



PHYTOCHEMICAL AND GC-MS ANALYSIS OF ETHANOL EXTRACT OF *SYZYGIUM AROMATICUM*

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ABSTRACT

A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. In the present study phytochemical and GC-MS analysis of ethanol extract of buds of *Syzygium aromaticum* was carried out. The extraction of plant materials was done following the method of Harborne. The phytochemical analysis of the ethanol extract of buds of *Syzygium aromaticum* revealed the presence of alkaloids, carbohydrates, proteins, amino acids, fixed oils, flavonoids and phenolic compounds. The GC-MS analysis of the ethanolic extract of buds of *Syzygium aromaticum* revealed the presence of 19 compounds. This study forms a basis for the phytochemical characterization of the extracts, the

isolation of responsible bioactive compounds and their biological activity are necessary for future studies.

KEYWORDS: *Syzygium aromaticum*, Phytochemical analysis, GC-MS, Eugenol.

INTRODUCTION

Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents. Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Phytochemicals may protect human from a host of diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are organic substances and could be obtained in both primary and secondary metabolic process; they also provide a source of medicine since the earliest time. The plant kingdom has proven to be the most useful in the treatment of diseases

and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development.^[1]

Syzygium aromaticum are used in Indian ayurvedic medicine. It is commonly called clove, belongs to the family Myrtaceae. It is native to Molucca Island of Indonesia. The major clove-producing countries are Indonesia, Tanzania, Sri Lanka, Madagascar and on a limited scale, India. In India it is grown in Kerala, Tamilnadu, Karnataka, Andaman and Nicobar Island over an area of 1735 hectares.^[2] The stem, unopened buds and leaves are normally used for extraction of essential oil.^[3]

Clove bud oil has biological activities, such as antibacterial, antifungal, antiinflammatory, chemopreventive, hepatoprotective, neuroprotective, insecticidal, analgesic, antispasmodic, anticarminative and antioxidant properties.^[4,5] Clove oil include acetyl eugenol, beta-caryophyllene, vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, the flavonoids like eugenin, kaempferol, rhamnetin, and eugenitin; triterpenoids like oleanolic acid, stigmasterol and campesterol and several sesquiterpenes.^[6-8]

It contains good amount of minerals like potassium, manganese, iron, selenium and magnesium. Potassium is an important electrolyte of cell and body fluids that helps control heart rate and blood pressure. Manganese is used by the body as a co-factor for the antioxidant enzyme superoxide dismutase. Clove is a good source of vitamin-K, vitamin-B (pyridoxine), thiamin (vitamin B-1), vitamin-C and riboflavin. Consumption of foods rich in vitamin C helps body to develop resistance against infectious agents and scavenge harmful oxygen free radicals.^[9] In the present study phytochemical and Gas Chromatography – Mass Spectrum Analysis of ethanol extract of *Syzygium aromaticum* were studied.

MATERIALS AND METHODS

Buds of *Syzygium aromaticum* (Fig. 1) was collected from Govindraja Mudaliar Stores, Dealers and Government supplier of Indian Raw drugs and Herbals, Rasappa Chetty Street, Park town, Chennai-600 003. The plant materials obtained were tested for authenticity in the Department of Botany, Quaid-e-millath government college for women, Chennai-600 002. Vouchered specimen of *Syzygium aromaticum* was deposited at Department of Zoology, Quaid-e-millath government college for women, Chennai-600 002. Cloves were cleaned and

coarsely powdered. Solvent extraction was done by cold percolation method, by soaking the plant materials in ethanol successively in an air tight bottle for 48 h.^[10] It was filtered by Whatman Filter paper No.1. The solvent was removed by distillation using Evapor rotary evaporator and the extracts were concentrated and dried in Vacuum desiccator. Concentrated extract was used for Phytochemical screening and Gas Chromatography – Mass Spectrum Analysis.

Phytochemical screening of ethanol extract of *Syzygium aromaticum*

Qualitative tests were performed to assess the nature of phytochemicals present in ethanol extracts of *Syzygium aromaticum*.^[10]

(A) Test for alkaloids

Solvent free 50 mg extract was stirred with few ml of dilute HCl and filtered. The filtrate was tested carefully with various alkaloid reagents as follows:

1. **Wagner's test:** To a few ml of filtrate, few drops of Wagner's reagent were added along the sides of the test tube. A reddish – brown precipitate confirmed the test.
2. **Hager's test:** To a few ml of filtrate 1 or 2 ml of Hager's reagent were added. A prominent yellow precipitate indicated the test as a positive

(B) Test for Carbohydrates and Glycosides

1. **Molish test:** To 2ml of filtrate two drops of alcoholic solution of α -naphthol were added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.
2. **Fehling test:** One ml of filtrate was boiled on water bath with 1ml each of Fehling solution A and B. A red precipitate indicates the presence of sugar.
3. **Barfoed's test:** To 1ml filtrate, 1ml of Barfoed reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicates the presence of sugar.
4. **Benedict's test:** To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 min. A characteristic colored precipitate indicates the presence of sugar.

