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# Antimicrobial activity and phytochemical screening of *Callistemon citrinus* L. leaf extract

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**Abstract**---Phytochemical investigation was carried out on the crude chloroform, ethanol and aqueous extracts of the leaves of *Callistemon citrinus* L. belonging family Myrtaceae. The extracts were subjected to screening for determination of their potent antimicrobial activity against various microorganisms like *Bacillus subtilis*, *Bacillus pumilis* and *Escherichia coli* etc. Streptomycin (25 µg/ml) was used as a standard drug. Results were subjected to minimum inhibitory concentration assay by two-fold dilution method. The chloroform extract exhibited moderate to significant antimicrobial activity against all the tested microbial strains. The alcoholic extract exhibited moderate antimicrobial activity. The aqueous extract was devoid of any antimicrobial activity. The results showed that the chloroform extract was more potent than the ethanolic extract.

**Keywords**---*Callistemon citrinus* L., Anti-Microbial activity, Phytochemical Screening, Cup Plate Agar Diffusion Method.

## Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and near East, but it is doubtless an art as old as mankind <sup>1</sup>. Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites <sup>2, 3</sup>. Many people in Indian rural areas depend on the traditional

medicine for the treatment of their ailments and since prehistoric times, various parts of plants have been used in the treatment and prevention of various diseases <sup>4</sup>. Different plants have been used as a source of inspiration in the development of novel drugs either in a pure compound form or their extract form and it provides unlimited opportunities to develop a variety of new drugs because they are relatively safer than the synthetic alternatives.

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction <sup>5</sup>. However, over the past few decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages <sup>6</sup>. Therefore, increase in failure due to chemotherapeutics and antibiotic resistance leads to screening of several medicinal plants for their antimicrobial effect <sup>7, 8</sup>. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethano-medicinal uses <sup>9</sup>.



Figure 1: Plant images (*Callistemon citrinus* L.)

*Callistemon citrinus* L., which is commonly known as 'Crimson Bottle Brush', is an evergreen tree or shrub, belonging to the family Myrtaceae. It grows upto 6-15 m in height and 1.3-1.5 m in girth with sharp pointed mid-green leaves <sup>10</sup>. (Figure 1)

The different parts of this herb have been used in common remedies for treatment of diarrhoea, dysentery and rheumatism. It is used as a water accent, anticough, antibronchitis and insecticide in folk medicine <sup>11</sup>. The plant is rich in polyphenols <sup>12</sup>. In view of the medicinal importance of *Callistemon citrinus* in the indigenous system, it was decided to work on the phytochemistry and antimicrobial investigations on *Callistemon citrinus* L.

## Materials and Methods

### Plant material

The leaves of *Callistemon citrinus* L. were collected from local region of Bargarh district of Odisha, India and were authenticated by Dr. K. B. Satapathy, emeritus Professor, Centurian University of Technology and Management, Bhubaneswar, Odisha, India. A voucher specimen was deposited at the herbarium in the institute.

### Preparation of Plant Extracts

Leaves were shade dried, coarse powdered and passed through # 20. The coarse powdered materials were extracted with chloroform, ethanol and water for 72 hours using soxhlet apparatus<sup>13</sup>. The filtrates were collected and concentrated at 40°C under reduced pressure using a Rotary evaporator. All the extracts were transferred into clean and dried airtight vials and stored at 4°C until further use for various evaluations. These extracts were used to conduct the phytochemical and pharmacological evaluation of *Callistemon citrinus* leaves. Details of Soxhlet Extraction were mentioned in Table 1.

### Preliminary Phytochemical Analysis

The leaf extracts were screened for the phytochemical components using standard method<sup>14,15</sup>. Phytochemical screening is done for analyzing secondary metabolites which are responsible for curing ailment and to detect the presence or absence of certain bioactive compounds. Details of Preliminary Phytochemical investigation was mentioned in Table 2.

Table 1: Details of the Soxhlet Extraction

| Plant Material | Solvent used | Volume of the Solvent | Weight of the Extract | % Yield |
|----------------|--------------|-----------------------|-----------------------|---------|
| Leaf (0.4 kg)  | Chloroform   | 750ml                 | 9gm                   | 2.25    |
|                | Ethanol      | 750ml                 | 7gm                   | 1.75    |
|                | Water        | 750ml                 | 2gm                   | 0.5     |

### Table 2: Preliminary phytochemical investigation:

The extracts prepared were tested for the type of chemical constituents present by known qualitative tests. The results are given as follows.

| S.N. | Name of the test                            | Chloroform extract | Ethanol extract | Aqueous extract |
|------|---------------------------------------------|--------------------|-----------------|-----------------|
| 1    | Libermann burchard (for terpenes & steroid) | +                  | -               | -               |
| 2    | Salkowski (for terpenes & steroid)          | +                  | -               | -               |
| 3    | Mayer's (for alkaloids)                     | +                  | -               | -               |
| 4    | Molisch (for carbohydrates)                 | -                  | -               | +               |
| 5    | Fehling's (for carbohydrates)               | -                  | -               | +               |

|   |                                             |   |   |   |
|---|---------------------------------------------|---|---|---|
| 6 | Baljet's (for glycosides)                   | - | + | + |
| 7 | Legal's (for glycosides)                    | - | + | + |
| 8 | Test for phenolics (FeCl <sub>3</sub> test) | - | + | - |
| 9 | Shinoda (for flavonoids)                    | + | + | - |

Note: (+): Presence, (-): Absence

#### **Preparation of test and standard:**

Initially 10 mg samples were weighed accurately and dissolved in 10 ml of Dimethyl Sulphoxide (DMSO) to get a concentration of 1000 µg/ml. The stock solution of reference standards (Streptomycin) was prepared at a concentration of 25 µg/ml by using sterile water <sup>16</sup>.

