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Examination of Clove (*Syzygium aromaticum*) pods using GC-MS for antimicrobial, larvicidal, phytochemical, and other purposes

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Abstract

Background: The myrtle family includes the aromatic spice cloves. An extremely precious spice, cloves are harvested before flowering. Plus, cloves are a health-promoting spice that should not be overlooked. They have a sharp taste and a slightly woody appearance. Cloves are full of helpful properties, such helping with digestion and eliminating foul breath.

Methods: The antibacterial activity of clove ethanolic extract was assessed against pathogenic bacteria such as *S. aureus*, *Salmonella typhi* (Sal. typhi), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and larvae of *Aedes aegypti* mosquito. The phytochemical analysis, GC- MS were conducted identifying the chemical composition of clove pods.

Results: According to the findings, cloves contain a large number of phytochemicals, each of which has a variety of medicinal applications. In addition, the findings of the GC-MS analysis revealed the existence of twenty-one chemical compounds, the compound eugenol being the most prevalent among them, with a percentage of 58.86%. The absence of nitrogenous and chlorinated compounds emphasizes the organic nature.

Conclusion: Clove pods have many chemical constituents which possess antimicrobial, larvicidal, properties, adding weight to the idea that clove pods could be a rich source of natural therapeutic ingredients.

Introduction

A member of the Myrtaceae family, the valuable spice clove (*Syzygium aromaticum*) contains antibacterial and antioxidant qualities that have made it useful as a food preservative and in medicine for ages. With an estimated 1200–1800 species, Syzygium is the most numerous genera of flowering plants in the Myrtaceae family. These plants are found all over the world, from the tropics and subtropics to Madagascar, Africa, and the Pacific and Oceanic regions.

The aromatic volatile oil found in cloves is primarily concentrated in the plant's aerial portions and is used for flavoring both food and medicine. It is believed that factors such as growing conditions, genetics, chemotypes, geographical origins, and variations in the nutritional status of the plant are associated with the varying yield and content of volatile oil [1-4]. The active ingredient compositions present in clove elicited varied mortality responses [5].

The utilization of clove essential oil (EO) as an environmentally friendly safe against *Anopheles stephensi* is favored over its primary component, eugenol. Given the lower cost and the presence of multiple components in the EO, which reduces the likelihood of resistance, the entire EO can be recommended as an effective larvicide [6].

Researchers have found that clove pods contain many different phytochemical substances that give them their many health benefits, including their ability to kill microbes and fungi and to reduce inflammation and pain [7,8,9,10]. The active ingredients in clove pods can be found and separated by experts. This will help them make new natural antibiotics that can treat bacterial infections more effectively and specifically.

Antibiotic resistance has become a major global health issue, but this method could cut the risk of it by a huge amount. Our study sought to discover the phytochemical and GC-MS (Gas Chromatography-Mass Spectroscopy) components of clove pods and determine their antibacterial capabilities.

Methods

Plant and microorganism specimen

The study investigated the potential antibacterial properties of S. aromaticum cloves, obtained from a local market, and compared them to pathogenic bacteria like *E. coli, K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *Sal typhi*.

Extracting plant material

Twenty grams air-dried powder and 150 milliliters filtered water were mixed to start processing. Two hours later, the mixture gently boiled. The fluid was centrifuged at 5,000 × g and filtered through muslin cloth for 10 minutes. Collect the surface liquid. This process has two runs with two-hour supernatant collection intervals. Six hours of consolidation concentrates it to 25%. Precision and thorough attention to detail ensure complete and damage-free target compound extraction in the methodical technique.

Clove antibacterial activity test

According to Sulieman et al. [12], the cup-plate agar diffusion methods were used with a few small changes to test the antibiotic activity of the extract that was made. Mueller Hinton (for bacteria) were introduced using a clean cotton swab. 10^8–10^9CFU/ml were taken from a stock solution and put into Petri dishes.

