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Purity of *Cardiospermum Helicacabum Linn* by Using HPLC Technque in Different Solvents

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A B S T R A C T

In this study we report the HPLC Purity of *Cardiospermum helicacabum* Linn, extract of different solvents, the purity of the biologically active fractions i.e. methanol, chloroform, ethanol and acetone fractions were determined by using in house developed HPLC method and % purity wad reported in the tables 1-4. The corresponding chromatograms The HPLC chemical investigations has resulted in the identification of the chromatogram, together the crude extract of the Cardiospermum helicacabum Linn flow and stop-flow HPLC analyses rapidly assisted in the identification of the major component , as well as to obtain sufficient quantities for biological testing.

Keywords: *Cardiospermum halicacabum* Linn. HPLC, Purity, extract.

A R T I C L E I N F O

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1. Introduction

Cardiospermum halicacabum Linn., commonly known as Ballon vine, is an important medicinal herb belonging to family Sapindaceae. It is an annual or sometimes perennial climber, found as a weed throughout India, ascending up to 1200 m in the hills. [1] The herb is used as diuretic,

stomachic, rubefacient, in rheumatism, lumbago and nervous disorders and in preparing hair oil for curing dandruff and alopecia. The juice of the herb is used to cure earache and asthma. The decoction is administered in piles. The pungent vapors from the crushed leaves are inhaled to relieve headache. The root is mucilaginous and considered emetic, laxative and anti-rheumatic. It is effective in didymitis and early. Stages of hydrocela and is used in asthma and colic. It is useful in amenorrhoea, gonorrhoea, nervous diseases, haemorrhoids and erysipelas. [2-4]

High performance Liquid Chromatography (HPLC):

HPLC is a type of liquid chromatography (Basset et al., 1986; Macek 1972) that employs a liquid mobile phase and a very fine divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch. The rate of distribution of drugs between stationary and mobile phase is controlled by diffusion process, if diffusion is minimized, a faster and effective separation can be achieved. The technique of high performance liquid chromatography is so called because of its improved performance when compared to classical column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation. The HPLC is the method of choice in the field of analytical chemistry, since this specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

- Speed (many analysis can be accomplished in 20 min or less). Greater sensitivity (various detectors can be employed)
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Ideal for the substances of low viscosity
- Easy sample recovery, handling and maintenance.
- Instrument leads itself to automation and quantification (less time and less Labour)
- Precise and reproducible
- Integrator itself does calculations.

2. Experimental

Preparation of Plant Extracts:

Petroleum ether extract:

The coarsely powdered shade dried roots of *Cardiospermum helicacabum* (50g) was extracted with ether by hot extraction process (Soxhlet) for 4 h. After completion of extraction the solvent was removed by distillation and Concentrated.

Methanol extract:

The marc left after the acetone extraction was dried and extracted with 95% ethanol by hot extraction process (Soxhlet) for 4 h. After completion of the extraction the solvent was removed by distillation and Concentrated

Chloroform extract:

The marc left after benzene extraction was dried and extracted with chloroform by hot extraction process (Soxhlet) for 4 h. After completion of extraction the solvent was removed by distillation and Concentrated.

Acetone extract:

The marc left after the extraction of the chloroform extraction was dried and extracted with acetone by hot extraction process (Soxhlet) for 4 h. After completion of extraction the solvent was removed by distillation and Concentrated.

Ethanolic extract:

The marc left after the acetone extraction was dried and extracted with 95% ethanol by hot extraction process (Soxhlet) for 4 h. After completion of the extraction the solvent was removed by distillation and Concentrated.

HPLC method development

(Mayer & Varenika 1985):

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling.

a. The important factors, which are to be taken into account to obtain reliable quantitative analysis, are
b. Careful sampling and sample preparation.
c. Appropriate choice of the column.
d. Choice of the operating conditions to obtain the adequate resolution of the mixture.
e. Reliable performance of the recording and data handling systems.
f. Suitable integration/peak height measurement technique.
g. The mode of calculation best suited for the purpose. By using the following chromatographic conditions the purity of all the fractions were determined

Chromatographic conditions:

Column: X Bridge C18 (150mmX4.6mm, 3.5µm)
Mobile Phase: A: 0.05% Formic acid in Acetonitrile
B: 0.05% Formic acid in Water
Gradient: Time/ %A: 0/10, 3/10, 8/90, 14/95, 18/95, 19/10, 20/10
Column Temp: Ambient,
Flow Rate: 1.0 ml/min
Diluent: ACN: H2O (70:30)
Wavelength of detection: 220nm & 254nm

3. Results and Discussion

Purity of the fractions:

The purity of the biologically active fractions i.e. methanol, chloroform, ethanol and acetone fractions were determined by using in house developed HPLC method and % purity wad reported in the tables 1-4. The corresponding chromatograms were also shown in the figures 1-4.

