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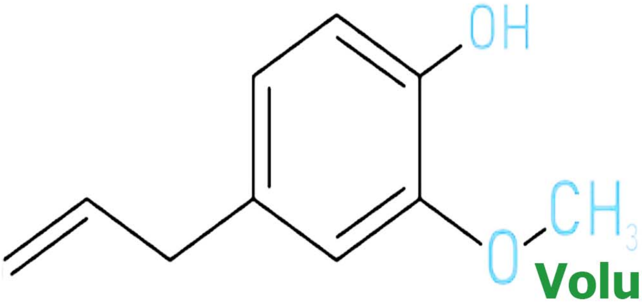

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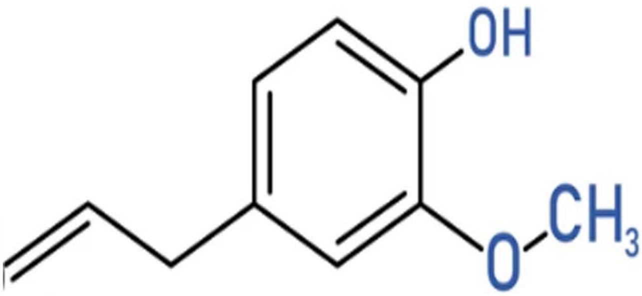

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Essential oils identification and its antimicrobial potential of Syzygium aromaticum (L.) Merr. buds

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Research Article

Essential oils identification and its antimicrobial potential of Syzygium aromaticum (L.) Merr. buds

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ABSTRACT

Syzygium aromaticum, often referred to as clove, is a spice used in cooking that also has medicinal applications. Clove finds its use in cosmetics, medicine, gastronomy, and agriculture, owing to its rich content of bioactive compounds including gallic acid, flavonoids, eugenol acetate, and eugenol. Aim of the present study of essential oil obtained by hydro-distillation from *S. aromaticum* (L.) Merr. buds were analyzed by Gas Chromatography - Mass Spectrometry (GC-MS) and its antimicrobial potential of *S. aromaticum* (L.) Merr. Buds studied by disc diffusion methods. Results of the present study, obtained the essential oils of *Syzygium aromaticum* (L.) Merr buds analysed by thirty- eight active components were identified. The main components of essential oils of *Syzygium aromaticum* (L.) Merr buds were present in the eugenol (63.52%), Eugenyl acetate (11.4%), β- caryophyllene (2.35%) and α-cadinol (2.43%). The antimicrobial activity of essential oils of *S. aromaticum* (L.) Merr buds were maximum activity active against *S. typhi, A.niger* and *A.flavous*. The findings of the present study indicate that the essential oils derived from *S.aromaticum* (L.) Merr buds were exhibit in the significant antibacterial and antifungal properties.

Keywords: *Syzygium aromaticum* (L.) Merr., bud oils, essential oil composition, GC-MS, eugenol.

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1. INTRODUCTION

Syzygium aromaticum (L.) Merr. is belonging to the Myrtaceae, which is the largest genus of Mirtaceae family, comprising of about 1200 to 1800 species of flowering plants, which are widely distributed in tropical and subtropical areas of Asia, Africa, Madagascar, and throughout Pacific and Oceanic regions (Cock and Cheesman, 2018). Clove is known by different vernacular names in different languages. It is known as qaranful (Arabic), Karamfil (Bulgarian), Ding xiang (Chinese), Kruidnagel (Danish), Garifalo (Greek), Mikhaki (Georgian), Nelke (German), Szegfu (Hungarian), Cengkeh (Indonesian), Choji (Japanese), Jeonghyang (Korean), Krustnaglinas (Latvian), Lwaang (Nepalese), Carvo de India (Portuguese), Mikhak (Persian), Kala (Pashto), Gvosdika (Russian), Clavo (Spanish), Carenfil (Turkish), Garn ploo (Thai), Dhing huong (Vietnamese), and Laung (Urdu/Punjabi/Hindi) (Milind and Deepa, 2011). Clove oil has been used as a topical anesthetic and flavoring for very long years ago. It is a medicinal plant, which is promising for its antimicrobial purposes. The oil extracted from *Syzygium aromaticum* buds (cloves) comprises primarily of eugenol, which is known to have pain relieving properties. Biting or chewing a clove bud is a traditional remedy for toothaches. Clove tea can be taken for curing stomach ailments. Essential oil produced from cloves can be applied externally for toothaches, headaches, colds and rheumatism. Clove oil has been examined its biological activities, including antibacterial, antifungal, antiviral insecticidal, and antioxidant properties are in the several authors (Chaieb et al.,2007;Slameňová et al.,2009; Abdullah et al.,2015;Khan et al.,2012). Therefore, the present study was investigated in the identification of essential oils and it's antimicrobial properties of the essential oils of *S. aromaticum* (L.) Merr. Buds.

