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# IN VITRO ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF *CARDIOSPERMUM HALICACABUM* L.

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**Abstract**

The present studies designed as *In vitro* antimicrobial and phytochemical activity of whole plant of *Cardiospermum halicacabum*. The extracts and seed oil exhibited antibacterial activities with zones of inhibition ranging from 7mm to14mm for ethanolic, ethylacetate extracts and seed oil, 7mm to 10mm for butanolic and 7mm to 9mm for aqueous extracts against the gram positive and gram negative bacterial strains. Crude ethanol, aqueous extracts and seed oil exhibited appreciable fungal activity against, *Candida albicans* while *Aspergillus niger* was only active against ethanolic extract with significant zone of inhibition 18mm. phytochemical analysis revealed the presence of tannins, saponins terpenes and sugar in the crude extract.

## Introduction

Green plants are the symbol of a reservoir of resourceful chemotherapeutics that provide important source of natural antimicrobials (Balandrin *et al*., 1985; Satish *et al*., 1999). *Cardiospermum halicacabum* L. is one of the members of soapberry family, *Sapindaceae*. It is an herbaceous climber widely distributed in tropical and subtropical regions. It is originated all through the plains of Africa, America, Bangladesh, India, Malacca and Pakistan. Common names are balloon vine, heart vine, heart pea, love-in-a-puff, and heart seed. The whole plant is diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific in folk. It is also used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite (Joshi *et al*., 1992; Gopalkrishnan *et al*., 1976; Chopra *et al*., 1980; Nadkarni, 1976; Abulla, 1973; Jafri, 1966). Variety of chemical constituents have been isolated from its *Viz.* β- arachidic acid, apigenin, apigenin-7-O-glucuronide, chrysoeriol-7-O-glucuronide and 80 luteolin-7-O-glucuronide (Khan *et al*., 1990; Subramanyam *et al*., 2007). Number of fatty acids were also isolated from seed oil (Chisholm & Hopkins, 1958).The plant was reported as antiulcer (Sheeba *et al*., 2006), analgesic (Muthumani *et al*., 2010), antiparasitic (Boonmars *et al*., 2005), antimalarial (Wakko *et al*., 2005), antifilarial (Khunkitti *et al*., 2000), and antipyretic action (Asha & Pushpangadan, 1999). Plants have been used as curative mediator from the most primitive day of human's survival (Shellard, 1987) and made it obligatory to study them in details in order to classify the kinds, working for different purposes (Ghani, 1986). Therefore, in present study we were screen the whole plant for its microbial and preliminary phytochemical analysis.

## Materials and Methods

**Plant material:** *Cardiospermum halicacabum* plant was cultivated in the Pharmacognosy medicinal plant garden adjacent to the Research Institute of Pharmaceutical Sciences in December 2003, whole plant was collected during June 2004. The plant was recognized by Prof. Dr. Ghazala H. Rizwani, Faculty of Pharmacy, University of Karachi, while the Voucher specimen No.036 was deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

**Preparation of extract:** Air dried whole plant (2Kg) was roughly powdered and percolated in ethanol at room temperature, for 20 days. The filtrate was filtered through Wahtman No.1 filter paper and concentrated on rotary evaporator under controlled conditions (Eyela, Japan). Semisolid brown ethanol extract (25.2gm) was subsequent separated first with water and then through 2 organic solvents, ethyl acetate and presaturated butanol. The filtrates were concentrated as above and yielded as ethyl acetate (11.4gm), butanol (5.2 gm) and aqueous (7.9 gm) extracts.

**Phytochemical analysis:** Ethanol extract of plant was examined for the presence of secondary metabolites including tannins, alkaloids, saponins, steroids, triterpenes, sugar and flavanoids in accordance to the (Shareef *et al*., 2010.)

