

Phytochemical and Anti-Microbial Studies on the Leaves Extracts of *Cardiospermum halicacabum* Linn.

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Abstract: The phytochemical screening and *In-vitro* anti-microbial activity leaves of *cardiospermum halicacabum linn* (Sapindaceae) were investigated. The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, proteins and saponins. The extracts exhibited marked anti-microbial activity against both Gram +ve and Gram-ve bacteria. When the concentration of the extracts was increased the zone of inhibition also increased.

Key words: *Cardiospermum halicacabum* Linn • Phytochemical Studies • Anti-Microbial Activity

INTRODUCTION

Plant-based drugs have been used worldwide in traditional medicine for the treatment of various diseases. Approximately 60% of world's population still relies on medicinal plants for their primary healthcare. According to a survey by NCI, USA, 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during 1981-2002 were inspired by natural products [1] Plant species still serves as a rich source of many novel biologically active compounds, as very few plant species have been thoroughly investigated for their medicinal properties [2]. Thus, there is renewing interest in phytomedicine during last decade and nowadays many medicinal plant species are being screened for pharmacological activities [3].

When we reviewed the number of articles published on the antimicrobial activity of medicinal plants in PubMed during the period between 1966 and 1994, we found 115; however, in the following decade between 1995 and 2004, this number more than doubled to 307. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine [4], essential oils [5] or isolated compounds such as alkaloids [6], flavonoids [7], sesquiterpene lactones [8], diterpenes [9], triterpenes [10] or naphthoquinones [11] among others. Some of these compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the part of the plant [12].

Cardiospermum halicacabum (Linn), family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed through out the plains of India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; 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 diarrhea, dysentery and headache and as a poultice for swellings. Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant [13]. Most likely confused with: *Physalis* spp. (ground cherry), *Clematis occidentalis*, *Clematis virginiana*, *Campsis radicans*, *Adlumia fungosa* [14]. The aim of the study was to show that leaves of *cardiospermum halicacabum linn*...have antimicrobial activity and preliminary phytochemical analysis was also evaluated

MATERIALS AND METHODS

Materials: Dried leaves powder of *C. halicacabum linn*, ethanol (SD Fine Chemicals, Mumbai, India), chloroform water (SD fine, Mumbai, India.), beef extract (Merck Ltd, India), acid hydrolysate casein (biomark laboratories, Maharashtra, India), starch (rajlal and ons ndore, India), agar-agar (Marine Chemicals, Kerala, India), sodium hydroxide (s.d. Fine Chem.), hydrochloric acid (Merck Ltd, India), ciprofloxacin, distilled water.

Plant Collection and Identification: Leaves of the plant *C. halicacabum linn* collected 20 km away from erambalur, Tamilnadu, India. This collection of the plant was authenticated by Dr. Kosiba B.S., M.S, (Govt.Regd. no: 1209) Assistant siddha medical officer, Government district head quarters, perambalur-621 212.

Extraction of Plant Material: The leaves of *cardiospermum halicacabum Linn* were dried in the shade. Then the dried leaves were powdered to get a course powder. About 25g of dry powder was extracted with 200ml of ethanol (95%) or 200ml distilled water and 10ml chloroform by hot continuous percolation method using soxhlet apparatus. The extractions were continued for 72 hrs at 50°C. The extract were filtered and concentrated to a dry mass by using vacuum distillation method. The extract stored in desiccators [15].

Qualitative Phytochemical Evaluation: The shade dried powder and various extracts of the leaves of *C. halicacabum linn* were subjected to chemical tests for its active constituents. Identification of the chemical constituents was carried out on the same extracts used in pharmacological tests according to the methodology proposed by Makonnen *et al.* [16] and parekh and Sumitra [17].

Cup Plate or Cylinder Plate Method: Cup plate method using Mueller-Hinton agar medium was employed to study the antibacterial activity of the extracts against *S. aureus*, *B. substilis* and *E. coli* [19]. 0.2ml of known concentration 100µg (What was that conc.??) of inoculums was spread on the surface of the medium. Cups (cavities) were made by using sterile borer (4mm) and 0.2ml of ciprofloxacin was poured into the cups of agar

plate. oth aqueous and alcoholic extracts (sample) in different concentrations were placed. Then the Petridish incubated at 37°C for 24 hrs [20, 21]. Ciprofloxacin (50mg/ml) was used as a standard drug to compare different concentrations (50mg/ml, 75mg/ml and 100mg/ml) of the sample.

RESULT AND DISCUSSION

The antimicrobial activity of the leaf extract was assayed by Cup plate or cylinder plate method method against 3 bacterial species *S. aureus*, *B. substilis* and *E. coli* [19]. Table 2 shows the microbial growth inhibition of both aqueous and methanol extracts of the screened plantspecies. The aqueous extract showed antimicrobial activity (*Cardiospermum helicacabum linn...*).

