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Research Article

Phytochemical Analysis of Clove (*Syzygium aromaticum*) Dried Flower Buds Extract and its Therapeutic Importance

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Abstract

The current study objective was to look into phytochemical analysis of *Syzygium aromaticum* dried flower buds. Clove has long been known to have positive health effects. The majority of the clove spice consumed worldwide comes from home kitchens. One of the most expensive spices, clove (*Syzygium aromaticum*) has historically been used for both food preservation and for several therapeutic goals. Clove is a native of Indonesia, although it has been cultivated throughout the world, including India. The dried bud powder was successively extracted with water, methanol, ethyl acetate and petroleum ether. The following phytochemicals are present in *Syzygium aromaticum*, Carbohydrates, lipids, alkaloids, flavonoids, tannins, sterols and triterpenes are all found, according to the phytochemical examination. Clove, also known as *Syzygium aromaticum* (Family Myrtaceace), is the most important and second-most precious spice. The utilization of herbal medicines is a significant source for the development of novel pharmaceutical compounds to treat severe ailments. It offers a wide range of medical benefits including antibacterial, analgesic, antioxidant, anti-inflammatory and antiseptic properties. In clove dried flower bud extract the identified compounds are Quercetin, Ursolic acid and Gallic acid, all these compounds are responsible for the medicinal properties of clove bud.

Keywords: Clove bud extract, Phytochemical, Soxhlet, LCMS, Health benefits.

INTRODUCTION

Cloves are fragrant plants that belong to the Myrtaceae family. It is a member of the *Syzygium* genus. The largest genus in the Myrtaceae family is called *Syzygium*. Cloves are a species of the Syzygium genus that have exceptional economic importance. Cloves are known by the scientific name *Syzygium aromaticum* [1]. Traditional medicine, which relied on using herbal medicines, continues to be crucial to the health care system. Due to the belief that natural medications from medicinal plants have fewer adverse effects and greater efficacy than their synthetic counter parts, these products have gained increasing recognition in recent decades [2]. Currently, traditional medicines represent the primary source of primary health care for about 80% of the world's population [3]. Numerous herbal plants have bactericidal, veridical and fungicidal effects, they are employed in embalming and food preservation, they also have anti-inflammatory, antibacterial, sedative, analgesic and local anesthetic effects. Herbal medicines have been acknowledged as a significant source for finding unique pharmacological compounds that have been employed to treat severe ailments up to this point. The discovery of these phytochemicals has been regarded as a great breakthrough in the search for potent and novel medications. Herbal medicines have been acknowledged as a significant source for finding unique pharmacological compounds that have been employed to treat severe ailments up to this point. The discovery of these phytochemicals has been regarded as a great breakthrough in the search for potent and novel medications [4]. Clove, also known as *Syzygium aromaticum*, is a dried flower bud from the Myrtaceae family that is native to the Maluku islands in Indonesia but has recently been grown in many locations throughout the world. The commercial section of the clove tree is made up of leaves and buds, and four years after planting, flowering buds start to be produced. Following that, they are harvested either manually or with the aid of a natural phytohormone during the pre-flowering stage [5]. Clove is one of the spices that may be used as preservatives in various dishes, notably meat, and is interestingly utilized commercially for many therapeutic uses as well as in the perfume industry, due to its antioxidant and antibacterial qualities, processing, to replace chemical preservatives [6]. Clove's effectiveness in preventing a variety of degenerative diseases is linked to the presence of certain chemical components that are highly concentrated and have antioxidant activity [7]. Traditional uses for clove essential oil (CEO) include the treatment of burns and wounds, as well as relieving dental discomfort, tooth infections, and toothaches. Additionally, its use in numerous industrial applications has been described, and it is widely employed in perfumes, soaps, and as a washing agent in histology work [8]. Chinese and Indian traditional medicine employs cloves as a warming and stimulating stimulant [9]. Cloves have historically been used to cure a variety of conditions, including nausea, liver, intestine and stomach ailments, as well as to stimulate the nervous system. Cloves have been shown to treat several pathogens in tropical Asia, including scabies, cholera, malaria and