The plates were incubated at 37°C for 24 to 48 hours. Clove extract was put on clean discs in three different amounts: 1 mg, 2 mg, and 3 mg/disc. It was done three times with each quantity and each species. After incubation, the width of the growth-inhibiting zones was measured, averaged, and the mean values were found. The molecules ampicillin and amphotericin B were used as standards.

Determination of M.I.C. by microdilution

A 96-well plate assay was used to measure the M.I.C for plant extract antibacterial activity according to Barry et al. [13]. The technique begins with plate preparation, using 50µl Mueller-Hinton broth for bacteria and adding 50µl of a stock solution containing the tested extracts to the first row.

Twofold serial dilutions are conducted across the plate, utilizing a concentration range of 100–0.1953 mg/ml, with ten microliters of inoculum given to each well. The positive control comprises an elevated inoculum of 1.5X10^8 CFU/mL, whilst the plant extract functions as a positive control and the medium inoculum acts as the negative control.

Larvicidal Activity

Larvae breeding and bioassay

Mosquito larvae (early third and fourth instars) were reared in the laboratory at room temperature (27°C). The bioassay was performed in accordance with the WHO procedure, employing 20 larvae in 200 ml of tap water, with three replicates for each extract concentration. Mortality was documented after a 24-hour period.

Phytochemical analysis

We used the chemical procedures suggested by Almuzaini et al. [14] and Banso and Adeyemo [15] to extract phytochemical components from clove pods. These techniques are well-known for their capacity to isolate individual chemicals, which guarantees the reliability and repeatability of study results.

GC-MS Analysis

The GC-MS analysis of clove pod ethanol extract was used to determine its chemical constituents, retention length, base peak, molecular weight, formula, and compound names. The NIST 14S library was used to identify research compounds.

The analytical settings included a split ratio of 10:1, an oven temperature program of 280°C for 25 minutes, and 0.7 mL/minute helium flow. Full-scan mass spectra were collected and analyzed. Pharmacology, food science, and studies of natural products all rely on this date. This information is very useful for the food industry, medicinal research, and natural product studies.

Statistical analysis

The data underwent descriptive analysis, with significant cases identified using the least significant difference (L.S.D.) analysis, represented by letters reflecting different levels of significance. Larvicidal Activity was performed by Porbit analysis conducted using Excel 2016 to determine the lethal concentrations for 50% of the mortality (LC50) and 95% of the mortality (LC95) at 24 hours after treatment. Logarithms were added at various concentrations (+6) to convert the logarithmic concentration to a positive value.

The mortality rate was transformed into Probit units using the Finney table. The concentrations were expressed logarithmically in X Probit units. The Y points were graphically depicted, and the toxicity threshold was visually indicated.

Results

Antibacterial properties of Clove pods

The document presents the results of a study on *the antibacterial activity of clove ethanolic extract—the study utilized in vitro testing against harmful bacteria, including S. aureus, Sal. typhi, P. aeruginosa, E. coli, and K. pneumoniae*. The results in Tables 1 and 2 indicate that the clove extract exhibited antibacterial activity at higher (3 µg/disc) and lower (1 µg/disc) concentrations. At the higher concentration, the mean inhibition zones for the tested bacteria ranged from 13.67±0.57 mm to 15.0±0.0 mm, while at the lower concentration, the mean inhibition zones ranged from 6.33±0.57 mm to 11.0±1.0 mm.

Notably, the extract showed the highest inhibition against *S. aureus*, with a mean inhibition zone of 15.0±0.0 mm at the higher concentration.