**Test organisms**: Microorganisms were obtained from the Laboratory of Microbiology, Department of Microbiology, University of Karachi, Pakistan. a. Gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus aureus*AB188, *Staphylococcus epidermis*, *Streptococcus pyrogenes, Streptococcus fecalis*, *Bacillus cereus, Bacillus subtilis*, *Bacillus steadher*, *Micrococcus luteus*, *Corynebacterium xerosis, Cornebacterium hofmanu, Phenmococci.* b. Gram negative bacteria: *Shigella boydii, Shigella dysenterae, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Shigella flexneri, Proteus mirabilis, Proteus vulgaris, Escherichia coli, Klebsiella pneumoniae, Enterobacter, Pseudomonas aerogenosa.*

Three different types of pathogens were used for antifungal activity:

**a. Human pathogens:** *Trichophyton longifusis, Trichophyton tonsurans, Aspergillus flavus, Aspergillus niger, Candida albicans*.

**b. Animal pathogens:** *Micosporum canis, Micosporillum gypsiccus, Trichophyton mentagrophyte*.

**c. Plant pathogens:** *Fusarium* sp., *Rhizopus* sp., *Saccharomyces cervisiae, Pencilllium* sp.

**Antibacterial assay:** Well diffusion technique described by (Farrukh *et al*., 2008) was used to examine the antibacterial activity. Ampicillin and Amoxicillin were used as a standard drug. The medium was nutrient agar and plates were inoculated with 18-24 hrs old bacterial culture having approximately $10^8$-$10^6$ colony forming unit (CFU/ml). The zone of inhibition of bacterial growth was calculated and compared with the standard.

**Antifungal activity:** Antifungal activity of plant extract was carried out according to Mahmud *et al*., 2009 by agar dilution method. Miconazole, Amphotericin B and Benlate were used as standard drugs. The culture of organisms was grown on Sabouraud dextrose agar (SDA). The broth was incubated at 37°C for 24 hours. Inoculums were prepared by dilution of 24 hours old culture in saline. The zone of inhibition of fungal growth was measured and compared with standard drugs.

**Result and Discussion**

Natural crops either extract or pure compounds provide infinite prospects for the progress of new drugs due to availability of chemical diversity (Cos *et al*., 2006). Preliminary phytochemical analysis of whole plant extract of *Cardiospermum halicacabum* revealed the presence of tannins, saponins, sterols and sugars which are shown in Table 1. The antimicrobial activities of plant extracts have created the starting point of many applications, including raw and processed food formation, pharmaceuticals, alternative medicine and natural therapies (Hammer *et al*., 1999). The study represents the antimicrobial activity of different fractions and oil of *Cardiospermum halicacabum* against twelve Gram positive and negative bacterial strains. In case of gram positive bacteria the ethanolic fraction showed significant activity against *Staphylococcus epidermis* and *Streptococcus fecalis with* zone of inhibition 14.0 and 13.0mm respectively. While the same extract also represent moderate activity against remaining gram positive strains i.e., *Staphylococcus aureus, Staphylococcus aureus AB188, Micrococcus luteus* and *Corynebacterium hofmanu.* The most pronounced activity with inhibition zones 14.0, 11.0 and 11.0mm was shown by ethyl acetate extract against *Streptococcus fecalis, Staphylococcus epidermis* and *Micrococcus luteus.* Among tested strain the left over showed restrain inhibition with the similar extract against *Staphylococcus aureus AB188, Bacillus steadher* and *Pnemococci.* The butanol fraction showed strong antimicrobial activity against *Micrococcus luteus* with inhibition zones 10.0mm and moderate activity against *Staphylococcus epidermis* and *Streptococcus fecalis.* The aqueous fraction exhibited modest antimicrobial activity against *Staphylococcus epidermis, Streptococcus fecalis, Micrococcus luteus* and *Pnemococci.* While the oil of *Cardiospermum halicacabum* also showed antibacterial activity against almost all tested gram positive bacterial strains but showed significant activity against *Streptococcus fecalis, Bacillus cereus, Bacillus steadher* with zone of inhibition 10.0 mm for each and 14.0 mm inhibition zone against *Micrococcus luteus*. The oil showed significant activity against *Shigella boydii, Salmonalla typhi, Klebseilla pneumonia* with zone of inhibition 10 mm each and *Enterobactor* showed slight highest value i.e 14mm. In case of gram negative bacteria the ethanolic extract showed pronounced activity against *Salmonella typhi, Salmonella paratyphi B, Klebsiella pneumonia* and *Enterobacter.* The ethylacetate fraction showed significant activity against *Klebsiella pneumonia* with zone of inhibition 10mm and considerable antibacterial activity against *Shigella boydii, Salmonella typhi,* and *Enterobactor.* The butanol fraction of *Cardiospermum halicacabum* represents strongest antibacterial activity against *Salmonella paratyphi B* and *Enterobactor* having inhibition zone 10 mm. While the same fraction also showed modest activity against *Salmonella typhi, Salmonella paratyphi A* and *Klebsiella pneumonia*. Among all the tested fractions of *Cardiospermum halicacabum*, the aqueous fractions represent moderate antimicrobial activity against *Shigella boydii, Shigella dysentery, Salmonalla paratyphi B, Protus vulgaris* and *Klebseilla pneumoniae.* The different cultures responded to standard antibiotics in a variable manner, resulting in zones of inhibition of 11.5-17mm. The result revealed that the ethanolic fraction and oil of plant was almost equally effective against some of the gram positive bacterial strains as compared to standard antibiotics (Ampicillin and Amoxicilline). The oil of *Cardiospermum halicacabum* was also nearly effective against some gram negative bacterial strains as compared to reference antibiotics (Table 2).