tuberculosis. Additionally, clove has long been used in America to cure viruses, worms, candida and several bacterial and protozoan illnesses by blocking food-borne pathogens [10]. Clove bud, also known as *Syzygium aromaticum*, is one of the oldest and most expensive spices in history used as a digestive aid to boost hydrochloric to reduce stomach acid and to enhance peristalsis [11]. Due to advancements in the nutritional and medical fields, the importance of plants to human life has been growing daily. Spices are the dried roots, seeds, bark, fruits, or flowers of plants that have a variety of uses, such as flavoring, food coloring, food additives, food preservatives and medication. The discovery of spices during prehistoric times was an exciting moment because they are utilized as flavoring ingredients [12]. Spices have long been an essential component of cuisine around the world. Due to their medicinal qualities, these spices have been utilized since the Ayurveda to treat a variety of ailments. Spice's therapeutic benefits have been attributed to a number of phytochemicals [13]. Additionally, they featured a number of pharmacological and phytochemical qualities that made them useful in the creation of numerous medicines.

Taxonomic classification

Kingdom –	Plantae
Sub kingdom –	Tracheobionta
Super division –	Spermatophyta
Division –	Magnoliphyta
Class –	Magnoliopsida
Subclass –	Rosidae
Order –	Myrtales
Family –	Myrtaceae
Genus –	Syzygium
Species –	aromaticum (L.)

The different names of clove (*Syzygium aromaticum*) are as given below:

Common Name: Clove

Botanical Name: *Syzygium aromaticum*

Local names : Lavanga, Devapuspa, Varala, Bhadrasriya (Sanskrit), Laung, Lavang (Hindi), Krayampu, Grampu (Malayalam), Luvang (Marathi), Grambu, Kirampu, Kirambu (Tamil), Lavanga, Daevakusuma (Kannada), Lavangalu, Devakusumamu (Telgu), Lavanga (Bengali), Lavang (Gujarati), Laung (Punjabi), Labanga (Oriya), Laung, Loung (Urdu).

International Names : Kabsh qarunfil (Arabic), Karamfil (Bulgarian), Ding xiang (Chinese), Kruidnagel (Dutch), Nellike (Danish), Giroflier (French), Gewürznelke , Nelke (German), Garifalo (Greek), Mikhaki, Mixaki (Georgian), Szegfu (Hungarian), Cengke, Cengkeh (Indonesian), Chiodo di garofano (Italian), Girofla, Choji, Kurobu (Japanese), Jeonghyang (Korean), Krustnaglinas (Latvian), Lwaang (Nepalese), Nellik (Norwegian), Cravo de India (Portuguese), Mikhak (Persian), Kala (Pashto), Gvosdika, Pazhitnik grecheski (Russian), Clavo (Spanish), Kryddnejlika, Kryddnejlikor, Nejlikor (Swedish), Carenfil (Turkish), Khan plu, Garn ploo (Thai), Dhing huong (Vietnamese).

MATERIALS AND METHODS

Collection and identification of plant material

The flower buds of clove plant used in this study were purchased from local market of Indore (M.P) India. The flower buds were identified by Dr. Navin Kumar Jain at the Department of Botany, Govt. Autonomous Holkar Science College Indore.

Extraction of Phytochemicals

Prepared powdered plant parts were extracted with decreasingly polar solvents for the extraction of phytochemical compounds.

Preparation of plant extracts

Syzygium aromaticum (Clove) dried buds were collected, dried, powdered and then extracted in a soxhlet apparatus with water, methanol, ethyl acetate and petroleum ether respectively.

Prepration of extract by soxhlet extractor

On dried powder, soxhlet extraction was carried out to create the extracts. As solvents, petroleum ether, methanol, and ethyl acetate were all employed. Each sample's dry powder was placed in the Soxhlet apparatus' thimble. 250 ml of a different solvent was administered to each sample. Plant material's phytochemicals are released from the thimble along with clean drops of solvent. For multiple cycles, the solvent continues to drop down on the plant material, evaporate, and condense. Weighed extracts were kept in sealed containers for a preliminary phytochemical investigation.

Preliminary Phytochemical analysis

These extracts were tested in order to find out the presence of active compounds by use of following standard methods.

Test for Carbohydrates:

(a) Molisch's test: 1 ml of plant extract was added to 0.4 ml of Molisch's reagent. Afterwards, 1 ml of conc. Sulphuric acid was added along the side of the test tube. A purple colour indicates the presence of carbohydrates (starch).

