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# Antimicrobial activities of *Syzigium aromaticum* L. (Clove)

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## Abstract

*Bioactive compounds from Syzigium aromaticum were extracted by Soxhlet using methanol and extracts were examined for its phytocomponents along with Clove oil. These bioactive plant compounds were screened for possible antimicrobial activities against six strains of MDR S. aureus. Antioxidant activities of extracts and oil was examined by free radical scavenging method.*

**Keywords**: *Syzigium aromaticum,* Clove, MRD, *S. aureus*, DPPH.

## Introduction

Natural plant products have been used for therapeutic purposes since the time immemorial and their use is of a greater demand nowadays. Majority of the users rely on herbal medicines for health care because the other treatment options available are more expensive and they are often thought to be more associated with serious side effects. Most of these plants have been ingested indiscriminately by many local populations for the management of various disease states without neither knowing how relief is brought about nor having adequate information on the safety/toxicity risks associated with their use. For proper enlightenment and guidance of the populace, especially users of these natural products, there is need for scientific documentation on the safety/toxicity profile of these acclaimed medicinal plants. There is therefore a need for continuous search for new, effective and affordable antimicrobial agents. In recent times, there has been renewed interest on plants as sources of antimicrobial agents due to their use historically and the fact that a good portion of the world's population, particularly in developing countries, rely on plants for the treatment of infectious and non-infectious diseases. Indeed, clove oil is commonly use as an anaesthetic in the relieve of toothache in dentistry. It is also used as a carminative, rubefacient and serves as a preservative in herbal recipes, signifying possible antimicrobial properties[1]. Historically, medicinal plants have been a source of novel drug compounds. Green pharmacy may became the base for the development of medicines by providing a pharmacophore which could be used for the development of new drugs with novel mechanism of action. Many scientists across the globe have reported antimicrobial properties of several medicinal plants but still a very meager portion of this tremendous potential drug-repertoure has been scientifically screened[2]. Syzygium species have been reported to possess antibacterial and anti-inflammatory activity. It was reported that the buds of *Syzygium aromaticum* were used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with carminative and stimulant activity. In addition, the antimicrobial activity of clove essentials oil have been studied against a large number of multi-resistant *Staphylococcus epidermidis* and oral pathogens[3].

## Material and Methods

Clove is purchased from local market of Lucknow Uttar Pradesh in June 2013. Plant material is washed thoroughly with tap water followed by sterile water and air dried and then in hot air oven. Dried plant material was then blended into fine powder and stored in dry place at room temperature for further use.

Solvent extraction method was used to obtain different extracts. Organic solvent like ethanol, methanol, ethyl acetate, acetone and chloroform are used for organic extraction. The solvent can be used for organic solvent extraction was 80% methanol. 5.0Gm of powder of dried clove was mixed in 50ml of organic solvent. The mixture was kept in dark at room temperature for 4-5 days. The slurry obtained after 4-5 days was filtered with Whatmann filter paper no. 1 and filtrate was concentrated by evaporation in hot air oven. The dried extract was dissolved in dimethyl sulphoxide.

Soxhlet apparatus was used for the extraction procedure 20.0Gm of sample was filled in main chamber *i.e*. extractor of Soxhlet apparatus. The extractor was placed on boiler containing 200ml of organic solvent and a condenser is placed over it . the condenser ensures the methanol vapour cool and drips back down to extractor containing the sample. The temperature of the apparatus was set at 70-80°C and allowed to run for 2 day. After 2 days extract was collected by evaporation in hot air oven and dried extract was dissolved in dimethyl sulphoxide. The antibacterial activity of different clove extract was determined by agar well diffusion method (Kirby Bauer method) against *Staphylococcus aureus*. Nutrient agar media

was prepared and autoclaved. Each bacterial species was inoculated by pore plate method (µl of media) to get a confluent growth. The media was poured in sterile and autoclaved Petri plates and allowed to solidify for 1hr. When the media was solidified wells are made in it and marked as. The plates are then incubated at 37°C For overnight. After 24 hrs the antibacterial activity was expressed in terms of the diameter of zone of inhibition (in mm) of each bacterial species by different samples.

Plant samples may contain significant phytochemicals that can be analyzed by several biochemical and analytical tests from solvent and extracts of each plant material and also the powdered form of plant as well as powdered extract of samples.

**Test for Reducing sugars:** 10 ml of deionized water was added to 1ml or 1.0g of sample in a test tube and followed by addition of few drops of Fehling solution and heat at 40°C in water bath. Brick red precipitate indicates positive result for reducing sugar.

**Test of Tannins:** 2.0 gm of aqueous extract was taken in test tube, after addition of few (2-3) drops of 5% ferric chloride green colour is observed, and is confirmation of presence of tannins.

