Anti-oxidation and Anti-microbial efficiency of *Cardiospermum halicacabum* leaf

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Abstract

In this study we extract the total phytochemical substances from leaf of *C.halicacabum* using Petroleum ether, qualitatively identify the secondary metabolites in Petroleum ether leaf extract and extract the total Alkaloid content from the leaf of *C.halicacabum.* We studied the Anti Oxidant capacity of the Leaf extract and the Alkaloid by Invitro methods measuring Spectrophotometrically using Hydrogen peroxide Sacvenging activity, Super Oxide Dismutase auto oxidation inhibition and we determined the microbicidal potency of the leaf extract and the Alkaloid of *C.halicacabum* leaf by Well diffusion method for Antibacterial activity and Antifungal activity. In the present study we revealed that the petroleum ether leaf extract of *Cardiospermum halicacabum (Linn)* have contained Phytochemical constituents like Alkaloid, , Flavonoids, Glycosides, Phenol, Tannin and resin Extracts have showed significant Anti-oxidant activity - H2O2 and SOD activities both by the leaf extract and the Alkaloid. The Phytochemical analysis, antioxidant potential of *Cardiospermum halicacabum* showed positive in all aspects. This may be due to the presence of various compounds present in *Cardiospermum halicacabum* and this may be the source for the wide activity of the plant against such activities. The present study reveals the potentiality of the plant taken for the study and extensive study with a particular area of focus in future may form a basis for a new drug discovery.

Keywords: Herbals, *Cardiospermum halicacabum,* Antioxidant, Antimicrobial

INTRODUCTION

The herbal plants are the major sources of our nation which has many biomedical applications such as Antibacterial, antifungal, cardioprotective, hepatoprotective, cardioprotective,

antidiapetic (1-5), hypo lipedemic, anticancer activity against different types of cancer cell lines (6-11). The *Cardiospermum halicacabum* shows the antidiarrheal activity when compared with standard drugs due to the presence of tannins, flavonoids alkaloids, saponins, reducing sugars, sterols and triterpenes, this plant shows antidiarrheal activity. Tannins induce an antidiarrheal effect while the flavonoids hinder intestinal motility and hydro-electrolytic emission (12)

Cardiospermum halicacabum is a plant belongs to the family sapindaceae. It is a deciduous climber growing upto 3 meters. The ground stem carries alternate double triad leaves 3 to 6 cm long, the tiny radiate flowers. Stems are 5 to 10 cm in length. The fruits are tiny green baloon shaped; spherical capsule containing the characteristics seeds with their heart shaped white markings. It is used in the treatment of arthritis (13), nervous disease, stiffness of the limbs and snake bites. The leaves are applied as poultice in the treatment of rheumatism. The tea made from this is used in the treatment of itchy skin, salted leaves are used as poultice on swellings, and the leaf juice has been used as a treatment for earache. It is also used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite (14)

TAXONOMY

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae – green plants
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Rosanae
Order	Sapindales
Family	Sapindaceae – soapberries
Genus	*Cardiospermum* L. – balloonvine
Species	*Cardiospermum halicacabum* L. – balloo nvine

The main aim of this study is to investigate the Auto-oxidation and Anti-microbial efficiency of *Cardiospermum halicacabum* leaf.

MATERIALS AND METHOD

COLLECTION OF PLANT MATERIALS:

Cardiospermum halicacabum Linn. (Fam.Sapindaceae), commonly found as a weed throughout India. The plant leaves was collected during the month of Janauary Then it was dried in shade, powdered, weighed and stored in a clean, dry and airtight polythene pack. The powder was subjected for successive extraction in Petroleum ether at room temperature for 24 hours in closed condition.

PREPARATION OF EXTRACTS:

The dried leaf of *Cardiospermum halicacabum* Linn were subjected to extraction using the solvent -petroleum ether in increasing order of polarity. The prepared extracts were then subjected to preliminary phytochemical analysis.Th extract were filtered using filter paper and the filterate concentrated in an hot air oven at 45° C and the extract yield % calculated gravimetrically.

QUALITATIVE DETERMINATIO OF PHYTOCHEMICAL SUBSTANCES

DETECTION OF FLAVONOIDS:

ALKALOID:

Wagner's Test (Iodine in Potassium Iodide)

Treat the acid layer with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

TRITERPENOIDS (OR) TERPENOIDS:

Salkowski Test

To the extract few drops of concentrated H_2SO_4 and 2ml chloroform and shaken then allow standing, appearance of golden yellow colour indicates the presence of triterpenes.

ZINC-HYDROCHLORIC ACID REDUCTION TEST:

The alcoholic solution was treated with a pinch of zinc dust and few drops of concentrated hydrochloric acid. Formation of magenta colour after few minutes indicates the presence of flavonoids

DETECTION OF PHENOL COMPOUND:

Ferric chloride test: The extract (50 mg) was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenols.

