

***In vitro* Antibacterial Activity Of Ethanolic Extract of ‘*Calliandra Haematocephala*’ against Selected Bacterial Strains**

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ABSTRACT

Calliandra haematocephala commonly known as powder puff has wide range of therapeutic properties like anti inflammatory, anticonvulsant, immunomodulatory and blood purifier. Previous Studies showed the antibacterial activity of the flowers and bark of *Calliandra haematocephala*, So this study is designed to evaluate the antibacterial activity of the ethanolic extract of the leaves of “*Calliandra haematocephala*” against selected gram positive and gram negative strains. The leaves of *Calliandra haematocephala* was subjected to soxhlet extraction using organic solvent of ethanol. The antibacterial activity of *Calliandra haematocephala* extract was done using standard disc diffusion method using Muller Hinton agar (MHA) against ten strains of bacteria (Three gram positive and Seven gram negative strains). Ampicillin is the standard positive control and 20µl of varying concentrations (1000 µg, 750µg and 500 µg) of the extract sample were tested. The antibacterial effect was determined by measuring the diameter of the zone of inhibition. The ethanolic extract of *Calliandra haematocephala* showed the moderate antibacterial activity against gram negative bacterial strains like *Salmonella typhi* (12mm) *Serratia marcescens* (9mm), *Shigella flexneri* (8mm) and gram positive bacterial strains like *Staphylococcus aureus* (9mm), *Bacillus subtilis* (10mm), *Micrococcus luteus* (10mm) as measured by the zone of inhibition produced by the plant extract when compared with the control drug Ampicillin. However it showed maximal activity against *Pseudomonas aeruginosa* (12mm). In our study the ethanolic extract of *Calliandra haematocephala* has shown to have good antibacterial activity against both gram negative and positive bacteria in high concentration.

Keywords: *Calliandra haematocephala*, Antibacterial activity, agar disc diffusion, zone of inhibition.

INTRODUCTION

Herbals have been used as therapeutic agents for longer period in our Traditional medical system. Considering the emergence of the multidrug resistance, cost and adverse effects of the antibiotics these natural sources provide nice opportunities for new drug leads. Also the therapeutic potential, least side effects and cost draw the researchers attention to identify phytochemicals with antibacterial activity that will cure the bacterial infections.

Calliandra haematocephala (family Mimosaceae), is a spreading well-branched shrub growing to a height of 1 to 3 meters. It has silky leaflets which are glossy copper when new, turning to a dark metallic green, The profuse, fragrant bloom is the main reason for its popularity, with big puffs, two to three inches across, of watermelon pink, deep red, or white silky stamens, produced during warm months¹

Native to North America, but it has been grown in Indian garden and park for longer period. Studies show that *Calliandra haematocephala* has anti-inflammatory, gastroprotective, [2] anticonvulsant, immunomodulatory, and antiulcerogenic properties. The decoction of the flower extract is used as blood purifier and tonic everywhere because of its antioxidant property [3]. Its roots are used for haemorrhoids [4]. It is reported that betulinic acid in *Calliandra* is responsible for its antitumor, anti-HIV [5] and anti-rotaviral activity [6]

Traditionally it is used as antibacterial agent [7] and studies showed about its antibacterial activity of bark and flowers. One study showed the antibacterial activity of silver nanoparticles synthesized using the leaf extract of *Calliandra haematocephala* against *E. coli*. Gallic acid present in the leaf extract plays a major role in synthesis of silver nanoparticles that leads to its antibacterial activity [8]. Also fractionation of the bark of *Calliandra*

haematocephala by chromatographic methods identified the compounds p-hydroxybenzoic acid, caffeic acid, protocatechuic acid, astilbin, neo-astilbin, and catechin-3-O-rhamnoside. These compounds showed varied antibacterial activity [7]. In another study in vitro investigation of extract obtained from flowers of the shrub *Calliandra haematocephala* showed greater activity against gram positive bacteria *Staphylococcus aureus* [9]. Disc diffusion method is one of the reliable, most commonly used in vitro screening methods to evaluate the antibacterial activity of various drugs and phytochemicals [10].

