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Analysis and bioactivities of essential oil of the flower buds of *Syzygium aromaticum* (L.) Merr. et LM Perry

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Abstract
Syzygium aromaticum L. (Myrtaceae), known as clove, is an aromatic dried flower bud grown in India and other tropical countries. It is aromatic, analgesic, antiseptic, spasmodic and stimulant and used to relieve cough, indigestion, toothache, arthritis, rheumatism and to manufacture perfumes, soaps and toothpastes. This research is principally focused on evaluating the effect of the flower bud on some acnegenic pathogens and anti-inflammatory and anti-oxidant activities. The GC-MS analysis of the clove bud essential oil showed the presence of eugenol (55.6%) as a major volatile constituents followed by eugenol acetate (12.5%), chavicol (6.5%), methyl acetate (5.9%), β-caryophyllene (5.2%) and *α*-caryophyllene (4.9%). The essential oil (1 %, v/v) exhibited significant antibacterial activity against *Propionibacterium acne* (17.8 mm) and *Staphylococcus epidermis* (16.8 mm) using Clindamycin as a standard. The essential oil showed an excellent scavenging activity against DPPH radical relative to Vitamin C (standard) at $P<0.05$. Dose dependent anti-inflammatory response was observed with increasing concentration of the clove bud oil. However, the essential oil (1%, v/v) exhibited significant effect comparable results to that of Diclofenac taken as reference standard. These observations justify the use of clove for the treatment of acne and pimples due to its marked antimicrobial, anti-oxidant and anti-inflammatory activities.

Keywords: *Syzygium aromaticum*, flower buds, essential oil, anti-acne effect, anti-oxidant, anti-inflammatory activities

Introduction
Syzygium aromaticum (L.) Merr. et. L.M. Perry, syn. *Caryophyllus aromaticus* L., *Eugenia caryophyllata* Thunb, Jambosa *caryophyllus* (Thunb.) Nied, *Myrtus caryophyllus* Spreng. (family Myrtaceae), known as clove and lavang, is grown in Indonesia, India, Malaysia, Sri Lanka, Madagascar, Tanzania and Brazil. It is an evergreen tree, up to 8–12 m tall, with large leaves and crimson flowers grouped in terminal clusters; flower buds have a pale hue initially, gradually turn green, then to a bright red when ready for harvest (Fig. 1). Cloves are used as an anodyne anthelmintic, antiseptic, aromatic, carminative, stimulant; used to treat arthritis, asthma, bronchitis, bruises, burns, cholera, colds, colic, coughs, diarrhoea, digestive disorders, earaches, gum diseases, headaches, hypertension, impotence, inflammation of the pharynx, intestinal worms, nausea, rheumatism, toothache, ulcers, vomiting and wounds [1, 2]. Clove oil is used as an anodyne, and in aromatherapy to produce bath salt, soaps, and perfumes.

Fig 1: Clove buds and clove plant

The main constituents of the clove essential oil are phenyl propanoids such as eugenol, carvacrol, thymol, cinnamaldehyde, eugenol acetate, β-caryophyllene, α-humulene, β-pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate [1-10]. Clove contained phenolic acids viz., gallic, caffeic, ferulic, ellagic and salicylic acids, tannins, kaempferol, quercetin, its glycosides and sesquiterpenoids [1, 2, 11, 12]. The buds yielded limonin, ferulic aldehyde, eugenol, tamarixetin 3-O-β-D-glucoside, ombuin 3-O-β-D-glucoside and quercetin [4, 13].

The clove extracts and the isolated flavonoids showed strong antioxidant and phytotoxic and activities [14 – 18]. An ethanol extract of clove exhibited remarkable hepatoprotective activity against paracetamol-induced liver injury in female rats [13]. The clove extracts elicited antibacterial, anit-Herpes simplex, anti- hepatitis C viruses, anticarcinogenic, antifungal and antiviral activities [2, 11, 19-25]. The clove essential oil possessed antiinflammatory, antimicrobial, antinociceptive, cytotoxic, insect repellent, insecticidal, fumigant, antioxidant and anaesthetic properties [1, 2, 10, 26 – 35]. Clove oil reverses learning and memory deficits in scopolamine treated mice [36].

The purpose of this work is to study chemical composition of the bud essential oil of *S. aromaticum* available in Delhi and to evaluate antimicrobial, anti-oxidant and anti-inflammatory activities of the clove extracts.

