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A comparative study on the synergistic activities of fractions and crude essential oil of *Syzygium aromaticum*

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Abstract

Background *Syzygium aromaticum* has been used for over a century because it has biological qualities for several health-related problems, and the crude essential oils are known to contain several components that could be responsible for synergistic or antagonistic potentials in relation to their biological uses. The study was designed to compare the biological activities of the crude essential oil from the bud of *Syzygium aromaticum* and fractions obtained from column chromatography.

Materials and methods The essential oil was extracted by hydrodistillation, and two prominent fractions, C1 and C2, were obtained from the column chromatography separation of the crude essential oil. The quantitative phytochemical analysis was done by standard methods, the antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging assays. The antifungal activities were assessed by the agar diffusion method, while the anti-inflammatory activity was done using a lipoxygenase inhibitory assay. The major component observed in the crude and fractions was determined by gas chromatography–mass spectrometry (GC–MS) analysis.

Results The phytochemical analysis showed that phenolic acid, tannin, saponin, flavonoids, alkaloids, cardiac glycosides, and terpenoids were present in variable quantities in all of the fractions and crude essential oil. The half-maximal inhibitory concentration (IC_{50}) value for DPPH scavenging activity ranged from 226.1 to 301.2 µg/mL while that of ABTS scavenging activity ranged from 211.0 to 316.0 µg/mL. The lowest value of IC_{50} for both DPPH and ABTS was observed in fraction C1. The crude essential oil with a minimum inhibitory concentrations (MIC) value of 8.00 mg/mL was the most efficient against *Candida albicans*, *Aspergillus penicillum*, and *Aspergillus niger*. The anti-inflammatory assay showed C1 to have the lowest value of IC_{50} for the lipoxygenase assay. The major component observed in the crude essential oil as well as fraction C1 was eugenol, while fraction C2 had β-caryophyllene as the major component.

Conclusions The result showed that all fractions as well as the crude essential oil exhibited antioxidant, antifungal, and anti-inflammatory activities, and the crude essential oil was synergic only with respect to its antifungal activity, while fraction C1 was more efficient as an anti-inflammatory and antioxidant agent.

Keywords Medicinal, *Syzygium aromaticum*, Essential oil, Synergic, Fractions

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Background

Africa is blessed with a vast array of plant species, of which several have been used since time immemorial for the treatment of ailments. In recent times, the use of natural products as well as their constituents has become a major source for the development of new drug entities (Awojide et al. 2023a). In spite of the present focus on synthetic medications, the primary source of medications has always been and will continue to be medicinal plants (Amal et al. 2021). Eighty percent or more of the world's nation, which resides primarily in underdeveloped nations, is still thought to rely on herbal remedies as their main healthcare provider today (Msomi and Simelane 2017). Essential oils (EOs), in particular, are plant extracts that contain a number of phytochemicals with a range of physiological effects on the body (Al-Mijalli et al. 2022). EOs are obtained via distillation from oil glands in some parts of plants, such as leaves, roots, and stem (Baldino et al. 2020). Numerous medical applications for the secondary metabolites exist, which includes anticancer, antifungal, antioxidant, anti-inflammatory, antiviral, and antiprotozoal (Ma and Yao 2020). Several studies have been conducted on the effect of antimicrobial effects from isolated components such as alkaloids, phenolics, lactones, terpenes, naphtoquinones, flavonoids, and phenolics derived from medicinal plants or their extracts and EOs (El Omari et al. 2022). This approach, known as "bioguided isolation and identification of phytochemicals," was employed after previously detecting microbial activities on the part of the plant. The previous information obtained is how some of these phytochemicals were found after the plant's antibacterial effect was first discovered (Nada et al. 2022). Additionally, current research has helped to identify the pathways by which components in plants help produce the various activities observed. In fact, natural antimicrobial compounds can function on a variety of levels, including intracellular signaling of microbes, subcellular, and protein synthesis (Acedo et al. 2018; Natalia et al. 2021).

Essential oils are known to contain several components that are responsible for the different activities observed. Synergistic activity is observed when the effect of the combined components of the crude essential oil is higher than what was observed when the crude essential oil is separated into different fractions that have components different from what was observed in the crude essential oil (Awojide et al. 2023b).

Syzygium aromaticum is a dried flower bud that is indigenous to the Maluku Islands and belongs to the *Myrtaceae* family in Indonesia, however, recently grown around the world (Batiha et al. 2019, 2020). Four years after planting, the clove tree's commercially valuable parts—its leaves and buds—begin to develop flower buds; afterward, during the pre-flowering stage, they are picked manually (Cortés-Rojas et al. 2014; Batiha et al. 2020). Clove is one of the spices that, interestingly enough, due to its antioxidant and antibacterial qualities, is utilized as preservatives in various foods, notably when preparing meat, to substitute artificial preservatives (Zainol et al. 2017; José et al. 2021). In addition to being used as a spice, it is also used in the perfume industry and in commercial medicine (Cortés-Rojas et al. 2014; Otunola 2022). Several researchers have shown that certain fragrant herbs, such as clove have antifungal, antibacterial, and antiviral properties (Zainol et al. 2017; Maggini et al. 2024). However, due to its powerful antibacterial and antioxidant properties, clove has attracted a considerable interest compare to other spices (Liangwei et al. 2023). Clove's potent ability to prevent several diseases is credited to the numerous chemical components which have antioxidant activity (Astuti et al. 2019; Aziz et al. 2023). Clove essential oil has long been used for healing wounds and relief of pain during dental procedures (Zainol et al. 2017; Banerjee et al. 2020). It is employed for use in several domestic industries while Indian and Chinese frolic medicine employ cloves as a stimulating and warming medication (Batiha et al. 2019). Clove essential oil had found use traditionally as a stimulant for nervous system as well as treatment for nausea, vomiting, flatulence, liver, intestine, and stomach disease (Zainol et al. 2017; Batiha et al. 2020). In tropical Asia, the use of cloves in the treatment of some pathogens such as tuberculosis, scabies, malaria and cholera has been proved (Batiha et al. 2020). Additionally, the use of eugenol is frequently utilized in the treatment of the teeth since it can reach the bloodstream through the pulp tissue in the teeth (Martínez-Herrera et al. 2016; De Grado et al. 2023).

