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Phytochemical Analysis and Antimicrobial Properties of *Eugenia caryophyllata (Syzigium aromaticum* L. Myrtaceae*)*

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ABSTRACT

This study is aimed at analyzing the phytochemicals and elucidating the antimicrobial properties of *Syzygium aromaticum* (Clove) which is one of the most popular and widely used spice that has been reported to possess high nutritional value, antimicrobial properties. 100% aqueous and 20:80% (water and alcohol) for hydroethanolic and hydromethanolic extracts were prepared. About 13 phytochemical compounds were detected qualitatively in all of the three different extracts which include phenolic compounds, tannins, ascorbic acid e.t.c. Sensitivity disc method was used to test the antibacterial and antifungal properties of *Syzygium aromaticum* (clove) was found that all pathogenic bacteria and fungus were inhibited by the Clove. *Staphylococcus aureus* was more sensitive with greater zone of inhibition of (12.33±0.88 mm) than the other bacteria. *Candida albicans* had no activity at concentration of 0.25 mg/mL in comparison with standard antibiotic (amoxicillin). The clove methanolic extract produced same zones of inhibition with amoxicillin (12.33±1.20 mm), which was used as a control and minimum inhibitory concentration was found to be 0.25 mg/mL for both gram positive and Gram-negative bacteria, respectively, and 0.5 mg/mL for *Candida albicans*.

INTRODUCTION

Natural products have a long history of usage in the treatment of a wide range of ailments in many regions of the world, including the United States. Many different natural items have been utilised in traditional medicine for thousands of years for a variety of purposes. Because of their antibacterial, antiinflammatory, cytostatic, antioxidant, anti-fungal, and antiviral properties, many herbal medicines have been utilised for a variety of ailments [1–5]. The World Health Organization (WHO) believes that traditional medicine is used nearly exclusively by 80 percent of people living in poor nations, according to their data [6]. It has been observed that certain Syzygium species exhibit antimicrobial and anti-inflammatory properties, respectively. *Syzygium aromaticum* (Clove) buds were recorded to be used in folk medicine as a diuretic, odontalgic, stomachic, tonicardic, aromatic condiment with carminative and stimulant action, as well as an aromatic condiment with carminative and stimulant activity. Indeed, cloves are one of the most highly coveted spices in the world, their medicinal and culinary properties being widely known across the world. Cloves are the "flower buds" of an evergreen rain-forest tree that grows naturally in Indonesia's Moluccas spice islands. Cloves may also be found in India, the West Indies, Tanzania, Sri Lanka, Brazil, and Madagascar, where they are harvested commercially. Cloves, with their sultry sweet fragrant fragrance and potent essential oil components, have been used as a nutritious spice for food and a treatment for a range of health issues for hundreds of years. They are a member of the ginger family.

Clove flowers and clove oil have been used extensively in traditional medicine for more than 2,000 years in both Indian and Chinese cultures. Because of their capacity to preserve goods and hide the scent of poorly-kept foods, cloves were extremely popular as a therapeutic flower in Europe during the fourth and eighth centuries A.D. Arabic traders introduced the buds to the continent during the fourth and eighth centuries A.D. Clove belongs to the family of *Myrtaceae* and the genus *Sygyzium*of and species name *Sygizium aromaticum*. Internationally the name is varied for example Laung, Laumg or Lavang in Hindi, Kabsh qarunfil or kabsh qaranful in Arabic, Ding xiang in Chinese, Clavos de olor in Spanish, Clous de girofle and Kaninfari in Nigeria (Hausa) [7–14].

When it comes to spices, cloves are one of the most often utilised in Indian cuisine. It has been demonstrated to be a powerful chemopreventive agent, and it has been utilised by traditional Ayurvedic physicians in India to treat respiratory and intestinal problems since ancient times. Among the essential oils found in clove flower buds are acetate, eugenol, caryophyllene, alpha-terpinyl methyl eugenol, alpha-humulene, eugenyl, actyl eugenol, chavicol, naphthalene, methyl salicylate pinene, heptanone, vanillin, sesquiterpenes, and naphthal [9,11]. Clove has a variety of chemical components, the most important of which are sesquiterpenes, volatile oil (eugenol), caryophyllene, tannins, and gum. Eugenol is the most important component in essential oils, accounting for 81.1 percent of the total by weight of the oils. Additionally, trans-cryophyllene and isoeugenol may be found in small amounts (7 percent and 10.1 percent, respectively) in the mixture [15].

Because of its various applications, this spice must be widely planted in most of the places where climatic conditions are conducive to its optimal growth and development. In this way, a maximum yield of its many useable pieces might be reached, allowing for the production of the greatest possible number of commodities of varying natures for the benefit of mankind [7–14]. Therefore, taking into consideration all of the information, it is necessary to establish its medicinal, pharmacological, and other diverse qualities that are beneficial to humans. The current research sought to identify and quantify numerous phytochemical components, as well as physiochemical and antibacterial activity, in distinct *Syzygium aromaticum* extracts (Clove).

