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**Abstract**

**Objective:** Volatile oil and extracts of the dried unripe buds of *Syzygium aromaticum* L. syn. *Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison (family Myrtaceae) have been used as a natural therapeutic agent in traditional medicine to treat inflammation, pain, and antimicrobial properties from ancient times.

**Materials and Methods:** Research is principally focused on evaluating the effect of the unripe flower bud on some acnegenic pathogens as well as its anti-inflammatory and antioxidant effects. Antimicrobial study was conducted on two microorganisms, i.e., *Propionibacterium acne* and *Staphylococcus epidermis*. *In vitro* studies were conducted for anti-inflammatory and antioxidant activities.

**Results:** Volatile oil studies of the clove bud showed the presence of seven volatile constituents. The oil was characterized by a large amount of eugenol (61.17%) and followed by eugenol acetate (20.37%). The maximum antibacterial activity was observed with 1% v/v of volatile oil obtained from clove oil buds on *P. acne* (17.8 mm) followed by *S. epidermis* (16.8 mm). An excellent scavenging activity against 2,2-diphenyl-1-picrylhydrazyl radical relative to Vitamin C (standard) at *P* < 0.05 was also observed in cloves buds extracts (alcoholic 10% w/w) and volatile oil (1% v/v). A remarkable anti-inflammatory activity observed by alcoholic extract (10% w/w) of unripe flower buds of clove against diclofenac sodium (standard). However, the entire findings of this research scientifically justify the use of clove in acne and pimples due to its strong antioxidant and anti-inflammatory activities.

**Key words:** Anti-inflammatory, antimicrobial, antioxidant, dried unripe flower buds, eugenol, *in vitro* activities, *Syzygium aromaticum*, volatile oil

**INTRODUCTION**

Essential oils are potential sources of novel antimicrobial compounds, especially against bacterial pathogens.[¹] Edible, medicinal and herbal plants, and spices such as clove, basil, turmeric, oregano, rosemary, thyme, sage, basil, ginger, garlic, nutmeg, mace, savory, and fennel have been successfully used either alone or in combination with other preservation methods.[²]

Clove buds (1.5–2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals which form a small ball in numerous groups of terminal clusters. The flower buds are at first of a pale color and gradually become green, after which they develop into a bright red, when they are ready for collecting. Cloves are harvested when 1.5–2 cm long, and consist of a long calyx, terminating in four spreading sepals, and four unopened petals which form a small ball in

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the center. Approximately 3000 essential oils are known till now. Essential oils are one of the plant extracts that have been used for the treatment of various medical and dental problems since ancient times. These are secondary metabolites produced by various medicinal plants and possess antibacterial, anti-inflammatory, and antioxidant properties. Earlier reported laboratory findings distilled clove bud oil contained beside 70.1% of eugenol, β-caryophyllene (4.8%), α-humulene (0.55%), α-terpenyl acetate (0.1%), methyl eugenol (0.2%), humulene epoxide (0.2%), and chavicol (0.3%). On the other hand, a number of compounds sesquiterpenic hydrocarbons, alcohols and oxides, methyl ketones, aliphatic alcohols, and esters are present in trace amounts.

### EXPERIMENTAL

**Plant Material**

Dried unripe flower buds of *S. aromaticum* were procured from local market Cherthala, Distt. Alappuzha, Kerala, India. The material was identified by ex. Chief Scientist, Department of Botany, Dr. H. B Singh and presently working in the Department of Herology, AIMIL Pharmaceuticals (I) Ltd. A voucher specimen is preserved in the herbarium of School of Pharmacy, Sharda University, Greater Noida, U. P.

**Isolation**

The clove buds (500 g) were steam distilled according to the method recommended in British Pharmacopoeia, 2009. The dark green oil so obtained was dried over anhydrous sodium sulfate and stored at 4°C in the dark. The yield was 1% v/w based on fresh weight of sample.

