



PRELIMINARY PHYTOCHEMICAL AND WOUND HEALING ACTIVITY OF *CARDIOSPERMUM HELICACABUM LINN*

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(Received : 23.07.2014; Revised : 02.08.2014; Accepted : 04.08.2014)

ABSTRACT

The present study deals with preliminary phytochemical and pharmacological investigation of *Cardiospermum helicacabum* (Linn). The stem were subjected to soxhalation using petroleum ether alcohol, water, chloroform, acetone and the extracts thus obtained were studied for preliminary phytochemical screening various classes of chemicals viz, Tannin, Flavonoids, Terpenoids, cardiac glycosides, steroids and phenols, pharmacological studies were carried out to evaluate the stem extracts of *Cardiospermum helicacabum* Linn. The *Cardiospermum helicacabum* Linn stem extract alcohol, *Cardiospermum helicacabum* Linn stem alcohol extract showed very good-wound healing activity when compared to the standard drug Soframycine. The present study emphasis will be laid on the pharmacological screening of the plant with special reference to the wound healing activities.

Key words: *Cardiospermum helicacabum*, Phytochemical, Pharmacological, Wound healing activity.

INTRODUCTION

Plant are integral part of nature. Nature reflects the creative power of living god. Plants have an almost endless variety of uses to human beings. India is birth place of indigenous medicine such as sidda, Ayurveda and unani. It is enriched with flora and fauna and therefore the plants have been used since ancient times for treatment of human ailments. Wound healing is a process that is fundamentally a connective tissue response. The initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules, which are later remodeled to form a scar¹. The plants is well known for its antioxidant and anti-inflammatory activity². Other uses of the plant are against syphilis, leucorrhoea, bronchitis, chronic rheumatism, urinary diseases, leprosy, leucoderma and as purgative, diaphoretic diuretic, antipyretic and ant diarrheal^{3,4}. It has also been used in combination with other drugs for snake bite⁵. The plant is used in traditional medicine for biliousness, respiratory disorders, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite and burning sensation^{6,7}. Medicinal herbs are moving from fringe to mainstream use with greater number of people seeking remedies and health benefits free from side effects. Recently, considerable attention has been paid to utilize eco-friendly and

bio-friendly plant based products for the prevention and cure of different human diseases including microbial infection⁸. Biological studies are very much essential to substantiate the therapeutic properties of medicinal plants and drugs mentioned in Ayurveda on scientific lines. The plant kingdom represents an extraordinary reservoir of novel molecules. The potential of higher plants, as source for new drug is thus still largely unexplored⁹. Bio-technologists suggested the “use of cell and tissue culture technology rather than to use the whole plant” for the extraction of certain secondary metabolites¹⁰. The use of plants by man to treat common ailments is time immemorial and many of the traditional medicines are still included as part of the habitual treatment of various maladies¹¹, about 60% of the total global population remaining dependent on traditional medicines for their health care system¹², in India thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times¹³. Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in the modern medicine^{14,15}. It is well known that even the most synthetic drugs have their origin from plant products¹⁶. In the present study, stem of *Cardiospermum helicacabum* were selected to assess the wound healing activity.

EXPERIMENTAL

Material and methods

Plant material

The roots of *Cardiospermum helicacabum* was collected during December 27 from Tirupati Andhra Pradesh state, India. The samples were authenticated by Dr. Madhava chetty, Assistant Professor of Botany, S.V. University College, Tirupati, India.

Preparation of plant extracts

The fresh stem are used for extraction the stem were extracted by 12 hrs with petroleum ether, 24 hrs chloroform, acetone, ethanol, and water, by hot continuous using Soxhlet apparatus, the extract and fraction were concentrated in a rotary evaporator at reduced pressure.

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedure described by 100 gm of powdered stem material was subjected to successive solvent extraction using petroleum ether, chloroform, acetone, ethanol, and water based on increasing polarity using soxhlet apparatus. The extracts were concentrated under vacuum using rotary vacuum evaporator; dried and weighed each extract was tested for the presence of phytoconstituents viz flavonoids, terpenoids, steroids, phenols, and cardiac glycosides

Test for Flavonoids

5.0 mL of the dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated H₂SO₄. A yellow coloration observed in extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Test for steroids

2.0 mL of acetic anhydride was added to 0.5 g ethanol extract of sample with 2 mL H₂SO₄. The colour changed from violet to blue or green in sample indicating the presence of steroids.

Test for cardiac glycosides

1.0 mL of plant extract was treated with 1 mL of glacial acetic followed by treatment of 1 drop of 5% ethanolic ferric chloride solution. After this, added 1 mL of concentrated H₂SO₄ poured along the sides of the test tubes. Appearance of a brownish ring between the two formed layers with lower acidic layer turning blue green upon standing indicated the presence of cardiac glycosides.