While there have been numerous studies on the therapeutic properties of essential oil from the bud of *S. aromaticum*, there are some schools of thought that contend that the different constituents may be the cause of synergic activity. Other schools of thought contend that certain compounds may have countereffect and may result in antisynergic properties (Zainol et al. 2017; Awojide et al. 2023b). This study was conducted to ascertain whether the components of the crude essential oil will possess synergistic activities in relation to some medicinal properties.

Methods

Materials

The clove bud (*S. aromaticum*) were obtained from a local market in Osogbo, Oja Oba market Osun State, Nigeria. The bud from clove was identified using a specimen from the herbarium, IFE-17940, at the Department

of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Sample pre-treatment

The *S. aromaticum* bud was air-dried after which it was size reduced to powder using pestle and mortar. The powder was kept in an airtight container until required for usage.

Extraction of the essential oil

The essential oil of the *S. aromaticum* bud was extracted for 4 h using the hydrodistillation method. The essential oil was dried with anhydrous sodium sulfate and stored in an airtight, labeled glass container at 4 °C in a refrigerator.

Fractionation

The crude essential oil (38 g) was separated into various fractions using a 3 × 100 cm column size. A range of 0.063- to 0.200-mm-sized particles of silica gel were used as the stationary phase. Hexane and ethyl acetate were used to elute the sample using a polarity gradient (100/00, 80/20, 60/40, 40/60, 20/80); and an additional ethyl acetate/methanol combination system was also used (100/0, 80/20, 100/0). Each time, the column was filled with a measured volume (100 mL) of each solvent mixture using a separating funnel. The eluted fractions were collected in test tubes in aliquots of 10 mL. Fractionation monitoring was done on a thin-layer chromatographic (TLC) aluminum plate. The fractions were spotted on pre-coated silica gel (F254) and developed using the solvent ratio that was used for the elution. The plate was taken out and dried with a hot air dryer. The TLC plate was visualized by spraying with H_2SO_4 reagent and subjecting the plate to heat in an oven at 110 °C for 5 min. After solvent evaporation on a rotary evaporator at 40 °C, the resulting fractions were categorized based on their chromatographic profiles, and a total of two fractions (C1 and C2) were obtained.

GC–MS analysis

The Agilent 6890N instrument containing a flame ionization detector and a capillary column HP-5MS (30 m, 0.25 mm, 0.25 m), coupled with an Agilent Technologies mass spectrometer, model number 5973N, was used. The injector temperature was left at 270 °C, and 1 µL 1:10 split ratio injections were used for the samples. Helium, the carrier gas, flowed at 1.0 mL min^{-1} The temperature in the GC was 60 °C for 1 min, ramped up to 180 °C for 10 min at a rate of 10 °C per minutes, and then increased to 280 °C for 15 min. The scans were done at 20 to 550 m/z at 2 scans per seconds. The various compounds, and their constituents' identities were verified by relating their Kovat's retention indices with respect to C_8–C_{32} n-alkanes and making use of samples or information from NIST 2008, as well as publications and the library.

Quantitative phytochemical analysis

Determination of total phenols

The method of Folin–Ciocalteu was used (Awojide et al. 2023b). A 125 µL crude essential oil or fractions was mixed with distilled water and Folin–Ciocalteu's reagent. The mixture was allowed to stand for 6 min before the addition of 7% sodium carbonate. The absorbance was measured at 760 nm using a SpectrumLab70 spectrophotometer after 90 min of standing, and the results were provided as gallic acid equivalents (GAE).

Determination of saponin

Spectrophotometric method (Awojide et al. 2023b) was employed. About 2 g of the essential oil was measured into a beaker and mixed with but-2-ol. The mixture was transferred into a beaker containing a 40% magnesium carbonate solution after filtering through No. 1 Whatman filter paper held the mixture. One mL of the solution was added to two mL of $FeCl_3$. At a wavelength of 380 nm, the absorbance was read using a SpectrumLab70 spectrophotometer after being left to stand for 30 min to allow the color to develop.

Determination of tannins

The crude essential oil or fractions (0.5 mL) was dissolved in 80% ethanol (2 mL), and Folin–Ciocalteu reagent (2 mL) was mixed with 0.1 mL of the diluted sample. The solution was left for 8 min, after which 7.5 mL of Na_2CO_3 (7%) was added and left to incubated for two hours. The tannic acid curve was used as a reference to determine the absorbance at 760 nm and the tannin concentration (Awojide et al. 2023a).

Determination of flavonoid

A 1 mL of distilled water was used to dissolve approximately 0.25 g of the crude essential oil or fractions; the resulting mixture was added to 0.150 mL of freshly made $AlCl_3$, 5% $NaNO_2$ solution, and 1 M NaOH solutions. A SpectrumLab70 was used to measure the absorbance at 510 nm after 5 min. The result was expressed as milligrams per gram (mg/g) (Awojide et al. 2023a).

Determination of alkaloid

To 0.3 g of ethanol which consist of 10% acetic acid was added 0.3 g of the crude essential oil or fractions; it was filtered after 4 min and the filtrate was then concentrated in a water bath, after which dropwise addition of NH_4OH was done till precipitation was complete. Washing of the

precipitate was done with dilute NH_4OH. The residue obtained was weighed after drying (Awojide et al. 2023b).

Determination of cardiac glycosides

Chloroform was mixed with 2 mL of the extract in a conical flask and filtered. Into the filtrate obtained, a mixture of 2 mL 29% sodium nitroprusside and pyridine was added and stirred for 10 min, after which a solution of 20% NaOH was added for color formation. Absorbance was then read at 510 nm on a SpectrumLab70 (Awojide et al. 2023a).

Quantitative test for terpenoids

The crude essential oil or fractions of 100 mg was added to 9 mL of ethanol and left for 24 h (Awojide et al. 2023a). A separating funnel was used to extract the filtrate obtained using 10 mL of petroleum ether. The ether extract was split into glass vials that were precisely weighed and allowed to dry completely (W_f). The total terpenoids' contents were measured by the formula:

$$(S_i - S_f/S_i \times 100)$$

where S_i is the initial weight and S_f is the final weight.