2. Materials and Methods

2.1 GC-MS Analysis of essential oils

Plant materials of *Syzygium aromaticum* (L.) Merr. buds were collected from Local Market of Palayamkottai, Tamilnadu. 50g of air-dried samples of *S. aromaticum* (L.) Merr. buds were hydro- distillation for Clevenger-type apparatus used for 1hrs and essential oil obtained and the residual moisture was removed with anhydrous sodium sulfate and kept in a refrigerated at -5°C. The essential oil yield was calculated by relating the volume of oil obtained and the material mass used in the extraction process on a dry basis (Santana de Oliveira et al.,2021; Ferreira et al.,2020). The collected essential oil was analyzed by GC-MS-FID on an Agilent 7890A GC system inert XL MSD with triple-axis detector and Agilent 7693 autosampler. GC was equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm, film thickness of 0.25 mm). The injector temperature was 240°C and the column temperature was initiated at 60°C, increased at 3°C/min to 240°C, and held for 5 min. The carrier gas was helium and the injection volume was 5µL (splitless). Mass conditions were mass range of 50-550 m/z, filament delay of 3.50 min. The MS conditions were mass range of 50-550 m/z, filament delay of 3.50 min. The oil components were identified by comparing their mass spectra with the NIST Library as well as with authentic compounds. This was confirmed by comparison of their retention indices with those of authentic compounds as well as with data published in the literature (Adams,2001).

2.2 Antimicrobial activity

2.2.1 Antibacterial activity

The antibacterial activity was investigated in the essential oil of aromaticum buds against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae*. Antibacterial activity of essential oils of buds was evaluated according to the antibacterial activity studied by disk diffusion methods (Bauer et al.,1966).

2.2.2 Antifungal activity

Antifungal Activity of essential oils of buds was evaluated according to the antifungal activity studied by disk - diffusion method (Bauer et al.,1966). Both tested fungus of *Candida albicans* and *Aspergillus niger* were reactivated with Sabouraud Dextrose Broth (SDB). The fungal suspensions were prepared by choosing five colonies with a diameter of approximately 1 mm after incubation of 24 h of the tested both fungi. Taking 5 colonies were suspended in 5 mL of sterile saline (0.90% saline), and the resultant suspension was homogenized on a vortex shaker for 15 seconds. Subsequently, a saline solution was added to obtain the turbidity equivalent to the standard solution of the McFarland 0.5 scale to obtain a standard yeast suspension containing approximately 10^5 fungi per 1mL. Assays were performed using 20mL of sterile Sabouraud Dextrose Agar (SDA) culture medium at 65° C in Petri dishes (50 ×10 mm) and were allowed to solidify. Different concentration of essential oils of buds was added to the different agar plates, obtaining different concentrations as shown in Table 3. A volume of 10µL of the microorganism suspension was then inoculated onto the agar and spread with the aid of a Drigalski loop over the entire surface of the plate. 'e plates were incubated at 36 ± 1°C in a greenhouse for 48 h for the *Candida albicans* and for 5 days at 27 ± 1°C for *Aspergillus niger*. The filter paper discs were impregnated with the extracts and placed individually on the SDA with flamed forceps and gently pressed down to ensure contact with the agar surface (Bauer et al.,1966).

3. RESULTS AND DISCUSSION

3.1 Essential Oils

The oil yields based on dry weight of samples were 0.048-0.096% (w/w). The results of the present study, identified by the active compounds from *S. aromaticum* (L.) Merr. buds were represented in the table-1. The major components of oil were found to be eugenol (63.52%), Eugenyl acetate (11.4%), and β- caryophyllene (2.35%). Previous studies, eugenyl acetate, β-caryophyllene, and α-humulene are other essential oils components, typically present in lesser quantities (El-Saber Batiha et al.,2020; Haro-González et al.,2021). For the reasons of different factors influence the essential oils composition, including the distillation method and plant varieties (Hastuti et al.,2016). The buds represent the plant partition with the highest content of essential oils, but it can also be obtained from leaf through distillation methods (Maugini et al.,2014). According to Cortés-Rojas *et al*.,(2014) reported that clove represents in the one of the most important sources of phenolic compounds, such as kaempferol and quercetin, caffeic acid, ellagic acid, and ferulic acid.