**Table 1. Preliminary phytochemical analysis of *Cardiospermum halicacabum* Linn.**

| Tests | Color indication | Observations |
|---|---|---|
| **Tannins** | | |
| (a) Ferric chloride test | (a) Bluish black color | + |
| (b) Lead acetate test | (b) Precipitation | + |
| **Saponins** Frothing test | Frothing persistence for 5-7 min | - |
| **Flavonoids** Conc.Hydrochloric acid | Pink tomato red color | - |
| **Terpenes/Sterols** Libermann's Burchard test | Bluish green ring for steroids and pinkish purple ring for triterpenoids | + |
| **Alkaloids** Dragendorff's reagent | Orange precipitate | - |
| **Ketones-** 40% sulphuric acid | Bottle green color | - |
| **Sugars** α –naphthol | Purple color | + |

- = Absence, + = Presence

**Table 2. Antibacterial activity of plant *Cardiospermum halicacabum* Linn., and its fractions.**

| Bacterial strains | *Cardiospermum halicacabum* Linn. | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | Standard drugs | |
| | CH | EtoAc | BuOH | Aqe. | Seed oil | Ampicillin | Amoxicillin |
| **a. Gram positive bacteria** | Zone of inhibition (mm) | | | | | | |
| *Staphylococcus aureus* | 10 | 7 | - | 7 | 7 | 12 | 11.5 |
| *Staphylococcus aureusAB188* | 10 | 9 | 7 | 7 | 7 | 12 | 12 |
| *Staphylococcus epidermis* | 14 | 11 | 9 | 9 | 8 | 16 | 15 |
| *Streptococcus pyogenes* | 7 | 7 | 7 | 7 | 8 | 13 | 12 |
| *Streptococcus fecalis* | 13 | 14 | 9 | 9 | 10 | 15 | 16 |
| *Bacillus cereus* | 7 | 7 | 7 | 7 | 10 | 12 | 14 |
| *Bacillus subtilis* | - | - | - | - | 9 | 13 | 12 |
| *Bacillus steadher* | - | 9 | - | - | 10 | 12 | 13 |
| *Micrococcus luteus* | 9 | 11 | 10 | 9 | 14 | 15 | 16 |
| *Corynebacterium xerosis* | - | - | - | - | - | 11 | 12 |
| *Corynebacterium hofmanu* | 9 | - | 8 | 7 | 9 | 11 | 11 |
| *Pnemococci* | 7 | 9 | - | 9 | 7 | 13 | 11 |
| **b.Gram negative bacteria** | | | | | | | |
| *Shigella boydii* | 7 | 9 | 7 | 9 | 10 | 11 | 13 |
| *Shigella dysenterae* | - | - | - | 9 | - | 11 | 10.5 |
| *Salmonella typhi* | 9 | 9 | 9 | 8 | 10 | 11.5 | 11 |
| *Salmonella paratyphi A* | - | - | 9 | 8 | 7 | 12 | 11 |
| *Salmonella paratyphi B* | 9 | 7 | 10 | 9 | 7 | 12 | 11 |
| *Shigella flexneri* | 7 | 8 | - | - | - | 10 | 10 |
| *Proteus mirabilis* | - | - | - | 7 | - | 11 | 11 |
| *Proteus vulgaris* | - | - | - | 9 | - | 12 | 10 |
| *Escherichia coli* | 7 | 7 | - | - | 9 | 12 | 11 |
| *Klebsiella pneumoniae* | 9 | 10 | 9 | 9 | 10 | 13 | 11.5 |
| *Enterobacter* | 9 | 9 | 10 | 7 | 14 | 16 | 17 |
| *Pseudomonas aerogenosa* | - | 8 | 7 | 7 | 9 | 11 | 12 |

