

Original Article

## COMPARISON OF PHYTOCHEMICALS IN THE FLOWER BUDS, PEDICELS AND LEAVES OF *SYZYGIUM AROMATICUM* (L.) MERRILL AND PERRY

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### ABSTRACT

**Objective:** To analyse and compare the major chemical components in the flower buds, pedicels and leaves of *Syzygium aromaticum* by Gas-Chromatography Mass spectrometry technique.

**Methods:** Healthy and mature flower buds, pedicels and leaves were shade dried and pulverized using a mechanical grinder. The powder was successively extracted with ethanol (40-60o C). The extracts were concentrated under reduced pressure in a rotary evaporator. The ethanolic extracts of the plant parts such as leaves, pedicels, and buds were used for GC-MS analysis.

**Results:** The major constituent is eugenol. Pedicels contain 79.75% eugenol, buds contain 74.12% eugenol and leaves contain 51.03% eugenol. In addition to eugenol, other important components are Acetyl eugenol, Caryophyllene, Humulene and Caryophyllene oxide.

**Conclusion:** Eugenol has a wide range of medicinal properties such as antiseptic, anaesthetic, analgesic anti-inflammatory. Commercially pedicel is not used for eugenol extraction. Present study has revealed that it could be used as a promising one in pharmaceutical industry in addition to flower buds.

**Keywords:** GC-MS, Clove buds, Pedicel, Eugenol, Caryophyllene.

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### INTRODUCTION

*Syzygium aromaticum* is a tree of the Family Myrtaceae and its aromatic dried flower buds are of economic importance. They are native to Maluku Islands in Indonesia and are commonly used as a spice. Cloves are commercially harvested in India, Pakistan, Indonesia, Madagascar, Zanzibar, Sri Lanka, and Tanzania. The term Clove is derived from the French word 'Clou' and the English word 'Clout', both meaning 'nail' from the likeness of the flower bud of the clove tree to a broad-headed nail. *Syzygium aromaticum* is an evergreen tree which grows to a height ranging from 8-12m having large leaves and sanguine flowers in numerous group of terminal cymose clusters [1]. Flowers are hermaphrodite and self-pollinating. Each peduncle bears 3-4 stalked flowers at the end. The flower buds are at first of a pale colour and gradually become green, after which they develop into a bright red when they are ready for harvest and collection. Cloves are harvested when 1.5-2 cm long consist of a long axis terminating in four spreading sepals and four unopened petals which form a ball at the centre. Then these flower buds of cloves are sundried.

Good quality clove buds contain 15-20% essential oil. The oil is dominated by eugenol (70-85%), eugenyl acetate (15%) and  $\beta$ -Caryophyllene.(5-12%) which together make up 99% of the oil [2]. Clove is one of the major vegetal sources of phenolic compounds such as hydroxyl benzoic acid, hydroxyl cinnamic acid, and hydroxyl phenylpropenes. Phenolic acids and gallic acids are the main compounds found in higher concentration [3]. Other phenolic compounds are caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin are present in lower concentration. Another important compound is  $\alpha$  humulene other volatile compounds present in a lower concentration in clove oil are  $\beta$ -pinene, limonene, farnesol, benzaldehyde and 2-heptanone.

Clove leaves yield 3.0-4.8 % essential oil. The essential oil content especially eugenol increases during different stages of leaf growth from 38.3 to 95.2 % with maturity while the contents of eugenyl acetate and caryophyllene decrease [2]. Clove bud oil and leaf oil contain various classes of compounds eg: monoterpenes, Sesquiterpenes, aldehydes, and ketones. Clove stem yield 6% volatile oil which contains 80.2% eugenol and 6.6%  $\beta$ -caryophyllene,

besides several minor components. Ripe fruits yields 2% of oil which comprised 50-55% eugenol.

Eugenol glucoside gallate, a Chromone C-glucoside, galloyl and hexahydroxy diphenyl esters of 2, 4-6, trihydroxy acetophenone-3-glucopyranoside have been isolated from clove leaves [4]. Further two ellagitannins namely syzygin A and syzygin B have also been isolated from leaves.