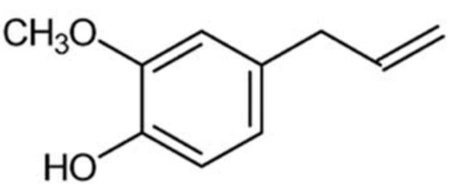
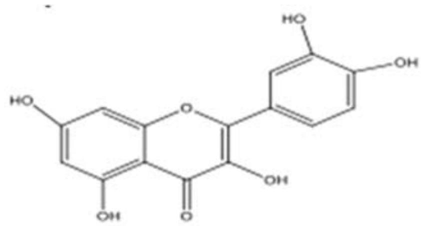
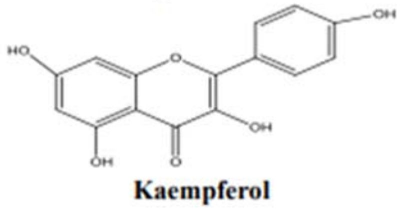


Fig.2: Chemical structure of eugenol (Nisar et al.,2021)



Quercetin



Kaempferol

Table-1: GC-MS analysis of essential oils of *S. aromaticum* buds

Sl.No	Component	Indices	Percentage	Identification Methods
1	Eugenol	1478	63.52	GC-MS
2	Eugenyl acetate	1321	11.4	GC-MS
3	β-Caryophyllene	1514	2.35	GC-MS
4	α-Humulene	1545	0.19	GC-MS
5	Caryophyllene oxide	1495	0.2	GC-MS
6	α-Copaene	1488	0.1	GC-MS
7	Chavicol	1458	0.31	GC-MS
8	Methyl salycilate	1377	0.08	GC-MS
9	Benzaldehyde	1253	0.07	GC-MS
10	Benzyl acetate	1188	0.05	GC-MS
11	2-Nonanone	1289	0.04	GC-MS
12	Benzyl benzoate	1371	0.02	GC-MS
13	Ethyl benzoate	1181	0.01	GC-MS
14	1,8-Cineole	1032	0.36	GC-MS
15	1,3,8-p-Menthatriene	1110	1.85	GC-MS
16	2-Heptanone	881	2.68	GC-MS
17	2-Heptyl acetate	1043	0.10	GC-MS
18	2-Nonanol	1098	0.93	GC-MS
19	6-Methyl coumarin	1549	2.91	GC-MS
20	Acetophenone	1078	0.03	GC-MS
21	Caryophyllene alcohol	1565	1.42	GC-MS
22	Epizonarene	1492	3.21	GC-MS
23	Germacrene D	1484	0.02	GC-MS
24	Methyl benzoate	1087	1.47	GC-MS
25	Methyl eugenol	1404	1.89	GC-MS
26	Methyl undecanoate	1420	0.58	GC-MS
27	N-Citronellyl butyrate	1332	0.01	GC-MS
28	Viridiflorol	1391	0.48	GC-MS
29	Z-Nerolidol	1334	0.03	GC-MS
30	α-Pinene	934	0.09	GC-MS
31	β-Cubebene	1322	0.12	GC-MS
32	β-Pinene	979	0.85	GC-MS
33	γ-Gurjunene	1475	0.41	GC-MS
34	δ-Cadinene	1501	0.65	GC-MS
35	ρ-Acoradiene	1141	0.12	GC-MS
36	ρ-Cymene	1125	0.65	GC-MS
		Total Percentage (99.2)		

3.2 Antibacterial activity

Essential oils have antibacterial activity due to their bioactive compounds, such as eugenol, thymol, and carvacrol, which can affect bacterial cell walls and membranes. In the present study was to investigated in the antibacterial activity of *S. aromaticum* active against such as *E.coli, P.aeruginosa, S.aureus, S.typhi* and *K.pneumoniae*. The maximum antibacterial activity of essential oils of *S. aromaticum* buds were observed that *S. typhi* and followed by *E. coli* and *P. aeruginosa.* The minimum level of antibacterial activity of essential oils of *S. aromaticum* buds were active against *K. pneumoniae* (Table-2). Essential oils of *Piper nigrum, Syzygium aromaticum, Pelargonium graveolens, Myristica fragrans, Origanum vulgare*, and *Thymus vulgaris* were evaluated for antimicrobial activity against twenty-five bacterial strains, including food borne, animal, and plant pathogens, considerable inhibitory action was observed by the volatile oils in a dose dependent behavior (Dorman and Deans,2000).