Experimental

Plant material

Dried unripe flower buds of *Syzygium aromaticum* were purchased from a local market, Khari Baoli, Delhi and authenticated by Dr. H. B Singh, Taxonomist, Division of Herbology, AIMIL Pharmaceuticals (I) Ltd, New Delhi. A voucher specimen is preserved in the herbarium of School of Pharmacy, Sharda University, Greater Noida, U.P.

Isolation

The clove buds (100 g) were steam distilled according to the method recommended in British Pharmacopoeia, 2009 (6). The dark green oil obtained was dried over anhydrous sodium sulphate and stored at 4^0C in the dark. The yield was 1.1 % v/w based on fresh weight of sample.

GC analysis

The gas chromatographic analysis of the essential oil was carried out on a GC-2010 (Shimadzu) equipped with a flame ionization detector (FID) and QP-2000 fused silica capillary column (60 m × 0.25 mm × 0.25 µm). The injector and detector (FID) temperatures were maintain at 250 and 270 °C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 ml/min with column pressure of 155.1 kPa. The sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60 to 230 °C at a rate of 30C/min and then held at 230 °C for 9 min, with a total run time of 55.14 min. Percentage of the constituents were calculated by electronic integration of FID peak areas.

GC- MS analysis

The GC-MS analysis was carried out on a GC-MS-QP 2010 Plus (Shimadzu) fitted with a column AB-Innowax (60 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was nitrogen at a flow rate 1.21 ml/min. The oven column temperature was initially kept at 60 °C for 10 min and increased up to 230 °C at a rate of 4 °C/min, then held at 230 °C for 10 min and increased up to 260 °C at a rate of 1 °C/min and then held at 260 °C for 10 min. The split flow was 101 ml/min. The split ratio was 1:80. The injector temperature was 240 °C and detector temperature was 280 °C. Injection volume was 0.3 µl. The ionization energy (voltage) was 70 eV and mass scan range (m/z) was 40-850 amu. The percentage composition of the oil was calculated automatically from the FID peak area without any correction.

Identification of compounds

The individual compounds were identified by comparing their Kovat's indices (KI) of the peaks on Innowax fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further identification was carried out by comparison of fragmentation pattern of the mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K L, WILEY 8 libraries and published literature [37, 38]. Relative amounts of identical components were based on peak areas obtained without FID response factor correction.

Anti-microbial activity

Alcoholic extraction of flower buds

The dried unripe flower buds of clove (100 g) were exhaustively extracted with ethyl alcohol (95%) in Soxhlet apparatus for 15 hrs. The extract was dried under reduced pressure to obtain a dark brown semisolid residue (9.5 g).

Preparation of standard drugs solution

Clindamycin was used as standard solutions for comparison of anti-bacterial studies. Both the standard drugs were taken in DMSO. The concentration of standard drug solutions was 0.10 mg. / ml.

Anti-microbial activity

The antibacterial activities of essential oil and alcoholic extract of clove buds were performed against *Propionibacterium acne* and *Staphylococcus epidermis* in the Department of Microbiology, School of Pharmacy, Greater Noida.

Media

Nutrient agar media was composed of beef extract (1.0 g), yeast extract (2.0 g), peptone (5.0 g), sodium chloride (5.0 g), agar (15.0 g) and distilled water (1.0 L). Sabouraud dextrose agar media was composed of dextrose (40.0 g), mycological peptone (10.0 g), agar (15.0 g) and distilled water (1.0 L).

Preparation of media

Nutrient agar medium (28 g) was accurately weighed, suspended in 1000 ml of distilled water in a conical flask and heated to boiling to dissolve the medium completely. The conical flask containing the nutrient agar medium was plugged with a non-absorbent cotton plug and covered properly with an aluminum foil. It was sterilized by autoclaving at 15-lbs/in2 pressure (121 °C) for 15 min. Sabouraud dextrose agar medium (65 g) was accurately weighed, suspended in 1000 ml of distilled water in a conical flask and heated to boiling to dissolve the medium completely. The conical flask containing the sabouraud dextrose agar medium was plugged with a non-absorbent cotton plug and covered properly with an aluminum foil. It was sterilized by autoclaving at 15-lbs/in2 pressure (121 °C) for 15 min.