Clove is a naturally occurring food flavour and is extensively used in fragrance and flavour formulations for its spicy aroma. Clove oil is found to be more effective against some foodborne pathogens [5]. It is used as a food preservative. Saponins are also present in cloves [6]. It is also used in the soap industry.

The most prominent use of clove oil is in dental care. The germicidal properties of the oil make it very effective for relieving dental pain, toothache sore gums and mouth ulcers [7]. Gargles with diluted clove oil help in easing the throat. The characteristic smell of clove oil helps removing bad breath. As a result clove oil is added to numerous dental products and medications including mouthwashes and toothpaste.

### MATERIALS AND METHODS

Healthy and mature flower buds, pedicels and leaves were collected from clove trees in Kollam district of Kerala. During the months of November-January, they were shade dried and pulverized using a mechanical grinder. The powder was successively extracted with ethanol (40-60o C). The extracts were concentrated under reduced pressure in a rotary evaporator. The ethanolic extracts of the plant parts such as leaves, pedicels, and buds were used for GC-MS analysis.

### GC-MS analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer System and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: Column Elite-I fused Silica capillary column (30 mm  $\times$  0.25 mm ID  $\times$  1  $\mu$ m film thickness, composed of 100% Dimethyl poly Siloxane) operating in electron impact mode at 70 eV; Helium (99.99%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2  $\mu$ l was employed (Split ratio

of 10:1) Injector temperature 250 °C; Ion source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C/min, to 200 °C, then 5 °C/minute to 280 °C, ending with a 9 min. isothermal at 280 °C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 min. Interpretation on mass spectrum GC-MS was conducted using the

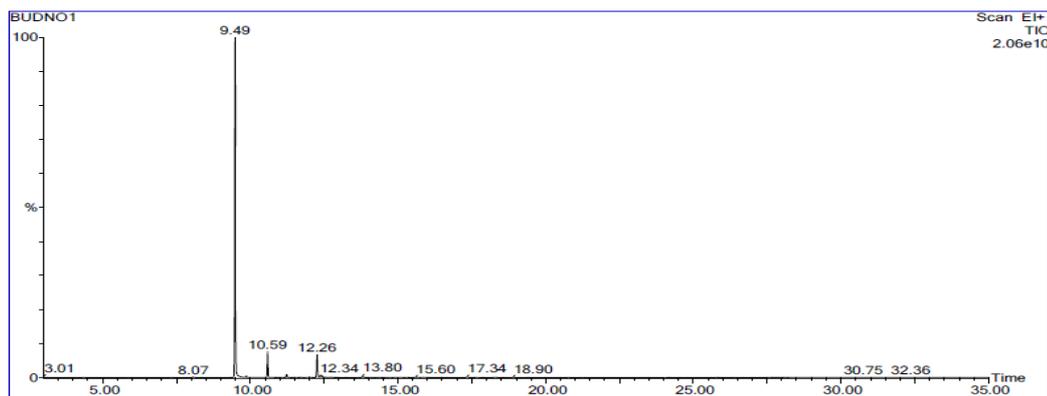
database of NIST. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