(C) Test for proteins and Amino Acids

1. **Biuret test:** An aliquot of 2 ml of filtrate was treated with one drop of 2% copper sulphate solution. To this 1 ml of ethanol (90%) was added, followed by excess of

potassium hydroxide pellets. Pink colour in ethanol layer indicated the presence of proteins.

- 2. Ninhydrin test:** Two drops of ninhydrin solution were added to 1 ml of aqueous filtrate. A characteristic purple colour indicated the presence of amino acids.

(D) Test for Fixed Oils and Fats

- 1. Spot test:** A small quantity of extract was pressed between two filter papers, oil stain on the filter paper indicated the presence of fixed oil.

(E) Test for Phenolic compounds and Tannins

- 1. Ferric chloride test:** The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of Phenolic compounds.

(F) Test for flavonoids

- 1. Alkaline reagent test:** An aqueous solution of extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

Analysis of ethanol extract of *Syzygium aromaticum* by Gas Chromatography–Mass Spectrum Analysis (GC–MS)

GC–MS analysis of the effective fractions were performed using GC–MS–QP 2010 (Shimadzu) and gas chromatograph interfaced to a mass spectrometer (GC–MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, film thickness : 0.25 μm composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 2 μl was employed (split ratio: 20) with injector temperature of 200 °C and ion–source temperature of 200 °C. The oven temperature was programmed from 70 °C (isothermal for 2 min), with an increase of 300 °C for 10 min. Mass spectra were taken at 70 eV; a scan interval of 0.5 sec with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC–MS solution ver. 2.53. Identification of phytocomponents.

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST08) and WILEY8. The spectrum of the unknown

components was compared with the spectrum of known components stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

In the present study phytochemicals analysis and Gas Chromatography – Mass Spectrum Analysis of ethanol extract of *Syzygium aromaticum* were studied.

Phytochemical analysis of ethanol extract of *Syzygium aromaticum*

The preliminary phytochemical studies are important because the crude extracts possess varied composition of secondary metabolites.^[11,12] The phytochemical test of the ethanol extract of buds of *Syzygium aromaticum* revealed the presence of alkaloids, carbohydrates, proteins, amino acids, fixed oils, flavonoids and phenolic compounds. (Table 1). Ethanol extract of *Syzygium aromaticum* revealed the presence of bioactive constituents which are known to exhibit medicinal properties. Biologically active components have always been of huge interest to scientists working on infectious diseases.^[13] Chemical constituents from natural sources have contributed significantly to the development of new drugs from medicinal plants.^[14,15] Medicinal plants have therapeutic properties due to the presence of various complex chemical substances of different composition which are found as secondary plant metabolites.

Analysis of ethanol extract of *Syzygium aromaticum* by Gas Chromatography–Mass Spectrum Analysis (GC–MS)

The studies on the active principles in the buds of ethanol extract of *Syzygium aromaticum* by GC-MS analysis clearly showed the presence of nineteen compounds. The active principles with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area%) are presented in Table 2. The total numbers of compounds identified in ethanol extracts were, the GC- MS. retention time (RT) and percentage peak of the individual compounds. The results revealed that Phenol, 4 (2-propenyl), Eugenol, Benzaldehyde 3-hydroxy-4-methoxy, Carophyllene, Humelene, Phenol, 2 methoxy -4 (2-propenyl acetate), Naphthalene, 1,2,4a, 5,8,8a-hexahydro-4,7, dimethyl-1 (1-methyl ethyl), Naphthalene, 1, 2,3,4 tetrahydro-1,1,6-trimethyl, 3-nonenic acid, Copaene, Carophyllene oxide), 2, 3, 4 trimethoxy acetophenone, 4a-7-methano-49h-naphth (1,8a-b) oxirane, Octahydro- 4,4,8,8 – (tetra methyl), Anethole, Phenol -2 methoxy -6 – (1-propenyl), Phenol -2 methoxy - 4 – (2-propenyl acetate), Squalene, Tetratetracontane and di- a- tocopherol.