- = No activity, CH = Ethanolic extract of whole plant, EtOAc = Ethyl acetate extract, BuOH = Butanol extract, Aqe. = Aqueous extract

The significant antifungal activity revealed. Ethanol extract was found 33.33mm against *Saccharmyces cervisiae* and 18 mm next to *Aspergillus niger* but in case of *Candida albicans* ethanol, aqueous and oil extracts recorded a moderate activity 15mm, 13mm and 13mm inhibition zone respectively. Seed oil is also active against animal pathogens with 12mm and 13mm beside the *Micosporillum gypsiccus* and *Trichophyton mentagrophyte* Table 3. The activity of the fungal strains against the *Cardiospermum halicacabum* is significant due to the involvement of fungi in the development of opportunistic infections in case of immunosuppressant patients (Portillo *et al*., 2001).

Finally it is concluded that the whole plant extract of *Cardiospermum halicacabum* L. may possibly be a great potential source of active antimicrobial agents due to the presence of number of chemical constituents which can be the part of new and novel bioactive compounds.

**Table 3. Antifungal activity of plant *Cardiospermum halicacabum* Linn., and its fractions.**

| Fungal strains | *Cardiospermum halicacabum* Linn. | | | | | |
|---|---|---|---|---|---|---|
| | CH | EtoAc | BuOH | Aqueous | Seed oil | Standard drug |
| **a. Human pathogens** | Zone of inhibition | | | | | |
| *Aspergillus flavus* | - | - | - | - | - | Amphotericin B |
| *Aspergillus niger* | 18 | - | - | - | - | Amphotericin B |
| *Candida albicans* | 15 | - | - | 13 | 13 | Miconazole |
| *Trichophyton longifusis* | - | - | - | - | - | Miconazole |
| *Trichophyton tonsurans* | - | - | - | - | - | Miconazole |
| *Micosporum canis* | - | - | - | - | - | Miconazole |
| **b. Animal pathogens** | | | | | | |
| *Micosporillum gypsiccus* | - | - | - | - | 12 | Miconazole |
| *Trichophyton mentagrophyte* | - | - | - | - | 13 | Miconazole |
| **c. Plant pathogens** | | | | | | |
| Fusarium sp. | - | - | - | - | - | Benlate |
| *Rhizopus* sp. | - | - | - | - | - | Benlate |
| *Saccharomyces cerevisiae* | 33.33 | - | - | - | - | Benlate |
| *Penicillium* sp. | - | - | - | 7 | - | Miconazole |

- = No activity, EtOH = Ethanolic extract, EtOAc = Ethyl acetate extract, BuOH = Butanol extract, Aqe. = Aqueous extract

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