Sample information

Sample name: Methanol extract
Date Acquired: 31-12-2014
Injection Volume: 3.00µl
Instrument ID: ANL-MCL3-HPLC-001
Vial: 2Proc.Chnl.
Descr: Channel Name 220.00nm, 254.0nm.

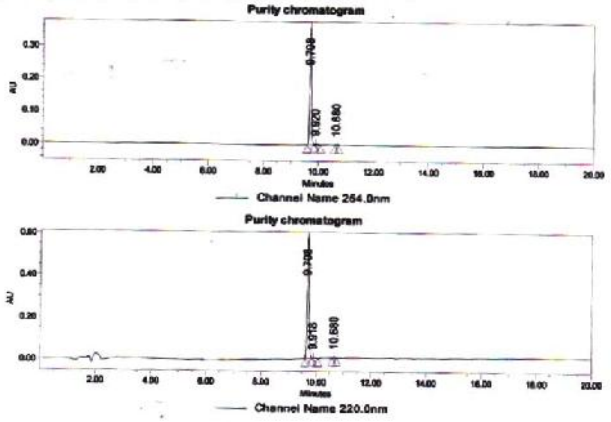

Figure 1: Chromatogram showing the Purity of Methanol extract

Table 2: Area and % Areas of Peaks obtained in the HPLC Chromatogram of Methanol extract processed channel: PDA 220.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

S.No	Retention Time (Min)	% Height	Area	% Area
1	9.71	98.52	27352	97.86
2	9.92	0.99	46205	1.65
3	10.68	0.48	`13732	0.49

Processed channel: PDA 254.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

S.No.	Retention Time (Min)	% Height	Area	% Area
1	9.71	98.62	172768	98.31
2	9.92	1.07	24174	1.38
3	10.68	0.31	`5509	0.31

Sample information

Sample name: Chloroform extract

Date Acquired: 31-12-2014

Injection Volume: 3.00µl

Instrument ID: ANL-MCL3-HPLC-001

Vial: 3 Proc.Chnl. Descr: Channel Name 220.00nm, 254.0nm

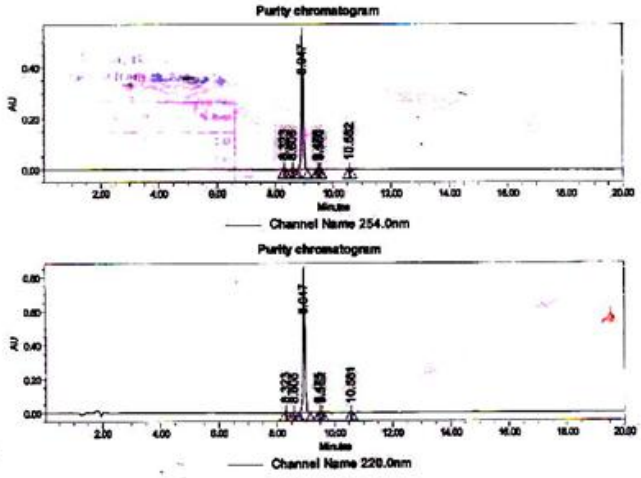

Figure 2: Inhibition of wriths by each treatment

Table 3: Area and % Areas of Peaks obtained in the HPLC Chromatogram of Chloroform extract processed channel: PDA 220.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

S.No	Retention Time (min)	% Height	Area	% Area
1	8.32	0.67	27970	0.69
2	8.61	0.33	12798	0.31
3	8.95	98.13	4006512	98.19
4	9.48	0.24	9090	0.22
5	9.55	0.32	11382	0.28
6	10.58	0.32	12537	0.31