Eugenol is the major component present in ethanol extract of *Syzygium aromaticum*. Eugenol has been reported to have various biological properties like anthelmintic, antiviral, antioxidant, anti-inflammatory, antigenotoxic, antiseptic, antifungal, analgesic and antibacterial activity.^[16-21] Eugenol is reported to possess anticancer activity against various cancers. Additionally, the molecular mechanism of eugenol-induced apoptosis in melanoma, osteosarcoma, leukemia, gastric, skin tumors and mast cells has been well documented. Eugenol and six of its derivatives for its antiproliferative activity against primary melanoma cell lines.^[22] The chemopreventive potential of eugenol in an experimental skin carcinogenesis mice model system.^[23] Srivastava have isolated and identified two anti-platelet components, eugenol and acetyleneugenol which inhibit platelet aggregation induced by arachidonate, adrenaline and collagen.^[24] Phytochemical study showed the presence of phytochemicals such as alkaloids, carbohydrates, proteins, amino acids, fixed oils, flavonoids and phenolic compounds in ethanol extract of *Syzygium aromaticum* which might be responsible for their therapeutic effects. It further reflects a possibility for the development of many more novel chemotherapeutic agents or templates from the plant which in future may serve for the production of improved therapeutic plant based drugs.



Fig. 1: Buds of *Syzygium aromaticum*.

Table 1: phytochemical analysis of ethanol extract of *Syzygium aromaticum*.

S.no	Test for Phytochemical	Results
1.	Hager's Test	Presence of alkaloids
2.	Fehling Test	Absence of sugar
3.	Molish Test	Presence of Carbohydrate
4.	Biuret Test	Absence of Proteins
5.	Ninhydrin Test	Presence of Amino acids
6.	Spot Test	Presence of Fixed oils
7.	Ferric chloride test	Presence of Phenolic compounds
8.	Alkaline reagent Test	Presence of Flavonoids

Table 2: Phytoconstituents identified in ethanol extract of *Syzygium aromaticum* by GC-MS.

S.No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area
1	9.35	Phenol, 4 (2-propenyl)	C ₉ H ₁₀ O	134	0.50%
2	11.29	Eugenol	C ₁₀ H ₁₂ O ₂	164	88.71%
3	12.07	Benzaldehyde 3-hydroxy-4- methoxy	C ₈ H ₈ O ₃	152	0.18%
4	12.57	Carophyllene	C ₁₅ H ₂₄	204	1.09%
5	13.21	Humelene	C ₁₅ H ₂₄	204	0.26%
6	14.12	Phenol, 2 methoxy -4 (2- propenyl acetate)	C ₁₂ H ₁₄ O ₃	206	2.81%
7	14.26	Naphthalene, 1,2,4a, 5,8.8a-hexahydro-4,7, dimethyl-1 (1-methyl ethyl)	C ₁₅ H ₂₄	204	0.66%
8	14.34	Napthalene, 1, 2,3,4 tetrahydro-1,1,6-trimethyl	C ₁₃ H ₁₈	174	0.30%
9	15.89	3-nonenoic acid	C ₉ H ₁₆ O ₂	156	0.22%
10	16.15	Copaene	C ₁₅ H ₂₄	204	0.15%
11	16.85	Carophyllene oxide)	C ₁₅ H ₂₄ O	220	0.33%
12	17.01	2 3 4 trimethoxy acetophenone	C ₁₁ H ₁₄ O ₄	210	1.56%
13	20.15	4a-7-methano-49h- naphth (1,8a-b) oxirne, Octahydro- 4,4,8,8 - (tetra methyl)	C ₁₅ H ₂₄ O	220	0.29%
14	27.81	Anethole	C ₁₀ H ₁₂ O	148	0.31%
15	29.35	Phenol -2 methoxy -6 – (1-propenyl)	C ₁₀ H ₁₂ O ₂	164	0.22%
16	30.41	Phenol -2 methoxy - 4 – (2-propenyl acetate)	C ₁₂ H ₁₄ O ₃	206	0.21%
17	31.17	Squalene	C ₃₀ H ₅₀	410	0.19%
18	33.42	Tetratetracontane	C ₄₄ H ₉₀	618	0.75%
19	34.28	di- a- tocopherol	C ₂₉ H ₅₀ O ₂	430	0.33%

CONCLUSION

Discovery of new therapeutic substances of natural origin, with possibly least toxicity to humans will protect from chemical inputs. The natural compounds derived from plants are more stable as these are mostly plant secondary metabolites synthesized over long synthetic ones and therefore are a source of low molecular weight structures active against a wide range of target agents and this diversity can preclude the occurrence of resistance. The phytochemical and Gas Chromatogram Mass spectrometry (GC-MS) analysis and of the ethanol extract of buds of *Syzygium aromaticum* revealed the presence of alkaloids, carbohydrates, proteins, amino acids, fixed oils, flavonoids and phenolic compounds and nineteen chemical constituents have been identified from the present study. Eugenol is the major component present in ethanol extract of *Syzygium aromaticum*. Eugenol has many potent activities and more research has to done to study about the Eugenol mechanisms. This

study explores the goodness of the buds of *Syzygium aromaticum* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance. Furthermore considering its versatile medicinal uses, there is an ample scope for future research.

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