Microorganism	One µg/disc	2 µg/disc	3 µg/disc
S. aureus	10±0.0 [a]	13.0±0.0 [a]	15.0±0.0 [a]
Sal. Typhi	10.33 ±0.57 [a]	11.67±0.57 [b]	13.67±0.57 [b]
P. aeruginosa	7.0±0.0 [b]	12.67±0.57 [ab]	14.33±0.57 [ab]
E. coli	11.0±1.0 [a]	12.67±0.57 [ab]	14.33±0.57 [ab]
K. pneumoniae	6.33±0.57 [c]	10.33 ±0.6 [c]	14.67±0.57 [a]

Table 1: The inhibition zone of Clove pods extract (mean±SE) Letters showed varied significance values relative to the mean+SE

The provided document outlines the results of a study conducted to determine the M.I.C. and M.B.C. values of clove extract against specific microorganisms. The M.I.C. values for the clove ethanolic extract (Table 2) ranged from 6.25 to 50 µg/mL for the tested bacteria. It was observed that the clove extract demonstrated a bacteriostatic profile against *S. aureus* and *P. aeruginosa*, with an MBC/MIC ratio of less than 4. Conversely, the same extract exhibited bactericidal action against *K. pneumoniae* and *E. coli*, with an MBC/MIC ratio greater than or equal to 4.

Microorganism	MIC	MBC	Ratio*
S. aureus	25	50	2
Sal. Typhyi	25	25	1
P. aeruginosa	25	25	1
E. coli	6.25	25	4
K. pneumoniae	6.25	25	4

Table 2 : Determination of MICs, MBC, and ratios of the tested Clove pods extract against the selected microorganisms expressed in µg/ml.

Larvicidal Activity

The effect of clove pod extract on *Aedes aegypti* mosquito larvae was evaluated after 24 hours of exposure at the doses of 0.0028, 0.0042, 0.0060, 0.0069, 0.0080 and 0.0083 ml/L is indicated in Figure 1. The resulting percentage mortalities were 55, 65, 75, 80, 85 and 95, respectively (Table 3). The doses reflected an LD50 of 0.0028 ml/L, and LD95 of 0.011 ml/L. The lowest dose (0.0028 ml/L) produced. 55% mortality, whereas the highest dose (0.0080ml/L) reflected 95% mortality. The R-square was 0.7945. The standard error of the log dose SE(Y) was 1.47 whereas SE(X) was 0.65.

DOSE		MORTALITY %		PROBIT	
ml/L	Log	Tested	Corrected	Tabulated	Calculated
0.0028	-2.55	55.0	55.0	5.13	5.00
0.0042	-2.37	65. 0	65.0	5.39	5.46
0.0060	-2.22	75.0	75.0	5.67	5.85
0.0069	-2.16	80.0	80.0	5. 84	6.00
0.0080	-2.09	85.0	85.0	6.04	6.18
0.0083	-2.08	95.0	95.0	6.64	*6.21*

Table 3: The effect of clove pods extract against the *Aedes aegypti mosquito* larvae.
Control mortality was 0.0% in all cases
Regression equation: $Y = 11.56 + 2.75X$
SE(Y) = 1.47
SE(X) = 0. 65
R-square = 0.794501, LD50 = 0.0028 ml/L, LD 95 = 0.011 ml/L

The phytochemical screening

The phytochemical screening results revealed the presence of flavonoids and steroids but the absence of glycosides and alkaloids. Additionally, it highlights the relatively high concentration of flavonoids and terpenoids within the clove pods. This information is essential for understanding the chemical composition of clove pods and their potential pharmacological or therapeutic uses. The presence of flavonoids and steroids could have implications for the medicinal properties of clove pods, while the absence of glycosides and alkaloids provides insight into their chemical makeup.

Damage larvae body | Bright color larva | Larvae damage disconnected head | Larva with disconnected alimentary canal and head

Figure 1: The damage caused by clove pods extract on *Aedes aegypti* mosquito larvae after 24 hour.