**Test of Phlobatannins:** 10ml of aqueous extract was boiled with few drops of 1% conc. hydrochloric acid for 10 min., deposition of red precipitate at the base of the test tube indicates presence of phlobatannins.

**Test of Seponins:** 1.0 gm of aqueous extract was added into a test tube followed by addition of 5.0 ml of deionized water, tubes was shaken vigorously, allowed it for few minutes. If froth remains for 15min, it means saponins are present.

**Test of Terepenoids:** To the 5.0 ml of aqueous extract, 2.0 ml of chloroform was added, followed by addition of 3.0 ml concentration $H_2SO_4$. The reddish brown interface indicates the presence of terpnoids.

**Test of Steroids:** The development of greenish colour when 2.0 ml of extract dissolved in 2.0 ml of chloroform and treated with $H_2SO_4$ and acetic acid.

**Test of Glucosides:** 2.0 ml of extract was dissolved in 2.0 ml of $CHCl_3$, 2.0 ml $H_2SO_4$ was added carefully and shaken gently. A reddish brown colour interface is the indication for the presence of glucoside.

**Minimal inhibitory concentration:** The extracts were subjected to determination of minimal inhibitory concentration (MIC) by micro-dilution agar double layer method. 10.0ml of autoclaved nutrient agar media for bacterial species was poured in Petri plate placed on slant surface. When slants were solidified another 10.0ml of media containing plant extract was poured over it. 20µl of test organism was spread on the surface of second layer ant the plates were then incubated at 37°C for overnight. After incubation of hrs, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial stain.

Elicitors are those compounds which exhibit the effectiveness of extract phytochemicals. As an elicitors we used primary and secondary metabolites, some metal ions etc. glucose, galactose, sucrose, lactose, maltose, dextrose were used as primary metabolites where as magnesium ($Mg^{++}$), zinc ($Zn^{++}$), calcium ($Ca^{++}$), copper ($Cu^{++}$) ferrous ($Fe^{++}$) used in form of elicitor of metal ions.

**Examination of antioxidant activity:** Antioxidants are those metal ions which having the properties of detoxifications of free radicals formed by the oxygen molecules and generally exist in free radical forms.

Fresh DPPH (2,2-diphenyl-1-picrylhydrazyl) stock solution (5ml) at a concentration of 2.5M/ml was prepared on each day of analysis. The stock solution of L-ascorbic acid (1Mm/ml) was prepared in methanol and stored at -20°C. The samples of A. marvels were prepared in methanol.

Free radicals scavenging capacity of methanolic and ethyl acetate extracts were evaluated with DPPH stable radicals. Briefly 0.1Mm solution in methanol was prepared in 2ml of this solution was added to 0.3ml of different extract concentration (1-100µg/ml) and allowed to react at room temperature. After 30 minutes the absorbance value were measured at 517nm against blank, which did not contain extract. The L-ascorbic acid was used as positive control. The radicals scavenging (% inhibition) was expressed as percentage of DPPH radicals elimination, calculated according to the following equation

% inhibition = A control – A sample/ A control

Where, A control = Absorbance of negative control and A sample = Absorbance of reaction mixture.

**Antiulceric properties:** The antiulceric properties of plant extract were analyzed in terms quantification Gallic acid (a known potent antiulceric compound) were perform through high performance liquid chromatography[4].

HPLC was performed by isocratic system (Shimadzu, Japan) having single pump (LC20AD) fitted with U.V. detector (SPD20A) with manual injector (Rheodyne manual injector; 7725i, with loop size 20µl). Data was analyzed by LC solution software. Column used was phenomenox Luna, RP,$C_{18}$, 240×4.6MM, 5µ.

All the solvent and standard compound of Gallic acid was purchased from Merck (HPLC grade solvent) and contain water-acetonitrile-acetic acid (88:10:2) was mix thoroughly by sonicating at room temperature for 10min.

Standard of Gallic acid was prepared by dissolving 10mg of Gallic acid in 10ml of water and was filtered via syringe filter of pore size 0.22µ.standard was diluted up to 10ppm in HPLC grade water before injection.

Soxhlet extracts of leaves and pulp was analyzed for antiulceric properties. The sample were prepared at concentration of 1.00mg/ml and were filter via syringe filter of pore size 0.22µm before injection. Flow rate of solvent was kept at 1.00ml/min with run time of 10.0min detection was monitored at 280nm.

## Results and Discussion

The extracts from *Syzygium aromaticum* obtained by different methods varied in yield and texture as given below.

**Table-1**
**Yield in (g/100g of sample) of each extract of *Syzygium aromaticum***

| Extraction Method | Yield (per 100g of sample) | Color of the extract |
|---|---|---|
| Methanol suspension | 21.4 | Dark brown |
| Methanol Soxhlet | 45 | Dark brown |
| Pure oil | 100.00% | Transparent, light cream |

Different phytochemicals were analysed for their presence or absence in plant material and we found a remarkable pattern in terms of proportion of various constituents among the plant.