Antioxidant analysis

DPPH scavenging assay

Method described by Awojide et al. (2023a) was adapted. The reaction was observed at 517 nm. 1 mL, 0.3 mM DPPH ethanol solution was mixed with 2.5 mL of different concentrations of the crude essential oil or fractions and standards of 50, 100, 150, 200, and 250 µg/mL) at room temperature, and let to incubate in the dark. Readings of absorbance was taken after 30 min at 517 nm. The DPPH served as the negative control, while ethanol was used as a blank. The degree of DPPH color change from purple to yellow revealed the studied samples' level of scavenging effectiveness. Every test was run in triplicate and average were obtained. Inhibitory concentration at 50% (IC_{50}) was calculated. The following equation was used to determine the DPPH free radical scavenging activity percentage.

$$\begin{aligned}\text{DPPH radical scavenging activity}(\%) =&[(\text{Abs control}\\ &-\text{Abs sample})]\\ &/(\text{Abs control})\\ &\times 100\end{aligned}$$

Abs control: absorbance of DPPH radicals + methanol, Abs sample: absorbance of DPPH radical + sample or standard.

ABTS radical scavenging activity

The ABTS activity was evaluated using method described by Awojide et al. (2023a). A 5 mL of 4.9 mM $K_2S_2O_8$ solution of 14 mM ABTS were combined and kept in a dark place for 16 h at ambient temperature. Before use, it was mixed with ethanol then absorbance read at 734 nm giving a value of 0.700. After homogenizing the crude essential oil and fractions each with 1 mL of the ABTS solution at varying concentrations, absorbance at 734 nm was taken. Each test included running ethanol blanks. The IC_{50} (g/mL) value for ABTS scavenging was used. Using the following formula, the inhibition percentage of the ABTS radical was determined:

$$\text{ABTS scavenging activity}(\%) = \{(S_0 - S_1)/S_0\} \times 100$$

S_0: Absorbance of the control and S_1: absorbance of the sample.

Antifungal screening

The fungal isolates used in this study were *Candida albicans* (NCIB 1161), *Aspergillus* penicillium (NCIB 1131543) and *Aspergillus niger* (NCIB 380704) which were obtained from the Central Research Laboratories, University of Ilorin. In order to observe fungus growth, the stock fungus isolates were revived by culture in nutrient broth and agar. Three distinct fungal species were put into petri dishes filled with potato dextrose agar. By running each extract through a 0.22 m Millipore VG filter paper, the extracts were sterilized (Millipore USA). To inoculate the petri plates, each extract (at a concentration of 100 mg/mL) was placed into spherical paper dishes with a radius of 0.8 cm. After 48 h of fungal colony growth at 28 °C, the inhibition zone (activity) surrounding the disk was assessed. As a positive control, fluconazole (FLU), 1 mg/mL was utilized.

Preparation of standard fungal organisms

On Sabouraud dextrose agar, the fungus standard cultures were kept alive for 4 days while being incubated at 25 °C. Harvested fungal growth was then rinsed with sterile normal saline. It was afterward suspended in 100 mL of the solution, and kept in the refrigerator until it was needed, assessing the antifungal effectiveness of the extract in vitro. The method of Oyewole et al. (2023) was used. According to this approach, 100 mL of sterile Mueller–Hinton agar that was kept at 45 °C was well mixed with 1.0 mL of the isolated, standardized, and fungal stock suspension (108–109 C.F.U per ml). On sterile petri dishes, 20 mL aliquots of the inoculated Mueller–Hinton agar were applied. The agar was allowed to set

before four cups (10 mm in diameter). The agar disks in each of these plates were cut out with a clean cork borer (NO.4). Using a standard fine adjustable automatic pipette, 100 µL of samples of each extract was placed in different cups then kept at room temperature for two hours to disperse. The plates were then kept at 37 °C for 18 h while standing upright. Each extract's effectiveness against each of the examined organisms was tested three times. The addition of methanol served as a positive control in place of the extract at the same time. After the incubation period was up, the diameter of the inhibitory zones that had formed was measured and averaged, and the mean values were calculated.

Lipoxygenase inhibitory assay

The technique by Awojide et al. (2023b) was used to suppress lipoxygenase with a little modification. 5 ml of enzyme solution (50,000 units/ml) in borate buffer were added to 5.0 ml of essential oil solution in DMSO (which provided 50, 25, 12.5, 6.2, 3.1, and 1.55 g essential oil/ml reaction mixture) (0.2 M, pH 9.2). 250 mL of linoleic acid solution was added to the reaction, along with 15 mL of ethanol and 15 mL of a borate buffer solution (0.2 M, pH 9.2), all while vigorously shaking. In a spectrophotometer, the rise in absorbance at 234 nm was monitored for 5 min (Spectrumlab 752S). As a positive control, quercetin was employed at the same concentration as the essential oils. As a negative control, the reaction mixture containing 5.0 mL of DMSO in place of the essential oil solution was employed. The IC_{50} was determined using the Prism 5.0 software after each test was run in triplicate.

Statistical analysis

The data were analyzed using SPSS software version 21, and comparisons of the means were determined by one-way analysis of variance (one-way ANOVA), followed by the Tukey test. Values with $p < 0.05$ were considered statically significant. All tests were run in triplicate, and the results are expressed as mean ± SD. Figure 1 depicted the procedure of extracting crude and fractionated essential oil from the bud of *S. aromaticum*.