MATERIALS AND METHODS

Chemicals

All chemicals used in this research work were of analytical reagent grade and were obtained from Biotechnology Department laboratories of Sharda University, Greater Noida, Utter Pradesh (UP), India.

Source of Plant Material

The clove was purchased from Spencer Ansal Plaza, Greater Noida, Uttar Pradesh, India and authenticated by Botanist of Life Science Department.

Preparation of aqueous extract of clove

The dried clove was grinded into a fine powder with the help of mixing grinder. About 50 g of clove powder was weighed using an electronic weighing balance and dissolved in 200 mL of distilled water and was warmed on a hot plate at 55 ºC for 2 hours. The mixture was regularly shaken at interval for 2-3 days, after which was filtered using filter paper. The aqueous extract (filtrate) was finally concentrated in hot air oven at 50 ºC and then stored in refrigerator at 4 ºC for further use.

Preparations of hydroethanolic and hydromethanolic clove extract

The ratios of water (hydro) to that of alcohol (ethanol and methanol) for the preparation of hydroethanolic mixtures were 20:80 respectively. 50 g of clove powder was weighed using electronic balance and dissolved in mixture containing 20 mL of distilled water and 180 mL of 70% ethanol. And this followed by regular shaking at intervals for 2-3 days and was then filtered using filter paper to recover the hydroethanolic extract. The extract was finally concentrated in hot air oven at 50 ºC and then kept at refrigerator at 4 ºC for further utilization. The same explained procedure above applied in hydromethanolic extract preparation.

Phytochemical Profiling

Preliminary screening for the presence of phytoconstituents (Primary and Secondary metabolites) of all the extracts was carried out using standard conventional procedures [16].

Pharmacognostical and fluorescence analysis

Pharmacognostical parameters such as foreign organic matter, loss on drying, total ash, acid insoluble ash, water soluble ash, moisture content and crude fibres contents were performed as per Indian Pharmacopoeia. Fluorescence analysis of the Clove powder was carried out with different chemical reagents in day (254 nm) and UV light (365 nm) [17].

Screening of antibacterial activity:

The bacterial species used for the test were *Staphylococcus aureus* ATCC (2592), *Escherichia coli* ATCC (25922) and *Klebsiella* ATCC 15/7 and the fungus species used for the test was *Candida albican*. All the strains were obtained from microbiology laboratory of Sharda university, Greater Noida, India

Culture media and inoculums preparation

Nutrient agar/ broth was used as the media for bacterial culture and potato dextrose agar was used for the fungus. To 1mL of mother culture of respective bacterial strain were inoculated in nutrient broth in aseptic condition and then incubated at 37 ºC for 24hr. Mac Conkey agar and potato dextrose agar were further used for preparation of plates.

Test sample preparation

The concentration of (0.25, 0.50, 0.75, & 1.0 mg/mL) of all the three different extract of clove (aqueous, hydroethanol & hydromethanol) were prepared. The concentration was prepared in their respective solvents.

Disc diffusion techniques

Using the Kirby- Bauer method, the nutrient agar was produced using normal microbiological procedures and carefully put into sterile Petri plates before solidifying. After solidifying, the dishes were streaked with clinical isolates (test organisms) in accordance with their protocol. In order to create positive and negative controls, one disc of each of the three clove extracts with the appropriate potency [(100, 200, 400, 600, 800, 1000(mg/disc)] was selected for each concentration and aseptically put on the plates; positive and negative controls were also prepared. After that, the plates were incubated aerobically at 35 ºC for 16-18 hours. After that, the diameter of the zone of inhibition (in millimetres) was measured .

Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the three clove extracts were evaluated using micro dilution methods in Mueller Hilton broth. Preparation of the inocula included increasing the density to 0.5 Mcfarland turbidity standards [10^8 colony forming units (CFU)/mL] and diluting it by a factor of 10 for the broth microdilution process. MICs were determined after 24 hours of incubation at 37 ºC in microtiter plates, and the results were recorded[14,18].

Statistical analysis

The inhibition zones were calculated as mean±SD (n=3).

RESULTS AND DISCUSSIONS

Yield of extracts from successive extraction of *Syzygium aromaticum*

The yield of successive extracts (g) is shown in **Table 1**. The amount of the aqueous extract obtained from the extraction was 4.015g yield, hydroethanolic extract was 12.972g yield and hydromethanolic extract was 13.972g.

Table 1. Phytoprofile and yield of extracts from various solvent extraction of *Syzygium aromaticum* (50 g).

