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ARTICLE

Fractionation and Characterization of the Bioactive Compounds of the Extracts of Buds of *Syzygium aromaticum*

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Abstract
This research focused on the separation and identification of bioactive components of the methanolic extracts of the buds of *Syzygium aromaticum*. A bioassay and phytochemical screening were performed on the various solvent fractions, and the most active fraction was subjected to spectroscopic analysis using infrared, mass spectroscopy, and nuclear magnetic resonance to determine the structure of the active compounds present. The methodology involved extracting the flower buds of *Syzygium aromaticum* using methanol, fractionating the plant extract using three solvents—n-hexane, ethylacetate, and methanol, and performing a bio Using the agar well diffusion method, the antibacterial properties of the three solvent fractions were ascertained. The analysis' findings revealed that each of the three solvent fractions had tannins, alkaloids, flavonoids, and reducing sugars; the only fraction to contain saponins was the ethylacetate fraction, and the only fraction to have glycosides. The results of the study further showed that the ethylacetate fraction had the strongest antimicrobial activity against the test organisms, inhibiting the growth of bacteria such as *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans* at concentrations of 200 mg/mL. The structural elucidation of the active compounds responsible for the antimicrobial was done through spectroscopic analysis using infrared, nuclear magnetic resonance and mass spectroscopy. The antimicrobial activities of this plant highlighted the significance of the extracts in traditional drug preparations, according to the study's findings, which suggested that the antimicrobial properties of the flower buds of Syzygium aromaticum may be due to the synergetic or individual effects of the phytoconstituents found.

Keywords: Antimicrobial, characterization, fractionation, screening, *syzygium aromaticum*

1. Introduction

Numerous chemical substances that plants produce have therapeutic benefits in the treatment of ailments. Different plant parts with beneficial chemical constituents have been extracted and biologically tested to demonstrate their therapeutic potentials [1]. Additionally, the modern medications utilized in conventional treatment have been derived from plants [2]. Therefore, it is not surprising that medicinal plants are widely used in the treatment of a variety of illnesses, such as measles, hepatitis, arthritis, rheumatism, burns, scalds, abdominal colic, peptic ulcer, diarrhea, and dysentery [3]. Proper bioassays need to be conducted to establish the biological activity shown by plant extracts as simple isolation and elucidation of chemical structures of plant extracts may not be enough to identify the medicinal

importance of the plants [4].

Because of its benefits to healthcare, medicinal plants have been the subject of scientific study in many nations. Exploring methods for collecting the necessary plant materials and analyzing their constituents has been sparked by the ongoing interest and quest for natural plant products that can be used as medications [5]. Many of the commercially successful medications used in contemporary medicine were first employed in undeveloped forms in conventional or folk medicine, or for other uses that revealed potential biological utility. The main advantages of employing plant-derived medicines are that they are generally safer than synthetic equivalents, provide significant therapeutic advantages, and are more cost-effective than other forms of therapy.

Syzygium aromaticum or cloves, is a plant in the Myrtaceae family that are indigenous to Indonesia's Maluku Islands. An evergreen tree with broad leaves and terminal clusters of crimson flowers, S. aromaticum can reach heights of 8 to 12 meters. The color of the flower buds changes from pale to gradually green to vivid red when they are ready for harvest. Cloves are picked when they are 1.5 to 2.0 cm long and are made up of four unopened petals that create a compact central ball and a long calyx that ends in four spreading sepals. Traditional healers have employed the plant *Syzygium aromaticum* to treat a variety of illnesses like toothache, a burning sensation in the tissue, pains in the body, and used to improve peristalsis [6].

Clove has been utilized in modern aromatherapy to treat illnesses like anxiety, sadness, sexual dysfunction, and weariness. It also works to balance hormones and tone the nervous system [7]. It is also employed in the flavoring of food and pharmaceutical products [8], as well as an antiseptic [9]. This study is consequently embarked upon with the intention of carrying out fractionation, antimicrobial screening, and characterisation of the bio-active chemicals on the methanolic extract of buds of *Syzygium aromaticum* in order to advance research on the pharmacological significance of the clove plant.

1.1 Statement of the problem

The use of different plant parts to cure illnesses is a global phenomenon that has been more prevalent in recent years as scientific evidence of herbal medicines' efficacy has become more publicly available [10]. In order to defend themselves from antimicrobial treatments, bacterial and fungal pathogens have developed a variety of defensive mechanisms. As a result, resistance to both old and new medications is increasing. The majority of medications, including antibiotics, are no longer effective against the intended pathogens. As a result, we see the development of bacteria that are resistant to antibiotics. The majority of conventional medications also cost a lot of money and have many negative side effects for the consumers. The cost of managing patients is very high, particularly in underdeveloped nations. Discovering and identifying new safe drugs without severe side effects has become an important goal of research in biomedical science.

The plant *Syzygium aromaticum* has been recognized as a potential treatment for human illnesses like toothaches, hormonal imbalances, sexual dysfunction, and exhaustion [7]. It has also been recognized as a significant source for the discovery of novel pharmacologically active compounds, from which many drugs can be derived either directly or indirectly [1]. In order to find the compound(s) responsible for the plant's numerous pharmacological and medicinal activities, it is therefore essential to advance the research on the plant by conducting spectroscopic analyses on the plant with reference to its methanolic extract, which is the motivation behind this study.

1.2 Aim and objectives of the study

The study's aim include methanolic extract fractionation, antimicrobial screening, and characterisation of the bioactive components from *Syzygium aromaticum* buds. The study's objectives are as follows:

1. To extract the bioactive components of *Syzygium aromaticum* using methanol
2. To fractionate the plant extract using n-hexane, ethylacetate and methanol
3. To determine the phytochemicals present in the different solvent fractions and to conduct the

antimicrobial screening of the fractions

4. To subject the pure compound of the most active fraction to spectroscopic analysis using infrared, mass spectroscopy and nuclear magnetic resonance with a view to ascertaining the structure of the compounds present.

1.2.1 Scope of the study

1. The fractionation of the methanolic plant extract of *Syzygium aromaticum* using n-hexane, ethylacetate and methanol.
2. The phytochemical screening of the different fractions of the flower buds of *Syzygium aromaticum* to determine the presence of alkaloids, tannin, glycosides, saponins, flavonoids and reducing sugars using standard analytical procedures.
3. Antibacterial screening of the solvent fractions using gram positive *Staphylococcus aureus* and *Bacillus subtilis,* and gram negative *Escherichia coli* and *Salmonella typhi*
4. Antifungal screening of the fungi *Aspergillus nigger* and *Candida albicans*
5. Spectroscopic analysis of the pure compound of the most active fraction using Infrared (IR), Mass Spectroscopy and Nuclear Magnetic Resonance (NMR).
6. Identification of the active chemical compounds from the flower buds of *Syzygium aromaticum* that exhibit highest bioassay actions against micro-organisms.