Results

Table 1 shows the percentage yield of the fractions obtained. Fraction CI had the highest yield of 61.13%. In Figs. 2, 3, and 4, chromatogram of the crude essential oil and fractions C1 and C2 of *S. aromaticum* is shown. The major components from the GC–MS analysis of the crude essential oil and fractions C1 and C2 of *S. aromaticum* are obtained in Tables 2, 3, and 4. The analysis revealed eugenol (76.13%) as the major component of the crude essential oil, after which comes β-caryophyllene (14.45%), while eugenyl acetate (1.22%) was the least

Table 1 Mass yield of fractions of *S. aromaticum*

Fractions	Yield (%)
C1	61.13
C2	19.0

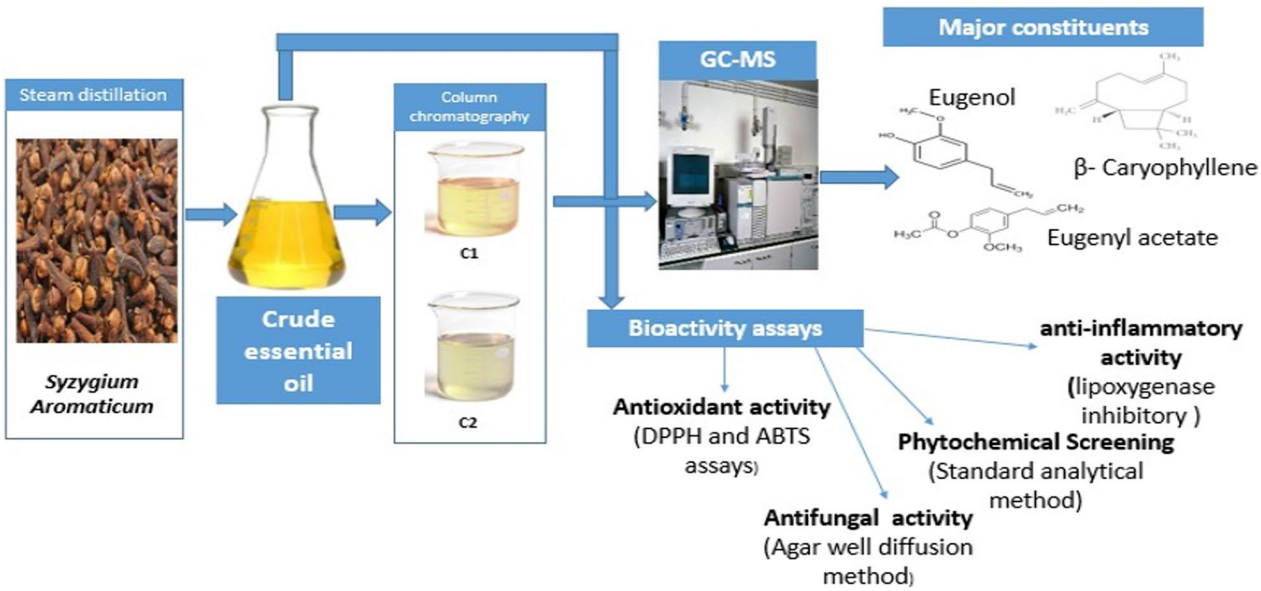

Fig. 1 Scheme showing the summary of the experimental procedure

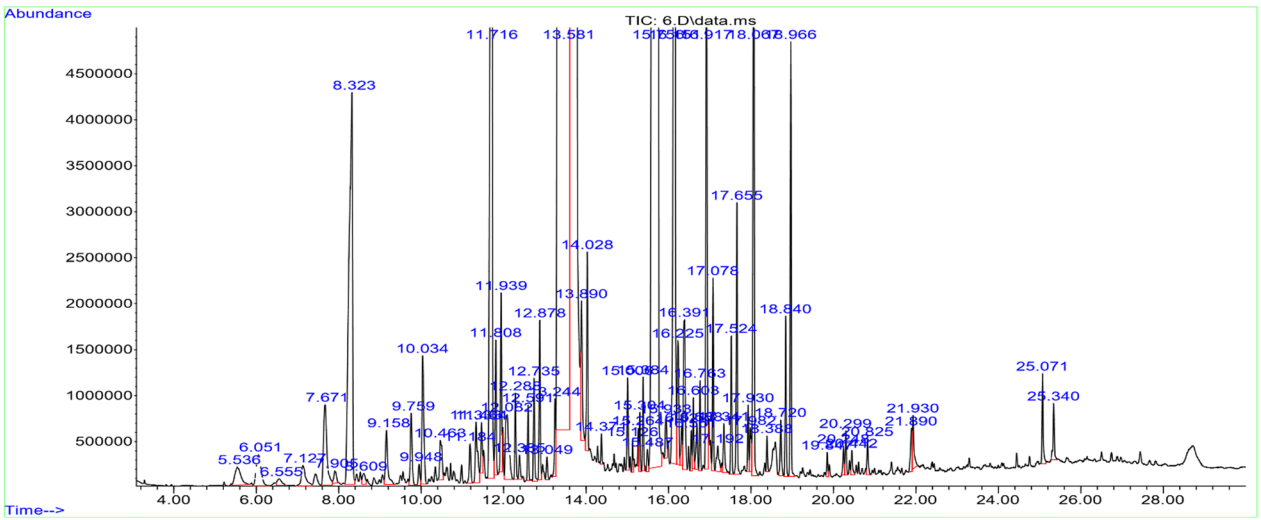

Fig. 2 Chromatogram of the crude essential oil

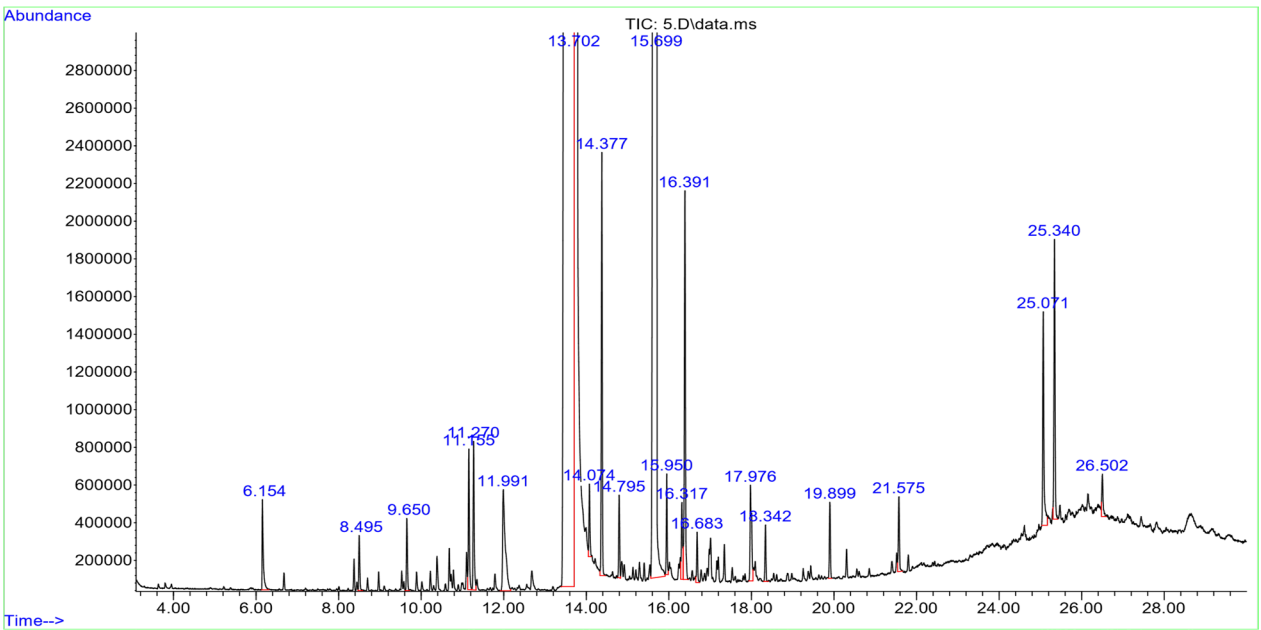

Fig. 3 Chromatogram of fraction C1

major component observed. Eugenol (90.20%) was the highest component observed in fraction C1, and fraction C2 indicated β-caryophyllene (63.19%) as the most predominant in the fraction.