S. No	Name of Extract	Colour	Consistency	Odour	Nature	% Yield (w/w)
1	aqueous	reddish brown	oily	aromatic	solid	8.03
2	hydroethanolic	dark brown	oily	aromatic	solid	25.94
3	hydromethanolic	dark brown	oily	aromatic	solid	27.94

Fluorescence Characteristics of different extracts of *Syzygium aromaticum* (Clove)

The result of fluorescence studies of the extracts is compiled in **Table 2**. Different chemical components found in plant material show the phenomena of fluorescence, which is a significant feature of their behaviour. When properly lit, a wide range of phytochemical fluorescence can be observed. Depending on the chemical, the fluorescence colour will be different. When a nonfluorescent chemical is combined with fluorescent impurities, the nonfluorescent compound might become fluorescent. Some components glow in the visible spectrum of day light, which indicates that they are alive. Many natural compounds (for example, alkaloids such as berberine) exhibit fluorescence when exposed to ultraviolet light, which is not visible in natural light. It is possible that some crude medicines will be evaluated qualitatively in this manner even though the chemicals themselves are not fluorescent; as a result, some crude drugs are frequently evaluated qualitatively in this manner, and it is an essential criterion of pharmacognostical evaluation.

Table 2. Fluorescence Characteristics of different extracts of *Syzygium aromaticum* (Clove).

S. No	Name of Extract	Under Ordinary Light	Under UV light (280 nm)
1	Aqueous	Reddish Brown	Yellowish Brown
2	Hydroethanolic	Dark Brown	Orange
3	Hydromethanolic	Dark Brown	Light Brown

Phytochemical Screening of successive extracts of *Syzygium aromaticum* (Clove)

Through the use of Phytochemical screening methods using various chemical reagents, it was possible to determine the presence or absence of various phytoconstituents such as carbohydrates, glycosides, proteins, tannins, saponins, flavonoids, and terpenoids. Phytochemical analysis revealed the presence of saponins and alkaloids in the successive extract of *Syzygium aromaticum* (Clove), as well as phytosterols, flavonoid pigments, tannins, terpenoids and steroids, ascorbic acid, carbohydrates and amino acids, and fatty acids in the subsequent extract of *Syzygium aromaticum* (Clove). The results of the Phytochemical analysis are presented in **Table 3**. All of these phytochemicals have strong antioxidant properties, and they have been shown to have a variety of biological effects, including anti-inflammatory and anti-tumor properties, according to research.

Table 3. Qualitative Phytochemical screening of various extracts of *Syzygium aromaticum* (Clove).

Plant Constituents	Tests Performed	Aqueous Extract	Hydroethanolic Extract	Hydromethanolic Extract
Alkaloids	Mayer's Test		+	+
Saponins	Frothing Test		+	+++
Phenolic Compounds	Ferric Chloride Test	+++	++	++
Phytosterols	Libermann-Burchard's Test	_	+	+
Flavanoids	Ammonia Test	+	_	+
Terpenoids	Salowski Test	+	++	++
Tannins	Ferric Chloride Test	+++	+++	+++
Steroids	Lieberman Test	++	+	+
Cardiac Glycoside	Keller Killani Test	+++	+++	+++
Phlobatannins	Hydrochloride Test	_	_	+
Ascorbic Acid		+++	+++	+++
Carbohydrates	Fehling Test	+	+	+
	Barfoed Test	+		+
Proteins and Amino Acids	Ninhydrin Test	–	–	–
Fats and Oils	Stain Test	++	+++	+++

Sensitivity disc method was used to test the antibacterial and antifungal properties of *Syzygium aromaticum* (clove) was found that all pathogenic bacteria and fungus were inhibited by the Clove (**Tables 4** to **6**). *Staphylococcus aureus* was more sensitive with greater zone of inhibition of (12.33±0.88 mm) than the other bacteria. *Candida albicans* had no activity at concentration of 0.25 mg/mL in comparison with standard antibiotic (amoxicillin).The clove methanolic extract produced same zones of inhibition with amoxicillin (12.33±1.20 mm), which was used as a control and minimum inhibitory concentration was found to be 0.25mg/mL for both gram positive and Gram-negative bacteria respectively and 0.5mg/mL for Candida albicans.