**Gas Chromatography (GC) Analysis**

Analytical GC was carried out on a Varian 3300 gas chromatograph fitted with a silicon DB-I capillary column (30 m × 0.25 m), film thickness 0.25 μm, carrier gas nitrogen, flow rate 1.5 ml/min, split mode, and temperature programmed 80–250°C at 4°C/min. Injector temperature and detector temperature were 250°C and 300°C, respectively. Detector used was FID. Injection volume for all samples was 0.1 μl.

**GC–Mass Spectrometry (MS) Analysis**

Analytical GC–MS was carried out on a QP 2000 instrument at 70 eV and 250°C GC column Ulbon HR-1 equivalent to OV-1 fused silica capillary 0.25 mm × 50 m with film thickness 0.25 μ. The initial temperature was 100°C for 6 min and then heated at a rate of 10°C per min to 250°C. Carrier gas He, flow rate 2 ml/min, detector used was FID.

**Identification**

The volatile components were identified by comparing their retention time of GC chromatograph with those of literature. Further, identification was done by GC–MS.

The fragmentation patterns of mass spectra were compared with those of the spectrometer database using the NBS 54 AL and Wiley L-built libraries and also with those reported in the literature. Many constituents were identified by comparing their retention indices with those of authentic standards available in the author’s laboratory [Table 1].

**Antimicrobial Activity**

**Dried alcoholic extraction**

The dried unripe flower buds of clove (500 g) were exhaustively extracted with ethyl alcohol (95%) in Soxhlet apparatus for 50 h. The extract was dried under reduced pressure to obtain a dried residue (35.0 g).

**Preparation of standard drugs solution**

Clindamycin used as standard solutions for comparison of antibacterial studies. Both the standard drugs were taken in dimethyl sulfoxide. The concentration of standard drug solutions was 0.10 mg/ml.

**Antimicrobial activity**

The antibacterial activities of volatile oil and alcoholic extract of clove buds were performed in the Department of Microbiology, School of Pharmacy, Greater Noida.

The identification of microbial strains was based on morphological, cultural, and biochemical tests. The microbes were procured from Microbial Type Culture Collection and Gene Bank, CSIR-Institute.
of Microbial Technology, Sector 39-A, Chandigarh - 160036, India. The in vitro antimicrobial activities of volatile oil and dried alcoholic extract of flower buds were studied by the cup plate method[14-17] against various microorganisms mentioned in Table 1. Clindamycin was used as standard and the activities of volatile oil and dried alcoholic extract were corresponded with corresponding concentration of standard drugs. The plates were incubated at 37 ± 2°C for antibacterial activity, after 48 h of incubation. The Petri dishes were taken out from the incubator and the antimicrobial activity of volatile oil and alcoholic extract of dried unripe flower buds of clove was compared by measuring the diameter of the zone of inhibition [Table 2].

**In vitro Evaluation of S. aromaticum Linn.**

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [Figure 1]

**Preparation of aqueous extract**
The air-dried and coarsely powdered material (500 g) was exhaustively extracted with distilled water in a reflux condenser for 4–5 h. The extracts obtained were dried under reduced pressure to obtain a brownish colored residue (28 g).

**Preparation of volatile oils concentrations**
The volatile oil (0.1% v/v, 0.5% v/v, and 1% v/v) and dried alcoholic extract (5.0% w/v) were dissolved in hydroalcoholic mixture (50:50) activity.

**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 min to complete the reaction.

**Determination of Antioxidant Activity**
1 ml of DPPH solution was added to 1 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 ml extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer [Table 3]. The percentage inhibition was measured by following formula.[18-20]

\%

\% inhibition = (Ac-At) ×100/Ac

Ac is the absorbance of control, At is the absorbance of test sample.

