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### Antibacterial Evaluation on Crude Bioactive Compounds of *Tridax Procumbens* Linn and *Cardiospermum Halicacabum* Linn

<sup>1</sup>A. Doss, <sup>2</sup>T. P. Kumari, <sup>3</sup>Pushpa Rani

Department of Microbiology Kamaraj College Tuticorin, Tamilnadu.

#### Abstract

Crude extracts and flavonoids of leaves of *Tridax procumbens* L. (Asteraceae) and *Cardiospermum halicacabum* L. (Sapindaceae) have been studied for their antimicrobial activities using disc diffusion assay, against two Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and five Gram negative bacteria (*Escherichia coli* MTCC 2961, *Salmonella typhi* MTCC 733, *Pseudomonas aeruginosa* MTCC 4676, *Klebsilla pneumoniae* MTCC 432, *Proteus mirabilis* MTCC 425). Both plantsexhibited broad spectrum antimicrobial activity. Among the 5 extracts tested, the flavonoids of *C. Halicacabum* and *T. Procumbens* were foundto be more potent. Results of the present study indicate that *T. Procumbent* sand *C. Halicacabum*can be exploited for future antimicrobial drugs.

**Keywords:** Flavonoids; Ethanol; Disc method; Antimicrobial activity; Microbial pathogens.

#### Introduction

Plants produce a good deal of secondary metabolites which have benefited mankind in various ways including treatment of diseases [1]. These metabolites serve different purposes in the plant, including growth regulation, allelopathy, defense against predators and infections or they may be waste products. Outside their intrinsic uses in the plant, these secondary metabolites have variously been shown to exhibit interesting biological and pharmacological activities and are important as prophylactics, chemotherapeutics or have served as the starting points in the development of modern medicines. In India herbal medicines have been the basis of treatment and cure for various diseases or physiological conditions in traditional methods practiced such as ayurveda, unani and siddha. Although reports of antibacterial activity of indigenous plants have been evaluated. Phenolics like flavonoids and tannins are widely distributed in plant kingdom, vegetables, flowers etc. For centuries preparations containing flavonoids as active constituents have been used to treat human diseases and in anti-infective research [2].

Flavonoids are phenolic substances widely distributed in all vascular plants. They are a group of about 4000 naturally compounds known, and have been shown to have contribute to human health through our daily diet. They are ubiquitous in plant foods and drinks such as fruits, vegetables, tea, wine, coffee and beer [3]. In a review, discussed by Harborne and Willians [4], many studies have suggested that flavonoids exhibit antioxidant, anti-inflammatory, antimicrobial, vascular activities and others medicinal properties. Many reports on the antimicrobial activity of flavonoids are available [5, 6]. Related studies of antimicrobial activity indicate that crude extracts containing flavonoids, triterpenes and steroids have showed significant activity against various strains of *Staphylococcus aureus*, *Streptococcus faecalis*and *Escherichia coli* [7].

*Cardiospermum halicacabum* (Linn), family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed throughout the plains of India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite [8]; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic and sudorific its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache [9] and as a poultice for swellings [8]. Phytochemical constituents such as flavonoids, alkaloids, steroids, fatty acids, terpenoids, saponins, sugars, sterol, an essential oil, resin, non-glucosidal bitter substance, tannin, large amount of potassium nitrate and other constituents have been reported from the various extracts of this plant [10, 11].



*Tridaxprocumbens* L. (compositae) is a common plant found in tropical areas of all countries, growing primarily during raining season. It is a common weed in Tamilnadu present along with economically important crops. It habitats waste places, road sides and hedges throughout India. The extracts of *T. Procumbens* have been reported to have various pharmacological effects, antimicrobial activity, wound healing property and immunomodulatory activity on the experimental animals [12, 13, 14]. Flavones and glycosides have been isolated from the leaves of the plant [15]. The present study intends to study about the antibacterial activity of the crude extracts and flavonoids of *T. Procumbens* and *C. Halicacabum* against selected microbes.

## Materials and Methods

### Plant Material

The botanical identity of *T. Procumbens* and *C. Halicacabum* were confirmed by Dr. S.P. Anand, PG & Research Department of Botany, National College, Tiruchirappalli, Tamilnadu. A voucher specimen has been deposited at the Department of Microbiology, Kamaraj College, Thoothukudi, Tamilnadu, India.