Table 4. Result of antimicrobial testing of three (3) different extracts of *Syzygium aromaticum* (Clove) samples against *Klebsiella.*

Extracts conc. Mg/mL	Zone of inhibition in diameter (mm±SE)			
	Control (Ampicillin)	Aqueous	Hydroethanolic	Hydromethanolic
0.25	12.33±0.33	11.67±0.33	10.33±0.33	11.67±0.33
0.50	12.67±0.33	8.33±0.33	11.33±0.33	10.00±0.58
0.75	12.33±0.33	11.00±0.58	12.00±0.58	11.00±0.58
1.00	13.00±0.58	8.67±0.67	9.67±0.33	10.33±0.67

Values are mean ± S.E (n=3), mm = millimeter & SE = Standard error

Table 5. Result of antimicrobial testing of three (3) different extracts of *Syzygium aromaticum* (Clove) samples against *Staphylococcus aureus.*

Extracts conc. Mg/mL	Zone of inhibition in diameter (mm±SE)			
	Control (Amoxicillin)	Aqueous	Hydroethanolic	Hydromethanolic
0.2	9.33±0.67	8.33±0.88	7.33±0.33	8.00±0.58
0.50	15.00±0.00	8.00±1.00	9.67±0.33	9.33±0.33
0.75	12.00±0.00	8.33±0.33	10.33±0.33	11.67±0.33
1.00	12.33±1.20	10.00±0.58	11.33±0.33	12.33±0.88

Values are mean ± S.E (n=3), Mm = millimeter & SE = Standard error

Table 6. Result of antimicrobial testing of three (3) different extracts of *Syzygium aromaticumum* (Clove) samples against *Candida albicans.*

Extracts conc. Mg/mL	Values are mean ± S.E (n=3) Zone of inhibition in diameter (mm±SE)			
	Control (fluconozole)	Aqueous	Hydroethanolic	Hydromethanolic
0.25	11.67±0.33	0.00±0.00	0.00±0.00	0.00±0.00
0.50	11.00±0.00	7.33±0.33	8.00±0.99	8.00±0.58
0.75	11.33±0.33	10.33±0.33	8.33±0.33	8.33±0.33
1.00	11.67±0.33	10.00±0.00	9.33±0.67	9.33±0.33

mm = millimeter & SE = Standard error

Based on different sources of literature consulted spices are used for traditional medicine for the remedy of many diseases. Today in many parts of the world herbal medicine is frequently used especially in developing countries by about 80% of their population as estimated by World Health Organization. Many researchers have been carried out in different types and various parts of medicinal plants. Several works carried out on *Syzygium aromaticum (*Clove) reported that it contained several phytochemical constituents which are responsible for the antioxidant, antimicrobial, anti-inflammatory, anti-viral, and anti-cancer properties of Clove. In this study, the ratio of water added to the various solvents used for extraction. 20:80 ratios of 70% ethanol and methanol to water were respectively used and 100% distilled water for the aqueous extract extraction. The findings of this work can be considered to suggest that *Syzygium aromaticum* (Clove) could be important as potential sources of anti-microbial. In one similar study, the methanolic extract of this plant was better than an ethanolic extract with the former exhibiting MIC values of 2.31, 0.385 and 0.01 mg/mL for *E. coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively [18].

Throughout history, spices have played a significant role in the lives of people in many areas of the world, particularly in the Middle East. Throughout history, they have played a variety of functions, including those of colouring agents, flavouring agents, preservatives, food additives, and medical treatments. The molecular foundation for this activity has been established by the active phytochemicals produced from various herbs and spices. Because of globalisation, these spices are now widely available, which is boosting their appeal [8,10,14].

According to established techniques, the dried clove was examined for phytochemical and antibacterial properties against pathogenic bacteria and fungi in this study. The qualitative chemical exams and tests for the detection of primary and secondary metabolites were all part of the phytochemical screening process. These metabolites are claimed to be beneficial to the plant itself, but they can be harmful to other creatures, including humans, according to some sources. The existence of these chemical components in this plant is a signal that, if adequately screened, the plant has the potential to produce medicines of medicinal value. A stronger argument for this is provided by the fact that members of the plant's family have been identified as being associated with ethnomedicine in the treatment of a variety of illnesses. Because of the wide range of active phytochemical components in this plant, it has been shown to have therapeutic qualities [9,13,18].

CONCLUSION

Medicinal plants are the richest bioresource of drugs for traditional systems of medicine, modern medicines, Nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. They are also the most diverse bioresource of drugs for traditional systems of medicine, modern medicines, Nutraceuticals, food supplements, and folk medicines. Clove is a fragrant herb that is used to make perfumes, flavours, cosmeceuticals, health drinks, and chemical terpenes, among other things. Medicinal plants are essential in pharmaceutical research and medication development because of their medicinal properties. The majority of the world's population (more than three-quarters) relies mostly on plant extracts for health-care purposes. It is estimated that one-fifth of the plants found in and around the glove are utilised for medicinal purposes. After everything is said and done, clove extracts were found to have antibacterial action against pathogens that are found in food. The hydromethanolic extract of clove was shown to be more effective against a Gram-positive bacteria (*Staphylococcus aureus*) at virtually all of the three different concentrations tested. Hydroethanolic and aqueous extract were shown to be more effective against gramme negative pathogens (*Escherichia coli* and *Klebsiella*) and fungus (*Candida albican*).

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