The results of a quantitative investigation of the phytochemical parameters of *S. aromaticum* fractions and crude essential oil are shown in Table 5. Both the crude essential oil and the fractions obtained contained phenolic acid; fraction C2 had the lowest value of phenolic acid (1.87 mg GAE/g), which is significantly different from the value obtained in the crude essential oil, while fraction C1 had the highest phenolic acid value of 2.65 mg GAE/g. Fraction C1 had the highest saponin content of 9.62 mg/g, whereas fraction C2 had the lowest saponin value of 5.73 mg/g. The value of tannin was found to be higher in fraction C1 (6.50 mg/100 g) and least in fraction C2 (3.20 mg/100 g). The quantity of flavonoid was highest in the crude essential oil of *S. aromaticum* (8.01 mg QE/g), and lowest in fraction C2 (6.31 mg QE/g). The highest amount of alkaloid

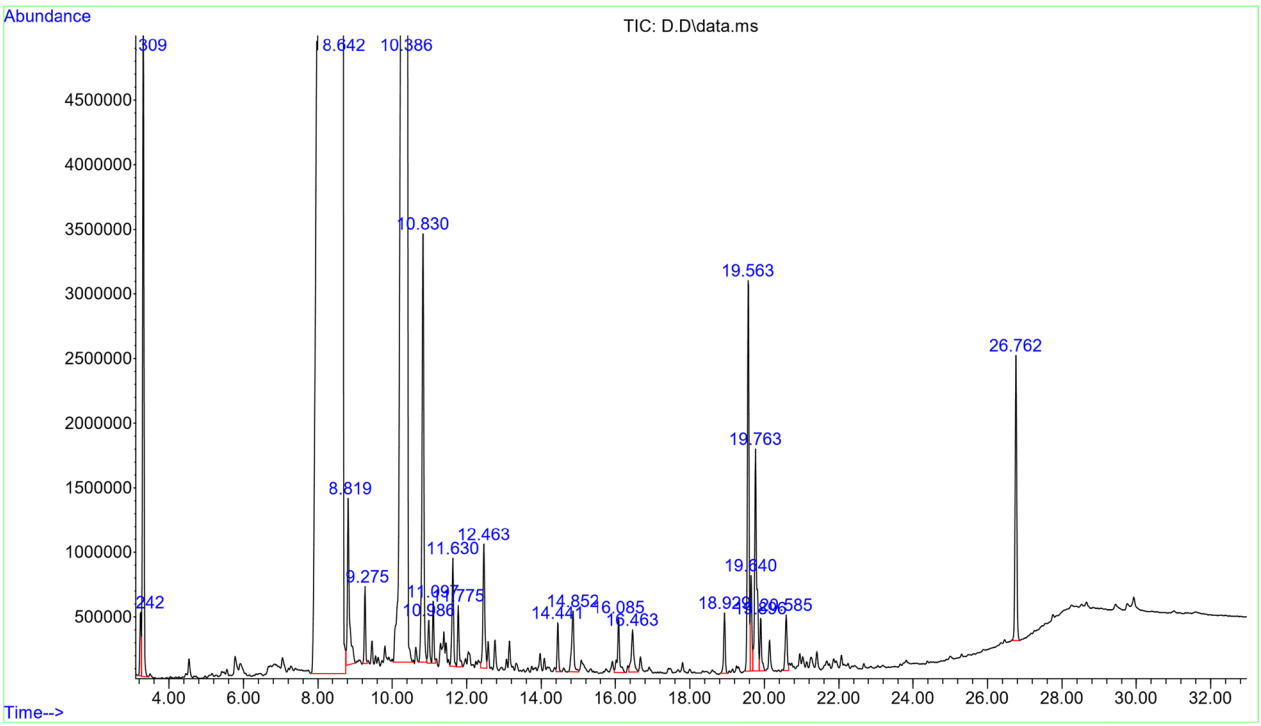

Fig. 4 Chromatogram of fraction C2

Table 2 Major constituents of the crude essential oil of *S. aromaticum*

Compounds	%	RI (cal)	RI (literature)
Eugenol	76.13	1326.3	1339.6
β-Caryophyllene	14.45	1411.2	1419.3
Eugenyl acetate	1.22	1473.3	1484.5