2. Methods

2.1. Plant material:

The *Syzygium aromaticum* flower buds were acquired from the Orie Igbo-Eze Market in the Udenu Local Government Area of Enugu State, Nigeria. A taxonomist from the Department of Plant Science and Biotechnology at the University of Nigeria, Nsukka, Mr. Onyukwu authenticated the flower buds, and the voucher specimen was kept for reference in the departmental herbarium.

2.2 Preparation of plant sample

Syzygium aromaticum flower buds were cleaned before air dried for a week at room temperature. They were ground with a hand grinder. After that, the powder was kept in an airtight container for the remainder of the project.

2.3 Solvent extraction

Methanol was employed in the extraction process. In a 2L sterile bucket, 500g of the powdered plant material was steeped in 1000mL of methanol. Stirred, covered, and left to stand for 48 hours at room temperature. Whatmann No. 1 filter paper was used to filter the mixture, and the filtrate was concentrated using a rotary evaporator before being kept in a refrigerator at 40°C until needed. After extraction, 168.4g of stock extract were obtained.

2.3 Determination of extractive value (percentage yield) of the crude methanol extract

The value of the extract produced by evaporating a solution of the dried flower buds is known as the extractive value of *Syzygium aromaticum* flower buds. To do this, the methanol extract was evaporated in a conical flask with a specified weight, and the difference was measured after the evaporation. The crude methanol extract's extractive value was 33.68%.

2.4 Fractionation of the methanol extract using separating funnel

The methanol extract of the flower buds of *A. Syzygium* was fractionated using ethylacetate, n-hexane, and methanol. The solvents were utilized for the fractionation using separating funnel in the following order of increasing polarity: n-hexane, ethylacetate, and methanol. Following fractionation, the various solvent fractions were gently dried using a rotary evaporator and kept in a refrigerator at 40 degrees Celsius until needed. The stock fractions obtained were 4.93g for the ethylacetate fraction, 5.52g for the n-hexane fraction, and 6.08g for the methanolic fraction.

2.5 Preparation of stock solutions of the different solvent fractions

The stock solvent fractions were used to create the stock solutions for the three solvent fractions, which included 200 mg/mL concentrations of the methanolic, ethylacetate, and n-

hexane fractions. This was accomplished by dissolving 2g of each fraction in 10ml of sterile distilled water. They were clearly labeled and kept at 40°C until needed.

2.6 Preparation of test organisms

The Department of Microbiology, University of Nigeria Nsukka, provided the stock bacterial and fungi isolates that were used in the experiment. The isolate cultures obtained on agar slants were used to create new plates of the test organisms. The isolates were sub-cultured into new, sterile nutritional broth for bacteria and Saboraud Dextrose Agar SDA for fungus, and incubated for 24 hours at 37°C for bacteria and 25°C for fungi, respectively. By comparing the turbidity to the 0.5 McFarland turbidity standard, they were standardised. To regulate the turbidity of the microbial solution so that the quantity of microorganisms will be within a specific range, the McFarland standard was employed as a guide. The 0.5 McFarland standard was made by continuously swirling 9.95 mL of 0.18M H_2SO_4 with 0.05ml of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$). The McFarland standard tube was kept in storage with a tight seal to prevent evaporation loss [11, 12, 13].

Colonies of the various isolated bacterial fresh cultures were selected using sterile wire loop, and they were then suspended in 5 mL of nutritional broth in sterile 10 mL bijou bottles with clear labels. They were incubated for 24 hours at 37^0C.

2.7. Determination of phytochemical constituents of the fractions

Chemical investigations of the various solvent fractions were conducted for the qualitative assessment of phytochemical contents as described by [14] and [15].

2.8. Determination of saponins

1 mL of the stock solutions (200 mg/mL) of each of the solvent fractions was added to five milliliters of distilled water before being heated. The mixture's soluble portion was heated up and then decanted into a test tube. The solution was used for the following tests:

2.9. Emulsion test

A test tube containing 1mL of the decanted solutions and two drops of olive oil was thoroughly shaken to see the emulsion.

2.10. Frothing test

A test tube containing 1mL of the filtrates and 3mL of distilled water was violently shaken to observe the presence of stable foam.

2.11. Determination of tannins

Acid test: Into a test tube, 3mL from the stock solution (200mg/mL) of methanol fraction was added to 2mL of 1% hydrogen chloride. The solution was observed for reddish brown ppt. The procedure was repeated for ethylacetate and n-hexane fractions.

Bromine water test: Into a test tube, 2mL from the stock solution (200mg/mL) of methanol fraction was added to 2mL of bromine water. The solution was observed for greenish-red colour. The procedure was repeated for ethylacetate and n-hexane fractions also.

Determination of alkaloids: 5mL of 2% hydrogen chloride acid were added to the methanol fraction (1mL) that was obtained from the stock solution of 200 mg/mL in the test tube. Whatmann No. 1 filter paper was used to filter the combinations after they had been heated in a water bath at 40^0C for 10 minutes. The following tests were performed on the filtrate:

Wagner's test: 1mL of Wagner's reagent was added to each filtrate (1mL) in a test tube. The mixture was thoroughly shaken, and a reddish-brown ppt color change revealed the presence of an alkaloid.

Meyer's test: 1 mL of Meyer's reagent was added to 1 mL of each filtrate in a test tube. After thoroughly shaking the combination, an assessment was done for the presence of an alkaloid-indicating cream color. The methanol fraction's alkaloid identification process was repeated for the ethylacetate and n-hexane fractions, respectively.

2.12 Determination of flavonoids

$FeCl_3$ test: To 1mL of methanol fraction got from 200mg/mL of the stock solution was added 1mL of 10% ferric chloride. The solution was mixed thoroughly and observed for colour change (green/black colour). This procedure was repeated for ethylacetate and n-hexane fractions respectively.

Lead acetate test: To 1mL of methanol fraction (from the stock solution of 200mg/mL) in a test tube was added 1mL of 10% lead acetate. It was mixed thoroughly. The mixture was observed for black colour or ppt, and the procedure repeated for n-hexane and ethylacetate fractions.