#### RESULTS AND DISCUSSION

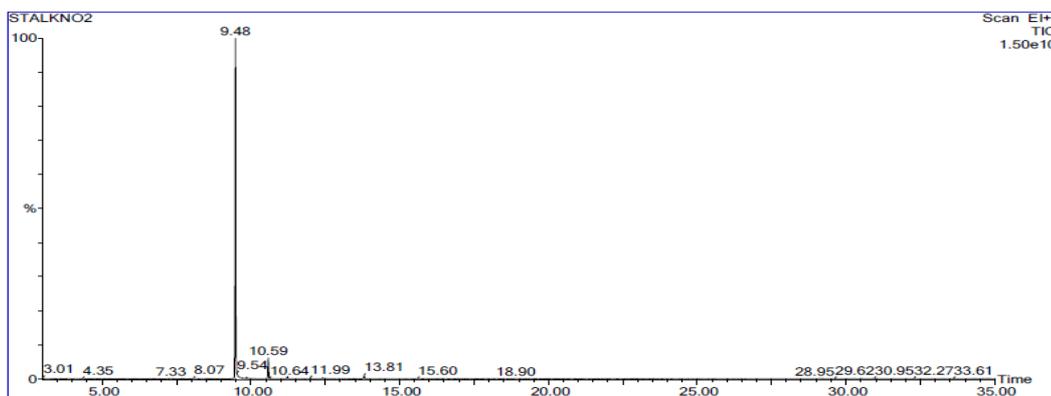
GC-MS analysis was carried out in the powdered clove buds, Pedicels and leaves. The results are given in the table 1 and graphs 1.4

**Table 1: Major chemical components of the flower buds, pedicels and leaves of *Syzygium aromaticum* (L.) merril and perry**

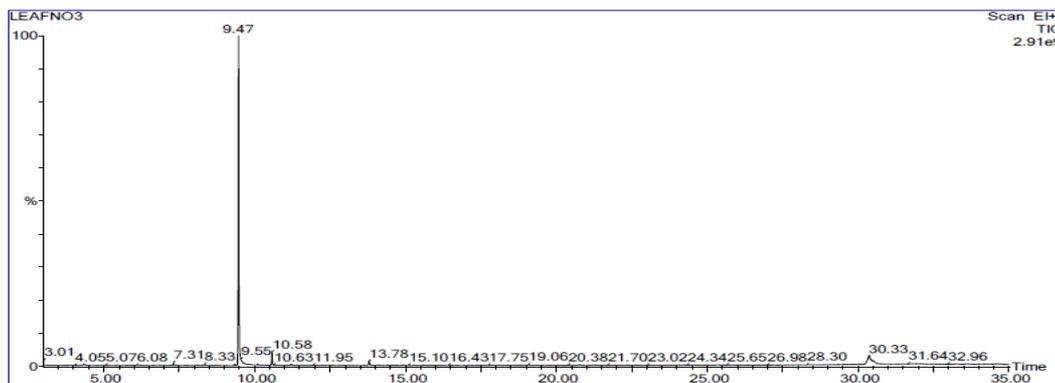
S. No.	Name of the compound	Plant parts (Area of %)		
		Bud	Pedicel	Leaf
1	Eugenol	74.12	79.75	51.03
2	Acetyeugenol	6.69	0.051	0.11
3	Caryophyllene	6.538	5.46	2.199
4	Humulene	0.871	0.685	0.29
5	Caryophyllene Oxide	0.482	1.131	1.088



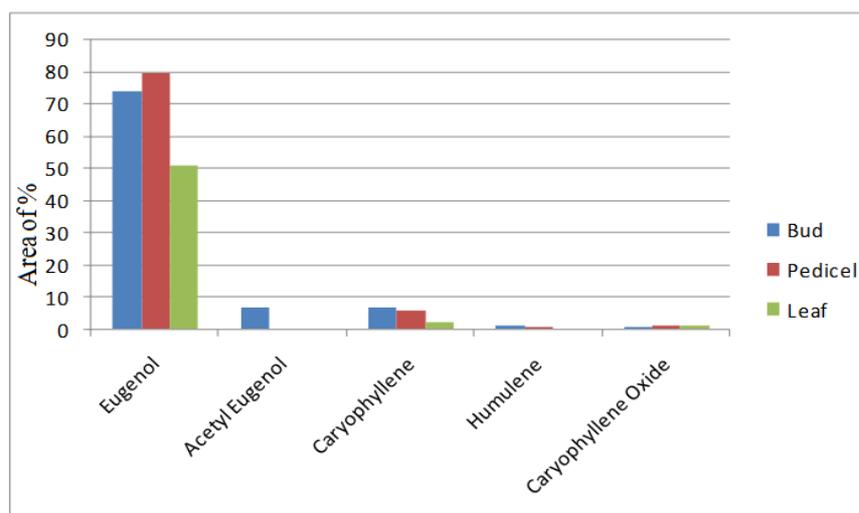
**Graph 1: GC-MS chromatogram profile of flower buds of *Syzygium aromaticum* (L.) Merrill and perry**