Table-2: Antibacterial activity of essential oils of *S. aromaticum* buds against some human pathogenic bacteria

Sl.No.	Concentrations (ug/ml^{-1})	Zone of Inhibition (mm)				
		Tested bacteria				
		E. coli	*S. aureus*	*P. aeruginosa*	*S. typhi*	*K. pneumoniae*
1	Control	0	0	0	0	0
2	25	9±0.3	11±0.3	8.0±0.3	11±0.3	7±0.3
3	50	12±0.3	13±0.6	11±0.3	14±0.3	9±0.6
4	75	14±0.3	15±0.3	14±0.3	15±0.3	13±0.3
5	100	17±0.3	16±0.3	17±1.2	18±0.7	15±0.3

MEAN ± S.E Values are triplicates

Previous studies, similar results were observed that ethanolic extract of the *Colocasia esculenta* leaves shows antibacterial activity against *S. aureus, P.aeruginosa, E.coli* and *Kleibsiella* (Sarmistha Dutta and Biswajit Aich,2017). According to Govindrajan et al., (2018) showed various solvent extracts of leaves of *Acalypha indica* exhibited inhibitory properties active against *S. aureus* (Govindrajan et al.,2018). Ashraf *et al*.,(2018) studied on the ethanolic extracts of several medicinal plants of *Punica granatum, Syzygium aromaticum*, *Zingiber officinales, Cuminum cyminum* and *Thymus vulgaris* were potentially active against *S. aureus*. According to Qader et al., (2013) reported that *Zingiber officinales* extract was active against *P. aeruginosa* and *K. pneumonia* while *Thymus kotschyana* was potentially effective against *S. aureus* and *E. coli*.

3.3 Antifungal activity

The antifungal activity of essential oils of *S. aromaticum* buds on the growth of *Candida albicans, Aspergillus flavus, Aspergillus niger* and *Aspergillus fumigatus* on Potato Dextrose Agar (PDA) medium and results are represented in the table-3. The maximum activity of essential oils of *S. aromaticum* buds active against *Aspergillus niger* (19±1.0) and followed by *A. flavus* and *A. fumigatus.* Previous studies on the antifungal activity of essential oils of *S. aromaticum* active against *Candida* species (Chaieb *et al*.,2007; Chaieb *et al*.,2007; Gayoso *et al*.,2005). Ethanolic extract of the *Colocasia esculenta* leaves shows good antifungal activity against *C. albicans* (Sarmistha Dutta and Biswajit Aich,2017). The conclusion of the present study observed that thirty-eight components were identified from *S. aromaticum* bud essential oil. The maximum antimicrobial activity of essential oils of *S. aromaticum* active against *S. typhi* and *A. niger*. The identification of main active components of The major components of oil were found to be eugenol (63.52%), eugenyl acetate (11.4%), and β- caryophyllene (2.35%) may be responsible for antimicrobial properties.

Table-3: Antimicrobial activity of *S. aromaticum* buds against some human pathogenic fungi

Sl. No.	Concentrations (ug/ml^{-1})	**Zone of inhibition (mm)**			
		Tested fungi			
		C. albicans	*A. flavus*	*A. niger*	*A. fumigatus*
1	Control	0	0	0	0
2	25	12.7±0.6	12.7±0.6	11.0±0.3	9.0±0.3
3	50	14.0±0.3	13.0±0.7	13.0±0.7	13.0±0.7
4	75	16.0±0.6	15.0±0.3	15.0±0.3	15.0±0.3
5	100	17.0 ±0.7	18.0±0.6	19.0±1.0	18.0±0.6
6	Ketoconazole	18.0 ±0.6	19.0±0.6	22.0±1.0	21.0±1.0

MEAN ± S.E Values are triplicates

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