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PHARMACOGNOSTIC STANDARDIZATION AND PHYTOCHEMICAL PROFILING IN *CARDIOSPERMUM HALICACABUM* L.

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Abstract

Therapeutic use of herbs is as old as human civilization and has been evolving along with it. This inevitably leads to the need of standardization of herbal drugs. Standardization is the process of prescribing a set of standards, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Since herbal drugs are mixtures of many constituents the establishment of phytochemical profiling of plant will help in standardization for quality and pharmacopoeial identification. Pharmacognostic standardization include physico-chemical evaluation which is meant for identification, authentication and detection of adulteration. The plant selected for the present investigation is *Cardiospermum halicacabum* L., belonging to the family Sapindaceae. The whole plant was shade dried and ground to fine powder and subjected to organoleptic, fluorescent and physicochemical analysis. The plant powder was subjected to Soxhlet extraction using methanol as the solvent. Phytochemical screening (Qualitative and Quantitative) of the plant extract was done according to the standard biochemical procedures. Antioxidant activity of the plant extract was tested using ABTS assay. The organoleptic analysis revealed characteristic colour, taste, odour and nature of the powder of *Cardiospermum halicacabum*. In fluorescence analysis, on treatment with different solvents, colour changes could be noticed in the plant powder. The results of the physicochemical analysis provided important parameters in detecting adulteration or improper handling of drugs. Qualitative and quantitative analysis in the plant confirmed the presence of many important phytochemicals such as tannins, saponins, flavonoids, terpenoids, alkaloids, simple phenolics, glycosides, coumarins, quinones, acids, lignin and flavanols. Bioactivity study indicated that the antioxidant potential of the plant is comparable to that of the standard Trolox. Further work should be carried out to isolate, purify, and characterize the active constituents responsible for the specific activity of the plant. Also, additional work is necessary to elucidate the possible mechanism of action of the extract.

Key words: Cardiospermum, Pharmacognosy, Fluroscence, ABTS, Antioxidant

Introduction

Medicinal plants have been playing an essential role in the development of human culture. As a source of medicine, these plants have always been at forefront of all cultures of civilizations (Dar *etal*., 2017). The use of these plants has been gradually refined over generations, and this has become known in many a context as traditional medicine. According to World Health Organization, approximately 80% of the world's population in developing countries depends on traditional medicines for primary health care because they are regarded as safe, cost effective and easily affordable. Besides this, modern treatment facilities do not reach aborigines or the people who live far away from the towns (WHO, 2002). Pharmacognosy is a vital link between the traditional and allopathic systems of medicine. It provides a system wherein the active principles of crude drugs derived from natural origin can be dispensed, formulated and manufactured in dosage forms acceptable to allopathic system of medicine (Kokate*etal*., 2012). The plant selected for the present study *Cardiospermumhalicacabum*, belongs to the family Sapindaceae (soapberry family), and is an herbaceous twiny climber. It is among the "Ten Sacred Flowers" of Kerala State in India, collectively known as 'Dashapushpam' (Warrier*etal*., 2002).

The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of the limbs, and snake bite; the decoction from its roots is used as a -

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diaphoretic, diuretic, emetic, laxative, and for sweating; the decoction of its leaves and stems is used in cases of diarrhea, dysentery, and headaches; a poultice of them as a cure for swelling. The juice of the leaves has even been used as a treatment for earache (Ragupathy et al., 2007).

The large number of medicinal plants used by traditional healers of remote villages and primitive aborigines has not been completely documented for their phytochemical constituents and pharmacological activities. It is very essential to have proper documentation of medicinal plants to know their potential for the improvement of health and hygiene through an eco-friendly system. Many valuable plant species are being ignored. This may be due to unrecognized nutritional and medicinal value and also poor consumer awareness.

It is evident from ancient literature and scientific research that *Cardiospermum halicacabum* is one such plant having high medicinal value. However, it is also observed from the existing literature that this plant has not been much explored in scientific research aspectSo, more research studies focusing on the phytochemical aspects are to be carried out to explore the true potential of the plant.

Materials and Methods

Plant Material

The plant selected for the study, *Cardiospermum halicacabum* was collected from Chengannur, Alappuzha. The whole plant was used for the present study.

I. Powder Analysis

Fresh plant of *Cardiospermum halicacabum* was collected in polythene bag. Dirt was removed from the collected material. It was shade dried and then powdered in an electric grinder and sieved with fine mesh sieve. The powder was then used for the organoleptic study and solvent extraction.

Organoleptic Study

Organoleptic (literally "impression on the organs") refers to the evaluation by means of the organs of sense and includes the macroscopic appearance of the plant material, its color, odor, and taste, occasionally the sound of 'snap' of its fracture and the 'feel' of the powder to the touch (Wozniak et al., 1997). The plant powder characteristics like the color, odor, taste and nature were evaluated.

Fluorescence Analysis

The crude drug powder was treated as such with eight different reagents. The solvents used were water, hydrochloric acid, sulphuric acid, nitric acid, sodium hydroxide, acetic anhydride, methanol and acetone. Each solution was loaded on an activated thin gel layer slide and the fluorescence under normal light, short UV (256 nm) and long UV (365 nm) was observed (Chase and Pratt, 1949).

Phytochemical characterization

Different physicochemical parameters were determined according to the official methods and guidelines on quality control for medicinal plant materials.
Foaming index (WHO, 1992),Foreign matter (Indian Pharmacopoeia, 1996),Loss on drying (Indian Pharmacopoeia, 1992),pH (Iqbal et al., 2010),Swelling index (WHO, 1992).