2.13 Determination of glycosides

Glycosides were measured by covering the powdered plant bud (1g) with adequate water in a 250mL conical flask. Picrate paper was suspended in the flask by a thread. The flask was heated for one hour at 40^0C in a water bath. It was noted that the picrate paper's color changed from yellow to brick-red.

2.14. Determination of reducing sugar

The amount of reducing sugar was determined by adding 7.5mL of Fehling's solution to 1g of powdered plant bud in a test tube. The mixture was heated in a water bath for 5 minutes at 40^0C while being watched for a change in color to brick-red.

2.15. Determination of preliminary antimicrobial activity of the different solvent fractions

Using the agar well diffusion technique, the antibacterial activity of the n-hexane, ethylacetate, and methanol fractions was assessed [16]. *Escherichia coli* and *Salmonella typhi* for gram positive bacteria; *Staphylococcus aureus* and *Bacillus subtilis* for gram negative bacteria; *Candida albicans* (yeast) and *Apergillus nigger* (mold) for fungi; and a panel of organisms representing the different classes of microorganisms were used to test the fractions. Each of the bacterial isolates was seeded onto a nutrient agar plate with 0.1 mL of an overnight broth culture, whereas each of the fungal strains was seeded onto a sabouraud dextrose agar plate with a comparable amount. The seeded plates were given time to set before being dried. Eight uniform wells of 8mm in diameter were drilled into the agar's surface using a sterile cork borer.

To each of the agar wells, 0.1mL of each of the solvent fractions of the stock solution (200mg/mL) were added. For the bacterial strains' positive and negative controls, the 7th and 8th wells were filled with 0.1 mL of chloramphenicol (25 mg/mL), while the fungal strains' positive and negative controls were fluconazole (50 mg/mL) and sterile distilled water. For the pre-diffusion step, the plates were left on the bench for 40 minutes. Then, for bacterial isolates, an overnight incubation at 37°C and for fungi isolates, an overnight incubation at 25°C were performed. Each solvent fraction's level of antibacterial activity was quantified by measuring the inhibition zone diameter in millimeters. In triplicates, the sensitivity test was conducted. The zone of inhibition for the specific bacterial and fungal isolates at the given concentration was determined to be the average of the three values.

2.16. Purification of the fraction with the highest anti-microbial activity

Gradient-elution chromatography was used to further purify the solvent fraction with the best anti-microbial activity. For the chromatography, various n-hexane and ethylacetate solvent systems were employed. At the end of use of each solvent system, a thin layer chromatography (TLC) also at a solvent system of 80% n-hexane and 20% ethylacetate was carried out on the eluates to evaluate the presence or lack of compound(s) under a UV light. The Gradient-Elution chromatography process at different solvent systems was carried out until there was no further elution, which was confirmed by subjecting the eluate to UV lamp of the TLC. The eluates that showed identical peaks under the UV lamp were combined together and labelled.

2.17. Spectroscopic analysis of the compound

Spectroscopic analysis was carried out on the fraction with the highest antimicrobial activity to determine the structure of its active component(s). The spectroscopic analysis carried out were Infrared Spectroscopy (IR), Mass Spectroscopy, and Nuclear Magnetic Resonance (NMR).

3. Results and Discussions

3.1. Percentage yield and macroscopic characteristics

Table 1 below shows the extractive yield and macroscopic characteristics of the crude extract of the flower buds of *Syzygium aromaticum.* The percentage yield of the crude extract of the plant was 33.68%, while its macroscopic characteristics showed that it was a solid substance, brown and

sticky in texture.

Table 1. Extraction yield and macroscopic characteristics of the crude methanolic extract

Extract	Percentage yield (%)	Macroscopic characteristics
Crude methanolic extract	33.68	A brown sticky substance

Table 2 below shows the extractive yield and macroscopic characteristics of the three solvent fractions. The % yield of the n-hexane fraction was 32.70%, had a dark green colour with sticky mass texture. The % yield of the methanol fraction was 33.56%. It had a dark brown colour with molten mass texture, while the ethylacetate fraction has a percentage yield of 24.90%. It was an oily mass substance, with greenish colour.

According to the results of the various solvent fractions, the methanol extract of *Syzygium aromaticum* flower buds had the highest extractive value (33.56%), was followed by the n-hexane fraction (32.70%), and had the lowest yield (24.90%). This finding demonstrates that methanol, which is used as the solvent in the fractionation process, has greater extrinsic and fractionating power than n-hexane and ethylacetate.

Table 2. Extraction yield and macroscopic characteristics of the three solvent (n-hexane, methanol and ethylacetate) fractions

Extract	Percentage yield (%)	Macroscopic characteristics
n-hexane fraction	32.70	A dark green colour with sticky mass texture
Methanol fraction	33.56	A dark brown substance with molten mass texture
Ethylacetate fraction	24.90	An oily mass substance, greenish in colour

3.2. Phytochemical screening

The findings of the phytochemical screening of the various fractions of the methanolic extract of *A. syzygium* were displayed in Tables 3, 4, and 5 above. The findings demonstrated that tannins, alkaloids, flavonoids, and reducing sugars were present in all of the fractions. Saponins were only present in the ethylacetate fraction, and glycosides were only found in the n-hexane fraction. Since they extracted all phytochemicals except for one—glycosides for ethylacetate and saponins for n-hexane—these solvents were discovered to be effective for extracting phytochemicals. Saponins and glycosides were not extracted using methanol.

The chemical makeup of the *Syzygium aromaticum* flower buds' three solvent fractions is revealed by phytochemical screening, which can also be used to look for bioactive substances that could be used to create very beneficial medications [17]. According to the study's phytochemical analysis of *Syzygium aromaticum's* flower buds, tannins, flavonoids, glycosides, saponins, alkaloids, and reducing sugars are all present. It demonstrates that the solvent fractions of this plant all include tannins, alkaloids, flavonoids, and reducing sugars, whereas only the ethylacetate fraction and n-hexane contain saponins and glycosides, respectively.

The findings, which excluded saponins from the phytochemical content of the methanol and n-hexane fractions, did not accord with the findings of [18], but they did with those of [19]. The geographical locations of the plant may be responsible for this variation. Different phytochemicals have the ability to inhibit microbial growth in various ways; for instance, tannins can work by robbing microbial cells of essential proteins like enzymes, hydrogen bonding, or iron [20, 21].

