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Phytochemical screening, Antimicrobial and Antioxidant activity of whole extract of *Cardiospermum halicacabum* Linn. (*Sapindaceae*)

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ABSTRACT

Man's existence on this earth has been made possible only because of the vital role played by the plant kingdom in sustaining his life. The three important necessities of life – food, clothing and shelter and a host of other useful products are supplied to a great extent by the plant kingdom. *Cardiospermum halicacabum* is one of the medicinally potential plants which is used in the treatment of rheumatism, lumbago, cough, hyperthermia, and nervous diseases. The present investigation was undertaken to screen the phytochemical analysis, antimicrobial and antioxidant activity of *Cardiospermum halicacabum* whole extract.

Keywords: Antimicrobial, antioxidant, *Cardiospermum halicacabum,* whole extract.

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INTRODUCTION

The modern system of medicine has made a tremendous progress and synthetic drugs and antibiotics have revolutionized the complete scenario. But their toxic side effects are also being realized which has attracted the attention of whole world towards natural system of medicine. Man has made use of plants in the treatment of diseases. A large number of drugs are of plant origin. Plants keep the body in tune with nature as nature intended and maintain proper balance. Natural sources help people to build their good health[1]. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for 'millennia' and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants; therefore, India has often been referred to as the medicinal garden of the world[2]. *Cardiospermum halicacabum* is a deciduous climber growing up to 3meters. The ground stem carries alternate double triad leaves 3 to 6 cm long, the tiny radiate flowers. Stems are 5 to 10cm in length. The fruits are tiny green balloon shaped; spherical capsule containing the characteristics. Seeds with their heart shaped white markings. The plant has been used in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, laxative, its leaves used in the treatment of diarrhoea, dysentery and headache. 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[3-5].

MATERIALS AND METHOD

The plant material of *Cardiospermum halicacabum* were collected seasonally and authenticated by the taxonomists Dr. S. P. Rothe from the Department of Botany, Shri Shivaji College Akola.

Chemicals

All the chemicals used in the study were obtained commercially and of analytical grade.

Microorganisms

The test organisms *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa* were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune 411 008.

Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in ethanol extract of test plant.

1) **Test for Sugar**

a) Molisch's Test: - Positive

b) Iodine Test: - Positive

2) **Test for Flavonoids**

a) Shinoda test: - Positive

b) H_2SO_4 test: - Positive

3) **Test for Sterols**

a) Salkowaski test:- Negative

b) Vanillin test:- Negative

4) **Test for Alkaloids**

a) Wagner's reagent test: - Positive

b) Mayer's reagent test: - Positive

5) **Test for Tannin**

a) $FeCl_3$ test: - Positive

b) Lead acetate test: - Negative

6) Test for Protein and Amino acid

a) Biuret test: - Positive

b) Xanthoprotein test: - Positive

7) **Test for Resin**

a) NaOH test:- Positive

MATERIALS AND METHOD

Soxhlet extraction method is used for the preparation of extracts of *Cardiospermum halicacabum* plant material. The coarse powders of *Cardiospermum halicacabum* plant material were extracted with water, ethanol and acetone solvents by using soxhlet apparatus. These extracts were concentrated at 40 ^oC using rotary evaporator. The extracts thus obtained were stored separately in air tight bottles for further study.

Study of Antioxidant Activity by DPPH[6]

The antioxidant activity of the ethanol extract was assessed on the basis 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test extract was prepared in ethanol. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was

calculated using the formula given below

$$\text{PERCENTAGE (\%) INHIBITION OF DPPH (\%AA)} = \frac{A-B}{A} \times 100$$

Where A = Optical density of the blank and B = Optical density of the sample

RESULTS AND DISCUSSION

The stock solution 1 mg/ml of ethanol extract was prepared. The required dilutions 0.11 mg/ml to 0.19 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity was calculated and reported in table 1.