DETECTION OF TANNINS:

Ferric chloride test: To extracts a few drops of 1% netural ferric chloride solution was added formation of blackish blue color indicatyes the presence of tannins.

GLYCOSIDES TEST:

Libermann's Test

To a 2g of extract, add 2ml of chloroform and concentrated acetic acid in an ice bath. And then add 2 drops of concentrated H_2SO_4. Formation of violet to the green colour presence of glycoside.

RESINS:

Dissolve extract in acetone and pour the solution in distilled water. Turbidity indicates presence of resins.

HYDROGEN PEROXIDE SCAVENGING EFFECT:

The scavenging activity of hydrogen peroxide by the petroleum ether leaf extract and Alkaloid of *C.halicacabum* was determined by the method of Ruch et al. (1989).

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Petroleum ether leaf extracts at the concentration of 10mg/10µl were added to 0.6ml of H_2O_2 solution. The total volume was made up to 3ml with phosphate buffer. The absorbance of the reaction mixture was recorded at 230nm. The blank solution contained phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging by the plant extracts was calculated as

$$\% \text{ scavenged hydrogen peroxide} = \frac{A_o - A_1 \times 100}{A_o}$$

Where,

Ao - Absorbance of control

A1 - Absorbance in the presence of plant extract

ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT (*C.halicacabum*)

PREPARATION OF PLATES:

For the method, Muller Hinton agar was prepared obtained from Himedia Mumbai LOT no. 0000102061 and sterilized 121ºC at 15 lbs pressure for 15 minutes. The sterile medium was poured in the petriplates and allowed for solidification.

PREPARATION OF CULTURE BROTH:

Bacterial samples respectively were inoculated in the sterile nutrient broth prepared obtained from Himedia Mumbai LOT no. 0000132645 and incubated for the overnight at 37ºC. The bacteria employed for the study include,

a. *Enterobacter faecalis MTCC 439*
b. Klebsiella pneumonia MTCC 9751
c. *Streptococcus pyogenes MTCC 1925*

The C.halicacabum leaf extract alone by well diffusion method using MHA as growth medium.The well were punctured aseptically and the extract with same concentration of the C.halicacabum leaf extract and Alkaloid used to indicate that the extract do not interfere bacterial inhibition (Figure 1).

ANTIFUNGAL ACTIVITY OF THE C.HALICACABUM LEAF EXTRACT:

PREPARATION OF PLATES:

For the method, Muller Hinton agar was prepared obtained from Himedia Mumbai LOT no. 0000102061 and sterilized 121ºC at 15 lbs pressure for 15 minutes. The sterile medium was poured in the petriplates and allowed for solidification.

PREPARATION OF CULTURE BROTH:

For fungal pathogen, Potato dextrose broth(Himedia) used and a loopful of the fungal pure isolate inoculated into the sterile broth and incubated at 28 ° C fro 48 hours and further used for the antifungal activity.

a. Aspergillus niger NCIM 1004
b. Mucor species NCIM 4563
c. Candida albicans NCIM 3100

The *C.halicacabum* leaf extract compared alone by well diffusion method using MHA as growth medium.The well were punctured aseptically and the extract with same concentration of

the *C.halicacabum* leaf extract used to indicate that the extract do not interfere bacterial inhibition.

The impregnated *C.halicacabum* leaf extract loaded plates were incubated at 37 °C for bacteria and 28°C for fungi overnight and the plates noted for its zone of clearance and measured using HiZone Inhibition scale (HIMEDIA) and the values recorded in mm.

