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Studies on the Phytochemicals, Antibacterial, and Antioxidant Effects of *Cardiospermum helicacabum* Leaf Extracts

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Abstract: *Cardiospermum helicacabum* was investigated in terms of the qualitative and quantitative properties of the phytochemical components. *Cardiospermum helicacabum* aqueous, ethanolic, and hexane extracts were tested for antibacterial activity against various bacteria, including at dosages ranging from 300 µg to 500 µg, it inhibited the development of *Escherichia coli, Proteus mirabilis, Salmonella typhi,* and *Enterobacter aerogenes*. In another experiment, α-tocopherol was utilised as a control to evaluate the possible antioxidant effects of those extracts with those of another drug. The study of the test results revealed presence of tannins, alkaloids, flavonoids, and saponins in *Cardiospermum helicacabum*. An extract of ethanol with a concentration of 500 µg/ml revealed 61.16 per cent of the antioxidant activity whereas 500 µg/ml of α--tocopherol, which served as the standard reference demonstrated 76.86 per cent.

Keywords: *Cardiospermum helicacabum,* Phytochemicals, Antibacterial, Antioxidant, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Enterobacter aerogenes*

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Introduction

Plants and their extracts contain antibacterial characteristics that can treat infectious disorders *in vitro* (Sofowora, 1983). Antibiotic-resistant bacteria are becoming more common due to antibiotic use, which could render some antimicrobials worthless for treating bacterial illnesses (Tanaka *et al*., 2006). Folk and traditional medicines incorporate herbal therapy.

Thus, critical scientific knowledge to folk medicine is applying to cure bacterial infections. Medicinal herbs can heal mild infectious diseases. They may also produce novel, strong antibiotics for disease strains without resistance. Recently, plant-derived medicines and nutritional supplements have become increasingly popular. Researchers in ethnopharmacology, botany, microbiology, and natural product chemistry are searching the medicinal flora for infectious disease treatments. Genus *Cardiospermum* of Asteraceae grows throughout southern India. Tribal South Indians utilise this leaf as an antibacterial (Gurudeva and Yoganarasimhan, 2009). *Cardiospermum halicacabum*, the balloon plant or love in a puff, is a tropical and subtropical African and Asian climbing plant. Antidiarrheal and homoeopathic properties have been explored in this plant found along highways and waterways. In this study *Cardiospermum helicacabum* was investigated in terms of the qualitative and quantitative properties of the phytochemical components. *Cardiospermum helicacabum* aqueous, ethanolic, and hexane extracts were tested for antibacterial activity against various bacteria.

Materials and Methods

Plant extract preparation:

Cardiospermum helicacabum leaf used in this study was obtained in June 2010 from Thirukkalukundram village, Kanchipuram District, Tamil Nadu, South India. The plant sample was refluxed in running tap water for 1-2 h before being shade dried at room temperature for 15 - 20 days. Using the soxhlet apparatus, for roughly 24 h, an aqueous, ethanolic, and hexane extract of *Cardiospermum helicacabum* was obtained (Hoffman *et al*., 2004). With the addition of $CaCl_2$, the extract was distilled and concentrated *in vacuo*. Antifungal, antibacterial, and antioxidant activities of lyophilized aqueous fractions were also investigated.

Chemicals and Microorganisms:

IMTECH, Chandigarh, India, supplied *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, and *Enterobacter aerogenes*. Himedia, Merck, and S.D. Fine-Chemicals in Mumbai provided the solvent and other chemicals utilised in this investigation.

Methods:

The screening methodologies used for phytochemicals were adapted from Oloyed (2005). This examination identifies the biologically active chemicals that contribute to the flavour, colour, and other properties of leaves.

Test for alkaloids: The ground sample was pounded separately. 0.2 g was heated with 5 ml of 2% hydrochloric acid on a steam bath for 5 min. After cooling, the fluid was filtered and split equally into three test tubes labelled A, B, and C. Two drops of each reagent were added to 1 ml of filtrate. Dragendroff's precipitate was red. Alkaloid was detected by Mayer's reagent's creamy precipitate (Harborne, 1973; Trease and Evans, 1989).

