

**IN VITRO ANTI-OXIDANT AND ANTI-BACTERIAL ACTIVITY OF SODIUM COPPER CHLOROPHYLLIN FROM *LEUCAS ASPERA* L.****GEETHA RANI. R<sup>\*1</sup> & BANU. N<sup>2</sup>**<sup>1</sup>Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Vels University, Chennai, Tamil Nadu, India.<sup>2</sup>Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Vels University, Chennai, Tamil Nadu, India.**ABSTRACT**

*Leucas aspera* L. commonly known as 'Thumbai' is distributed throughout India. The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity. The leaf extracts of *Leucas aspera* L. shows significant anti-oxidant and anti-bacterial activity against *Klebsiella pneumonia* and *Staphylococcus aureus*. The antioxidant activities of *Lucas aspera* L. were observed by a change in colour from yellow to green with different concentrations (100-600µl). The antibacterial activities of *Leucas aspera* L. Were screened against two infectious pathogens, by the agar disc diffusion method. Ampicillin (10 µg /ml) was used as positive control. The antibacterial activity of *Leucas aspera* L. showed active inhibition against the tested human pathogens.

**KEY WORDS:** Antibacterial, Antioxidant, *Leucas aspera* L.**GEETHA RANI. R**Research Scholar, Department of Biotechnology, Vels Institute of Science,  
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## INTRODUCTION

The plants with medicinal properties are the source of treating illness from the millinicum in the early civilization in India, eastern countries and China. The medicinal plants are still widely used in ethnomedicine around the world.<sup>1</sup> A wide range of medicinal plant parts are used to extract as raw drugs and they possess varied medicinal properties.<sup>2</sup> Medicinal plants represent a rich source of antibacterial and antioxidant compounds. Although hundreds of plant species have been tested for antibacterial activity the vast majority have not been adequately evaluated to screen local flora for antibacterial activity from *Leucas aspera* L. belonging to the family Lamiaceae.<sup>3</sup> *Leucas aspera* L. is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities.<sup>4</sup> *Leucas aspera* L. is used in the traditional medicine of Philippines to treat scorpion bites. It is also an antipyretic.<sup>5</sup> The juices of the flower can be extracted and used to help treat sinusitis, as well as headaches and also be used to treat intestinal worms in children. The entire plant is also used as an insecticide and indicated in traditional medicine for coughs, colds, painful swelling and chronic skin eruption.<sup>6</sup> Leaves of *Leucas aspera* L. are useful in chronic rheumatism, psoriasis, scabies, chronic skin eruptions and their juice used as antibacterial agent.<sup>7</sup> Compounds isolated from the plant include long chain aliphatic compound, triterpenes, sterols and novel phenolic compounds.<sup>8</sup>

## MATERIALS AND METHODS

### Collection of Plant Material

Fresh leaves of *Leucas aspera* L. were collected from in and around Chennai, Tamil Nadu. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water.

### Extraction of Sodium copper chlorophyllin

Ten grams of fresh leaves were taken and 1gm of sodium carbonate was added to neutralize the acidity. The plant material was ground with 50-100ml acetone and filtered using filter paper. This procedure is repeated until the residue becomes colourless. It was then washed with 50-150ml of diethyl ether to wash off acetone. The mixture was poured into a separating funnel and acetone was washed off using distilled water. This was repeated until a yellow colour separates off, which consists of flavones. The solution was poured into a bottle and 10-25ml of methanol saturated with potassium hydroxide pellets was added. The solution was shaken thoroughly and kept in icebox for overnight. The alkaline solution of chlorophyllin was poured into a separating funnel and 100ml diethyl ether was added and left for 30 minutes. Chlorophyllin separates off greenish layer which was removed. The ether layer was washed off with dilute potassium hydroxide and distilled water, to remove traces of chlorophyllin salts. The filtrate was evaporated to dryness in rotary evaporator and the extract was stored in ice box.<sup>9</sup>

### Purification

The extracted Sodium copper chlorophyllin was purified by column chromatography using silica gel by the elution with di ethyl ether and acetone in the ratio of 8 : 2 .

### Anti-Oxidant Activity

The Chlorophyllin of *Leucas aspera* L. were tested for the anti-oxidant activity by phospho molybdenum method . The purified extracts were taken in six different concentrations of 100 µl, 200 µl, 300 µl, 400 µl, 500 µl and 600 µl. This was mixed with the reagent of 5.88 ml of concentrated H<sub>2</sub>SO<sub>4</sub>, 0.49 gms of ammonium molybdate and 0.36 gms of sodium phosphate in the estimated concentrations. It was incubated at 95°C for 90 minutes. Formation of green colour shows the presence of antioxidant activity in *Leucas aspera* L. Optical density was recorded at 695nm.<sup>10</sup>

### Growth and Maintenance of Test Microorganism for Antibacterial Studies

Bacterial cultures of *Klebsiella pneumonia* and *Staphylococcus aureus* were collected from King's Institute of Preventive Medicine, Guindy, Chennai. The bacteria were maintained on a nutrient broth (NB) at 37°C. The gram positive of *Staphylococcus aureus* and gram negative *Klebsiella pneumonia* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C.