RESULT

Figure-1: Leaf of *C.halicacabum*

Table-1: Determination of Moisture

Leaf (g)	Dry leaf (g)	Moisture%
128.5	39.9	88.6

Table-2: Yield % of *C.halicacabum* leaf extract

Powder(g)	Total weight(g)	Empty weight(g)	Extract(g)	Yield%
15	55.85	55.4	3	20

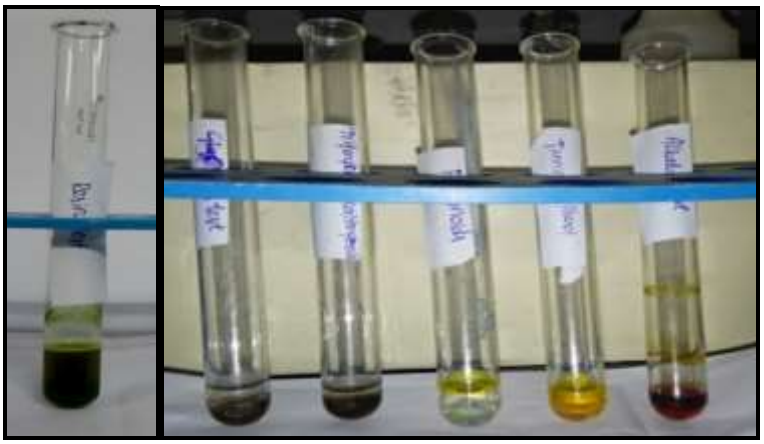

Figure-2: Screening test for phytochemical

Table-3: Phytochemical Screening – Leaf extract

Screening	Test	Inference
Alkaloid	Wagners Test	+++
Tannin And Phenol	Ferric Chloride Test	-
Flavonoids	Zine-Hcl Reduction Test	+
Triterpenids or Terpenids	Salkowshi Test	-
Glycosides Test	Libermanns Test	+
Rersin		++

Figure-3: Extraction of Alkaloid

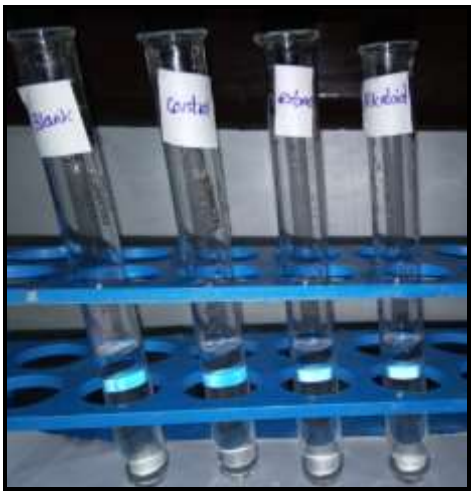

Figure-4: H_2O_2 Scavenging Activity of *C.halicacabum* leaf extract

Table-4: Determination of H_2O_2 Activity:

H_2O_2		
Preparation	**Absorbance, 230nm**	**% Inhibition**
Control	0.234	***
Extract	0.201	16.44
Alkaloid	0.017	92.72

Figure-5: Auto oxidation of SOD with *C.halicacabum* leaf extract

Table-5: Auto Oxidation capacity of *C.halicacabum* leaf extract:

SOD		**% Inhibition of Pyrogallol Auto-Oxidation**
	Absorbance, 270 nm	
Control	0.189	
Extract	0.168	
Alkaloid	0.154	
Total Extract		12
Oxidation-Alkaloid		18.6

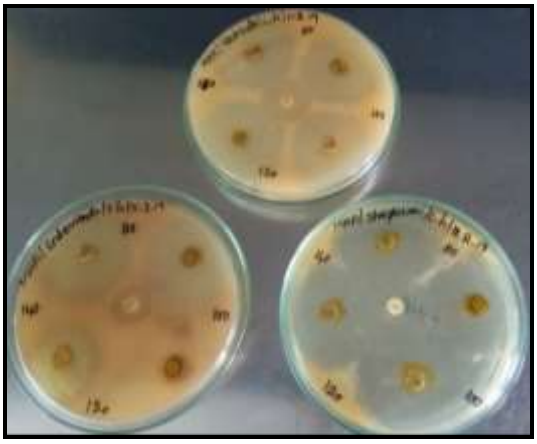

Figure-6: Antibacterial Activity of *C.halicacabum* leaf extract

Figure-7: Antifungal Activity of *C.halicacabum* leaf extract

Table-6: Antimicrobial Activity of *C.halicacabum* Petroleum ether Leaf extract:

Organism	Control	Concentration (mcg)				
		80	100	120	140	MIC(mcg/ml)
Klebsiella	27	38	40	40	43	38.3127
Enterobacter	20	45	10	25	40	255.495
Streptococci	20	40	39	40	45	40.332
C.albicans	10	22	25	29	22	45.6255
Mucor	13	23	26	22	25	45.8285
A.niger	13	25	30	25	25	44.7868