Since the above findings suggested for strong antibacterial activity, we designed this study to prove the antibacterial activity of the ethanolic extract of the leaves of *Calliandra haematocephala* against selected gram positive and gram negative microorganisms using the in vitro standard disc diffusion method.

MATERIALS AND METHODS

Plant Materials

The leaves of *Calliandra haematocephala* were collected in and around area-Madipakkam and certified by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, West Tambaram. (Reg no. PARC/2017/3442)



Table 1: Antibacterial activity produced by the various concentrations of *Calliandra haematocephala* ethanolic extract against selected bacterial strains compared with Ampicillin as a positive control

Organisms	MTCC No.	Zone of inhibition (mm)			Ampicillin (1mg/ml)
		1000	750	500	
<i>Salmonella typhi</i>	1169	12	11	11	13
<i>Micrococcus luteus</i>	7950	10	10	10	11
<i>Serratia marcescens</i>	3124	9	8	7	10
<i>Escherichia coli</i>	45	10	8	7	16
<i>Bacillus subtilis</i>	2763	10	9	7	11
<i>Staphylococcus aureus</i>	3160	9	8	7	10
<i>Shigella flexneri</i>	9543	8	8	8	9
<i>Vibrio parahaemolyticus</i>	451	11	10	8	14
<i>Pseudomonas aeruginosa</i>	2581	12	12	12	11
<i>Enterococcus faecalis</i>	3159	10	8	8	14

Phytochemical analysis

The phytochemical analysis shows the presence of alkaloids, tannins, flavonoids, saponins and glycosides.

were further condensed using rotary evaporator. The crude extract was stored at room temperature in airtight container for further analysis. A portion of the extract is used for antibacterial evaluation.

Extraction

The leaves of *Calliandra haematocephala*, cut into pieces, air dried and powdered. 25 gram of powdered sample were taken and extracted with 300ml of ethanol in soxhlet apparatus for 12 hours of time. The crude extract was filtered and the solvents

Bacterial culture & Preparation of inoculum

The bacterial strains used in the study are three specimens of gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*) and seven gram negative bacteria specimens (*Salmonella typhi*, *Serratia*