**Graph 2: GC-MS chromatogram profile of pedicels of syzygium aromaticum (L.) merril and perry**



**Graph 3: GC-MS chromatogram profile of leaves of syzygium aromaticum (L.) merril and perry**



**Graph 4: Comparison of major phytochemicals in flower buds, pedicels and leaves of *syzygium aromaticum* (L.) merril and perry**

Eugenol was found to be the main compound present in the buds, pedicels, and leaves. In bud eugenol was 74.12% in pedicel it was 79.75% and in leaves, it was 51.03%. In addition to this, Acetyl eugenol, Caryophyllene, Caryophyllene oxide, humulene are also present. Present study shows that the concentration of eugenol is highest in pedicel. Eugenol constitutes a potential antitumor compound against different kinds of Cancer cells depending up on their sensitivity towards it [8]. Eugenol isolated from clove buds show antiviral activity against Herpes Simplex Virus.

In addition to the above-mentioned compounds, certain other compounds are present in lower concentration. Some of them present in bud include Octadecane, alfa-copaene, ulangene, cubenene, isocaryophyllene, Bisa-bolane Naphthalene, Silane, GermacreneD Aromadendrene, Cis and trans calamenene, doconexent, farnesol, Milbemycin, Rhodopin, Toluene, 2, 6-dihydroxy acetophenone, Retinol, Strychane, arachidonic acid, hexa decanoic acid, Lysergic acid, propanoic acid, Tocopherol. Other compounds present in leaf includes 2, 6 dihydroxy acetophenone, Strychane, demecolcine, phentolamine, a-acorneol, hydrocortisone, dodecane, Malonic acid, Memantine, Myristic acid, arachidonic acid, stearic acid, Silane, allo aroma dendrane etc. Other compounds present in pedicel include dodecane, doconexent, farnesene, Memantine, quinolin, carvacrol, alfa copaene, ylangene, allo aroma dendrene, Cis and Tran's calamenene, Stearic acid, Formic acid, 2, 6-dihydroxy acetophenone.

Clove is rich in minerals such as Calcium, Iron, Mg, Pottassium, Magnesium Phosphorus, Zinc Copper, Manganese, Selenium, Vitamin A, B<sub>6</sub>, E and K[9]. One Kg of dried buds yield approximately 150 ml of eugenol. Eugenol cause toxic relatively small quantities as lower 5 ml [10]. Clove oil can be extracted from the leaves, Stem, and buds of clove plant. The main chemical component of clove oil are eugenol, eugenol acetate and caryophyllene [11]. Clove oil is a potential source of bioactive compounds against some bacteria in during nosocomial infections in neonate intensive care Units. [12] Clove oil shows potent insecticidal activity [13]. Clove and eugenol possess strong antioxidant activity.

Eugenol has a wide range of medicinal value such as antiseptic anaesthetic analgesic anti-inflammatory and antimicrobial activities [14]. Clove is used as a carminative to increase hydrochloric acid in the stomach and to improve peristalsis. Eugenol is powerful enough for preventing blood clots. Clove enhances memory retention. It is recommended for brain fog, lethargy and depressive state of mind.

#### CONCLUSION

The chemical composition of dried clove buds, pedicels and leaves were investigated. Pedicel shows highest concentration of eugenol than buds and leaves. Commercially pedicel is not used for eugenol

extraction. But this study has shown that pedicel can also be used as a promising one to the pharmaceutical industry in addition to buds.

#### AUTHORS CONTRIBUTIONS

All the author have contributed equally

#### CONFLICT OF INTERESTS

Declare none

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