Test for terpenoids

5.0 mL of plant extract mixed in 2 mL of chloroform, and concentrated sulphuric acid (3 mL) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Tests for Tannins and Phenolic Compounds:

- (i) To 2-3 mL of aqueous or alcoholic extract, add few drops of following reagents:
- (ii) 5% FeCl₃ solution: Deep blue-black colour is observed.
- (iii) Lead acetate solution: White precipitate is observed.
- (iv) Gelatin solution: White precipitate is observed.
- (v) Acetic acid solution: Red colour solution is observed.
- (vi) Potassium dichromate: Red precipitate is observed.
- (vii) Dilute iodine solution: Transient red colour is observed.
- (viii) Dilute HNO₃: Reddish to yellow colour is observed.
- (ix) Dilute NH₄OH and potassium ferricyanide solution: red colour solution is observed.
- (x) One drop NH₄OH, excess 10% AgNO₃ solution in a test tube. Heat for 20 min. in boiling water bath. White precipitate is observed, then dark silver mirror deposits on wall of test tube.
- (xi) Dil. Potassium permanganate solution: Deceleration of the solution is observed.

RESULTS AND DISCUSSION

Physicochemical analysis

Air dried stem material was made into fine powder and used for quantitative determination of physicochemical values. The powder fluorescence analysis of powder was carried out with different chemical reagents and observed under UV short, long and visible light. The physical properties and nature of extracts prepared by successive extraction method are recorded.

Table 1: Colour & consistency of *cardiospermum helicacabum* Linn. stem extracts

S. No.	Extracts	Colour	Consistency	Yield % w/w
1	Ethanol	Green	Amorphous	18.2
2	Chloroform	Orange	Flakes	19.4
3	Acetone	Light green	Amorphous	17.5
4	Pet ether	Light yellow	Amorphous	12.5
5	Water	Light yellow	Sticky	22.5

Table 2: Behavior of stem powder on treatment with different chemical reagents

S. No.	Reagent	Long (366 nm)	Short (265 nm)	Day light
1.	Powder + 50% H ₂ SO ₄	Black	Black	Brown colour
2.	Powder + 50% HNO ₃	Black	Red	Yellowish brown
3.	Powder + 5% NaOH	Gray	Yellow	Yellow
4.	Powder + 5% KOH	Dark Black	Whit	Pal Yellow
5.	Powder + Methanol	–	Yellow	Whit
6.	Powder + Con H ₂ SO ₄	–	Black	Yellow
7.	Powder + Ammonia	Black	White	Brown
8.	Powder + Con HNO ₃	–	Green	Brown
9.	powder + Con HCl	–	Light Brown	Gray
10.	Powder + FeCl ₃	Yellow	Green	Orange

Phytochemical screening was carried out to assess the qualitative chemical composition of stem extracts using commonly employed precipitation and coloration to identify the major natural chemical groups such as tannin, flavonoids, terpenoids, cardiac glycosids, steroids, phenols. General reactions in these analysis revealed the presence or absence of these compounds in the stem extracts tested. Phytochemical evolution of the extracts of the stem of *Cardiospermum helicacabum* Linn were done for the presence of tannin, flavonoids, terpenoids, cardiac glycosides, steroids, phenols, results were presented in the Table 3.

Table 3: Results of phytochemical screening of stem extracts of *Cardiospermum helicacabum* Linn.

S. No.	Test	Acetone	Chloroform	Ethanol	Pet ether	Water
1	Tannin	+	+	+	–	+
2	Flavonoids	–	+	+	–	–
3	Terpenoids	–	+	–	–	–
4	Cardiac glycosides	–	+	+	–	–
5	Steroids	+	+	+	+	–
6	Phenols	+	+	+	+	+

Pharmacological screening

Experimental animals

Adult rats of both strains (Albino and Wister) of either sex weighing 170-210 g were obtained from the animal house. Ethical Committee clearance was obtained from IAEC (institutional Animal ethics committee) of CPCSEA, Ref. No./IAEC/XII/06/SIPS/2011-2012. The animals were placed in a controlled room, in which temperatures were maintained at 25 ± 3 and 35-60% humidity. Normal rat feed and water was provided at regular intervals. All the animals were housed in polypropylene cages having a measurement of 43 x 27 x 15 cm. The animals were acclimatized to laboratory conditions before experimental procedures were started.

The experimental animals were anaesthetized using lignocaine 2% injection over the local selected region. The rats were depilated over the region excision wound was infected by cutting a way of 5 mm square thickness of skin from the predetermine area, the wound was left and rest to the open environment then the drugs reference standard (0.01% w/w Soframycine ointment) control (simple ointment BP) only *Cardiospermum helicacabum Linn* extract stem in methanol extract is applied. (10% w/w simple ointment) were applied till the wound was healed. This model was used to monitored by calculating the decreasing area.