### Preparation of Aqueous and Ethanol Extracts

The leaves of *T. Procumbens* and *C. Halicacabum* were air-dried, powdered and macerated in water for 2 h. The aqueous solution was boiled twice, each time for 2 hrs and then concentrated to give a final concentration of 1g/ml. Similarly the powder (1.0kg) was macerated in ethanol (70% w/v) and mixed well in a water bath for 3 h at 50<sup>0</sup> C. The solvent was then filtered and concentrated to give a final concentration

### Preparation of the Polysaccharides and Flavonoids

The hot water extract was prepared by boiling 200 g of fresh leaves with 1 liter of distilled water for 3 h. The final volume was reduced to 200 ml. The water extract was centrifuged and the supernatant was obtained. Excess of ethanol was added to the supernatant to precipitate the high molecular weight polysaccharide fraction, which was filtered and concentrated in vacuum and extracted with ethyl acetate. The ethyl acetate soluble fraction and the aqueous fraction (Proanthocyanidin) were obtained [16].

### Determination of Flavonoids

A small piece of magnesium ribbon was added to crude extracts of leaves of *T. Procumbens* and *C. Halicacabum*. This was followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to crimson are indicative of the presence of flavonoids.

### Bacterial Strains

Microorganisms were obtained from the Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. Amongst seven microorganisms investigated, two Gram-positive bacteria were *Staphylococcus aureus*, *Bacillus subtilis* while five Gram-negative bacteria were *Proteus mirabilis* MTCC 425, *Escherichia coli* MTCC 2961, *Pseudomonas aeruginosa* MTCC 4676, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733. All the microorganisms were maintained at 4<sup>0</sup>C on nutrient agar slants.

### Antimicrobial Activity

Antimicrobial activity was carried out by the disc diffusion method. Sterile paper discs (6 mm in diameter) prepared from Whatman No 1 filter paper was impregnated with drug, containing solution placed on the inoculated agar. Negative and Positive controls used ethanol and Chloroamphenical. The inoculated plates were incubated at 37<sup>0</sup>C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms.

## Results and Discussion

Resistance in microorganisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. Though a number of antibiotics are available but increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin. The presence of antibacterial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug [17].

The antimicrobial screening assay of the *T. Procumbens* and *C. Halicacabum* leaf extracts is shown in both ethanolic, polysaccharides and flavonoid extracts gave relatively wide inhibition zones against the test strain compound with the positive control. The relatively wide spectrum of activity of the flavonoid extracts over the positive control is significant from the disc diffusion assay. The inhibition zones of the *T. Procumbens* and *C. Halicacabum* leaf ethanolic (10 – 14



mm), polysaccharides (10 – 19 mm) and flavonoid extracts (12 – 26 mm) were greater than that of the positive control (13 – 19 mm). No inhibition zone was observed for the aqueousextract for all bacterial strains tested (Table 1 & 2). Best antibacterial activity against *S. Aureus* (20 mm), *B. Subtilis* (19 mm) and *S. Typhi* (18 mm) was observed in *T. Procumbens* whereas, flavonoids of *C. Halicacabum* showed excellent activity against *S. Aureus* (26 mm), *B. Subtilis* (24 mm) and *P. Mirabilis* (23 mm). From there results, it is possible that the flavonoids may be used as natural antimicrobial substance to replace antibiotics for controlling bacterial infections.

Bioactive potential of flavonoids have been proved by several workers [18, 19, 20]. The antibacterial effects of the flavonoid fraction from *C. Halicacabum* indicated that the plant has activity against all the tested bacterial strains. This observation agrees with the previous reports of Leven et al. [21], Scherbonvaski, [22] and Banso & Mann, [23] linked the antibacterial properties of plants to the presence of phytochemicals such as tannins, alkaloids, flavonoids and saponins. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [24]. Our findings confirm that the traditional therapeutic claims for these plants, in near future surely be able to replace the conventional antimicrobial agents to which there is increased incidence of drug interactions and the study suggests that this plant is promising for development of phytomedicine for antibacterial properties

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**Table. 1. Antimicrobial properties of certain bioactive compounds and crude extracts from *Tridax procumbens***

Compounds/Extracts	Zone of Inhibition (mm)						
	S.aureus	B.subtilis	P.aer.	S.typhi	E.coil	P.vulgaris	K.pne
Ethanol	14	11	9	10	9	-	-
Aqueous	11	10	-	-	9	-	-
Polysaccharides	14	19	-	16	17	9	10
Flavonoids	20	19	14	18	16	12	10
Positive control	21	24	18	23	27	16	17

**Table. 2. Antimicrobial properties of certain bioactive compounds and crude extracts from *Cardiospermum halicacabum***

Compounds/Extracts	Zone of Inhibition (mm)						
	S.aureus	B.subtilis	P.aer.	S.typhi	E.coil	P.vulgaris	K.pne
Ethanol	12	10	9	-	-	-	-
Aqueous	10	-	-	-	-	-	-
Polysaccharides	12	10	9	10	11	10	9
Flavonoids	26	24	21	21	14	23	12
Positive control	21	24	18	23	27	16	17