The retention indices revealed by Babushok et al. (2011) are represented by the acronym RI (literature). Component retention indices for the stationary phase of dimethylsilicon

Table 3 Major constituents of the fraction C1 of the essential oil of *S. aromaticum*

Compounds	%	RI (cal)	RI (literature)
Eugenol	90.20	1325.4	1339.6
β-Caryophyllene	8.42	1411.2	1419.3

The retention indices revealed by Babushok et al. (2011) are represented by the acronym RI (literature). Component retention indices for the stationary phase of dimethylsilicon

was recorded in fraction C1 (3.47 mg/g) while the least value of alkaloid was observed in the crude essential oil (3.00 mg/g). Cardiac glycoside was highest in fraction C1 (5.42 mg/g) and lowest in fraction C2 (4.73 mg/g). The highest concentration of terpenoid was found in

Table 4 Major constituents of the fraction C2 of the essential oil of *S. aromaticum*

Compounds	%	RI (cal)	RI (literature)
Eugenol	5.78	1331.1	1339.6
β-Caryophyllene	63.19	1411.2	1419.3
Eugenyl acetate	4.80	1479.3	1484.5
Benzyl acetate	4.21	1139.2	1141.1
Sabinol trans-	2.54	1126.3	1130.9

The retention indices revealed by Babushok et al. (2011) are represented by the acronym RI (literature). Component retention indices for the stationary phase of dimethylsilicon

fraction C1 (5.25 mg/g), while the lowest value of terpenoids was found in the crude essential oil (5.00 mg/g) with no significant difference in the value obtained in fraction C2.

Antioxidant activity

Using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ABTS (2, 2-azobis-2-aminopropane hydrochloride) methods, the antioxidant activity of the crude essential oil and fractions were assessed. Table 6 displays the values of the inhibitory concentration at 50% (IC_{50}) for DPPH, ABTS, and two positive standards, gallic acid and vitamin C. The highest radical scavenging activity

Table 5 Quantitative phytochemical component of *S. aromaticum*

Component	C_1	C_2	Crude essential oil
Phenolic acid (mg GAE/g)	2.65 ± 0.02^{c}	1.87 ± 0.03^{a}	2.00 ± 0.01^{b}
Saponin (mg/g)	9.62 ± 0.03^{c}	5.73 ± 0.01^{a}	9.06 ± 0.01^{b}
Tannin (mg/100 g)	6.50 ± 0.01^{c}	3.20 ± 0.01^{a}	5.70 ± 0.03^{b}
Flavonoid (mg QE/g)	7.28 ± 0.01^{c}	6.31 ± 0.02^{a}	8.01 ± 0.02^{b}
Alkaloid (mg/g)	3.47 ± 0.03^{c}	3.22 ± 0.03^{b}	3.00 ± 0.01^{a}
Cardiac glycoside (mg/g)	5.42 ± 0.02^{c}	4.73 ± 0.01^{a}	5.18 ± 0.02^{b}
Terpenoid (mg/g)	5.25 ± 0.03^{b}	5.03 ± 0.01^{a}	5.00 ± 0.03^{a}

The table's values are each represented as mean SD ($n = 3$). A substantial difference ($p < 0.05$) exists between values in the same column that are followed by a different letter

Table 6 DPPH radical scavenging activities of fractions and crude essential oil of *S. aromaticum*

Concentration IC_{50}(µg/ml)		
Fractions/Crude oil	**DPPH**	**ABTS**
C1	226.1 ± 12.0	211.0 ± 2.2
C2	301.2 ± 13.2	316.0 ± 3.0
Crude essential oil	235.3 ± 9.1	261.0 ± 2.7
Ascorbic acid	734.20 ± 10.3	342.2 ± 10.3
Gallic acid	482.9 ± 10.2	321.1 ± 10.2

The table's values are each represented as mean SD ($n = 3$). A substantial difference ($p < 0.05$) exists between values in the same role that are followed by a different letter

for DPPH and ABTS was reported in fraction C1 with an IC_{50} value of 226.1 µg/mL and 211 µg/mL, respectively, but the least activity was recorded in fraction C2 with IC_{50} values of 301.2 µg/mL and 316.0 µg/mL for DPPH and ABTS, respectively. Gallic acid, with an IC_{50} value of 482.9 µg/mL for DPPH and 321.10 µg/mL for ABTS had lower radical scavenging activities than the crude as well as the fractions. The IC_{50} values for the crude essential oil as well as the fractions showed that they have a greater radical scavenging potential than ascorbic acid.

Antifungal activity

Results of the crude essential oil's antifungal activity and fractions of *S. aromaticum* were examined using the cup-plate agar diffusion method against *C. albicans*, *A. penicillum*, and *A. niger*. Tables 7, 8, and 9 and Fig. 5 display the antifungal activities of the crude essential oil and fractions of *S. aromaticum* essential oil against three fungi. *C. albicans* is the only gram-positive fungus included in the study. Both the crude essential oil and the fractions recorded a zone of inhibition with a dose of 6.25 mg/mL. The concentration of 50 mg/mL produced the highest activity; fraction C2 recorded a zones of inhibition of 2.80 mm, which was lower than the zone of inhibition of the crude essential oil of 4.00 mm. The result of the antifungal activities of the crude essential oil and fractions of *S. aromaticum* against *A. penicillum* revealed that all samples recorded zone of inhibition with a dose of 6.25 mg/mL, the crude essential oil recorded a zone

Table 7 Antifungal activity of fractions and crude essential oil of *S. aromaticum* against *Candida albicans*

Concentration (mg/mL)	50.00	25.00	12.50	6.25	MIC
Fractions/Crude	**Diameter of zone of inhibition (mm)**				
C1	3.10 ± 0.10	3.20 ± 0.10	3.00 ± 0.10	2.10 ± 0.10	11.0
C2	2.80 ± 0.10	2.40 ± 0.10	2.20 ± 0.10	2.10 ± 0.10	12.5
Crude essential oil	4.00 ± 0.10	3.30 ± 0.10	3.10 ± 0.10	2.10 ± 0.10	8.00

