100	10.86±3.07^{a}	9.78±1.54^{a}	11.96±4.61^{ab}	15.22±0.00^{b}	29.35±7.69^{c}
200	22.83±1.54^{b}	16.30±1.54^{a}	20.65±4.61^{b}	20.65±1.54^{b}	42.30±3.07^{c}
400	29.35±4.61^{b}	22.83±1.54^{a}	28.26±3.07^{b}	29.35±1.54^{b}	53.26±1.54^{c}
800	36.96±3.07^{b}	30.43±3.07^{a}	36.96±3.07^{b}	33.69±1.54^{b}	62.64±1.54^{c}
1600	45.65±6.15^{c}	36.96±3.07^{a}	41.30±3.07^{b}	40.22±1.54^{b}	78.26±3.07^{d}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Table 17. IC_{50} Values for membrane stability assay of the Crude Essential Oils, Blend, and Standard.

EO/Standard	Concentration IC_{50}(µg/mL)
S. aromaticum	1134.90±7.12^{b}
M. myristica	2232.11±5.12^{e}
X. aethiopica	1942.35±6.20^{c}
Blend	2102.00±4.70^{d}
Ibuprofen	519.52±5.20^{a}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Xanthine Oxidase Inhibition

The inhibitory activities of the crude essential oils, the blend, and the standard (ibuprofen) against xanthine oxidase are presented in Table 18. It was observed that the higher the concentration of the essential oils, the higher the percentage inhibition. At a concentration of 1600 µg/mL, it was indicated that *X. aethiopica* (41.69%) had the highest percentage inhibition of xanthine oxidase, while *S. aromaticum* (33.01%) had the least xanthine oxidase inhibition. the blend, with an inhibition of 41.69%, had a higher activity than *S.aromaticum* and *M. myristica*. It was revealed that *S.aromaticum* with an IC_{50} of 1579.78 µg/mL had the least inhibition activity against xanthine oxidase (Table 19). The result showed that only *X. aethiopica*, with an IC_{50} of 1251.78 µg/mL, exhibited a higher inflammatory activity compared to other EOs.

Table 18. Percentage Inhibitory Activity of Xanthine Oxidase by the Crude Essential Oils, Blend, and Standard.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Ibuprofen
100	6.24±0.75^{b}	8.14±0.25^{c}	9.38±0.33^{c}	4.28±0.50^{a}	8.02±0.58^{c}
200	12.70±0.50^{a}	14.85±0.00^{b}	16.45±0.08^{b}	15.38±0.08^{b}	19.06±0.25^{c}
400	19.18±1.25^{a}	21.91±0.92^{a}	25.24±0.25^{b}	22.51±0.25^{a}	32.01±2.09^{c}
800	27.19±0.50^{a}	28.62±0.33^{a}	34.08±0.34^{c}	30.99±0.61^{b}	45.07±0.25^{d}
1600	33.01±1.34^{a}	35.27±0.50^{a}	41.69±0.16^{b}	37.71±0.41^{ab}	51.84±0.58^{c}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Table 19. IC_{50} Values for Xanthine Oxidase inhibition of the crude Essential Oils, Blend, and Standard.

EO/Standard	Concentration IC_{50}(µg/mL)
S. aromaticum	1579.78±4.12^{d}
M. myristica	1551.51±3.38^{d}
X. aethiopica	1251.78±2.31^{b}
Blend	1311.11±1.87^{c}
Ibuprofen	855.22±1.20^{a}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Antidiabetic Activities

α-amylase Inhibition

Table 20. revealed the percentage inhibitory activities of each of the crude essential oils, the blend, and the standard (Acarbose) against α-amylase. It was observed that with a concentration of 1600 µg/mL, the blend of the crude essential oils recorded a percentage α-amylase inhibition of 54.45% which was the highest obtained, while the essential oil of *M. myristica* recorded the least inhibitory activity of 30.04%. It was also observed that the standard acarbose (65.11%) had a higher percentage inhibition than all the crude essential oils and the blend. The result of the anti-diabetic properties of individual crude essential oils and blends using the α-amylase assay with the inhibition concentration at 50% (IC_{50}) is shown in Table 21. The lowest IC_{50} value of the essential oils against α-amylase was 1250.69 µg/mL obtained in the blend essential oil, while the highest IC_{50} of 2703.00 µg/mL was recorded for the essential oil of *M. myristica*. This indicated that the blend of essential oils had a better antidiabetic activity regarding α-amylase than the other essential oils.