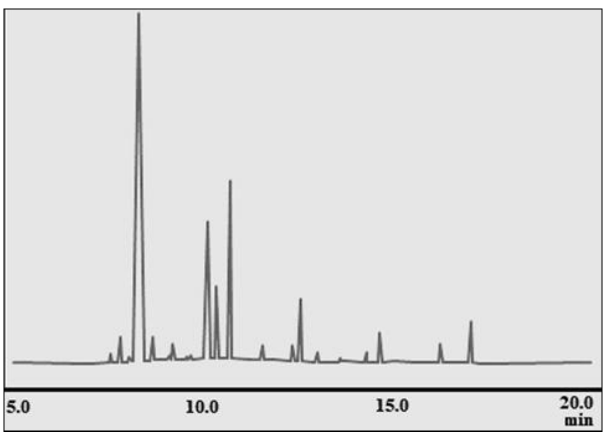

Figure 2: GC-MS chromatogram of clove (*Syzygium aromaticum*) pods.

GC-MS of Clove pods

The GC-MS analysis of clove pods (Figure 2) revealed the presence of 21 different compounds within a retention time range of 7.564 – 17.023 minutes. The primary constituent identified was eugenol, accounting for 58.86% of the compounds, followed by caryophyllene (14.72%), phenol, 2-methoxy-4-(2-propenyl)- acetate (9.60%), humulene (3.62%), eugenol acetate (3.13%), and n-hexadecanoic acid, methyl ester (2.05%). Notably, no nitrogen-containing or chlorine-containing compounds were detected. The analysis also identified three phenolic compounds and three esters within the clove pods, varying from 0.08% to 0.43%. Three phenolic compounds and three esters were detected within the clove pods. The phenolic compounds identified were Phenol, 4-(2-propenyl)-, 2-propenal, 3-phenyl-, and Phenol, 2-methoxy-4-(2-propenyl)—acetate, with concentrations ranging from 0.21% to 0.41%. The detected esters were Docosanoic acid, ethyl ester, Acetic acid, phenylmethyl ester, and n-hexadecanoic acid, methyl ester.

Discussion

Antibacterial properties

Standardized techniques and accurate measurements allowed the plant extract's antibacterial capabilities to be reliably assessed, revealing its antimicrobial potential. This study highlights the need for comprehensive testing of plant extract-derived antibacterial agents for therapeutic use.

The clove ethanolic extract showed variable antibacterial efficacy against dangerous microorganisms, according to the study. These findings imply that clove (*Syzygium aromaticum*) pods are a promising natural source of antibacterial agents. The extract appears to be antimicrobial, notably against *S. aureus*. The tables' quantitative inhibitory zone measurements reveal the extract's efficacy at varied concentrations. These findings help explain clove extract's potential pharmaceutical uses, particularly against bacterial infections. Cloves have been shown to prevent bacterial infections, and other researchers have shown its potential in various sectors [16,17]. This research improves human health and helps us comprehend phytochemistry. It could change the pharmaceutical sector by developing natural alternatives to standard drugs. To combat antibiotic resistance, clove and other plants and herbs must be fully explored.

Larvicidal Activity

The effect of clove pods extract against the *Aedes aegypti* mosquito larvae was determined LC50 is lower than the values reported in many studies against *Anopheles stephens* and other species which means clove pods are more potent *against Aedes aegypti*. Examples of plants with high levels of *Lawsonia inermis* (69.40ppm) [18], *Cionura erecta* (77.30ppm) [19], *Cypressus arizonica* (79.30ppm) [20], and *Zhumeria majdae* (61.34ppm) [21]. Nevertheless, in certain other studies, the calculated LC50 values are lower than the LC50 value we reported. For example, *Bunium persicum* had an LC50 of 27.72ppm [21], *Tanacetum persicum* and *Achillea kellalensis* had LC50 values of 48.64ppm and 35.42ppm respectively [22], *Satureja bachtiarica* had an LC50 of 24.27ppm [23], and Citrus aurantium had an LC50 of 31.20ppm [24].

Phytochemical constituents of Clove pods

Phytochemical screening of clove pods revealed the presence of flavonoids and steroids, but the absence of glycosides and alkaloids using normal chromatographic and spectroscopic methods. The clove pods' high flavonoids and terpenoids concentrations reveal their chemical composition and possible uses. These chemicals' antioxidants, anti-inflammatory, and immunomodulatory characteristics may explain clove pod extracts' antibacterial actions [25]. These discoveries may help researchers, pharmaceutical businesses, and healthcare practitioners use clove pods for medical or therapeutic purposes and comprehend their phytochemistry. Clove pod essential oils are antibacterial, antiviral, and anti-inflammatory, supporting their use as a natural medicine [26].