**Anti-inflammatory Activity**
The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen’s egg), 2.8 ml of phosphate-buffered saline, pH 6.4, and 2 ml of varying concentrations of alcoholic extract of clove oil so that final concentrations becomes 100, 200, 400, 800, and 1000 µg/ml. Similar volume of double-distilled water served as control. Then, the mixtures were incubated at 37 ± 2°C in biochemical oxygen demand 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 reference drug [Table 4]. The percentage inhibition of protein denaturation was calculated using the following formula [Figure 2].[21-23]

\%

\% inhibition = (Abs control - Abs test sample) *100/ Abs control

Whereas Abs - Absorbance.

**RESULT AND DISCUSSION**
The volatile components of dried unripe flower buds of S. aromaticum Linn. are listed in Table 4. Components are

<table>
<thead>
<tr>
<th>Name of Volatile component</th>
<th>RI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl salicylate</td>
<td>1190 (0.97)</td>
</tr>
<tr>
<td>Chavicol</td>
<td>1243 (1.00)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>1351 (61.17)</td>
</tr>
<tr>
<td>t-caryophyllene</td>
<td>1444 (11.64)</td>
</tr>
<tr>
<td>α-caryophyllene</td>
<td>1454 (2.91)</td>
</tr>
<tr>
<td>Eugenol acetate</td>
<td>1506 (20.37)</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>1572 (1.94)</td>
</tr>
</tbody>
</table>

RI: Retention index, total identified volatile components=07, total % of identified volatile components=100, total monoterpenes=01, total % of monoterpenes=1.0, total sesquiterpenes=03, total % of sesquiterpenes=16.49%, non-terpenic/aromatic components=03, total % of non-terpenic/aromatic components=82.51

**Table 2: Antimicrobial activity of volatile oil, alcoholic extract, and aqueous extracts of dried unripe bud flowers of Syzygium aromaticum Linn. (clove)**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Conc. of volatile oil</th>
<th>Zone of Inhibition in mm*</th>
<th>Dried alcoholic extract 5.0% w/v</th>
<th>Standard clindamycin (0.1 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1%v/v</td>
<td>0.5%v/v</td>
<td>1.0%v/v</td>
<td></td>
</tr>
<tr>
<td>P. acne</td>
<td>16.4</td>
<td>17.1</td>
<td>17.8</td>
<td>15.8</td>
</tr>
<tr>
<td>S. epidermis</td>
<td>16.0</td>
<td>16.4</td>
<td>16.8</td>
<td>15.4</td>
</tr>
</tbody>
</table>

*An average of triplicate, clindamycin - against acengetic microbes, P. acne: Propionibacterium acne, S. epidermis: Staphylococcus epidermis
arranged to GC elution on QP-2000 column. The seven volatile constituents were identified from dried unripe flower bud of clove having one monoterpene (1.0%), chavicol. There were three sesquiterpenes (16.49%): The major sesquiterpene was \(\tau\)-caryophyllene (11.64%), followed by caryophyllene (2.91%) and caryophyllene oxide (1.94%); there were three non-terpenic/aromatic compounds (82.51%): The major non-terpenic/aromatic component was eugenol (61.17), followed by eugenol acetate (20.37%) and methyl acetate (0.97%). Antimicrobial activities of dried alcoholic extract and different concentration of volatile oil of clove buds were summarized in Table 2. The maximum antibacterial activity was observed with 1% v/v of volatile oil obtained from clove buds on \textit{Propionibacterium acne} (17.8 mm) followed by \textit{Staphylococcus epidermis} (16.8 mm). DPPH radical scavenging activity was summarized in Table 1. It was observed that the scavenging activity of volatile oil of clove buds at 0.1%, 0.5%, and 1.0% at all concentrations from 10 to 250 µg/ml was dose-dependent. The results demonstrated that the alcoholic extract at concentration 10% w/w showed maximum scavenging activity, and among the oils, 1% v/v showed maximum inhibition of reactive oxygen species with EC_{50} value of 15 µg/ml. Anti-inflammatory activities were summarized in Table 3. Dose-dependent anti-inflammatory response was obtained with increasing concentration of clove bud oil. However, 1% v/v of volatile oil and 10% w/w alcoholic extract showed significantly higher percentage of inhibition comparable results to that of diclofenac sodium taken as reference standard. Denaturation of tissue proteins is one of the well-documented causes of inflammation.