The methanol extracts of the investigated plants showed maximum antimicrobial activity against Gram-negative *K.pneumoniae*. Similar results were also reported byParekh, J., *et al.* (17), Babu Ananth, D. The studied plant were most active against *E. coli*. The extracts of Preliminary phytochemical analysis revealed the presence of alkaloids (+ve test for alkaloids and saponins. The other secondary metabolites like saponin, protein, lignin, etc. were present in trace amounts in some of the plant (Table 1). It is not surprising that there are differences in the antimicrobial effects of plant species, due to thephytochemical properties and varying among concentration.. The drying process may havecaused conformational changes to occur in some of thechemical constituents found in these plants. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to thedevelopment of a phytomedicine to act against microbes.Plant-based antimicrobials have enormous therapeutic potential as

Table 1: Phytochemical screening of leaves extract of *C. halicacabum linn*

S. No	Chemical test	Aqueous extract	Alcoholic extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Fixed oils and fats	-	-
4	Saponins	+	+
5	Tannis and phenolic compounds	-	-
6	Proteins and free amino acids	+	+
7	Gums and mucilage	-	-
8	Flavinoids	-	-
9	Lignin	+	+
10	Phytosterol	+	+
11	Glycosides	-	-

Table 2: zone of inhibition

<i>Cardiospermum helicacabum linn.</i>		Zone of inhibition(mm)		
		<i>S. aureus,</i>	<i>B. subtilis</i>	<i>E. coli</i>
Aqueous	50µg	16	17	20
	75µg	20	19	22
	100µg	24	21	24
Alcoholic	50 µg	15	12	15
	75 µg	17	15	17
	100µg	19	18	19

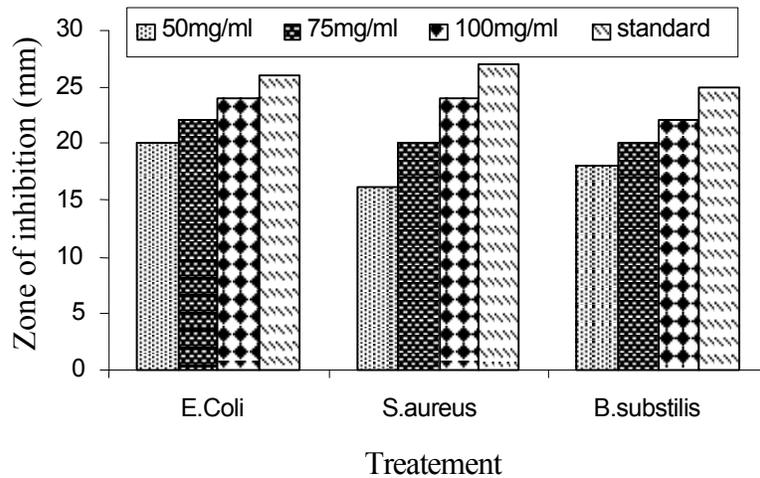


Fig. 1: Zone of inhibition of various concentrations of aqueous extract of *C. halicacabum linn*

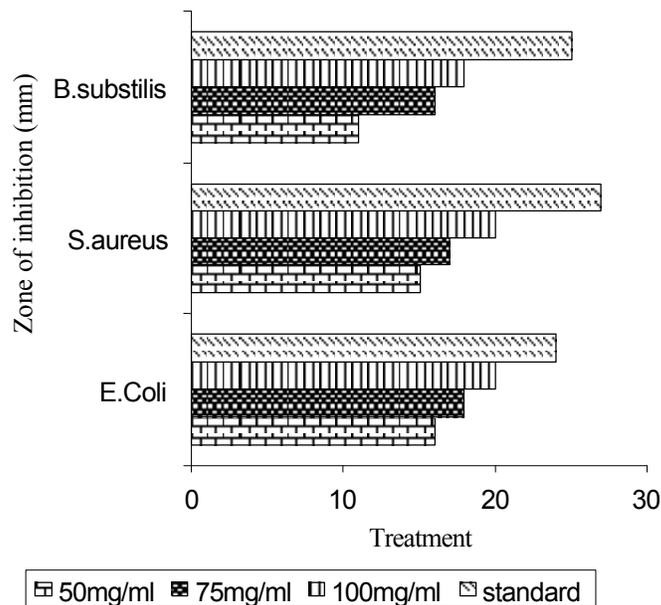


Fig. 2: Zone of inhibition of various concentration of alcoholic extract of *C. halicacabum linn*.

they can serve the purpose with lesser sideeffects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999). However, the present study of antimicrobial evaluation shows zone of inhibition was more in

aqueous extract compared to alcoholic (Fig 2 and 3). In conclusion, (*Cardiospermum helicacabum linn...*) broad spectrum of activity against a panel of bacteria responsible for the most common bacteria.

Extraction Procedure: 25 g of air-dried powder was taken in 200 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume (12) and stored at 4 °C in airtight bottles.

Phytochemical Screening: Preliminary phytochemical analysis revealed the presence of alkaloids (+ve test result for Wagner's), carbohydrates (+ve test result for Fehling's test and Benedict's test), proteins (+ve test result for Biuret test) and saponins. The other secondary metabolites like steroids, cardiac glycosides, etc. were present in trace amounts in the extracts (Table 1).

Anti-Microbial Study: The average zone of inhibition (18 mm) of aqueous extract (50 mg/ml) was smaller (26 mm) than that of the standard drug (50mg/ml). But it was too low in alcoholic extract (14mm) (Fig. 1 and 2). According to the results aqueous extract was effective than the alcoholic extract.

Plants continue to be used world-wide for the treatment of disease and novel drug entities continue to be developed through research into their constituents. In the present study, preliminary qualitative phytochemical tests revealed the presence of alkaloids, sterols, carbohydrates, protein, lignins and saponins in the extracts of *C. halicacabum linn*. Both aqueous and alcoholic extracts have significant antibacterial activity. But the aqueous extract showed better anti-bacterial activity than the alcoholic extract. When the concentration was increased the zone of inhibition also increased.

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