Tannins are widely known for their diuretic, calming, anti-inflammatory, and anti-microbial activities, as well as their antioxidant and anti-microbial capabilities [22]. Tannin-containing plants are astringent in nature and are used to treat gastrointestinal conditions like diarrhea and dysentery [23]. This could also explain why *Syzygium aromaticum* is used as a traditional treatment for typhoid and digestive problems [24].

Only the ethylacetate fraction, which has the most anti-microbial activity, tested positive for saponins, which are responsible for several pharmacological activities [25]. The majority of the biological effects that have been seen are attributed to saponins, which are regarded as a vital component of traditional Chinese medicine [26]. They reduce cholesterol and have anti-diabetic, anti-carcinogenic, and anti-cancer activities [27]. Additionally, saponins have expectorant, antitussive, and hemolytic properties [15, 28].

The most revered phytochemicals, alkaloids, are thought to be pharmacologically active; they are believed to affect the autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract, uterus, malignant illnesses, infections, and malaria [27]. Alkaloids also have antibacterial, analgesic, and antispasmodic properties [22]. All solvent fractions of plant material contain alkaloids, which have a characteristic poisonous nature that boosts their biological capabilities and their activities against cells of foreign species [21]. Additionally, flavonoids are said to be a key antibacterial component [29, 30], and are potent polyphenolic antioxidants [19].

Table 3. Phytochemical properties of methanolic fraction of flower buds of *Syzygium aromaticum.*

	Phytochemical test	Observation	Methanolic fraction
1.	**Tannins**		
	Acid test	Reddish brown precipitate observed	+
	Bromine water test	Greenish red colour observed	+
2.	**Saponins**		
	Frothing test	No thick persistent froth observed	-
	Emulsion test	No emulsion observed	-
3.	**Alkaloids**		
	Wagner's test	Reddish-brown precipitate observed	+
	Meyer's test	Cream colour precipitate observed	+
4.	**Flavonoids**		
	Lead acetate test	Black colour or precipitate observed	+
	$FeCl_2$ test	Green/black colour observed	+
5.	**Glycosides**		
	Picrate paper test	No brick red colour observed	-
6.	**Reducing sugars**		
	Fehling's test	Brick-red precipitate observed	+

\+ = Present
\- = Absent

Table 4. Phytochemical properties of n-hexane fraction of flower buds of *Syzygium aromaticum.*

	Phytochemical test	Observation	n-hexane fraction
1.	**Tannins**		
	Acid test	Reddish brown precipitate observed	+
	Bromine water test	Greenish red colour observed	+
2.	**Saponins**		
	Frothing test	No thick persistent froth observed	-
	Emulsion test	No emulsion observed	-
3.	**Alkaloids**		
	Wagner's test	Reddish-brown precipitate observed	+
	Meyer's test	Cream colour precipitate observed	+
4.	**Flavonoids**		
	Lead acetate test	Black colour or precipitate observed	+
	$FeCl_2$ test	Green/black colour observed	+
5.	**Glycosides**		
	Picrate paper test	Brick red colour observed	+
6.	**Reducing sugars**		
	Fehling's test	Brick-red precipitate observed	+

\+ = Present
\- = Absent

Table 5. Phytochemical properties of ethylacetate fraction of flower buds of *Syzygium aromaticum.*

	Phytochemical test	Observation	Ethylacetate fraction
1.	**Tannins**		
	Acid test	Reddish brown precipitate observed	+
	Bromine water test	Greenish red colour observed	
2.	**Saponins**		
	Frothing test	Thick persistent froth observed	+
	Emulsion test	Emulsion observed	
3.	**Alkaloids**		
	Wagner's test	Reddish-brown precipitate observed	+
	Meyer's test	Cream colour precipitate observed	
4.	**Flavonoids**		
	Lead acetate test	Black colour or precipitate observed	+
	$FeCl_2$ test	Green/black colour observed	
5.	**Glycosides**		
	Picrate paper test	No brick red colour observed	-
6.	**Reducing sugars**		
	Fehling's test	Brick-red precipitate observed	+

\+ = Present

\- = Absent

Table 6. Sensitivity analysis of the solvent fractions at concentration of 200mg/mL.

	Test organisms	Inhibition Zone Diameter, IZD (mm)			Standards	
		Methanolic fraction	n-hexane fraction	Ethylacetate fraction	Fluconazole (50mg/mL)	Chloramphenicole (25mg/mL)
1.	*Escherichia coli*	--	7	17		27
2.	*Salmonella typhi*	--	7	15		21
3.	*Staphylococcus aureus*	--	--	--		19
4.	*Bacillus subtilis*	11	15	7		13
5.	*Candida albicans*	--	20	24	22	
6.	*Aspergillus nigger*	--	--	--	19	

Key: **--** = No inhibition

Flavonoids have been proven to prevent the peroxidation of polyunsaturated fatty acids in cell membranes by studies of [31, 32]. Additionally, research has demonstrated that flavonoids from the *Syzygium* genus prevent the production of hydroxyl radicals and superoxide ions, two potent peroxidation agents [33]. While heated caramels made of reducing sugars have astringent and poisonous effects, glycoside works on the heart muscles and increases renal flow (diuresis) [34].

3.3 Anti-microbial screening of the fractions

The findings of the preliminary/sensitivity study of the fractions at concentrations of 200 mg/mL were displayed in Table 6 below. The outcome demonstrated that only *Bacillus subtilis*, with an inhibition zone diameter (IZD) of 11mm, was sensitive to the methanolic fraction. With IZDs of 7 mm, 7 mm, 15 mm, and 20 mm, respectively, the n-hexane fraction was sensitive to *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Candida albicans*. Further analysis revealed that the ethylacetate fraction had IZDs of 17 mm, 15 mm, 7 mm, and 24 mm for Escherichia coli, *Salmonella typhi*, *Bacillus subtilis*, and *Candida albicans*, respectively. The findings indicated that none of the fractions were sensitive to the development of the bacterium *Staphylococcus aureus* and the fungus *Aspergillus nigger*.

Comparing the results of the antibacterial activities of the different solvent fractions to that of the standards (Fluconazole for fungi and Chloramphenicole for bacteria), it could be observed that methanolic (11mm) and n-hexane (15mm) fractions exhibited antibacterial activities against *Bacillus subtilis* that could be related to the antibacterial activity of Chloramphenicole (13mm) against *Bacillus subtilis*, while n-hexane (20mm) and ethylacetate (24mm) fractions exhibited

anti-fungal activities similar to Fluconazole (22mm) against *Candida albicans*. From the above result, the three solvent fractions could be compared to the standards (Chloramphenicole and Fluconazole) as their inhibition zone diameter (in mm) is almost the same as that of the standards.