Test for flavonoids: 0.5 g of macerated *Cardiospermum helicacabum* was boiled in water in 10 ml of ethyl acetate for 1 min. The filtrate was used in the next experiment after filtering the mixture. 4 ml of filtrate was combined with 1 ml of 1 per cent aluminium chloride solution. In 1 ml dilute ammonia solution, flavonoids turned yellow, indicating their existence (Harborne, 1973).

Test for saponins: 0.1 g of the sample was boiled for 5 min in 5 ml of distilled water. The filtrate was used for the following tests after the mixture was filtered while hot (Trease and Evans, 1989). The filtrates were stirred with 2 drops of olive oil to make an emulsion. 1 ml of filtrate was diluted with 4 ml of distilled water. The liquid was violently agitated before being checked for stable foam on a stand (Trease and Evans, 1989).

Test for tannins: 2 g of pulverized material was heated for 5 min with 5 ml of 45 per cent ethanol. After cooling, the mixture was filtered. 1 ml of filtrate was mixed with 3 drops of lead sub acetate solution. Tannins were found as gelatinous precipitates. 0.5 ml of bromine water

was added to 1 ml of filtrate. Tannins formed pale brown precipitates (Trease and Evans, 1989).

Test for glycosides: 2 g of the sample was mixed with 30 ml of distilled water and boiled for 5 min on a water bath before being filtered and used. 5 ml of the filtrate was mixed with 0.2 ml of Fehling solution A and B until alkaline, then heated in a water bath for 2 min. Glycosides were absent because the precipitate was light blue instead of brick red (Oloyed, 2005).

Quantitative Analysis of Phytochemical Constituents:

Estimation of alkaloids: 0.5 g was dissolved in 96 per cent ethanol-20 per cent H_2SO_4 (1:1). After 5 min, 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (VI). After 3 h, 5 ml of 0.5% formaldehyde was added. Absorbance was read at 565 nm (Harborne, 1976).

Estimation of flavonoids: The test sample's flavonoid content was measured by acid hydrolysis spectrophotometry. Heat 0.5 g of processed plant material in 5 ml of dilute HCl for 30 min. Filtration followed cooling. 5 ml of ethyl acetate and 5 ml of 1 per cent NH_3 were added to 1 ml of filtrate. Absorbance was read three times from 420 nm to 520 nm (Harborne,1976).

Estimation of saponins: 0.5 g material was heated for 4 h in 20 ml of 1N HCl. The ether layer was formed by adding 50 ml of petroleum ether to the filtrate and evaporating it. 5 ml of acetone ethanol was applied to the residue. In three test tubes, each received 0.4 ml. They received 6 ml ferrous sulphate reagent and 2 ml concentrated H_2SO_4. It was well mixed after 10 min and the absorbance was measured at 490 nm (Oloyed, 2005).

Estimation of tannins: 5 g of ground sample was agitated with 3 ml of methanol for 1 min in a test tube, it was put into a Buchner funnel with the suction on. The tube's contents were immediately emptied into the funnel after being cleaned with 3 ml of methanol. 50 ml of water and the filter were tested within 1 h. The filtrate was combined with 50 ml of water after aqueous extractions. To 5 ml of extract, 3 ml of 0.1 ml $FeCl_3$ in 0.1 NH_4Cl was added, followed by a timed addition of 3 ml of 0.008 ml $K_2Fe(CN)_6$. The absorbance was determined spectrophotometrically at 720 nm (Onwuka, 2005).