(b) Benedict's test: : 1 millilitre of plant extract and 1 millilitre of Benedict's reagent were heated for 5 minutes. The presence of carbohydrates (disaccharides) was shown by the formation of an orange precipitate.

(c) Fehling's Test: Boiling and filtering 1 ml of plant extract with 2 ml of purified water. Then, 2 ml of Fehling's reagent were added to 2 ml of filtrate, which was then heated. Reddish brown precipitate indicates the presence of carbohydrate (glucose).

Test for Proteins:

(a) Xanthoprotic test: 0.25 ml of nitric acid was applied to 1 ml of plant extract. Appearance of white precipitate indicated the presence of proteins.

(b) Biuret test: 1ml of plant extract was taken in a test tube followed by 4% NaOH and 1% $CuSO_4$. Violet pink colour development indicated the presence of proteins

Test for lipids:

(a) Solibility test: 1 ml of plant extract was evaporated to dried powder. Few drops of petroleum ether were poured into the test tube and shaken well. Complete dissolution of extract identified the presence of lipids.

(b) Glycerol test: To 1 ml of 1% $CuSO_4.5H_2O$ solution, 5 drops of the plant extract were added and mixed thoroughly.

Then it received 5 drops of a 10% sodium hydroxide solution. A clear blue solution was obtained which indicates the presence of glycerol.

(c) Sudan III test: To 1ml of plant extract, few drops of Sudan III solution were added. Appearance of red colour indicated the presence of lipids.

Test for alkaloids:

(a) Mayer's Test: 1 ml of clove flower bud extract, 2 drops of chloroform, and 2 drops of Mayer's reagent were added. A positive alkaloid reaction resulted in the production of white deposits.

(b) Wagner's test: 1 ml extract was treated with Wagner's reagent, formation of brown reddish precipitate indicates presence of alkaloids.

(c) Dragendorff's test: 2 ml of Dragendorff's reagent was added to 1 ml of plant extract. Formation of orange white precipitate indicated the presence of alkaloids.

Test for Tannins:

(a) Gelatine test: To 500 µl of the filtrate, 1% gelatine solution was added. Formation of curdy white precipitate indicated the presence of tannin.

(a) Lead acetate test: To the filtrate, 5ml of 10% lead acetate solution was added. Formation of white precipitation indicates the presence of tannin.

(b) Ferric chloride test: Five drops of a 5% ferric chloride solution were added to the filtrate. Formation of blue green colour indicated the presence of tannin.

Test for Saponin:

(a) Foam test: 1 ml of plant extract was taken in a test tube with small amount of water. Sodium bicarbonate was added to it and shaken vigorously for 5 min. Formation of foam indicated the presence of saponins.

Test for Flavonoids: To 0.5 ml of plant extract, 5 ml dilute ammonia was added followed by the addition of 1 ml concentrated sulphuric acid. A yellow coloration that disappeared on standing indicated the presence of flavonoids.

Test for Resins:

In a dry test tube, to 0.5 ml of acetic acid and 2 drops of conc. Sulphuric acid were added. It was clear that resins were present because a purple color that turned violet in about 10 minutes.

Test for Sterols (Salkowski test)

2 ml of concentrated sulphuric acid and 2 ml of chloroform were applied successively from the side of the test tube to 0.5 ml of the plant extract. The test tube was shaken gently for few minutes. The development of red colour in the chloroform layer indicated the presence of sterols.

Test for Cardiac Glycosides (Keller Killiani test)

Approximately 5 ml of each plant extract was evaporated at 40°C and the residue was collected. A few mg of residue was suspended in 5 ml water. 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to it. This solution was underplayed with 1 ml of conc. sulphuric acid. A brown ring at the interface indicated the presence of deoxy sugar, a characteristic of cardiac glycosides

Test for Triterpenes:

Few milligrams of plant extract residue was mixed with 5 ml of chloroform and warmed for 30 min at 40°C. Few drops of conc. sulphuric acid were added and mixed well. The appearance of red colour indicated the presence of triterpenes.

Test for Anthraquinones:

2ml of each plant extract was shaken with 10ml benzene, and 5ml of 10% ammonia solution was added. The mixture was shaken in order to obatained the color of antraquinones. The ammonical layer acquiring pink colour indicated the presence of Anthraquinones.