Table 1. Optical density and percent antioxidant activity for ethanol extract. O. D. of blank DPPH = 0.565

CONC. mg/ml	1 mg/ml	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
O.D. OF SAMPLE	0.387	0.385	0.380	0.375	0.370	0.367	0.365	0.363	0.360	0.358	0.356
%AA	19.37	19.79	20.83	21.87	22.91	23.54	23.95	24.37	25.00	25.41	25.83

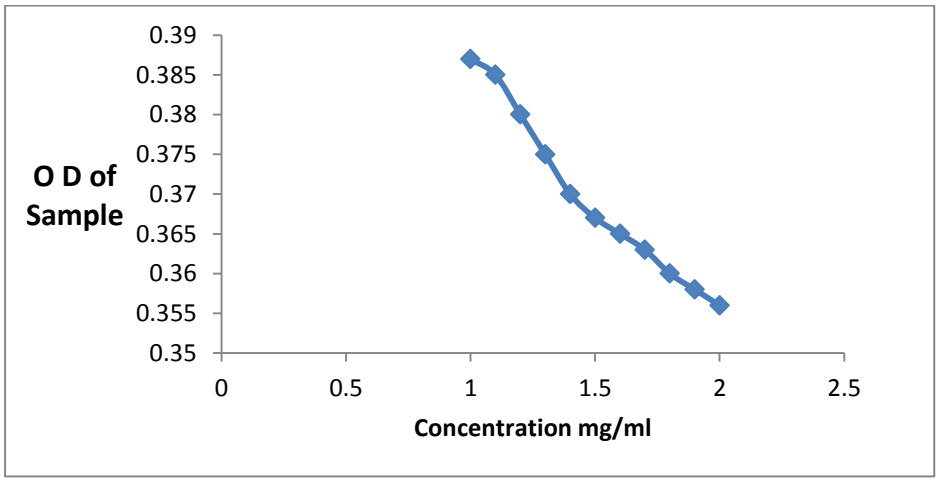

Figure 1: Decrease in O. D. of sample with increase in concentration of Ethanol Extracts

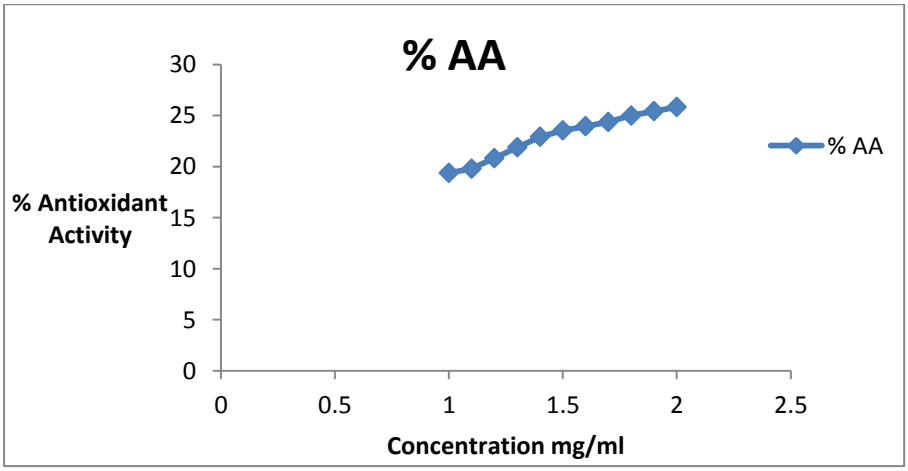

Figure 2: Increase in percent antioxidant activity with increase in concentration of Ethanol Extracts

Antimicrobial Assay[7]

The whole extracts of plant material in aqueous, ethanol and acetone solvents were screened for their antimicrobial potency by cup plate agar method against microbial species *viz. Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa*. The petriplates were prepared with 25ml sterile Mueller Hinton Agar. A sterile cork borer (8 mm) was used to make wells in each plate. 1 ml inoculums' suspension was swabbed uniformly over the agar medium to get uniform distribution of bacteria. After labelling the plates 100µl of each test compound (at concentration of 0.01 mol) was added aseptically into the wells. The petriplates were then incubated at 37°C for 24 hrs during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The negative control was prepared using respective solvent. *Ampicilin disc* (10 mcg/disc) and *Vancomycin disc* (30 mcg/disc) were used as positive control. The zones of inhibition were recorded in millimetres by using Himedia Zone Reader Scale.