3.4. Antimicrobial activity of the ethylacetate fraction

The ethylacetate fraction's antibacterial activity was displayed in Table 7 below at concentrations ranging from 200 mg/mL to 6.25 mg/mL. According to the results, the fraction exhibited antimicrobial effects on *Escherichia coli* at doses of 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, respectively. The inhibition zone diameters, or IZDs, of the fraction were 17 mm, 14 mm, 10 mm, 6 mm, and 4 mm. Additionally, the fraction inhibits *Salmonella typhi* at 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL, respectively, with IZDs of 15 mm, 11 mm, 9 mm, and 3 mm. Additionally, the ethylacetate fraction inhibited the growth of *Bacillus subtilis*, but only at high concentrations of 200 mg/mL and 100 mg/mL with IZDs of 7 mm and 5 mm, respectively. Conversely, the ethylacetate fraction had the highest inhibition against *Candida albicans*, with IZDs of 24 mm, 20 mm, 18 mm, 11 mm, 7 mm, and 5 mm at concentrations of 200mg/mL.

3.5. Antimicrobial activity of the n-hexane fraction

The antibacterial performance of the n-hexane fraction at concentrations ranging from 20 mg/ml to 6.25 mg/mL is shown in Table 8 below. The results revealed that the fraction had inhibitory effects against *Salmonella typhi* with IZDs of 7mm and 3mm at concentrations of 200mg/mL and 100mg/mL, respectively, but only at high concentrations of 200mg/mL and 100mg/mL with IZDs of 7mm and 4mm.

With IZDs of 15mm, 11mm, 9mm, 5mm, and 3mm at concentrations of 200mg/mL, 100mg/mL, 50mg/mL, 25mg/mL, and 12.5mg/mL, respectively, the n-hexane fraction likewise shown a significant inhibitory activity against *Bacillus subtilis*. With IZDs of 20mm, 17mm, 11mm, 9mm, 5mm, and 4mm at doses of 200mg/mL, 100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL, and 6.25mg/mL, respectively, the fraction showed the most inhibitory activity against *Candida albicans*.

3.6. Antimicrobial activity of the methanolic fraction

Table 9 below showed the antimicrobial result of the methanolic fraction. The result showed that the fraction had the lowest inhibition activity against the microorganisms with IZDs of 11mm, 8mm and 3mm at concentrations of 200mg/mL, 100mg/mL and 50mg/mL respectively against *Bacillus subtilis* only. From these results, it could be ascertained that the ethylacetate fraction had the highest antimicrobial activity, followed by n-hexane fraction and the least was methanolic fraction.

The ethylacetate fraction which has the highest anti-microbial activity was further purified using solvent system of 80% n-hexane and 20% ethylacetate. The resulting fraction was again tested against the test microorganisms and the result is shown in table 10 below.

3.7. Antimicrobial activity of the purified ethylacetate fraction

Table 10 below showed the antimicrobial activity of the purified ethylacetate fraction at 100mg/mL to 6.25mg/mL. The outcome demonstrates that the unpurified fraction and the purified fraction almost had the same activity. The antimicrobial results revealed that the fraction had inhibitory activities against *Salmonella typhi* with IZDs of 12mm, 11mm, and 4mm at concentrations of 100mg/mL, 50mg/mL, and 12.5mg/mL, respectively, while inhibiting the growth of *Escherichia coli* with IZDs of 15mm, 12mm, 8mm, and 4mm at concentrations of 100mg/mL, 50mg/mL, The purified ethylacetate fraction demonstrated significant inhibitory activity against *Bacillus subtilis* with IZDs of 9 mm, 7 mm, and 3 mm at concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL, respectively. Conversely, it demonstrated its greatest inhibitory activity against *Candida albicans* with IZDs of 22 mm, 19 mm, 13 mm, 10 mm, and 5 mm at concentrations of 100mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL respectively.

The n-hexane and ethylacetate fractions of the *Syzygium aromaticum* flower buds evaluated for antibacterial activity against disease-causing organisms showed substantial activity,

but the methanol fraction showed very little activity and exclusively against *Bacillus subtillis*.

Of the three solvent fractions, result showed that both n-hexane and ethylacetate fractions of *Syzygium aromaticum* had antimicrobial activity against all the microorganisms except *Aspergillus nigger* while the methanol fraction exhibited very little activity, and only against *Bacillus subtillis*.

The Syzygium aromaticum flower buds of n-hexane and ethylacetate fractions had little effect on *Aspergillus nigger* but had the strongest effect on *Candida albicans*, with zones of inhibition at 20 and 24 mm, respectively. The zone of inhibition for the methanol fraction's activity against *Bacillus subtillis* was barely 10 mm, and it had no effect on other microbes. This outcome relates to research on the effectiveness of *Syzygium aromaticum* flower buds against yeast microorganisms [35].

From the antimicrobial activity conducted, the ethylacetate fraction had the most inhibitory activity against the microorganisms used for the study, and thus the spectroscopic analysis and Gas Chromatography-Mass Spectroscopy (GC-MS) were carried out on it to determine the active compounds present.

3.8. Spectroscopic analysis of the purified ethyl acetate fraction

The result of the spectroscopic analysis of the ethylacetate fraction was interpreted as follows:

3.8.1. Infrared analysis

The IR result (Table 11) suggested that the isolated compound contains a carbonyl of ketone or aldehyde; a hydroxyl (OH) group band, an NH_2 band probably of an amide given the appearance of C = O band of amide at 1638cm-[1]. The fraction also contains an aromatic ring and aliphatic chains.

3.8.2. Proton Nuclear Magnetic Resonance (^{1}H NMR) analysis

Table 12 showed the results of the ^{1}HNMR of the isolated compounds. From the spectra obtained, signal at 1.29ppm indicated the presence of one hydrogen (1H, CH) singlet, while signal at 2.32ppm showed 3H of CH_3 singlet. Also, signals at 3.40-3.33ppm showed 6H of CH_3 multiplet; 3.84ppm showed 3H of CH_3 singlet; 3.88ppm showed 4H of $2CH_2$ singlet which were hydrogen of cycloalkanes, while 5.15-5.06ppm depicted 12H of $4CH_3$ multiplet.