Table 20. Percentage Inhibitory Activity of Alpha-amylase by the Crude Essential Oils, Blend, and Standard.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Acarbose
100	8.77±1.73^{b}	5.21±0.22^{a}	5.14±1.19^{a}	8.97±0.33^{bc}	14.19±0.54^{d}
200	17.64±2.60^{b}	11.43±1.19^{a}	17.41±1.08^{b}	18.17±0.54^{b}	24.54±0.22^{c}
400	23.75±1.52^{b}	18.56±1.52^{a}	28.14±0.98^{c}	29.45±0.87^{c}	37.88±0.65^{d}
800	34.13±1.19^{b}	30.75±1.63^{a}	34.59±1.19^{b}	47.47±0.98^{c}	50.69±0.54^{cd}
1600	44.40±2.49^{b}	30.04±0.65^{a}	44.94±1.74^{b}	54.45±1.58^{c}	65.11±0.76^{d}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

Table 21. IC_{50} Values for Alpha-amylase Inhibition of the Crude Essential Oils, Blend, and Standard.

EO/Standard	Concentration IC_{50}(µg/mL)
S. aromaticum	1799.52±2.60^{cd}
M. myristica	2703.00±1.70^{e}
X. aethiopica	1735.00±3.20^{c}
Blend	1250.69±3.10^{b}
Acarbose	1002.26±2.20^{a}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

α-glucosidase Inhibition

The percentage inhibitory activities of each crude essential oil, the blend, and the standard (Acarbose) against α-glucosidase are obtained in Table 22. It was observed that the percentage inhibition of α-glucosidase increased with concentration, while a concentration of 1600 µg/mL recorded the highest inhibition. The blend of essential oils had 76.80% inhibition with a concentration of 1600 µg/mL, which is

closely followed by the standard acarbose with a percentage inhibition of 58.67%. The least inhibition was observed in the essential oil of *X. aethiopica* at 33.38%. The IC_{50} values of α-glucosidase for each of the essential oils and the standard are recorded in Table 23. Essential oil of *S. aromaticum* with the highest IC_{50} value of 3908.00 µg/mL had the least anti-diabetic activity, while the blend essential oil with an IC_{50} of 1080.56 µg/mL had the highest α-glucosidase inhibition.

Table 22. Percentage Inhibitory Activity of Alpha-glucosidase by the Crude Essential Oils, Blend, and Standard.

Samples Concentration (µg/mL)	*S. aromaticum*	*M. myristica*	*X. aethiopica*	Blend	Acarbose
100	8.89±0.21^{c}	7.07±0.93^{b}	5.98±0.41^{a}	7.14±0.41^{b}	11.81±0.62^{d}
200	11.81±0.21^{a}	13.00±0.62^{b}	15.96±0.31^{b}	10.79±0.21^{a}	21.28±0.21^{c}
400	21.28±0.62^{a}	24.93±0.21^{b}	25.36±1.24^{b}	22.38±1.86^{a}	32.94±0.62^{c}
800	28.28±1.86^{a}	31.71±0.31^{b}	32.51±0.82^{b}	50.61±1.24^{d}	45.55±0.10^{c}
1600	33.38±0.83^{a}	40.45±0.10^{b}	38.19±1.13^{b}	76.80±0.41^{d}	58.67±0.52^{c}

*Values here are the mean ± standard deviation. Means with different letter in a row are significantly different at $p < 0.05$.

Table 23. IC_{50} Values for Alpha-glucosidase Inhibition of the Crude Essential Oils, Blend, and Standard.

EO/Standards	Concentration IC_{50}(µg/mL)
S. aromaticum	3908.00±0.80^{e}
M. myristica	1969.00±2.30^{c}
X. aethiopica	2112.22±8.12^{d}
Blend	1080.56±7.30^{a}
Acarbose	1173.45±10.20^{b}

*Values here are the mean ± standard deviation. Means with different letters in a row are significantly different at $p < 0.05$.

DISCUSSION

S. aromaticum essential oil extracted in this study yielded 11.34% which was higher than what was obtained by Selles et al. (2020), who obtained 10.34% yield. Amanda et al. (2021) reported a 3.8% yield. The yield of *M. myristica* essential oil obtained in this study was revealed to be 2.74% which was higher than what was obtained by Ekere et al. (2017), who reported a yield of 0.66% for the essential oil of *M. myristica* but not significantly different from what was obtained by Owokotomo & Ekundayo, (2012), who reported a yield of 2.16%. *X. aethiopica* was reported by Alphonse et al. (2018) to yield 4.2% which was higher than what was reported in this study for *X. aethiopica*, which yielded a value of 1.44%. The differences in the yield from other studies could be a result of harvest, geographical origin, extraction method, and particle size (Selles et al. 2020).

Essential oils are made up of a wide variety of components; these components may be responsible for the synergistic (positive combined effort) properties exhibited by the extract. In some situations, some components present in the extract may have anti-desire effects on the extracts, resulting in an antagonistic (reduced combined effort) effect on the extract activities (Manindra et al., 2011).