General formula as follows:

$$\text{RWH} = \frac{\text{Size of wound in surface area (mm}^2\text{) at Day 9}}{\text{Size of Wound in surface area (mm}^2\text{) at Day 1}} \times 100\% \text{ Reduction in Healing} = 100 - \text{RWH}$$

The effect of topically applied *Cardiospermum helicacabum Linn* Stem and root extract ointment on excision wound of mice is shown in Table 4.

The present work is the pharmacological studies on the extracts of *Cardiospermum helicacabum Linn*. The soxhalet extraction procedure carried out using coarse dried stem with by successive solvent petroleum ether, chloroform, ethanol, acetone and water. The preliminary chemical analysis indicates the presents of alkaloids, glycoside and phytosterols.

The wound healing activity was studied by using four groups. The groups are, Group I negative control simple ointment, In Group II positive control Soframycine. 0.01% w/v, Group III CHCE and Group IV CHSE.

The size of the wound in surface area, On the Day 1 (45.25) (45.44) (46.24) (45.25). On the Day 3 (45.25) (22.16) (30.42) (30.42). On the Day 5 (45.25) (12.24) (22.24) (22.24). On the Day 7 (25.42) (2.14) (10.14) (10.14). On the Day 9 (32.42) (0.585) (2.14) (2.14). The mean percentage closure of excision wound model on Day 9 (28.35) (98.71) (95.37) (95.27).

Contraction of the excision wound was promoted from Day 1 of the treatment till Day 9. The epithelization of wound in case of mice treated with extract was found to be quite earlier than control. It is also comparable with the marketed preparation. It suggests than the stem extract of *Cardiospermum helicacabum Linn* promoted wound healing activity. The excision wound model showed significant wound healing property of the stem extract of *Cardiospermum helicacabum Linn*, which was well compared with standard drug. The results are shown in Table 4.

Table 4: Effect of topically applied *Cardiospermum helicacabum Linn* Stem extracts on excision wound in mice

Group	Avg. wt	Drug/ formulation	Size of wound in surface area day 0 (mm ²)	Day 1 (mm ²)	Day 3 (mm ²)	Day 5 (mm ²)	Day 7 (mm ²)	Day 9 (mm ²)	Percent age of wound healing
I	170-210 g	Control	45.25	45.25	45.25	45.25	25.42	32.42	28.35
II		Soframycine	45.44	45.44	22.16	12.24	2.14	0.585	98.71
III		CHCE	46.24	46.24	30.42	22.24	10.14	2.14	95.37
IV		CHSE	45.25	45.25	30.42	22.24	10.14	2.14	95.27

CHCE: *Cardiospermum helicacabum Linn* Chloroform extract, CHSE: *Cardiospermum helicacabum Linn* stem extract

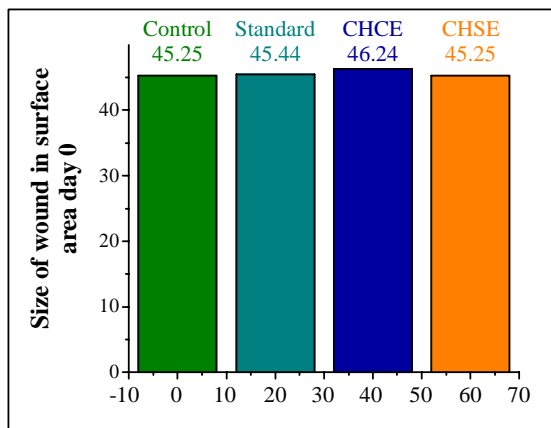


Fig. 1: Effect of Chloroform extract of *Cardiospermum helicacabum* Linn stem bark and its fractions on percentage of wound contraction in excision wound model on 0 day

(All the values represent the Mean \pm of data obtained from six different animals)

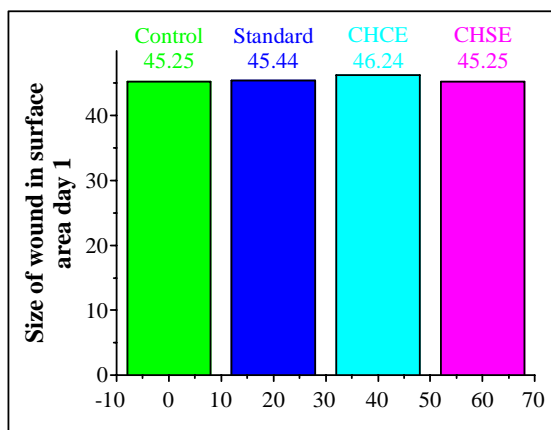


Fig. 2: Effect of Chloroform extract of *Cardiospermum helicacabum* Linn stem bark and its fractions on percentage of wound contraction in excision wound model on 1st day