**CONCLUSION**

From the results achieved in the present preliminary study, it can be concluded that clove bud can be used as a potential acne treatment due to its strong antimicrobial, antioxidative, and anti-inflammatory properties. Clove oil and eugenol might be a potential antiaging substance by preventing aging of skin through oxidative processes and inducing collagen synthesis. Moreover, they also possess antityrosinase activity, suggesting a skin whitening effect.

**Table 3:** DPPH Radical Scavenging Activity: volatile oil, alcoholic Extract and Aqueous extracts of dried unripe bud flowers of \textit{Syzygium aromaticum} Linn. (clove)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (µg/ml)</th>
<th>Vit C</th>
<th>% of volatile oil of dried unripe flower buds of clove oil (v/v)</th>
<th>Aq. Extract of clove buds</th>
<th>Alc. Extract of clove buds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1%</td>
<td>0.5%</td>
<td>1.0%</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>46.35±3.31</td>
<td>33.05±5.44</td>
<td>34.78±3.90</td>
<td>34.90±4.48</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>71.66±6.11</td>
<td>34.85±5.36</td>
<td>34.99±7.06</td>
<td>34.98±4.89</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>79.10±5.80</td>
<td>34.90±6.69</td>
<td>35.10±5.19</td>
<td>35.89±4.34</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>87.44±4.74</td>
<td>34.67±5.05</td>
<td>35.67±4.76</td>
<td>35.99±2.05</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>94.44±4.13</td>
<td>35.17±6.31</td>
<td>35.90±7.29</td>
<td>36.01±4.32</td>
</tr>
<tr>
<td>6</td>
<td>EC 50</td>
<td>14.9 µg</td>
<td>12.50 µg</td>
<td>164.90 µg</td>
<td>170.80 µg</td>
</tr>
</tbody>
</table>

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

**Table 4:** \textit{In vitro} anti-inflammatory activity of volatile oil, alcoholic, and aqueous extracts of dried unripe bud flowers of \textit{S. aromaticum} Linn. (clove)

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% of inhibition (10% w/w aq. ext)</th>
<th>% of inhibition (10% w/w alc. ext)</th>
<th>% of inhibition (0.1% v/v of v. oil)</th>
<th>% of inhibition (0.5% v/v of v. oil)</th>
<th>% of inhibition (1.0% v/v of v. oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1.51±0.87</td>
<td>1.89±0.45</td>
<td>3.11±1.89</td>
<td>4.02±0.93</td>
<td>5.43±0.86</td>
</tr>
<tr>
<td>200</td>
<td>1.78±0.74</td>
<td>1.99±0.79</td>
<td>3.66±0.90</td>
<td>4.67±0.96</td>
<td>5.68±0.58</td>
</tr>
<tr>
<td>300</td>
<td>1.90±0.67</td>
<td>2.01±0.59</td>
<td>3.78±1.43</td>
<td>5.40±1.42</td>
<td>6.47±1.91</td>
</tr>
<tr>
<td>400</td>
<td>2.09±0.72</td>
<td>2.78±0.90</td>
<td>3.92±1.45</td>
<td>6.62±1.67</td>
<td>6.72±1.75</td>
</tr>
<tr>
<td>500</td>
<td>2.87±0.89</td>
<td>2.96±0.69</td>
<td>4.40±0.70</td>
<td>6.80±1.78</td>
<td>6.90±1.65</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>7.04±0.98</td>
<td>7.04±0.98</td>
<td>7.04±0.98</td>
<td>7.04±0.98</td>
<td>7.04±0.98</td>
</tr>
</tbody>
</table>

\textit{S. aromaticum:} Syzygium aromaticum
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