Clove pod extracts contain phenols, tannins, alkaloids, flavonoids, terpenoids, sterols, cardiac glycosides, and saponins, which have several uses. Flavonoids may be healthy, phenols and tannins are antioxidants and antimicrobials, and alkaloids can be therapeutic [27].

GC-MS constituents

Clove pod GC-MS analysis revealed 21 components, with eugenol accounting for 58.86%. The composition's organic character is shown by the absence of nitrogen and chlorine molecules. Clove pods' chemical profile, including phenolic chemicals and esters at various quantities, illuminates their possible uses and biological effects. This detailed analysis helps us comprehend clove pods' chemical composition, paving the way for pharmacological, culinary, and aromatherapy studies. Compared to other researchers, Ahamad et al. [28] discovered 43 chemical components in clove essential oil, including eugenol (59.16%), β-selinene (9.34%), caryophyllene (7.68%), eugenol acetate (3.35%), and α-humulene (2.16%).

Using gas chromatography-mass spectrometry (GC-MS), the study identified and characterized various chemical constituents in the clove pod ethanol extract, revealing the ginger rhizome extract's chemical profile. The defined chemical composition has interesting applications in numerous fields, according to this study. It also supports product and process development using these chemicals. Hassan et al. [29] and Song et al. [30] noted that clove pods have long been utilized as antimicrobials and suggested they may be a promising source of natural antimicrobials for bacterial infections. Eugenol may activate clove oil, which disrupts the bacterial cytoplasmic membrane, allowing ion extravasation and protein loss, which kills the bacterium. Clove essential oil inhibited *S. aureus*, *E. coli*, *L. monocytogenes*, and *S. typhimurium* at 0.304 mg mL^{-1}. Microorganism membrane properties did not alter this effect [31].

In conclusion, the comprehensive study of clove pods through antibacterial, phytochemical, and GC-MS analyses showcases the incredible potential of these humble plant components. This research sheds light on plants' rich and diverse capabilities, offering innovative solutions to address numerous healthcare and environmental challenges. As we move forward, it is crucial to encourage further exploration into the immense benefits that nature's treasures can provide. By embracing the profound knowledge inherent in botany, we can unlock the keys to healthier living, more robust ecosystems, and a more sustainable global society.

Driven by the relentless pursuit of medical advancements and ecological sustainability, our efforts to highlight the undeniable value of natural remedies and the versatile attributes of plant-based substances will undoubtedly yield groundbreaking discoveries and revolutionary innovations. It is our responsibility as global citizens to continue championing the importance of botanical research and actively seek out novel applications for plants' medicinal and environmental benefits.

While the potential of clove pods has been highlighted in various fields, there are still significant challenges and opportunities for further research. One major issue facing researchers is replicating the natural conditions under which clove plants grow. This is crucial for accurately testing the effects of the plant's phytochemicals on human health and disease. Developing advanced in vitro and in silico models that mimic the complex interactions between clove compounds and humans can help reduce the need for labor-intensive and potentially harmful experiments. Additionally, exploring the potential of clove derivatives in treating other conditions, such as fungal infections, neurodegenerative diseases, and even cancer, could lead to exciting discoveries.

Author Contributions

A.E.S,: Conceptualization, writing—original draft preparation, methodology, writing—review and editing, funding acquisition; methodology, N.A.I., N.S.B.: writing—review and editing, formal analysis; N.A.; writing—original draft preparation, g, resource, validation, M.A. formal analysis, data curation: S.M.I funding acquisition, administration; Z.A. S.; resources, writing—original draft preparation, investigation, software All authors have read and agreed to the published version of the manuscript.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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