II. Phytochemical Screening

A.**Preparation and yield of extract** (Indian Pharmacopoeia, 1996)

About 15 g of the powdered plant material was subjected to extraction by Soxhlet apparatus using 100 ml methanol. The extract was concentrated under reduced pressure and preserved in refrigerator until further use. The percentage of the crude extract was determined using the following equation.

$$\text{Percentage yield (\%)} = \frac{Weight\ of\ the\ crude\ extract}{Weight\ of\ the\ sample} \times 100$$

B. Quantitative Analysis (Harbone, 1973)

Tannins, Saponins, Flavonoids, Alkaloids, Terpenoids, Phlobatannins, Glycosides, Simple phenolics, Coumarins,Quinones, Acids,Flavanols, Lignin, Steroids, Gums and muscilage

C. Quantitative Analysis

i. Determination of Alkaloids (Harborne, 1973)

ii. Determination of phenols (Spanos and Wrolstad, 1990)

D. Bioactivity study — Antioxidant activity

1. ABTS [2, 2'-Azino - Bis (3—Ethyl Benzothiazoline—6 Suphonic acid) Radial Scavenging activity (Miller and Rice—Evans, 1997)

Trolox standards of different concentrations were prepared. The ABTS substrate working solution was prepared by adding 25 µl of 3 % hydrogen peroxide solution to 10 ml of ABTS substrate solution. The assays were prepared in 96 wells plate. In the wells for the Trolox standard, 10 µl of Trolox and 20 µl of myoglobin working solution were added. In the wells for the test samples, 10 µl (20-80 µg/ml concentration) of plant extracts and 20 µl of myoglobin working standard were added. Then the solution was incubated for 5 minutes at room temperature. The stop solution (100 µl) was added to each well. The end point absorbance was read at 405 nm using a plate reader.

$$\text{Percentage of inhibition (\%)} = \frac{\text{Abs } control - \text{Abs } sample}{Abs\ control} \times 100$$

Result and Discussion

Powder Analysis

Organoleptic study

Colour: Olive green
Smell: Characteristic woody
Taste: Tasteless
Texture: Rough

Fluorescence Analysis

The dry powder was subjected to fluorescence analysis with different reagents in normal light, short UV and long UV. The colour changes are summarized (Table 1)

Phytochemical characterization

A total of five physicochemical parameters were evaluated in *Cardiospermum halicacabum* (Table 2). The plant moisture content was reported in low amounts. Forming index was more than 100 ml. The pH was found to be 5.8. Foreign matter and swelling were not observed.

Phytochemical Screening

Yield of extract

The methanol extract was prepared by Soxhlet extraction. The yield of the methanol extract was 4.5%.

Quantitative analysis

A total of 15 phytochemicals were qualitatively analysed in methanol extract of the plant. Most of the compounds were present in the extract.Phlobatannins,gums and mucilage and steroids were absent in the plant extract. The phytochemical screening tests are provided (Table 3 and Plate 2).

Quantitative analysis

The quantitative analysis of two phytochemicals was done in the methanol extract of *Cardiospermum halicacabum* (Table 4) by standard procedures. The amount of phenol was higher than the amount of alkaloids.

Bioactivity study—Antioxidant activity Antioxidant activity ABTS [2, 2'-Azino - Bis (3—Ethyl Benzothiazoline—6 Suphonic acid) Radial Scavenging activity

The scavenging activity of the extract was compared with that of Trolox standard and percentage inhibition was calculated . The percentage inhibition of the ABTS radical by the methanol

extract of the plant increased with increase in concentration. In this assay, by Probit analysis the methanol extract of had good ABTS scavenging activity with IC_{50} value of 38 µg / ml. The IC_{50} value of the standard Trolox was 36 µg / ml. The results are shown in Table 5 .

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Table 1. Fluorescence analysis of *Cardiospermum halicacabum*

Powder + Reagent	**Visible (400-800 nm)**	**UV short (256 nm)**	**UV long (365 nm)**
Powder as such	Dark green	Green	Brownish green
Powder + H_2O	Green	Green	Black
Powder + Conc. HCl	Brownish green	Green	Black
Powder + Conc. H_2SO_4	Black	Black	Black
Powder + Conc. HNO_3	Green	Green	Black
Powder + NaOH	Green	Black	Greenish black
Powder + Acetic anhydride	Green	Grey	Black
Powder + MeOH	Dark green	Dark green	Black
Powder + Acetone	Dark green	Green	Black

Table 2. Physicochemical characters of *Cardiospermum halicacabum*

Parameters	**Values**
Loss on drying	12.02 % ± 0.06
Foaming index	>100 ml
Swelling index	NIL
Foreign matter	NIL
pH	5.8

Table 4. Quantitative estimation of *Cardiospermum halicacabum*

Sl. No.	**Phytochemicals**	**Amount (mg/g)**
	Alkaloids	0.62
	Phenols	22.5

Table 3. Phytochemicals tested in *Cardiospermum halicacabum*

S.NO.	PHYTOCHEMICALS	PRESENT/ABSENT
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Terpenoids	+
6	Phlobatannins	-
7	Glycosides	+
8	Simple Phenolics	+
9	Coumarins	+
10	Quinones	+
11	Acids	+
12	Flavanols	+
13	Lignin	+
14	Steroids	-
15	Gums and mucilage	-

Table 5. ABTS antioxidant assay of *Cardiospermum halicacabum* extract

Sl. No.	Concentration of extract (µg / ml)	Trolox (percentage of inhibition) (%)	Plant extract (percentage of inhibition) (%)
1	20	40	37
2	40	58	55
3	60	87	85
4	80	98	94

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