The research reported eugenol (75.08%) in the essential oil from the bud of *S. aromaticum*, isocaryophyllene (29.36%) in the essential oil from the seeds of *M. myristica*, isospathulenol (8.67%) in the essential oil from the pod of *X. aethiopica*, and eugenol (34.25%) in the blend essential oil as the major components. Several studies have determined the chemical composition of the three essential oils. Ainane et al. (2019) reported that eugenol (17.60%), 1,1,4,8-tetramethyl-cis, cis,4,7,10-cycloundecatriene (27.7%), Caryophyllene oxide (24.3%), Caryophyllene (4.22%) and Humulene epoxide II (3.93%) were the five main components of a sample of *S. aromaticum* essential oil from morocco; Kaur & Kaushal, (2019) reported eugenol with a component of 76.8% very close to what was reported in this study, 75.10% was also reported by Simiat et al. (2017). Awojide et al. (2024) reported eugenol (76.13%) as the major compound found in the essential oil of *S. aromaticum*, which was not significantly different from what was observed in the essential oil of *S. aromaticum* of the present study. Isocaryophyllene (29.36%) and Germacrene D-4-ol (20.31%) were reported by Ainane et al. (2019) to be the major compounds found. The essential oil of *M.myristica* in a sample from Nigeria, Awojide et al. (2023), reported sabinol (20.95%), n-hexadecanoic acid (7.66%), linalool (9.11%), and Trans-13-octadecenoic acid (25.18%) as the major constituents of the essential oil of *M. myristica*. Alphonse et al. (2018) revealed that the main components of *X. aethiopica* essential oil were β-pinene (32.16%), β-phellandrene (10.71%), Z-γ-bisabolene (10.07%), and α-pinene (7.39%). The provenance of the fruits, which was not always known, could be one of the causes of this variation; Awojide et al. (2023) mentioned that the treatments of the fruits received after harvesting may be another factor responsible for the variations.

Phenolic contents were detected in all the essential oils, with the highest phenolic constituent observed in *S.*

aromaticum (182.46 mg GAE/g Extract), this was higher than what was observed in the work of Trifan et al. (2021), who reported a total phenolic content of 113.86 mg GAE/g Extract. El Ghallab et al. (2019), also reported the presence of phenolic content in *S. aromaticum.* Nkwocha et al. (2018) reported the presence of six phytochemicals obtained in the study of the essential oil of *M. myristica* (flavonoid was not determined). The values of phenolic content in this study were found to be greater than the values obtained for flavonoids; the same was reported by Hemalatha et al. (2016) and Nkwocha et al. (2018). Aguoru et al. (2016) also reported the presence of alkaloids, saponins, tannins, flavonoids, and steroids in the seeds of *X. aethiopica*. The phytochemicals present in the crude essential oils were present in the blend of the essential oils, but the values obtained for proanthocyanin, saponin, and terpenoid were the least in the essential oil blend. The blend of the essential oils may either increase or reduce the biological activity due to interaction between the components, which may be responsible for the variable values obtained (Romulo et al., 2020).

Antioxidants are known to be responsible for the defense mechanisms of the body against diseases associated with free radical attacks. Plant extracts have been documented to help prevent diseases caused by oxidative stress (Moharram & Youssef, 2014). Antioxidants work either by breaking down antioxidants, which react with lipid radicals and convert them into more stable products (Miguel, 2010) or by capturing free radicals and stopping the chain reactions (Moharram & Youssef, 2014). The result of this research indicated that while the blend resulted in a synergistic response as an antioxidant with respect to some of the essential oils, it was antagonistic to some. In the assessment of the DPPH scavenging ability of the essential oil and the blend, the blend was only synergistic to *M. myristica*. The blend had 61.26% inhibition of DPPH, higher than 60.95% obtained for *M. myrisitca*, but significantly not different; the same trend was reported for nitric oxide inhibition. The lower value of phenolic content present in the blend and *M. myristica* compared with those of other essential oils may be responsible for the lower DPPH scavenging activity observed. According to Zeb (2020), phenolic compounds have been attributed to have a strong antioxidant potential. The higher DPPH scavenging ability of *X. aethiopica* could be a result of the compound chavibetol observed in the essential oil. Chavibetol has been reported by Singh et al. (2009) to possess antioxidant properties, which could also have been responsible for the higher terpenoid observed in the essential oil, which is also known to have a wide spectrum as an antioxidant (Gutiérrez-del-Río et al., 2021). The presence of chavibetol in the blend could also contribute to the higher activities displayed than the essential oil of *M. myristica*.