Processed channel: PDA 254.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

S.No	Retention Time (min)	% Height	Area	% Area
1	8.32	0.58	13911	0.53
2	8.61	0.47	12052	0.46
3	8.95	98.00	2593102	98.16
4	9.48	0.26	6471	0.24
5	9.55	0.30	6730	0.25
6	10.58	0.37	9351	0.35

Sample information

Sample name: Ethanol extract

Date Acquired: 31-12-2014

Injection Volume: 3.00µl

Instrument ID: ANL-MCL3-HPLC-001

Vial : 2

Proc.Chnl. Descr: Channel Name 220.00nm, 254.0nm.

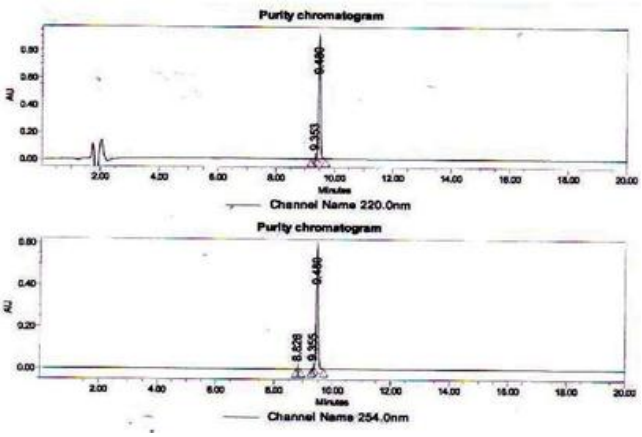

Figure 3: Chromatogram showing the Purity of ethanol extract

Table 4: Area and % Areas of Peaks obtained in the HPLC Chromatogram of Ethanol extract processed channel: PDA 220.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

	Retention Time min)	% Height	Area	% Area
1	9.35	0.46	22758	0.56
2	9.48	99.54	4049288	99.44

Processed channel: PDA 254.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

	Retention Time min)	% Height	Area	% Area
1	8.83	0.18	4867	0.18
2	9.35	0.50	10436	0.39
3	9.48	99.32	2643864	99.42

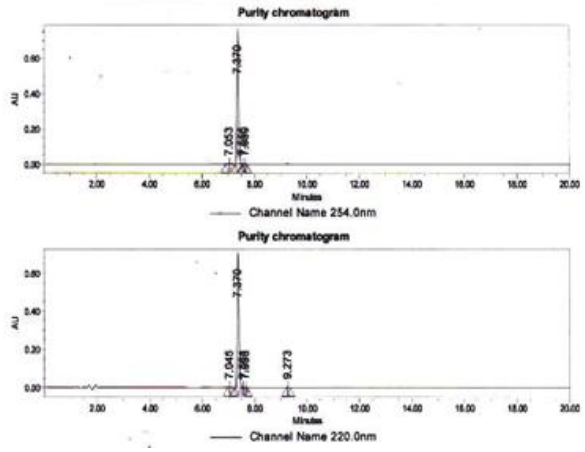

Figure 4: Chromatogram showing the Purity of Acetone extract

Table 5: Area and % Areas of Peaks obtained in the HPLC Chromatogram of Acetone extract processed channel: PDA 220.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

	Retention Time (min)	% Height	Area	% Area
1	7.05	0.23	14409	0.48
2	7.37	99.76	2978246	98.43
3	7.56	0.35	13559	0.45
4	7.66	0.25	7563	0.25
5	9.27	0.41	11924	0.39

Processed channel: PDA 254.0 nm (PDA 220.0 to 400.0 nm at 1.2 nm)

	Retention Time (min)	% Height	Area	% Area
1	7.05	0.24	16380	0.18
2	7.37	99.56	3201639	99.34
3	7.56	0.09	1817	0.06
4	7.65	0.11	2976	0.09

4. Conclusion

A rapid, simple, precise, accurate and specific HPLC method for C. *halicacabum* has been developed and validated. *By using* mobile phase Acetonitrile: Formic acid (70:30) was carried out. The data could be used as a quality control standard. The activity of a plant extract is always influenced by the quantity of active principle present in the extract. it is essential to develop a standardization method from which one can optimize its quantity in the herbal formulations.

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