### Anti-bacterial Activity

The Sodium copper chlorophyllin of *Leucas aspera* L. were tested by the agar disc diffusion method. The test microorganisms were seeded into respective medium by a spread plate method using 10 µl of the 24h cultures of bacteria grown in nutrient broth. After solidification the sterile discs (0.5cm) impregnated with the extracts were placed on test organism-seeded plates. Ampicillin (10 µg /ml) used as positive control and diethyl ether was used as negative control. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.<sup>11</sup>

## RESULTS

The result of the present study of Chlorophyllin from *Leucas aspera* L. indicates the presence of anti-oxidant property. Anti-oxidant activity showed the formation of green colour in different concentrations used in this study. Dark green colour was observed in 600µl at the peak value of 1.980 (Table 1). The present study revealed that the Chlorophyllin of *Leucas aspera* L. posses potential antibacterial activity against *Klebsiella pneumonia* and *Staphylococcus aureus* when tested by the disc diffusion method, the extracts of *Leucas aspera* L. showed significant activity against *Klebsiella pneumonia* with a zone of inhibition around 15mm, *Staphylococcus aureus* around 11mm, which were compared with positive control (20mm) (Table 2) (Fig 1&2).

Table1

Antioxidant activity of Chlorophyllin from *Leucas aspera* L.by Phospho-molybdenum method

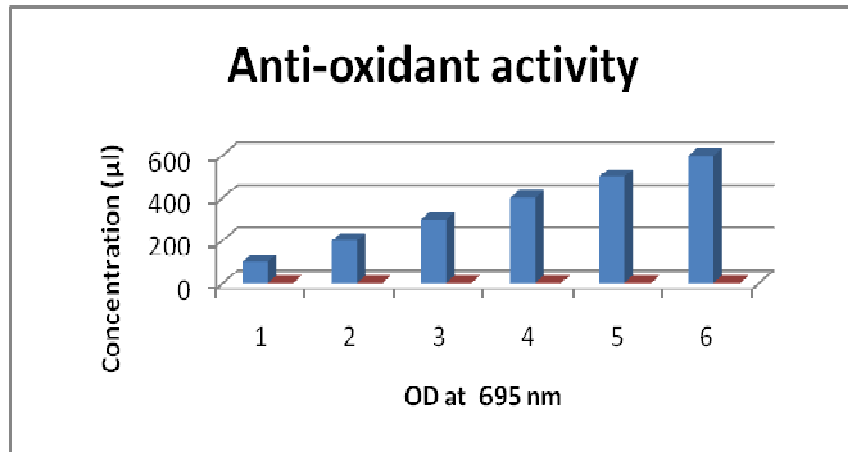


Figure1  
*Klebsiella pneumonia*

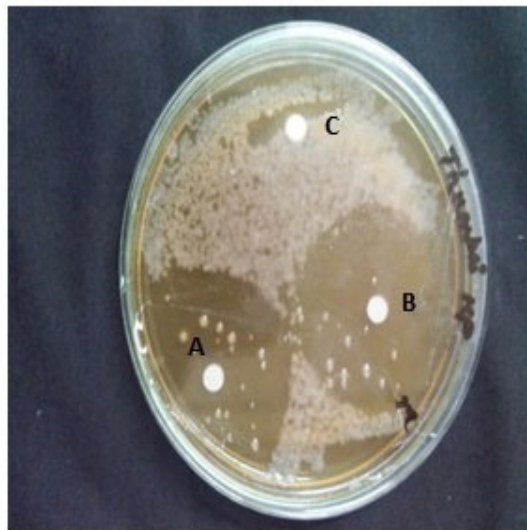
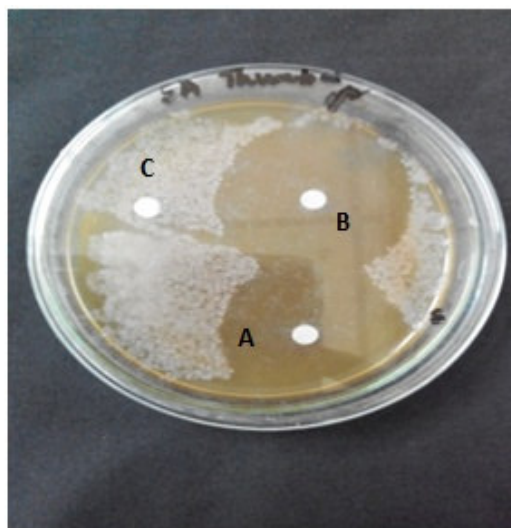


Figure 2  
*Staphylococcus aureus*



**Anti-bacterial activity**

- A-Sample
- B-Positive control (Ampicillin)
- C-Negative control (Di ethyl ether)

**Table 2**  
**Antibacterial activity**

Sl.No	Bacterial species	Zone of inhibition(mm)	
		Positive (Ampicillin-10µg/ml)	Sample (10µg/ml)
1	<i>Klebsiella pneumonia</i>	20mm	15mm
2	<i>Staphylococcus aureus</i>	20mm	11mm

## DISCUSSION AND CONCLUSION

Plants are an important source of potentially useful sources for the development of new chemotherapeutic agents. The first process for this goal to know that the antibacterial activity assay<sup>12</sup> Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antioxidant and anti-inflammatory properties of plants.<sup>13,14,15,16,17</sup> Some of these observations have helped in identifying the active principle that are responsible for such activities in developing drugs for the medicinal use in human beings. Anti-oxidant activity of Chlorophyllin was proved by the formation of green colour in different concentrations. Dark green colour was observed in 600µl at the peak value of 1.980, which is a good indicator of potential anti-oxidant activity. In the present study, the Chlorophyll derivative of Chlorophyllin from *Leucas aspera* L.

showed antibacterial activity against *Klebsiella pneumonia* and *Staphylococcus aureus*. It showed significant activity against *Klebsiella pneumonia* with a zone of inhibition around 15mm and *Staphylococcus aureus* around 11mm, positive control showed 20mm. The results of present investigation clearly indicates that the antioxidant and antibacterial activity vary with the species of the plants and plant material used. Thus, this study confirms the medicinal value of *Leucas aspera* L. as an antifungal and antibacterial sources.

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