Antibacterial Effect:

Agar well diffusion was used to assess *Cardiospermum helicacabum*'s antibacterial properties (Chung *et al*., 1990). Petridishes were filled with Muller Hinton agar medium. A sterile cotton swab was used to inoculate the medium with a mid-log phase bacterial culture. With a sterile cork borer, wells were made in the agar medium (10 mm diameter). The wells were filled with the test material and incubated at 37°C for 24 h. The experiment was repeated five times under strict aseptic conditions. The organism's sensitivity was measured by the inhibitory zone diameter. The mean value was utilised to analyse each experiment, which was done five times. The control experiment includes antibiotics like streptomycin and chloramphenical (Shobana *et al.*, 2009).

Antioxidant Effect:

Aqueous, ethanolic, and hexane *Cardiospermum helicacabum* extracts were investigated for antioxidant activity using ferric thiocynate (Mistuda et al., 1996). 100, 200, 300, 400, and 500 µg/ml extracts were diluted in 99.5 per cent ethanol. A screw-top vial contained 2 ml of sample in 99.5 per cent ethanol, 2.052 ml of 2.51 per cent linoleic acid in ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0), and 1.948 ml of water. This sample solution received 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocynate. The absorbance of the red colour formed in 3 min was measured at 500 nm after adding 0.1 ml of 2 x 10-2 M ferrous chloride in 3.5 per cent hydrochloric acid to the reaction mixture (Mokbel and Hashinaga, 2005). The control and standard received solvent and the same amount of α-tocopherol (reference component), respectively (Yildirim *et al*., 2001). Lipid peroxidation suppression per cent was calculated as:

$$\% \text{ Inhibition} = 1 - (A1/A2) \times 100$$

Table 1: Qualitative analysis of different extracts of *Cardiospermum helicacabum* for Phytochemicals

S. No.	Name of the phytochemical	Different extracts of *Cardiospermum helicacabum*		
		Aqueous extract	Ethanolic extract	Hexane extract
1.	Alkaloids	+ve	+ve	+ve
2.	Flavonoids	+ve	+ve	+ve
3.	Saponins	+ve	+ve	+ve
4.	Tannins	+ve	+ve	+ve
5.	Glycosides	-ve	-ve	-ve

+ve – present; -ve - absent

Table 2: Quantitative analysis of *Cardiospermum helicacabum* for phytochemicals

S. No.	Name of the phytochemical	*Cardiospermum helicacabum*	
		Dry sample	Wet sample
1.	Alkaloids (mg/100g)	46.68 ± 3.21	21.39 ± 2.17
2.	Flavonoids(mg/100g)	69.33 ± 4.14	56.18 ± 3.19
3.	Saponins(mg/100g)	03.77 ± 0.54	01.21 ± 0.84
4.	Tannins(mg/100g)	02.37 ± 0.76	01.67 ± 0.46

Where, A1 - absorbance of the test sample; A2- absorbance control reaction.

Results and Discussion

Phytochemical Analysis:

Phytochemical analysis assesses medicinal plant bioactives. Dry and moist materials were examined qualitatively and quantitatively. Alkaloids, flavonoids, saponins, and tannins were found in *Cardiospermum helicacabum* (Table 1). This shows great medicinal and nutritional potential (Oloyed, 2005). Tannins, for example, are plant-specific but harmful to humans and livestock (Odebiyi and Sofowora, 1979). Antinutritional active components diminish feed palatability and digestibility (Odebiyi and Sofowora, 1979).

Table 2 illustrates phytochemical (bioactive) values. Moisture-free samples had higher bioactive chemicals. Non-volatile bioactive chemicals may have a high dry weight. The vegetable's biological antioxidant flavonoids (69.33 4.14 and 56.18 3.19) benefit cardiovascular health and oxidative stress. Singlet oxygen, super oxide, peroxyl, hydroxyl, and peroxynitrile are protected by antioxidants. Oxidative stress damages cells from antioxidant and reactive oxygen species imbalances (Burlon and Ingold, 1984). Oxidative stress causes cancer, atherosclerosis, inflammation, ischemic injury, and neurological disorders like Parkinson's and Alzheimer's (Palozza, 1998). Flavonoid, antioxidant vitamins, and enzymes may prevent some diseases. Epidemiological studies demonstrate flavonoids and carotenoids prevent coronary heart disease mortality and heart attacks (Donald and Cristobal,2006).