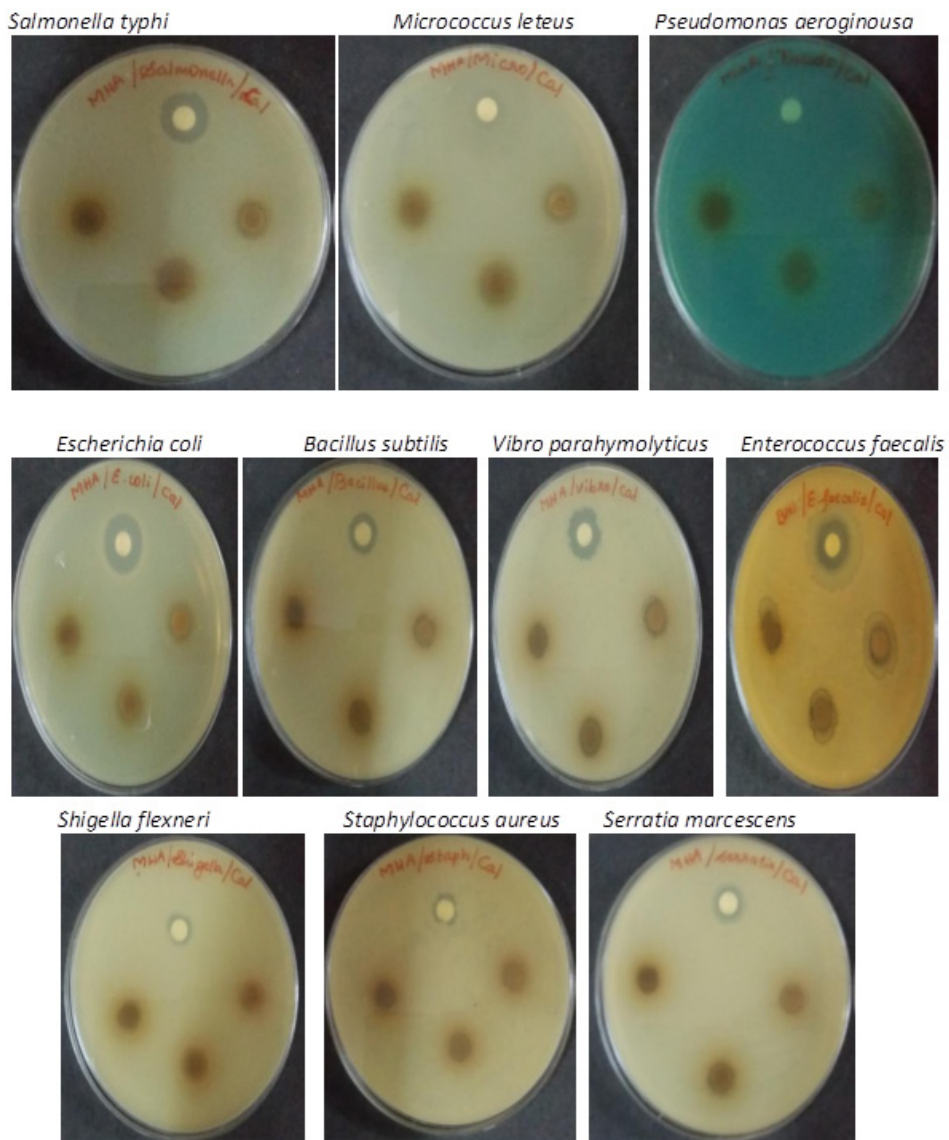


Fig.1: Zone of inhibition of bacterial colonies by different concentrations of *Calliandra haematocephala* in the Muller Hinton Agar (MHA) medium

marcescens, *Escherichia coli*, *Shigella flexneri*, *Vibrio parahemolyticus*, *Pseudomonas aeruginosa* and *Enterococcus*.) clinical isolates purchased from MTCC, Chandigarh. The isolates were identified by a standard method (Cowan and steel, 1993). Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loopful of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 24hrs at 37°C. The Assay was performed by agar disc diffusion method. The culture media and the Ampicillin discs were purchased from hi media labs, Mumbai, India.

Bacterial susceptibility testing

In vitro Antibacterial activity of extract was determined against three specimens of gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*) and seven gram negative bacteria specimens (*Salmonella typhi*, *Serratia marcescens*, *Escherichia coli*, *Shigella flexneri*, *Vibrio parahemolyticus*, *Pseudomonas aeruginosa* and *Enterococcus*.) by agar disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates

and 20µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc. The plates were incubated at 37°C for 24 hrs.. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition. The diameter of the zone of inhibition produced by each agent were measured with a transparent ruler in millimeter and compared with those produced by the commercial antibiotic Ampicillin (20µl/disc) served as a positive control. The diameters of the zone of inhibition less than 5mm were considered insignificant for analysis.

RESULTS

Results showed the antibacterial activity of *Calliandra haematocephala* leaves on all strains. They were effective against both gram positive and gram negative organisms in various concentrations (500,750 and 1000µg/ml) when compared with Ampicillin (1mg/ml). The antibacterial activity was assessed by measuring the zone of inhibition. The extract showed the maximum activity against *Salmonella typhi* (12mm), *Micrococcus luteus* (10mm), *Serratia marcescens* (9mm), *Bacillus subtilis* (10mm), *Staphylococcus aureus* (9mm) and *Shigella flexneri* (8mm). It showed maximum inhibition in higher dose (1000µg/ml) and moderate inhibition in 750 and 500µg/ml. But irrespective of the concentration, in all the three doses it showed the