For ABTS scavenging activities of the essential oils and the blend, the blend produces a percentage inhibition of 34.90% which was the least compared to other essential oils. The lipid peroxidation activity indicated that the blend exhibited a synergistic response, producing the highest lipid peroxide inhibition of 76.80%. The different responses of the indicators to the essential oils could be a result of the different mechanisms of action, which may be brought about by the different chemical components observed in the different essential oils (Miguel, 2010). *X. aethiopica* had higher DPPH scavenging activity than other essential oils, and the blend, *S. aromaticum*, was more effective in the inhibition of ABTS radical scavenger and nitric oxide oxidation, but the blend had the highest lipid peroxidation. Eugenol, having the highest composition in *S. aromaticum*, and the blend may be responsible for this activity observed, this was revealed in a study by Han and Parker (2017). In general, the antioxidant activities differ, depending on the test employed (Chebbac et al., 2013). The value of IC_{50} reported in this study for the DPPH assay was higher than that reported by Selles et al. (2020). Amanda et al. (2021) also reported the ABTS radical scavenging ability of *S. aromaticum* essential oils. Lucrece et al. (2018) reported that the evaluation of the antioxidant activity indicated that *S. aormaticum* had better antioxidant activity compared to *M. myristica*. This is in tandem with what was reported in this work. The synergistic and antagonistic antioxidant activity was evident in the blend.

Unlike in the case of antidiabetic activity, where the blend of the essential oils was more effective in the inhibition of the two different antidiabetic indicators, the essential oils subjected to the anti-inflammatory activity recorded a different trend for the three indicators used. For the lipoxygenases assay, the blend recorded the least inhibition of lipoxygenases (28.51%), while the highest activity of lipoxygenases inhibition was observed in the essential oil of *S. aromaticum* (67.11%). *S. aromaticum* also recorded the highest inhibition in the membrane stability at 45.65% but the least activity of 39.96% was exhibited by *X. aethiopica* essential oil. This trend in the variation of the effectiveness as an anti-inflammatory agent for the indicators was also observed by İlhami et al. (2012), who reported variation of the effectiveness of the different extracts on antioxidant indicators. This variation could be as a result of the different modes of action possibly produced by the differences in the chemical compounds of the different essential oils (Miguel, 2010). Eugenol, which was the most abundant component observed in the essential oil of *S. aromaticum*, may have been responsible for the anti-inflammatory activities observed here. According to Patlevič et al. (2016), eugenol was observed to exhibit anti-inflammatory activity effects in acute lung injury caused by lipopolysaccharide. The high abundance of flavonoids in the essential oil of *X. aethiopica* may have contributed to the anti-

inflammatory properties. Ginwala et al. (2019) reported that flavonoids provide anti-inflammatory activity by lowering the formation of reactive oxygen species. A high content of tannin present in *S. aromaticum* may have also contributed to the higher anti-inflammatory activity observed in two of the indicators (Patlevič et al., 2016; Ambreen & Mirza, 2020). The blend of the essential oils recorded the least activity in respect to lipoxygenases inhibition alone, but showed a higher activity than *M. myristica* for the membrane stability assay and higher activity than *S. aromaticum* and *M. myristica* in the xanthine oxidase activity. The blend, therefore, showed synergistic and antagonistic activities with respect to some anti-inflammatory indicators. The differences in the activities may depend on the composition and ratio of the components observed in each oil (Pandur et al., 2021).

The result of the antidiabetic activities of the different essential oils using α-amylase and α-glucosidase assay indicated that the blend of essential oils recorded the least IC_{50} value of 1250.69 µg/mL and 1080.56 µg/mL, respectively, compared to the other essential oils. This is indicative of a better antidiabetic potential of the blend of essential oils than observed for the crude essential oils of *S. aromaticum, M. myristica,* and *X. aethiopica*. The antidiabetic activity observed could be attributed to the presence of flavonoids, tannins, saponins, phenolic content, and steroids in the essential oils (Tadesse et al. 2017; Gowd et al., 2017). The higher antidiabetic activity recorded in the blend could be a result of the synergy between eugenol and chavibetol, an isomer of eugenol (Vandana & Shalini, 2014); both have been reported to possess antidiabetic activity (Al-Trad et al., 2019). *Piper betle,* which has been attributed to possess antidiabetic activity, has chavibetol to be the highest component (53.1%), and it was touted to be responsible for the activity (Rekha et al., 2014; Thirugnanasambandam et al., 2022).

CONCLUSION

In this study, the medicinal activities of the blend of the essential oils of *S. aromaticum, M. myristica* and *X. aethiopica* were compared with those of the crude essential oils. The study showed varied components in the blend, compared to the crude essential oils. The results obtained from this study showed that the essential oil blend has synergistic efficacy in several of the medicinal assays conducted, indicating a potential better therapeutic as antioxidant and anti-diabetic properties compared to the crude essential oils.

LIST OF ABBREVIATIONS

IC_{50}- Inhibitory Concentration at 50%; DPPH- 2,2 diphenyl-1-picryhydray; ABTS- 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid; EOs- Essential Oils; GC-MS- Gas Chromatography-Mass Spectrometry.

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