(All the values represent the Mean \pm of data obtained from six different animals)

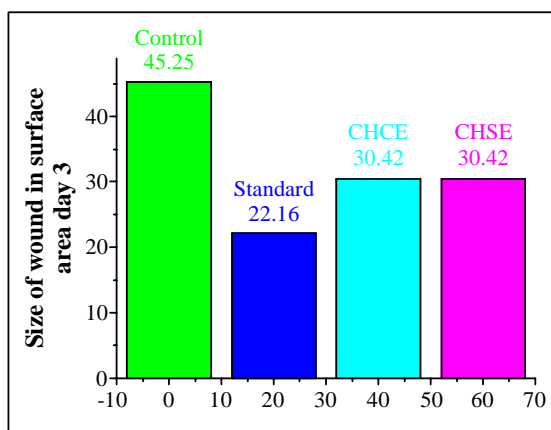


Fig. 3: Effect of Chloroform extract of *Cardiospermum helicacabum* Linn stem bark and its fractions on percentage of wound contraction in excision wound model on 3rd day

(All the values represent the Mean \pm of data obtained from six different animals)

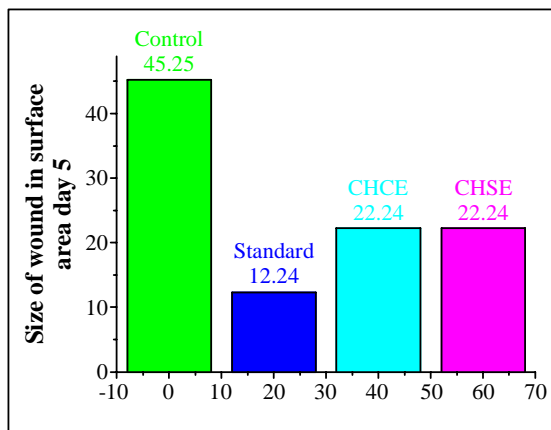


Fig. 4: Effect of Chloroform extract of *Cardiospermum helicacabum* Linn stem bark and its fractions on percentage of wound contraction in excision wound model on 5th day

(All the values represent the Mean \pm of data obtained from six different animals)

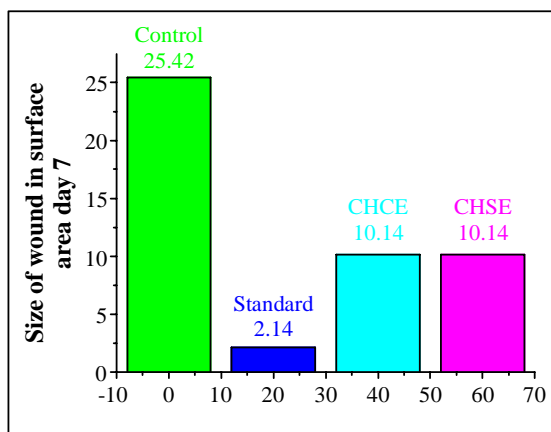


Fig. 5: Effect of Chloroform extract of *Cardiospermum helicacabum* Linn stem bark and its fractions on percentage of wound contraction in excision wound model on 7th day

(All the values represent the Mean \pm of data obtained from six different animals)

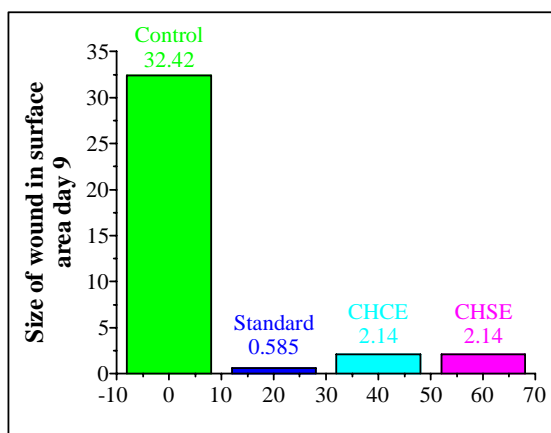


Fig. 6: Effect of Chloroform extract of *Cardiospermum helicacabum* Linn stem bark and its fractions on percentage of wound contraction in excision wound model on 9th day

(All the values represent the Mean \pm of data obtained from six different animals)

ACKNOWLEDGEMENT

The authors are thankful to Prof. K. Sudhakar Babu, Sri Krishnadevaraya University, Chemistry Department, Anantapuramu, for their valuable guidance and support in the completion of the study. The author thanks to the Department of Bio-technology, Sri Krishnadevaraya University College of Engineering & Technology Anantapuramu, for providing facilities to carry out the work.

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