Antibacterial Activity:

As indicated in Tables 3, 4, and 5, the aqueous, ethanolic, and hexane extracts of *Cardiospermum helicacabum* inhibited development only at dosages of 300 µg to 500 µg. Aqueous *Cardiospermum helicacabum* extracts were more sensitive to *E. aerogens* and *P. mirabilis* than ethanolic and hexane extracts. *S. typi* showed moderate resistance to all extracts compared to others (Table 3, 4, 5). Ethanolic and hexane extracts of *Cardiospermum helicacabum* were less toxic to bacteria than water extract, even at higher concentrations.

Table 3: Antibacterial activity of aqueous extract of *Cardiospermum helicacabum*

Aqueous extract	Concentration in µg	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Cardiospermum helicacabum	100	NS	NS	NI	NI
	200	NS	10.1	NS	NS
	300	10.3	12.6	NS	10.2
	400	13.1	16.1	11.8	14.7
	500	16.5	19.3	15.6	19.7
Streptomycin	100	21.6	20.8	19.1	22.1
Chloramphenicol	100	22.3	19.6	20.7	19.8

NI—No inhibition; NS – Non significant value (<10mm)

Table 4: Antibacterial activity of ethanolic extract of *Cardiospermum helicacabum*

Aqueous extract	Concentration in µg	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Cardiospermum helicacabum	100	NS	NI	NI	NS
	200	NS	NS	NI	NS
	300	12.7	11.2	10.2	11.1
	400	14.6	13.7	11.6	13.4
	500	15.3	16.2	14.4	16.3
Streptomycin	100	20.8	19.5	18.9	19.1
Chloramphenicol	100	20.1	18.6	19.3	20.7

NS – Non significant value (<10mm); NI – No Inhibition

Table 5: Antibacterial activity of hexane extract of *Cardiospermum helicacabum*

Aqueous extract	Concentration in µg	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Cardiospermum helicacabum	100	NI	NI	NI	NS
	200	NS	NI	NS	NS
	300	11.5	10.4	10.8	10.3
	400	13.1	12.8	12.3	11.9
	500	16.2	14.7	15.7	14.2
Streptomycin	100	19.9	20.1	18 .9	21.4
Chloramphenicol	100	19.1	19.9	20 .1	20.1

NS – Non significant value (<10mm); NI – No Inhibition

Table 6: Antioxidant activity of aqueous, ethanolic and hexane extracts of *Cardiospermum helicacabum*

S. No.	Extract	% of inhibition of lipid peroxidation				
		100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
1.	Water	12.31	21.12	35.73	45.34	57.75
2.	Ethanol	18.17	24.63	43.08	50.67	61.16
3.	Hexane	14.71	20.32	28.63	39.34	48.55
4.	α-Tocopherol	27.43	39.71	47.87	62.78	76.86

Antioxidant Activity:

Table 6 reveals *Cardiospermum helicacabum* aqueous, ethanolic, and hexane extract ferric thiocynate (FTC) antioxidant activity testing. The FTC method measured peroxide, which forms crimson ferric chloride ($FeCl_3$) with ferrous chloride ($FeCl_2$). Extract antioxidant activity reduces peroxide concentration. Aqueous, ethanolic, and hexane extracts at 100,200, 300,400, and 500 µg/ml demonstrated concentration-dependent antioxidant activity. Ethanol extract demonstrated 61.16% antioxidant activity and 76.86% α-tocopherol at 500 µg/ml. *Cardiospermum helicacabum* aqueous and hexane extracts inhibited lipid peroxidation. And increased antioxidant activity.

Conclusion

This study showed the antibacterial and antioxidant effects of leaf extracts of *Cardiospermum helicacabum*. The plant was investigated for the qualitative and quantitative properties of the phytochemical components. To understand such a characteristic, the responsible compounds present in the extract need to be isolated and studied further.

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