Preparation of organisms or inoculums The test organisms

were maintained on freshly prepared medium slants. The slants were incubated at 370C for 24 h. The organisms from the medium slants were washed using 3 ml of saline solution and incubated for 24 h at 37 ± 20C. The developed organisms from the nutrient media were washed using 50 ml of distilled water. A dilution factor was determined which gave 25% light transmission at 530 nm. The amount of suspension to be added to each 100 ml nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

The identification of microbial strains was based on morphological, cultural and biochemical tests. The microbes were procured from Institute of Microbial Technology, Chandigarh, The in-vitro antimicrobial activities of essential oil and dried alcoholic extract of the dried clove flower buds were studied by the cup plate method (14- 17) against various microorganisms mentioned in the Table-3. Clindamycin was used as standard and the activities of the essential oil and alcoholic extract were compared with corresponding concentration of standard drugs. The plates were incubated at 37 $\pm$ 2 ºC for antibacterial activity, after 48 hrs of incubation. The Petri dishes were taken out from the incubator and the antimicrobial activity of essential oil and alcoholic extract of dried unripe flower buds of clove were compared by measuring the diameter of the zone of inhibition. (Table-4).

DPPH Radical scavenging activity

Preparation of aqueous extract

The air dried and coarsely powdered material (100g) was exhaustively extracted with distilled water in a reflux condenser for 4 -5 hrs. The extracts obtained were dried under reduced pressure to obtain a red brown colored residue (8.3g).

Preparation of essential oil concentrations

The essential oil (0.1% v/v, 0.5 % v/v, 1% v/v) and dried alcoholic extract (5.0% w/v) were dissolved in aqueous alcoholic solution (1: 1).

Preparation of DPPH solution: Solution of DPPH (0.1 mm) in methanol was prepared by dissolving 1.9 mg of DPPH in methanol and volume was made up to 100 ml with methanol. The solution was kept in darkness for 30 minutes to complete the reaction.

Determination of antioxidant activity

1.0 ml of DPPH solution was added to 1.0 ml of different extracts and allowed to stand at room temperature for 30 min, and then absorbance was measured at 517 nm in a spectrophotometer. Similarly 1.0 ml of the extract in distilled water was added to 0.6 ml of hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer. The percentage inhibition was measured by following formula (18-20):

$$\% \text{ inhibition} = (Ac\text{-}At) \times 100/Ac$$

Ac is the absorbance of control, at is the absorbance of test sample

Anti-inflammatory activity

The reaction mixture (5 ml) was consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of alcoholic extract of the clove bud to obtain concentrations of 100, 200, 400, 800 and 1000 µg/ml. Similar volume of double-distilled water served as a control. Then the mixtures were incubated at (37±2) °C in a Biochemical oxygen demand (BOD) incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium (100 µg/ml) was used as a reference drug. The percentage inhibition of protein denaturation was calculated by using the following formula:

Percentage inhibition = (Abs control – Abs test sample) * 100/ Abs control

Whereas Abs = Absorbance.

Result and Discussion

The essential oil components of the flower buds of *S. aromaticum* are listed in Table-1. The components are arranged in order to GC elution on QP-2000 column. The oil was characterized by a large amount of eugenol (55.6%) and followed by eugenol acetate (12.5%), chavicol (6.5%), methyl salicylate (5.9%), β-aryophyllene (5.2%), α- caryophyllene (4.9%), *p*-allyl phenol (2.8 %), δ - cadinene (2.5 %), caryophyllene oxide (1.4%) and linalool (1.1 %). α-Pinene and p-cymene occurred in trace amounts.

Antimicrobial activities of the dried alcoholic extract and different concentrations of the essential oil are summarized in Table-2. The maximum antibacterial activity was observed with 1% v/v of the essential oil against *Propionibacterium acne* (17.8 mm) followed by *S. epidermis.* (16.8 mm).

The free radical scavenging activity of the essential oil was determined at all concentrations from 10 to 200 µg /ml and was significant with alcoholic extract (10 %) essential oil (0.5 %) of the flower buds (Table 3). The results demonstrated that the extracts of the *S. aromaticum* flower buds and isolated flavonoids have effective activity as hydrogen donors and as primary antioxidants by reacting with lipid radicals. Anti-inflammatory activities are summarized in Table - 4. Dose dependent anti-inflammatory response was observed with increasing concentration of the clove bud oil. However, 1% v/v of the essential oil exhibited significant effect comparable results to that of Diclofenac taken as reference standard.