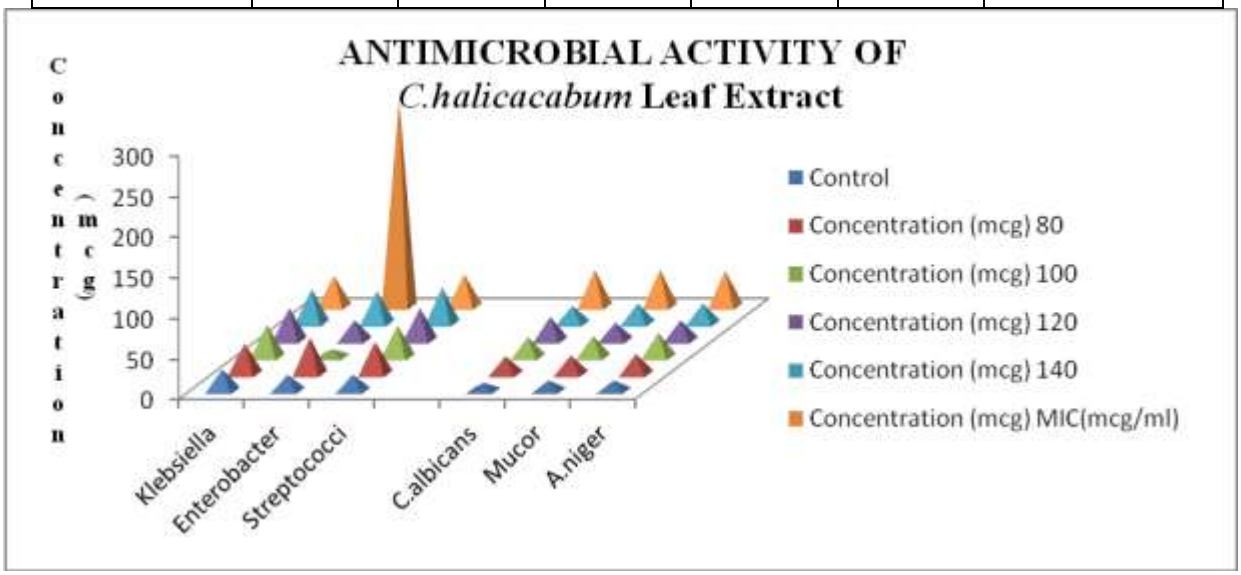

Figure-8: Antibiogram representation of *C.halicacabum* leaf extract

DISCUSSION

The leaf of *C.halicacabum* was extracted for its total phytochemical using petroleum ether as solvent and the yield % was calculated the 20g /100 g of the leaf powder and the moisture content found to be 88.60 % in the fresh leaf which tested for loss on drying immediately after collection. The petroleum leaf extract was identified for its potent active compounds qualitatively in which basic substances as Alkaloid, Flavanoid, Glycosides and Resin were present and Phenol, Tannin and terpenoids were absent in the petroleum ether extract, which have significant effect of the leaf in elucidating the different capacity as the study

revealed. Based on the qualitative screening the Alkaloid was extracted and used for further investigation in the study (Figure 2,3 and Table 1,2,3).

Figure 4,5 and Table 4 and 5 showed that *Cardiospermum halicacabum* extracts exhibited strong free radical scavenging activity and pyrogallol auto oxidation. Large quantity of compounds in *C.halicacabum* extract makes it a strong free radical scavenger, which indicates that the extract has good potential as a source for natural antioxidants to prevent free radical mediated oxidative damage. Naturally there could be few explanations for the decreases in both Hydrogen peroxide scavenges the free radical capacity of 16.44 % for the total extract and 92.72 % for Alkaloid and SOD antioxidant activity of the extracts showed 12 % and 18.6 % of auto oxidation of Pyrogallol during the process of drying that attributes the deactivation of the degradative enzymes.

The Figures 6-8 and Table 6 and 7 shows he anti microbial capacity of the petroleum ether leaf extract alone was evaluated for its microbicidal capacity by well diffusion method by applying both bacteria and fungi for the study. On bactericidal effect of the Leaf and Alkaloid the bacterium Streptococcus pyogenes exhibits a maximum of 45 mm and a MIC of 40 mcg/ml with the concentration of 28 mcg of leaf extract at 140 µl. Similarly the bacterium Klebsiella exhibited a inhibition zone of 43 mm with corresponding concentration of 28 mcg with an MIC of 38 mcg/ml and Enterobacter exhibited with a maximum MIC of 255 mcg/ml.

The fungicidal activity was evaluated to present a significant effect of 45 mcg/ml for C.albicans, Mucor and 44 mcg /ml for A.niger with a zone of Inhibition ranges from 22 mm to 25mm respectively. The plants extracts are widely used for many biomedical applications based on the antioxidant and antimicrobial properties, by using the same methods algae also used (15-20).

CONCLUSION

Further studies and investigation should be aimed at the isolation and characterization of the active principles responsible for the reported activities. This should include analysis of active principles by MS., I.R., N.M.R. spectroscopy, H.P.T.L.C and confirmatory studies like mechanism of action at cellular and anatomical levels in animal models. In this thesis, efforts are made to draw broad conclusions regarding some possible beneficial uses of the leaf extracts of

Cardiospermum halicacabum Linn.

The plant extractive studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The preset study verified the traditional use of *C. halicacabum* for human ailments and partly explained its use in herbal medicine as rich source of phytochemicals with the presence of tannins, phenols, saponins, steroids, flavinoids and terpenoids. Thus this plant can be utilized as an alternative source of useful drugs. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for industrial drug formulation.

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