



ANTI-BACTERIAL AND ANTI-OXIDANT ACTIVITY OF CHLOROPHYLLIN FROM TRIDAX PROCUMBENS (L)

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ABSTRACT

The leaf extracts of *Tridax procumbens* (L) shows significant anti-oxidant and anti-bacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The antibacterial activity of *Tridax procumbens* (L) collected from different localities of Chennai, Tamil nadu were screened against two infectious pathogen, by the agar disc diffusion method. Ampicillin (10 µg ml) was used as positive control. The antibacterial activity of *Tridax procumbens*(L) showed active inhibition against the tested human pathogens. The Anti oxidant activity of *Tridax procumbens* (L) were observed by changing in colour from yellow to green with different concentrations.

Key words: Antibacterial, Antioxidant, *Tridax procumbens*.

INTRODUCTION

The plants with medicinal properties are the source of treating illness from the millinieu in the early civilization in India, Eastern countries and China. The medicinal plants are still widely used in ethnomedicine around the world (Thomson, 1978. Stockwell, 1988). Medicinal plants represent a rich source of antibacterial and antioxidant compounds. Plants are used medicinally in different countries and are a different source of many potent and powerful drugs (Srivastava, Lambert and Vietmeyer, 1996).

A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties (Uniyal, Singh, Jamwal and Lal, 2006). 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 *Tridax procumbens* (L) belonging to the family Asteraceae (Balandrin, Klocke, *et al.*, 1985).

Leaves are used in therapeutic activities like anti-viral, anti-oxidant, antibiotic efficacies, wound healing, insecticidal, anti- fungal and anti - inflammatory activities.

MATERIALS AND METHODS

Collection of Plant Material:

Fresh leaves of *Tridax procumbens* (L) were collected from Chennai, Tamil nadu. The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water.

Solvent Extraction:

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

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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.

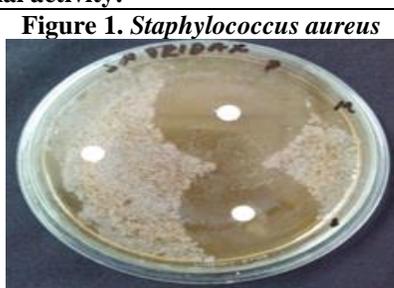
Growth and Maintenance of Test Microorganism for Antibacterial Studies:

Bacterial cultures of *Staphylococcus aureus* and *Klebsiella pneumonia* were collected from King's Institute of Preventive Medicine, Guindy, Chennai. The bacteria were maintained on 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 10 µl /well of Chlorophyllin of *Tridax procumbens* (L) were tested by the agar disc diffusion method. The test microorganisms were seeded into respective medium by spread plate method using 10 µl of the 24h cultures of bacteria grown in nutrient broth. After solidification the sterile discs 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.

Anti bacterial activity:



The diameters of the inhibition zones were measured in mm.

Anti-Oxidant Activity:

The Chlorophyllin of *Tridax procumbens* (L) were tested for the Anti-oxidant Activity by Molybdenum method (Prieto *et al.*, 1999). The extracts were taken in six different concentrations of 100µl, 200µl, 300µl, 400µl, 500µl and 600 µl. This was mixed with the reagent of Concentrated. H₂SO₄, Ammonium molybdate and 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 that particular plant. OD readings were measured at 695nm.

RESULTS:

Results obtained in the present study revealed that the Chlorophyllin of *Tridax procumbens* posses potential antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia*. When tested by the disc diffusion method, the extracts of *Tridax procumbens* (L) showed significant activity against *Staphylococcus aureus* with a zone of inhibition around 10mm and *Klebsiella pneumonia* around 13mm, which were compared with positive control (20mm) (Table 1) (Fig 1&2). Anti oxidant activity showed the formation of green colour in different concentrations used in this study. Dark green colour was observed in 600 µl at the peak value of 1.980. The result of the present study of Chlorophyllin from *Tridax procumbens* (L) indicates the presence of anti-oxidant property. (Table 2).



Figure 3. Antioxidant activity of Chlorophyllin from *Tridax procumbens* (L) by Phospho-molybdenum method:

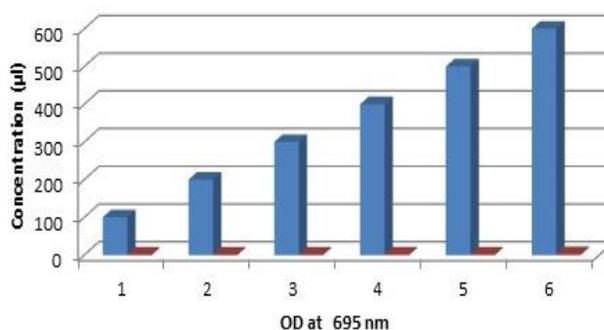


Table 1. Antibacterial activity

Bacterial species	Positive(antibiotic)	Sample
<i>Staphylococcus aureus</i>	20mm	10mm
<i>Klebsiella pneumonia</i>	20mm	13mm

DISCUSSION AND CONCLUSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first process for this goal to know that antibacterial activity assay (Tona *et al.*, 1998). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antioxidant and anti-inflammatory properties of plants (Samy *et al.*, 2000, Palombo *et al.*, 2001, Kumaraswamy *et al.*, 2002, Stepanovic *et al.*, 2003, Bylka *et al.*, 2004, Behera *et al.*, 2005, Govindarajan *et al.*, 2006). 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.

In the present study, the Chlorophyll derivative of Chlorophyllin from *Tridax procumbens* (L) showed antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia*. It showed significant activity against *Staphylococcus aureus* with a zone of inhibition around 10mm and *Klebsiella pneumonia* around 13mm,

positive control showed 20mm. Anti oxidant activity of the Chlorophyllin was proved by the formation of green colour in different concentrations. Dark green colour was observed in 600 µl at the peak value of 1.980.

The results of present investigation clearly indicate that the antibacterial and antioxidant activity vary with the species of the plants and plant material used. Thus, this study confirms the medicinal value of *Tridax procumbens* (L) used in ayurveda, which could be of considerable interest to the development of new drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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