Table 1: Essential oil constituents of the dried unripe flower buds of *S. aromaticum*

S. No.	Essential oil components	Kovat's indices	Area %
1	α - Pinene	925	0.9
2	p - Cymene	1026	0.7
3	Linalool	1102	1.1
4	Methyl salicylate	1190	5.9
5	*p* - Allyl phenol	1251	2.8
6	Chavicol	1255	6.5
7	Eugenol	1351	55.6
8	B- Caryophyllene	1444	5.2
9	*α* - Caryophyllene	1454	4.9
10	δ - cadinene	1483	2.5

11	Eugenol acetate	1506	12.5
12	Caryophyllene oxide	1572	1.4

Table 2: Anti-microbial activity of the essential oil, alcoholic extract and aqueous extract of the flowers buds of *S. aromaticum*

S. No.	Test Organism	Zone of Inhibition in mm[a]				
		Conc. of Volatile Oil			Dried Alcoholic Extract 5.0 %w/v	Standard Clindamycin (0.1 mg/ml)
		0.1 %v/v	0.5 %v/v	1.0 %v/v		
1	*Propionibacterium acne*	16.4	17.1	17.8	17.6	18.6
2	*Staphylococcus epidermis*	16.0	16.4	16.8	16.4	17.8

[a] an average of triplicate
Clindamycin - Against acnegenic microbes

Table 3: DPPH Radical Scavenging Activity of the essential oil and aqueous and alcoholic extralcts of flower buds of *S. aromaticum*

S. No.	Concentration (µg/ml)	Ascorbic acid	% of essential oil of *S. aromaticum* flower buds (v/v)			Aqueous extract of clove buds	Alcoholic extract of clove buds
			0.1%	0.5%	1.0%	10% w/w	10% w/w
1	10	46.35±3.31	33.05±5.44	34.78±3.90	34.90±4.48	32.05±1.04	34.06±1.90
2	50	71.66±6.11	34.85±5.36	34.99±7.06	34.98±4.89	32.35±3.55	34.54±2.89
3	100	79.10±5.80	34.90±6.69	35.10±5.19	35.89±4.34	32.90±6.56	34.87±2.07
4	200	87.44±4.74	34.67±5.05	35.67±4.76	35.99±2.05	33.67±3.89	35.67±6.04
5	250	94.44±4.13	35.17±6.31	35.90±7.29	30.01±4.32	33.17±7.85	35.17±5.09
6	EC 50	14.9 µg	158.50 µg	164.90 µg	170.80 µg	25 µg	15 µg

Values are expressed as mean ± S.D., n = 4

Table 4: *In vitro* Anti-inflammatory activity of essential oil *S. aromaticum* flower buds

S. No.	Conc. (µg/ml)	% of inhibition (10%w/w aq. ext)	% of inhibition (10% w/w alc. ext)	% of inhibition (0.1% v/v of v. oil)	% of inhibition (0.5% v/v of v. oil)	% of inhibition (1.0% v/v of v. oil)
1	100	1.51±0.87	1.89±0.45	3.11±1.89	4.02±0.93	5.43±0.86
2	200	1.78±0.74	1.99±0.79	3.66±0.90	4.67±0.96	5.68±0.58
3	300	1.90±0.67	2.01±0.59	3.78±1.43	5.40±1.42	6.47±1.91
4	400	2.09±0.72	2.78±0.90	3.92±1.45	6.62±1.67	6.72±1.75
5	500	2.87±0.89	2.96±0.69	4.40±0.70	6.80±1.78	6.90±1.65
6	Diclofenac	7.04±0.98	7.04±0.98	7.04±0.98	7.04±0.98	7.04±0.98

Conclusion

The essential oil of the flower buds of clove (*Syzygium aromaticum*) was constituted mainly of eugenol (55.6%), eugenol acetate (12.5%), chavicol (6.5%), methyl salicylate (5.9%), β-caryophyllene (5.2%) and α- caryophyllene (4.9%). The alcoholic extract of clove buds showed significant antibacterial activity against *Propionibacterium acne* and *Staphylococcus epidermis.* The clove essential oil exhibited strong free radical scavenging activity in comparison to standard ascorbic acid. The clove bud oil showed dose dependent anti-inflammatory response against reference standard Diclofenac sodium. These observations justify the use of clove for the treatment of acne and pimples due to its marked antimicrobial, anti-oxidant and anti-inflammatory activities.

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