Table 2: Antimicrobial activity of different extracts of the test plant

Sr. No.	Extracts	Concentration	Inhibitory zones in mm			
			Escherichia coli	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***
1)	Aqueous extract	100 mg/ml	12 mm	12 mm	10 mm	11 mm
2)	Ethanol extract	100 mg/ml	13 mm	11 mm	11 mm	12 mm
3)	Acetone extract	100 mg/ml	12 mm	10 mm	11 mm	13 mm
4)	*Ampicilin disc*	(10 mcg/disc)	14 mm	13 mm	14 mm	12 mm
5)	*Vancomycin*	(30 mcg/disc)	14 mm	16 mm	12 mm	13 mm

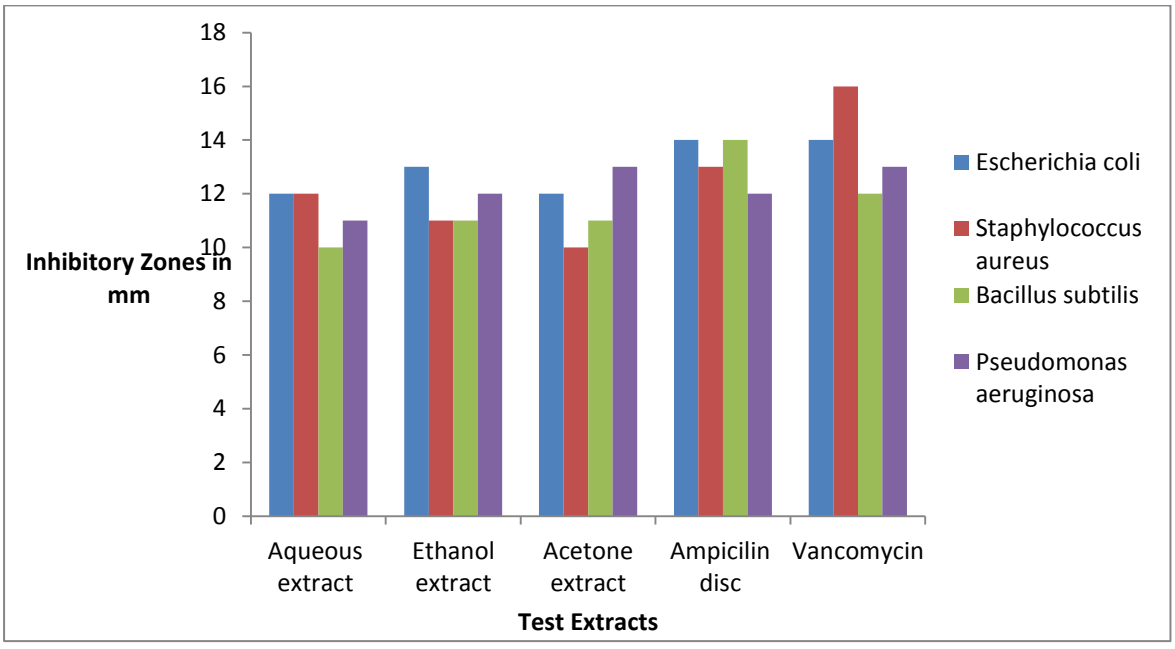

Figure 3: Antimicrobial activity of different extracts of the test plant

CONCLUSION

Remarkable decrease in the O. D. values of sample for three different solvent extract were observed indicating antioxidant activity of the fractions. Ethanol extract of the whole test plant showed good to moderate antioxidant activity which is evident from the graph. The IC_{50} value for ethanol extract is calculated from the figure 2 which is 1.4 mg/ml. The whole extracts of plant material in aqueous, ethanol and acetone solvents were screened for their antimicrobial potency which shows moderate to good activity as shown in figure 3.

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