Phytochemical profiling using Liquid Chromatography Mass spectroscopy (LC MS)

The plant extracts were dried and the residue was sent for LCMS analysis at Sophisticated Analytical Instrumentation Facility (SAIF), Indian Institute of Technology Mumbai (IIT Bombay), India.

RESULTS AND DISCUSSIONS

The phytochemical analysis of *Syzygium aromaticum* (clove) extract revealed the presence of carbohydrates, lipids, alkaloids, flavonoids, tannins, sterols and triterpenes while proteins, saponins, cardiac glycosides and anthraquinones were absent in table 1.

Carbohydrate test used three methods namely Molisch's test, Benedict's test and Fehling's test, all methods show positive result. In protein test two methods are used namely Xanthoprotic test and Biuret test, both methods show negative result. In Lipid test three methods are used namely Solubility test, Glycerol test and Sudan III test, two methods show negative results in solubility test it show positive result. In alkaloids test three methods used namely Mayer's test, Dragendroff's test and Wagner's test, all three tests show positive results. In saponins test only one method is used namely foam test and this test show negative result. Flavonoids test show positive results. Resin test also shows negative result. In Tannin's test three methods are used namely Lead acetate test, Gelatin test and Ferric chloride test. All three tests show positive results. In sterols test only one method used namely Salkowski method, it shows positive result. Sterols test also show positive result. Cardiac glycoside and Anthraquionone test show negative result. According to the findings of this study's investigation, clove bud extract included phytochemicals with established pharmacological action. This investigation indicated the existence of numerous medically significant phytochemicals in clove bud extract. Extract from clove buds included compounds with known pharmacological effects. This study showed that clove bud extract has a large number of phytochemicals that are important for health.

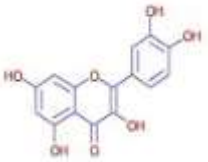
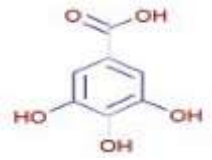
Table 1: Result of the Phytochemical analysis of clove flower bud extract

S. No.		Phytochemicals	Extracts			
			Aqueous	Methanol	Ethyl acetate	Petroleum ether
Primary metabolites						
1		**Carbohydrates**				
	a.	Molisch's test	-	+	-	-
	b.	Benedict's test	-	+	-	-
	c.	Fehling's test	+	-	-	-
2		**Protein**				
	a.	Xanthoprotic test	-	-	-	-
	b.	Biuret test	-	-	-	-
		Lipid				
	a.	Solubility test	-	-	-	+
	b.	Glycerol test	-	-	-	-
	c.	Sudan III test	-	-	-	-
Secondary metabolites						
1		**Alkaloids**				
	a.	Mayer's test	-	+	+	-
	b.	Dragendroff's test	-	+	-	-
	c.	Wagner's test	+	+	+	-
2		**Saponins**				
	a.	Foam test	-	-	-	-
3		**Flavonoids test**	-	+	+	-
4.		**Resins test**	-	-	-	-
5		**Tannins test**				
	a.	Gelatin test	-	+	+	-
	b.	Lead Acetate test	-	+	+	-
	c.	Ferric chloride test	+	+	+	-
6.		**Sterols**				
	a.	Salkowski test	+	+	+	-
7		**Cardiac Glucosides**				
	a.	Keller – Killiani test	-	-	-	-
8		**Triterpenes test**	-	+	+	-
9		**Anthraquinones test**	-	-	-	-

Note: **(+)** represents the presence of the constituents

(-) represents the absence of the constituents

Table 2: The phytochemicals identified through mzCloud™ spectral library from the *Syzygium aromaticum* (clove) extract that showed potential antimicrobial and antidiabetic activity.