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

Table 8 Antifungal activity of fractions and crude essential oil of *S. aromaticum* against *Aspergillus penicillum*

Concentration (mg/mL)	50.00	25.00	12.50	6.25	MIC
Fractions/Crude	**Diameter of zone of inhibition (mm)**				
C1	3.20 ± 0.10	2.80 ± 0.10	2.10 ± 0.10	1.10 ± 0.10	10.00
C2	3.00 ± 0.10	2.30 ± 0.10	2.00 ± 0.10	1.00 ± 0.10	12.50
Crude essential oil	3.40 ± 0.10	3.00 ± 0.10	2.10 ± 0.10	1.10 ± 0.10	8.00

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

Table 9 Antifungal activity of fractions and crude essential oil of *S. aromaticum* against *Aspergillus niger*

Concentration (mg/mL)	50.00	25.00	12.50	6.25	MIC
Fractions/Crude	Diameter of zone of inhibition (mm)				
C1	3.10 ± 0.10	2.80 ± 0.10	2.30 ± 0.10	1.00 ± 0.10	10.50
C2	2.80 ± 0.10	2.40 ± 0.10	2.10 ± 0.10	1.00 ± 0.10	11.50
Crude essential oil	3.80 ± 0.10	3.10 ± 0.10	2.10 ± 0.10	1.00 ± 0.10	8.00

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

Fig. 5 Sample images showing zone of inhibition of the fungal (The numbers in the figure indicates: 1. Standard 2. Crude essential oil 3. Fractions C1 4. Fractions C2)

of inhibition of 3.40 mm with a dose of 50 mg/mL and was the highest zone of inhibition observed, which was not different from those of the fractions. The MIC values recorded for fraction C2 was 12.50 mg/mL indicating fraction C2 had the least activity against *A. penicillum*.

The crude essential oil and the fractions C1 and C2 recorded the least zones of inhibition against *A. niger* with the least concentrations of 6.25 mg/mL. The highest activity of the zone of inhibition was recorded at a concentration of 50.00 mg/mL where the crude essential oil recorded the highest inhibition of 3.80 mm with an MIC value of 8.00 mg/mL indicating that it had the highest activity against *A. niger*.

The crude essential oil recorded the minimum inhibitory concentration (MIC) of 8.0 mg/mL for the three fungi, which indicated a higher activity against *C. albicans*, *A. penicillum*, and *A. niger* than all of the fractions.

Anti-inflammatory activity

Using the lipoxygenase inhibitory assays, the anti-inflammatory action was determined. The results of the anti-inflammatory activities of the crude essential oil and fractions of *S. aromaticum* are displayed in Table 10. Fraction C1 had the lowest IC_{50} of 33.8 mg/mL, indicating that when used as an anti-inflammatory agent, it worked better than fractions C2 and the crude essential oil of *S. aromaticum* in the inhibition of lipoxygenases. Ascorbic acid, with an IC_{50} value of 64.34 µg/mL had a higher inhibitory activity than the crude essential oil. The IC_{50} values for fraction C1 indicated that it has a higher anti-inflammatory effect than ibuprofen.

Table 10 Anti-inflammatory activities of fractions and crude essential oil of *S. aromaticum*

Sample	Concentration IC_{50}(µg/mL)
C1	33.80 ± 1.60
C2	168.00 ± 2.00
Crude essential oil	95.50 ± 1.80
Ascorbic Acid	64.34 ± 1.70
Ibuprofen	34.70 ± 1.80

Discussion

Numerous components make up essential oil, and these constituents are what give extracts their functions. Some components may interfere with the desired effects of the extracts under certain circumstances, which may be the cause of the extract's decreased activity (Awojide et al. 2023b).

The research identified eugenol (76.13%) and β-caryophyllene (14.45%) as the two most dominant

components of the crude essential oil of *S. aromaticum*. Numerous studies have identified the chemical composition of the *S. aromatiucm* essential oil. Ainane et al. (2019) reported five main components in a sample of *S. aromatiucm* essential oil from Morocco including 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%). Eugenol (80.5%) and eugenyl acetate (5.01%) were identified as the main compounds found in a sample of *S. aromaticum* from Nigeria, according to Fayemiwo et al. (2014). Eugenol (60.4%) and trans-β-caryophyllene (24.0%) were found to be the major constituent from another sample from Benin as reported by Alitonou et al. (2012). The presence of eugenol in all these studies correlated with the presence of eugenol in the fractions and crude essential oil. The higher value of eugenol in fraction C1 (90.20%) could be a result of the fractionation procedure, where some other components were removed, leaving behind a higher value of eugenol, while the lower quantity in fraction C2 also showed less eugenol during the fractionation procedure. According to Owolabi et al. (2009), the treatments given to the seeds after they were harvested or the place where the seeds were originally from may be a key factor for the variations in the essential oil components reported from different places. The fraction of the crude essential oil may account for the many components and the variation in the amount of each component.

The research reported flavonoid (8.01 mg QE/g) as one of the major phytochemicals from the crude essential oil of *S. aromaticum*. A higher value of 21.90 mg QE/g was reported by El Ghallab et al. (2019) for the essential oil of *S. aromaticum*.

The presence of phenolic acid was noted in the fractions and the crude essential oil. El Ghallab et al. (2019) likewise reported the same phytochemical for the essential oil of *S. aromaticum*. The highest amount of saponin was observed in fraction C1 (9.62 mg/g). Saponin was also reported by Fateh et al. (2017) to be present in the essential oil of *S. aromaticum*. Tannin was observed to be highest in fraction C1 (6.50 mg/100 g) and lowest in fraction C2 (3.20 mg/100 g), and tannin was reported by Karm (2019) to be available in the essential oil of *S. aromaticum*. Flavonoids were found in all of *S. aromaticum* fractions and crude essential oil, with the crude essential oil containing the highest component (8.01 mg QE/g), with these findings being equivalent to those of Cortés-Rojas et al. (2014). Alkaloid was present in *S. aromaticum* fractions and crude essential oil, with fraction C1 having the highest value (3.47 mg/g). The presence of alkaloids was observed in the findings of Lone and Jain (2022). The highest concentration of cardiac glycoside was found in fraction C1 (5.42 mg/g); Lone and Jain (2022) also identified this component in the essential oil of *S. aromaticum*. The highest concentration of terpenoid was found in fraction C1 (5.25 mg/g), which was comparable to other fractions and the crude essential oil of *S. aromaticum* but did not differ significantly in value. Plata-Rueda et al. (2018) also noted this phytochemical to be present in the essential oil of *S. aromaticum*.