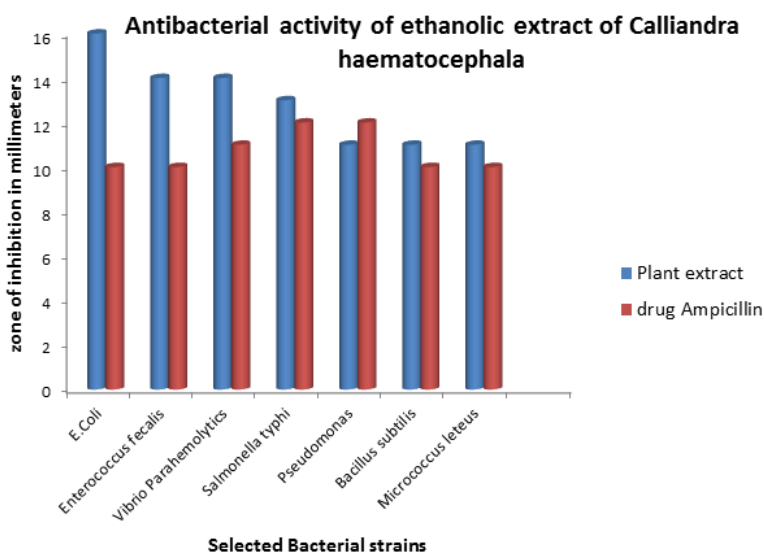


Fig. 2: Significant Antibacterial activity of ethanolic extract of *Calliandra haematocephala* against selected bacterial strains

same level of inhibition against *Micrococcus luteus* (10mm), *Shigella flexneri* (8mm) and *Pseudomonas aeruginosa* (12mm). The antibacterial activity of this plant extract showed moderate inhibitory activity against gram negative bacteria like *Escherichia coli* (10mm), *Vibrio parahaemolyticus* (11mm) and *Enterococcus faecalis* (10mm) in higher doses. However it showed the maximum activity against *Pseudomonas aeruginosa* (12mm) than the control drug.

DISCUSSION

The above results favour the antibacterial activity of *Calliandra haematocephala*. The phytochemical analysis of the leaf extract shows the presence of alkaloids, tannins, flavonoids, Saponin and glycosides; It is proved that both flavonoids and tannins possesses greater antimicrobial activities^[11-13]. Flavonoids are hydroxylated phenolic compounds known to be synthesized by plants in response to microbial infection^[11]. Three flavonoid aglycones, quercetin, kaempferol, myricetin were isolated and identified from aerial part *Calliandra haematocephala* and found to have strong antioxidant activities^[14,15]. Condensed tannins isolated from bark, stems, and leaves of *Calliandra haematocephala* were evaluated for in vitro antioxidant activities using BTS+ and FRAP methods and proved their stronger antioxidant activities. Tannins has great antibacterial activity by binding with proline rich protein that interfere with protein synthesis^[15]. Also, antioxidant galloylated glycosides isolated from leaves of *Calliandra haematocephala* exhibited moderate to strong radical scavenging properties on lipid peroxidation, hydroxyl radical, superoxide anion generation and DPPH radical in comparison with that of quercetin as a positive control *in vitro*

^[16]. In our study, the ethanolic extract of leaves of *Calliandra haematocephala* showed antibacterial activity against both gram positive and gram negative strains in all doses. Particularly it was more effective against *Pseudomonas aeruginosa* and showed moderate activity against *Salmonella typhi*, *Micrococcus luteus*, *Serratia marcescens*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Shigella flexneri*. The presence of flavonoids, tannins and the strong antioxidant activity could be the reason for the antibacterial activity. Also previous studies showed the antibacterial activity of flowers and bark of *Calliandra haematocephala* and identified the compounds responsible for that^[7,9]. So that strongly suggested the antibacterial potential of the leaves of *Calliandra haematocephala* and its therapeutic role in life threatening microbes. But we need further studies to evaluate the mechanism of action and extraction of phytochemicals that is responsible for its antibacterial activity. Nowadays hospital acquired infections^[17] is a major threat and particularly multidrug resistant *Pseudomonas aeruginosa* can be deadly for patients in critical care^[18]. Since the above results showed maximum activity against *Pseudomonas aeruginosa* and moderate activity against gram negative organisms, it could enhance the effectiveness of the antibiotics in life threatening infections.

CONCLUSION

So the above study proved the antibacterial activity of the ethanolic extract of *Calliandra haematocephala* leaves against both gram positive and gram negative bacteria and its promising adjuvant role in life threatening hospital acquired infections.

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