#### **Culture media:**

The media employed for the study was nutrient agar.

#### **Test Microorganisms:**

The test organisms were *Bacillus subtilis*, *Bacillus pumilis* and *E. coli*. All these organism's cultures were obtained from National Collection of Microorganisms, Pune, India.

#### **Sterilization of materials:**

The Petri dishes and pipettes packed into metal canisters were appropriately sterilized in the hot air oven at 170°C for 1 hour at each occasion. Solution of the extract and culture media were autoclaved at 121°C for 15 min.

#### **Preparation of culture media:**

All culture media were formulated according to manufacturer's specification. Basically, for nutrient agar, this involves appropriate weighing of nutrient agar, distributing into bijou bottles (in 50 ml) and then sterilization using autoclave at 121°C, 15 lbs/sq.inch

for 15 min; then allowed to cool to 45°C before pouring into the agar plate. The P<sup>H</sup> of the agar medium was maintained at 7.4.

#### **Maintenance and standardization of test organisms:**

The organism (*B. subtilis*, *B. pumilis*, *E. coli*) was maintained by weekly subculturing on nutrient agar slant. Before each experiment, the organism was activated by successive subculturing and incubation. Standardization of the test organism was according to previously reported method <sup>17</sup>.

#### **Evaluation of Antimicrobial activity: Determination of zone of inhibition by cup plate method:**

The cylinder plate assay of drug potency is based on the measurement of the diameter of zone of inhibition of bacterial growth surrounding cylinders (cups), containing various dilutions of test compounds <sup>18</sup>. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the microorganisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each extract and reference standards were added to the cups with a micropipette.

Table 3: Antimicrobial activity of *Callistemon citrinus* L. Leaf

| Bacterial Species  | Zone of Inhibition Mean of Diameter (MM) |                    |                 |                 | MIC ( $\mu$ G/ML) |                    |                 |                 |
|--------------------|------------------------------------------|--------------------|-----------------|-----------------|-------------------|--------------------|-----------------|-----------------|
|                    | Streptomycin                             | Chloroform Extract | Ethanol Extract | Aqueous Extract | Streptomycin      | Chloroform Extract | Ethanol Extract | Aqueous Extract |
| <i>B. subtilis</i> | 22                                       | 20                 | 12              | -               | 22                | 15                 | 9               | -               |
| <i>B. pumilis</i>  | 20                                       | 17                 | 13              | -               | 20                | 8                  | 7               | -               |
| <i>E. coli</i>     | 18                                       | 12                 | 9               | -               | 18                | 7                  | 7               | -               |

All the plates were kept in a refrigerator at 2 to 8°C for 2 hours effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antimicrobial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide (DMSO) which was used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded. Procedure was repeated in triplicate for accurate results. Resulting zone of inhibition was measured using a Hi media zone scale <sup>19</sup>.

#### Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration values were determined by broth dilution assay. Varying concentrations of the extracts (500, 250, 125, 62.5  $\mu$ g/ml) were prepared, along with this DMSO as control and streptomycin (25  $\mu$ g/ml) as standard. 0.1 ml of each concentration was added to each 9 ml of nutrient broth containing 0.1 ml of standardized test organism of bacterial cells.

The tubes were incubated at 37°C for 24 hours. Positive controls were equally set up by using solvents and test organisms without extracts. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration <sup>20</sup>. Results were subjected to minimum inhibitory concentration by two-fold dilution method. The results are reported in Table 3.

#### Results and discussion

The present study deals with the preliminary phytochemical screening and evaluation of anti microbial activity against *B. subtilis*, *B. pumilis* and *E. coli* by cup plate agar diffusion method. Streptomycin was used as standard and distilled water was used as negative control. As revealed from Table 3, the chloroform extract exhibited moderate to significant antimicrobial activity against all the tested microbial strains, and it showed significant inhibition on gram (+)ve compared to gram (-)ve. The ethanolic extract exhibited moderate antimicrobial activity. The aqueous extract was devoid of any antimicrobial activity. The chloroform extract showed maximum antimicrobial effect of the three extracts (Table 3). Antimicrobial activity varied significantly between different extracts of *Callistemon citrinus*. This credit to maximum activity of chloroform extract was supposed to chloroform being an organic solvent and will dissolve organic compounds better, hence liberate component required for antimicrobial activity <sup>21</sup>.

## Declarations

**Conflict of Interest:** No potential conflicts of interest are declared by the authors.

**Ethical Approval:** This article does not contain any experiments involving human subjects or animals that were conducted by the author.

## Conclusion

With this project, we can conclude that chloroform, ethanolic leaf extracts of *Callistemon citrinus* were exhibiting good antimicrobial activity against Gram (+) ve and Gram (-) microorganisms. Further studies are required to isolate the active compound from chloroform extract of *Callistemon citrinus* responsible for this significant antimicrobial effect which might be a lead compound in antimicrobial arena.

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