Research had revealed the antioxidant potential of β-caryophyllene (Lucas et al. 2022). β-Caryophyllene, which was present in the crude essential oil, may have greatly contributed to the flavonoid's content. Awojide et al. (2023b) reported that flavonoids have been known for their antioxidant properties.

Eugenol which was found to be the most prominent compound in faction C1, as well as the crude essential oil, might have greatly contributed to its antioxidant property. Liangwei et al. (2023) revealed that eugenol, which is a major phenolic acid compound, has several biological activities such as antioxidant, anti-inflammatory, and anticancer. Phenolic acid, which was found to be abundant in fraction C1, might have contributed significantly to its antioxidant property due to the presence of eugenol. Zeb (2020) revealed that phenolic substances are more secure than synthetic antioxidants and have stronger antioxidant properties.

Eugenol was the highest component in the crude essential oil of *S. aromaticum*. Didehdar et al. (2022) reported eugenol to possess antifungal activity. Flavonoids, and tannins which were reported to be in abundance in the crude essential oil, might have contributed to its antifungal activity as a result of eugenol observed. Mohammed and Suresh (2020) demonstrated that numerous species of plant extracts with high flavonoid content had antifungal effects. A previous report by Cotas et al. (2020) reported tannin to possess antifungal activities.

Saponin has been observed to be highest in fraction C1, and it has been observed to display a variety of anti-inflammatory potentials; Passos et al. (2022) reported that saponins have garnered a lot of interest as substantial natural anti-inflammatory components that can affect the activity of a number of inflammatory cytokines in a variety of inflammatory settings. Eugenol being the highest constituent in fraction C1 might have contributed to its anti-inflammatory potential. Patlevič et al. (2016) reported anti-inflammatory effects of eugenol on acute lung injury brought by lipopolysaccharide (LPS).

DPPH and ABTS assays were utilized to evaluate the fractions and crude essential oil antioxidant activity. The researchers observed that the IC_{50} for scavenging free radicals for DPPH and ABTS was 226.1 µg/mL and 261.0 µg/mL, respectively. This result was similar to what was reported by Behbahani et al. (2019), who recorded

a lower activity for both DPPH and ABTS at an IC_{50} of 600.00 µg/mL for both DPPH and ABTS. An earlier study by Alawiyah et al. (2019) found that *S. aromaticum* essential oil exhibits a highly potent antioxidant with an IC_{50} value of 8.224 µg/mL in the DPPH test. The higher value of flavonoid found in the crude essential oil (8.01 mg QE/g) and fraction C1 (7.28 mg QE/g) may account for the stronger radical scavenging potentials, which were demonstrated by their IC_{50} values of 235.3 µg/mL and 226.1 µg/mL for DPPH, respectively. The higher antioxidant activity of fraction C1 could also be explained by the existence of β-caryophyllene present (Lucas et al. 2020). The higher antioxidant activity of fraction C1 could be a result of the higher value of eugenol observed, as eugenol has been reported to possess antioxidant activity (Liangwei et al. 2023). This can also be explained by the greater value of phenolic acid that was observed in fraction C1. Phenolic acid has been observed to contribute to antioxidant properties (Zeb 2020).

In order to assess the antifungal activity, two gram-negative and one gram-positive fungi were used. The findings revealed that crude essential oil and fractions were effective against the three fungi strains and albeit to variable degrees as evidenced by the zone of inhibition and MIC values. Parikh et al. (2021) indicated that *S. aromaticum* essential oil had potent antifungal action against a wide variety of fungi. This outcome is consistent with the current research.

The MIC of the crude essential oil with the maximum activity against *C. albicans*, *A. penicillum*, and *A. niger* was observed to be 8.00 mg/mL. This is consistent with the results of Hamini-kadar et al. (2014), who reported that the essential oil of *S. aromaticum* exhibited several range of antifungal activity against the examined microorganisms. The highest antifungal activity displayed by the crude essential oil is possibly because of the higher combination of the components Awojide et al. (2023a) explained that the numerous compositions of the constituents may be synergistic in functions they exhibit.

The outcome of the anti-inflammatory test for the essential oil of *S. aromaticum* revealed that fraction C1 had the highest anti-inflammatory activity with an IC_{50} value of 33.8 µg/mL for the inhibitory activity of lipoxygenases. The high value of eugenol and saponin could be the cause of the anti-inflammatory found in fraction C1, as both have been observed to possess high anti-inflammatory properties (Passos et al. 2022). A similar study by Han and Parker (2017) identified eugenol, the primary active ingredient in clove essential oil, as having anti-inflammatory properties.

Conclusions

Evidently, *S. aromaticum's* essential oil contains a variety of components. The crude essential oil was fractionated, and this revealed that some components were more prominent in a given percent than others. The outcome clearly shows that the crude essential oil had synergistic effects on the antifungal activity. The antagonistic influence of some components, however, was visible in some select indicators, as evidenced by the reduced activity noted for the crude essential oil in several of the parameters established. This investigation demonstrated that synergistic action was more specific to the fungal activities against *C. albicans*, *A. penicillum*, and *A. niger*, while the compound's antagonistic effect was shown for other parameters. This outcome is as a result of the different degrees of components and fractions found in the crude essential oil and the fractions. The better activities of fractions as antioxidants and anti-inflammatory agents may be as result of some components that could have counter effects that were removed in the process of fractionation.

Abbreviations

DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
GC–MS	Gas chromatography–mass spectrometry
IC_{50}	The half-maximal inhibitory concentration
MIC	Minimum inhibitory concentrations
EOs	Essential oils
TLC	Thin-layer chromatographic
Abs	Absorbance

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Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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