Signals at 5.58ppm showed hydrogen of NH_3 which is singlet and broad, while signals at 5.92 – 6.02ppm showed 5H multiplet which could be aromatic hydrogen; peaks at 6.72 – 6.07ppm depicted 2H multiplet of aromatic hydrogen and peaks at 6.81 – 6.77ppm and 6.88 – 6.86ppm showed 1H multiplet and 2H multiplet respectively, both of which were aromatic hydrogen, while signals at 6.98 – 6.96ppm depicted 1H doublet which could be aromatic hydrogen.

Furthermore, the Correlation Spectroscopy (COSY) result showed that the protons at 3.40 – 3.83ppm were coupled to protons at 6.72 – 6.70ppm, 6.02 – 5.92ppm, and 5.15 – 5.06ppm, while the protons at 6.72 – 6.70ppm were coupled to protons at 3.83ppm. Also, the protons at 6.02 – 5.92ppm were coupled to the protons at 5.15 – 5.06ppm, while the rest of the protons were not coupled.

3.8.3. ^{13}C - Nuclear Magnetic Resonance (^{13}C – NMR) analysis

Table 13 below showed the results of ^{13}C-NMR of the isolated compound. From the spectra obtained, peaks at 169.01 showed the presence of carbonyl (C=O) group; peaks at 151.20, 146.46, 143.91, 139.02, 137.84, 137.05, 131.91, 122.52, 121.18, 120.67, 116.15, 115.51, 114.29, 112.73, 111.14 showed the presence of 15 aromatic, alkenyl or quaternary carbons, signals at 77.39 – 76.76 indicated the solvent peak, while signals at 55.85, 40.09, 39.89, and 20.67 showed the presence of four aliphatic carbons.

3.8.4.Gas Chromatography - Mass Spectroscopy (GC-MS)

The GCMS data revealed compounds which were identified following their fragmentation patterns. The fragmentation pattern of 2-methoxy-4-(prop-2-enyl) phenyl ethanoate is shown in figure 1.

The DEPT result further simplified the ^{13}C-NMR result as it showed that there were six quaternary carbons with signals at 169.01, 151.20, 146.46, 143.91, 139.02, and 131.91. The result further showed that there were eight C-H carbons with signals at 137.84, 137.05, 122.52, 121.18, 120.67, 114.29, 112.73,

111.14, while signals at 116.15, 115.51, 40.09, 39.89 indicated the presence of four CH_2 carbons and signals at 55.83 and 20.67 indicated the presence of two CH_3 carbons.

3.8.5. Structure Elucidation

Investigation of the pure ethylacetate fraction was done by the purification of the ethylacetate fraction over polyamide column and elution with solvent system of 80% n-hexane and 20% ethylacetate. The structure of the compounds present in the purified ethylacetate fraction were confirmed by interpretation and comparison of their spectral data.

Also, the fragmentation pattern of 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol is shown in figure 2.

Table 7. Antimicrobial activity of ethylacetate fraction.

Microorganisms	**Inhibition Zone Diameters(mm)**					
	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Escherichia coli	17	14	10	6	4	--
Salmonella typhi	15	11	9	3	--	--
Bacillus subtillis	7	5	--	--	--	--
Candida albicans	24	20	18	11	7	5

Key: -- = No inhibition

Table 8. Antimicrobial activity of n-hexane fraction.

Microorganisms	**Inhibition Zone Diameters(mm)**					
	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Escherichia coli	7	4	--	--	--	--
Salmonella typhi	7	3	--	--	--	--
Bacillus subtillis	15	11	9	5	3	--
Candida albicans	20	17	14	11	8	6

Key: -- = No inhibition

Table 9. Antimicrobial activity of methanolic fraction.

Microorganisms	**Inhibition Zone Diameters(mm)**					
	200mg/mL	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Bacillus subtillis	11	8	3	3	--	--

Key: -- = No inhibition

Table 10. Antimicrobial activity of the purified ethylacetate fraction (at 100mg/mL concentration).

Microorganisms	**Inhibition Zone Diameters (mm)**				
	100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL
Escherichia coli	15	12	8	4	--
Salmonella typhi	12	11	4	--	--
Bacillus subtillis	9	7	3	--	--
Candida albicans	22	19	13	10	5

Key: -- = No inhibition

Table 11. Infrared spectroscopy interpretation of the purified ethylacetate fraction.

Infrared Spectroscopy bands (cm^{-1})	Possible functional groups
3516, 3455	OH, NH_2
3177, 3077, 3003	C-H of aromatic ring
2842	C-H of aliphatic ring
1764	C=O of a ketone or aldehyde
1638	C=O of amide
1231, 1196, 1148, 1120, 1032	C-N, C-O

Table 12. Proton NMR (^{1}H NMR) (δ or ppm) interpretation of isolated compound.

1H NMR (δ or ppm)	Interpretations
1.29	s, 1H, CH
2.32	s, 3H, CH_3
3.40 – 3.33	m, 6H, CH_3
3.84	s, 3H, CH_3
3.88	s, 4H, $2CH_2$ of cyclo alkanes
5.15 – 5.06	m, 12H, $4CH_3$
5.58	s, broad, NH_3
6.02 – 5.92	m, 5H, ArH
6.72 – 6.70	m, 2H, ArH
6.81 – 6.77	m, 1H, ArH
6.88 – 6.86	m, 2H, ArH
6.98 – 6.96	d, J = 8.0 H_2, IH, ArH

Table 13: ^{13}C - NMR interpretation of the isolated compound.

^{13}C – NMR	Interpretations
169.01	C = O
151.20, 146.46, 143.91, 139.02, 137.84, 137.05, 131.91, 122.52, 121.18, 120.67, 116.15, 115.51, 114.29, 112.73, 111.14	15 Aromatic, alkenyl or quaternary carbons
77.39 – 76.76	Solvent peak ($CDCl_3$)
55.85, 40.09, 39.89, 20.67	Four aliphatic carbons
DEPT Interpretation	
169.01, 151.20, 146.46, 143.91, 139.02, 131.91	Six Quaternary carbons
137.84, 137.05, 122.52, 121.18, 120.67, 114.29, 112.73, 111.14	Eight C – H carbons
116.15, 115.51, 40.09, 39.89	Four CH_2 carbons
55.83 and 20.67	Two CH_3 carbons