Plant extract	Structure	Name	Formula	Mol. Wt.
Clove bud extract		Quercetin	$C_{15}H_{10}O_7$	302.0427
		Ursolic acid	$C_{30}H_{48}O_3$	456.3603
		Gallic acid	$C_7H_6O_5$	170.0215

This study found that clove bud extract included a number of phytochemicals. The current investigation, which was conducted on clove bud extracts, found that there are active components that have therapeutic properties. A collection of metabolites rather than a single component is frequently to blame for the medicinally beneficial effects of plants, which typically result from the secondary products present in the plant [14]. The results of a qualitative analysis of each of Clove's phytochemical active components are shown in Table 1. Carbohydrates, lipids, alkaloids, flavonoids, tannins, sterols and triterpenes are all screened. However, certain chemical compounds found in plants that have distinct physiochemical effects on the human body are what give them their therapeutic worth [15]. Difference Saponins protect against hypercholesterolemia, and phytochemicals have been identified to have a wide range of activities that may assist to protect against chronic disorders like heart disease and stroke [16]. The central nervous system activity is shown to be analgesic by steroids and terpenoids. Recently, researchers reported on the significance of alkaloids, saponins, and tannins in several antibiotics used to treat prevalent pathogenic strains[16]. Both in China and India, traditional medicine uses cloves very heavily. Cloves have historically been used to cure liver, intestine, and stomach diseases as well as nausea, vomiting and flatulence. Clove has been reported to be used for scabies, cholera, malaria and tuberculosis due to its antimicrobial effects. [17].

Medicinal uses

- Since clove has antibacterial characteristics, it is used to kill bacteria in a variety of mouthwashes, throat sprays, dental creams, and tooth pastes.
- Additionally, it helps to enhance general oral health and relieves the discomfort of sore gums. Cloves are aphrodisiacs, which are substances that stimulate or heighten sexual arousal or potency.
- Due to its high flavonoid concentration, clove is utilized as an anti-inflammatory.
- Pure clove oil is used by aroma therapists to treat the signs and symptoms of arthritis and rheumatism. To promote peristalsis, raise stomach hydrochloric acid, and act as a carminative, clove is employed.
- To treat acne, apply a paste made of clove powder and honey. Clove powder mixed with water to make a paste encourages quicker healing of bites and injuries. Many digestive issues can be easily treated with cloves. It has therapeutic properties that can treat nausea, indigestion, loose stools and flatulence.
- Cloves can be used for easing the symptoms of diarrhoea, irritation in the stomach and vomiting. By cleaning the blood and assisting in the battle against numerous diseases, cloves and clove oil strengthen the immune system.
- The treatment of athlete's foot and nail fungus with clove oil is successful.
- Good expectorants like cloves encourage the release of mucus and secretions from the respiratory tract.
- When inhaled, the fragrant clove oil can relieve several respiratory ailments like sinusitis, bronchitis, asthma, colds and coughs. Additionally, it aids in nasal passage way cleansing.
- Because clove oil promotes blood flow and circulation, it is beneficial for persons with chilly extremities.
- Additionally, cloves reduce the progression of macular degeneration and improve vision in old age by protecting the retina of the eye from damage. The basic method involves stopping the degradation of docosahexaenoic acid, which protects senior citizens' vision.
- Researchers discovered that inhaling the spicy scent of cloves lessens headaches, tiredness and irritation.
- Spiced fruits, hot spiced drinks, chocolate drinks, wines and liqueurs, puddings, sandwiches, cakes, curries and pickles are just a few of the foods and beverages that frequently use clove as a flavouring.
- It is a typical cooking spice that is used to flavour foods like tomatoes, onions, sausage, soups, salads and herbal teas.
- It is a significant spice used in Russian, Scandinavian, Greek, Indian and Chinese cuisines.

- The primary component of Indian masala tea is dried cloves.

CONCLUSION

A positive reaction with the appropriate test reagent during phytochemical screening of aqueous, methanol, ethyl acetate and petroleum ether extracts indicated the presence of carbohydrates, lipids, alkaloids, tannins, flavonoids, sterols and triterpenes. The three main components that are isolated from the clove bud extracts are quercetin, Ursolic acid and Gallic acid. Clove has pharmacological effects that include antimicrobial, antidiabetic, antioxidant, anti-inflammatory, analgesic, anti-cancer and anesthetic properties. These biological processes can be highly beneficial for illness prevention and therapy. The therapeutic properties of clove are strong, and it has a long tradition and history. Clove is beneficial for mental, emotional and physical health. One of Mother Nature's most potent antiseptics is cloves. The major ingredients that give clove buds their therapeutic qualities are quercetin, Ursolic acid and Gallic acid. According to global trade, clove is the most significant spice in the world.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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