**Table-2**
**Phytochemical quantification (in %) of *Syzygium aromaticum***

| Phytochemical Constituent | Result (Intensity) | Percentage ratio |
|---|---|---|
| Reducing sugar | + | 9.09 |
| Tannins | +++++ | 45.45 |
| Phylobatannins | - | |
| Saponins | ++ | 18.18 |
| Terepenoids | + | 9.09 |
| Steroids | + | 9.09 |
| Glucoside test | + | 9.09 |

Screening clove extracts for antimicrobial activity against all six strains were analyzed and following results were obtained.

**Table-3**
**Screening (in mm) of *Syzygium aromaticum***

| Strains | Methanolic Soxhlet | Methanolic suspension | Pure oil |
|---|---|---|---|
| 1. | 18 | 17 | 20 |
| 2. | 16 | 19 | 20 |
| 3. | 17 | 16 | 21 |
| 4. | 25 | 20 | 20 |
| 5. | 18 | 20 | 19 |
| 6. | 18 | 20 | 17 |

**Discussions:** Our data suggest that Soxhlet extraction is far better compared to other methods even for same solvent (table 1) and because of that for extraction of phytochemical this method is being utilized in several research projects. Again our data suggest that clove contains large amount of tannins followed by saponions, terepenoids, sugars, steroids and glucosides (table-2).

Most of the plant contain phytochemical that are able to inhibit several pathogens. Our plant also contain such compound that are able to inhibit drug resistant pathogens (table-3) among all (methanolic Soxhlet, methanolic suspension, and pure oil) oil was better in respect of antimicrobial property of clove (up to 21mm). All stains of. *S. aureus* selected in study were provided by MRD LifeSciences were too much drug resistant against several drugs[5]. Clove extract were found very much impressive in respect of antibacterial properties against those drug resistant pathogens. To understand better we analyzed antibacterial properties of clove extract along different elicitors including sugars and metal ions[6, 7]. All the elicitors selected in the study were did not responded well because there was no increase in zone of inhibition when added with plant extract (table 4).

All three extracts of clove extract of clove were analyzed for possible antioxidant properties and pure oil was found best among all with 33.47 ± 0.16% inhibition of DPPH free radicals[8, 9] (table-5)

Antiulceric properties were analyzed in terms of amount of gallic acid present in extract. Our data suggest that all the three plant sample contain almost negligible amount of gallic acid.

**Table-4**
**Effect of sugars (in mm) of *Syzygium aromaticum***

| Extracts | Methanolic Suspension | | | | | | Methanolic Soxhlet | | | | | | Pure Oil | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strains** | **1** | **2** | **3** | **4** | **5** | **6** | **1** | **2** | **3** | **4** | **5** | **6** | **1** | **2** | **3** | **4** | **5** | **6** |
| **Sucrose** | 14 | 11 | 13 | 11 | 11 | 16 | 11 | 13 | 14 | 11 | 14 | 13 | 15 | 15 | 12 | 15 | 20 | 12 |
| **Maltose** | 15 | 13 | 12 | 15 | 15 | 15 | 12 | 12 | 15 | 13 | 15 | 12 | 17 | 17 | 13 | 15 | 14 | 11 |
| **Lactose** | 12 | 10 | 11 | 14 | 14 | 13 | 11 | 12 | 15 | 12 | 13 | 13 | 15 | 14 | 12 | 15 | 16 | 13 |
| **Galactose** | 12 | 12 | 14 | 14 | 14 | 14 | 11 | 13 | 15 | 11 | 12 | 14 | 16 | 14 | 14 | 16 | 18 | 14 |
| **Dextrose** | 14 | 13 | 14 | 13 | 13 | 14 | 12 | 10 | 13 | 10 | 11 | 14 | 17 | 14 | 12 | 17 | 13 | 16 |
| **Glucose** | 13 | 12 | 12 | 15 | 15 | 13 | 12 | 11 | 13 | 13 | 13 | 11 | 18 | 14 | 14 | 16 | 16 | 17 |

**Table-5**
**Antioxidant quantification (in %) of *Syzygium aromaticum***

| Sr. No. | Extract | Antioxidant property |
|---|---|---|
| 1. | Methanolic suspension | 33.47± 0.16 |
| 2 | Methanolic Soxhlet | 18.36 ± 0.38 |
| 3 | Pure oil | 25.33± 0.25 |

## Conclusion

Our experimental data suggests that Clove has high activity of antimicrobial properties against pathogens isolated from hospital soils. Different elicitors responded well to increase this property and our data suggest that Clove has very good antioxidant properties and it could also used in the treatment of ulcers, coronary diseases, blood pressure as a herbal medicine[10].

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