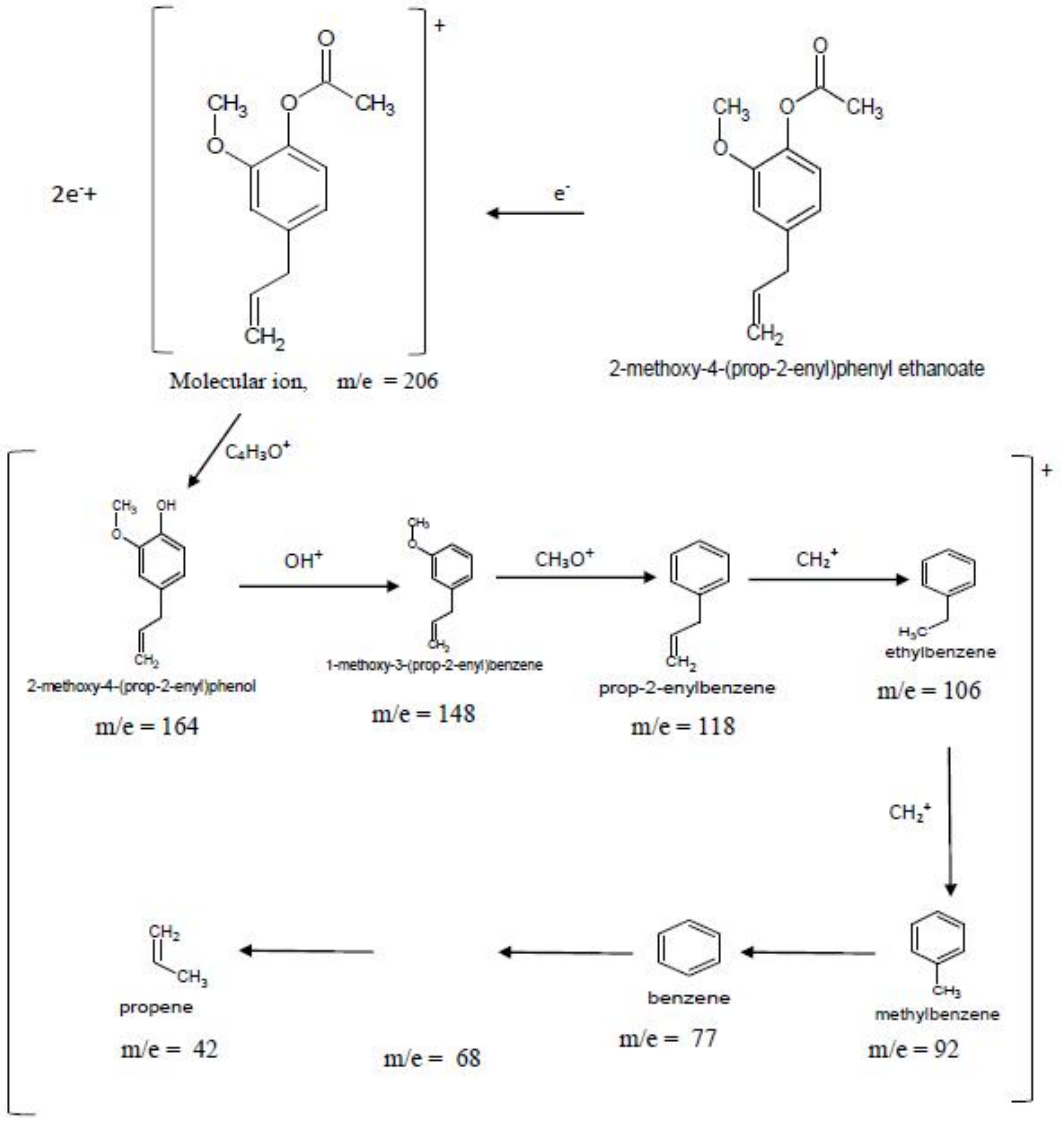

Figure 1. The GCMS fragmentation pattern of 2-methoxy-4-(prop-2-enyl) phenyl ethanoate

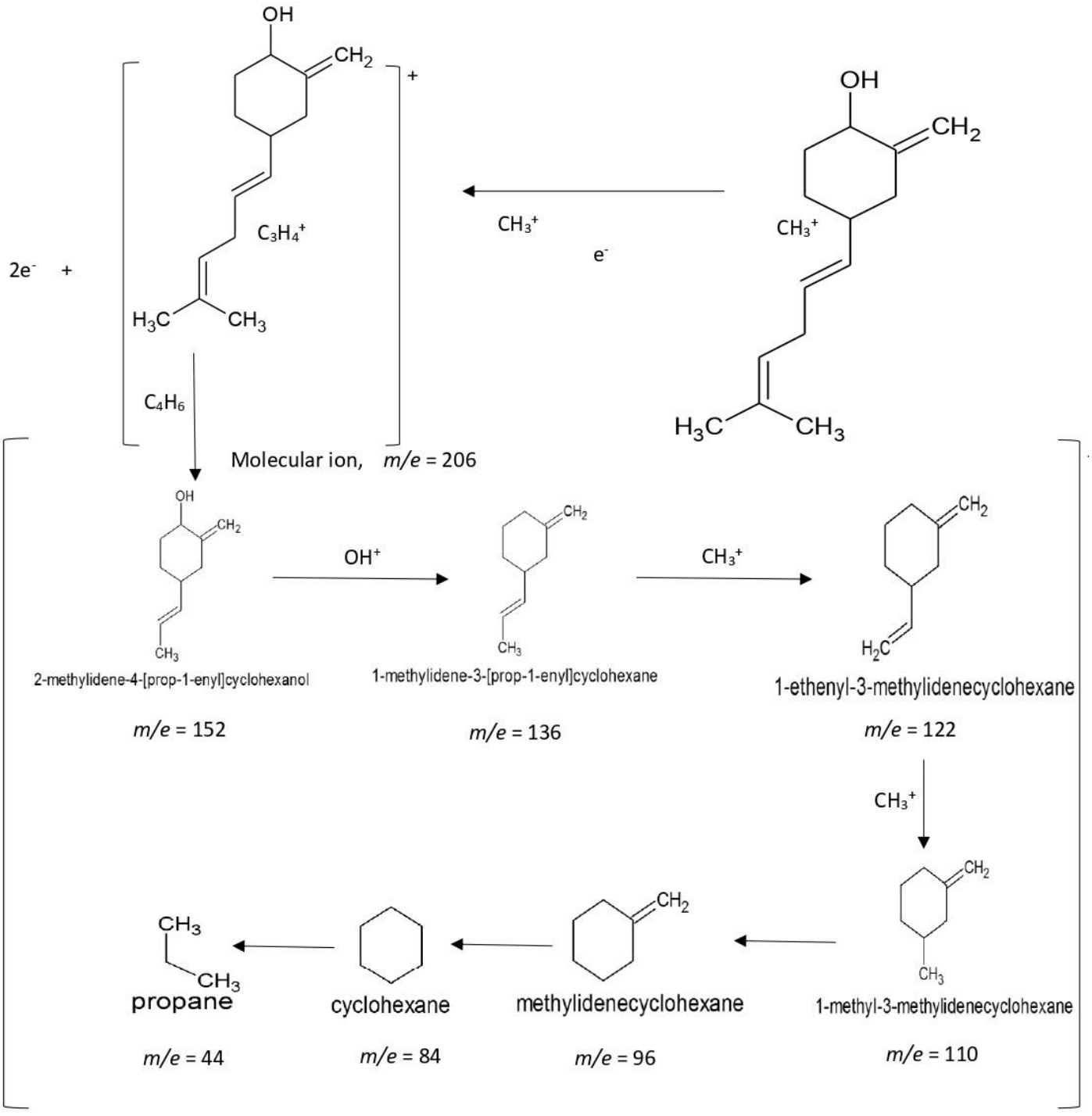

Figure 2. The GCMS fragmentation pattern of 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol

After analyzing the spectral data of the purified ethylacetate fraction, the compounds present were Aceteugenol with IUPAC name of 2-methoxy-4-(prop-2-enyl)phenylethanoate and 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol. These compounds were authenticated using their fragmentation pattern which agrees with their structures as was revealed by the Gas-Chromatography-Mass Spectroscopy carried out on them. Based on the total spectral analyses done, the structures of the compound are as shown in figure 3.

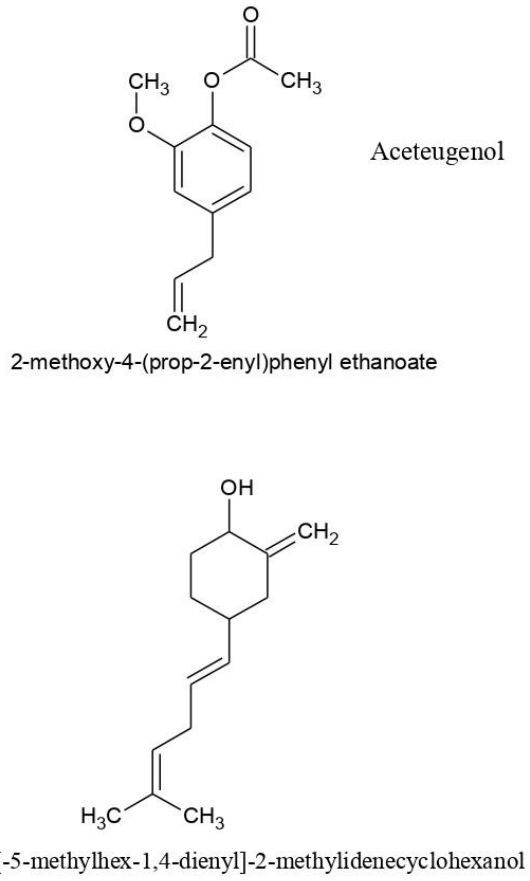

Figure 3. Structures of 2-methoxy-4-(prop-2-enyl)phenyl ethanoate and 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol

The discovery of these compounds, 2-methoxy-4-(prop-2-enyl)phenyl ethanoate and 4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol, as well as their inhibitory activities against microorganisms, support previous research on the benefits of Syzygium aromaticum cloves for oral health by authors like [36]. Their research showed that Syzygium aromaticum extracts had analgesic, anti-inflammatory, and biocidal properties against A. albopictus (tiger mosquitos), which aid in the prevention and treatment of malaria. The study's findings also agree with those of [6], which claimed that eugenol, which is present in clove oil from Syzygium aromaticum and has antioxidant properties, prevents cancer.

The study's finding is also in line with the study on the antibacterial properties of spices and herbs done by [37]. The research showed that *S. aureus*, *L. monocytogenes,* and *C. albicans* are all inhibited by Syzygium aromaticum extract, particularly clove oil. Additionally, eugenol, which has antibacterial, antifungal, anti-inflammatory, insecticidal, and antioxidant properties, is highly concentrated in *Syzygium aromaticum* flower buds, according to a study by [38] on the constituents of the essential oil from the plant's leaves and buds, and it is traditionally used as a flavoring agent and an antimicrobial.

This study therefore supports and justifies the traditional uses of Syzygium aromaticum flower buds for treating a variety of diseases. This is in line with the finding made by [38] who investigated the antibacterial activity of *Syzygium aromaticum* flower buds and came to the conclusion that the main ingredient in clove oil, eugenol, is widely used in folk medicine as an analgesic, anti-vomiting, antispasmodic, kidney-enhancer, antiseptic, diuretic, and aromatic agent [39].

4. Conclusion

The natural world contains herbs in abundance. Natural elements found in plants have the potential to improve health. According to the results of the current investigations, the antimicrobial properties of Syzygium aromaticum flower buds may result from the combined or individual effects of the phytoconstituents identified. This conclusion is further supported by extensive studies, the findings of which revealed that the phytochemicals present in the various solvent fractions of the plant include tannins, alkaloids, flavonoids, reducing sugars, saponins, and glycosides. As it inhibited the growth *of*

Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, and *Candida albicans* at a concentration of 200 mg/mL, the plant's ethylacetate fraction exhibited the highest antimicrobial activity against the test organisms. Its spectroscopic analysis using infrared spectroscopy, nuclear magnetic resonance, and mass spectroscopy for structural elucidation of the active compounds responsible for the antimicrobial inhibition revealed the presence of two compounds; 2-methoxy-4-(prop-2-enyl) phenylethanoate and

4[-5-methylhex-1,4-dienyl]-2-methylidenecyclohexanol.

The antibacterial properties of this plant brought to light the significance of the extracts in conventional medication formulations. Further investigation should be done to determine how the two compounds obtained from the ethylacetate fractions can be produced in large quantities without incurring much cost for their efficient integration into precursors used for drug production. This is because they inhibited the growth of some microorganisms.

Conflict of Interest

The authors declare no conflict of interest.

Authors' credit statement

Agu, C.L. conducted the research work, wrote the manuscript and has the main idea. Omeje N.O. revised the manuscript and provided suggestions.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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