



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)

Platform for Pharmaceutical Researches & Innovative Ideas
www.ijprd.com

CHEMICAL STANDARDISATION STUDIES ON *CARDIOSPERMUM HALICACABUM LINN*

K.Sughuna^{1*}, P.Brindha

¹Seethalakshmi Ramaswami College, Trichy-620019, TN, India

ABSTRACT

There is an increasing demand for herbal-based medicines as an alternative to modern medicines, as the later produces toxicity and side effects on prolong administration. World health organization has also recognized herbal based medicines and there is resurgence of interest in the study and use of traditional medicines in health care. But the greatest lacuna existing in herbal based medicine is lake of standardisation and validation. To fill up the lacuna it is essential to carry out the standardisation studies on herbal medicine and contribute towards its international recognition and acceptance. Hence in the present paper an attempt is made to standardize and validate a common herbal medicine botanically equated as *cardiospermum halicacabum* Linn used in the Indian traditional systems. The data of the results obtained were presented and discussed

Correspondence to Author



K.Sughuna

K.Sughuna, 3/369, Thirunagar,
Kattur (North), Trichy-19

Email

sughuna_guna@yahoo.co.in

Key Words

Ayurveda, herbal medicine and Luteolin.

INTRODUCTION

India has the rich heritage of two traditional systems of medicines such as Ayurveda and Siddha. These two systems are complementary to each other and have contributed significantly to the human health care. Herbal drugs recorded in these renowned systems have over the years have turned out to be potential and useful drugs. Recently these herbal drugs are blooming across the world as "GREEN MEDICINE " and there is an increasing demand for herbal-based medicines as an alternative to modern medicines, as the later produces toxicity and side effects on prolong administration. World health organization has also recognized herbal based medicines and there is resurgence of interest in the study and use of traditional medicines in health care. But the greatest lacuna existing in herbal based medicine is lack of standardisation and validation. To fill up the lacuna it is essential to carry out the standardisation studies on herbal medicine and contribute towards its international recognition and acceptance. Hence in the present paper an attempt is made to standardize and validate a common herbal medicine botanically equated as *cardiospermum halicacabum* Linn used in the Indian traditional systems.

The selected drug is a herbaceous climber with spiral tendrils, panicle inflorescence and capsular fruit. The whole plant is diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, rubefacient, stomachic and sudorific. It is used in the treatment of rheumatism, nervous diseases, stiffness of the limbs and snakebite (Chopra 1986). The leaves are rubefacient, they are applied as a poultice in the treatment of

rheumatism (Chopra 1986). A tea made from them is used in the treatment of itchy skin. Salted leaves are used as a poultice on swellings. The leaf juice has been used as a treatment for earache (Chopra 1986). The leaves are rubefacient and good for arthritis, otalgia and ophthalmodynia. The plant has sedative action on the central nervous system. The root is diaphoretic, diuretic, emmenagogue, laxative and rubefacient. It is occasionally used in the treatment of rheumatism, lumbago and nervous diseases. It also finds use in tumours. (Chopra 1986).

METHODS AND MATERIALS

Fresh plants of *Cardiospermum Halicacabum* Linn were collected from in and around Trichy (kattur) during the month of April 2008 and identified with the help of flora (Gamble, 1957) and authenticated with the voucher specimen kept at RAPHINAT HERBARIUM of St. Joseph College, Trichy. The whole plant was shade, dried and coarsely powdered and subjected to chemical standardization studies employing standard textual procedures. Preliminary phytochemical screening of the drug powder as well as extracts were carried out. Physicochemical constants were also worked out. Extracts were analysed for the Fluorescence features. Their chemical Composition were determined using GC-MS engine model GC Clarus 500, Perkin Elmer and Computer Mass Spectral Library. The capillary column was Elite-1 (5% phenyl-, 95% dimethyl polysiloxane). The temperature of a source is 280°C. Fourier Transform Infrared Spectrometry (FTIR) studies were also carried out. HPTLC analysis was also done.

RESULTS

Physico Chemical Constants determined were tabulated in table 1

S.No	Parameters (%)	Value % w/w
1	Loss on drying	19.1
2	Total Ash content	5
3	Acid Insoluble Ash	0.05

Extractive values

1	Ethanol	4.18
2	water	3.86

The data obtained of Behaviour of drug powder with Various chemical reagents were recorded in table -2

S.No	TEST FOR	REAGENTS	REACTION	RESULT
1	Saponin	Water shake	Foam	+Ve
2	Protein	Picric acid	Yellow	+ Ve
3	Tannin	Lead acetate solution	Yellow	+ Ve
4	Sterol	Chloroform+ Acetic anhydride+ Acetic acid+H ₂ SO ₄ .	Green	+ Ve
5	Terpenes	Tin+ Thionyl chloride	Pink	+ Ve
6	Sugar	Anthrone+ H ₂ SO ₄ .	Red	+ Ve
7	Flavonoids	Alcohol+Mg+Con.Hcl	Magenta	+ Ve
8	Coumarin	10%NaOH	Yellow	+ Ve
9	Quinone	Con H ₂ SO ₄ .	Red	+ Ve
10	Lignin	Alcoholic Phloroglucinol+Dil.Hcl	Red	- Ve
11	Alkaloid	Drangendroff's reagent+Acetic acid	Orange	+ Ve
12	Starch	Iodine	Blue	- Ve
13	Gum	Drops of water	Swells/torns adhesive	+ Ve

+ Ve- indicates positive

Ve- indicates positive

Preliminary Phytochemical screening of aqueous and alcohol extracts were recorded in Table -3

S.NO	TEST FOR	WATER	ALCOHOL
1	Saponin	+Ve	+Ve
2	Protein	+Ve	+ Ve
3	Tannin	+ Ve	+ Ve
4	Sterol	- Ve	+ Ve
5	Terpenes	+ Ve	+ Ve
6	Sugar	+ Ve	+ Ve
7	Flavonoids	+ Ve	+ Ve
8	Coumarin	+ Ve	+ Ve
9	Quinone	+ Ve	- Ve
10	Lignin	- Ve	- Ve

11	Alkaloid	+ Ve	+ Ve
12	Starch	- Ve	- Ve
13	Gum	- Ve	- Ve

+ Ve- indicates positive

-Ve- indicates positive

Table 4, 5, 6 and 7 will give the details of fluorescence features observed of the powder and extracts of Fluorescence analysis of powder after 24 hours selected plant drug.

S.No	Treatment	Day Light	UV Light
1	Drug Powder	Greenish yellow	Yellowish green
2	Drug Powder+Aq.1 N NaoH	Brown	Dark Brown
3	Drug Powder+ Alc 1N NaoH	Pale Green	Pale green
4	Drug Powder+1N Hcl	Greenish Brown	Greenish Brown
5	Drug Powder+50% H ₂ SO ₄	Dark Brown	Dark Brown

Fluorescence Analysis of Extracts after 24 Hours. Table-5

S.No	Name of Extracts	Day Light	UV Light
1	Hexane	Pale Green	Pale Green
2	Benzene	Dark Brown	Dark Green
3	Chloroform	Greenish Brown	Dark Brown
4	Ethyl acetate	Dark Green	Dark Green
5	Acetone	Dark Green	Dark Green
6	Ethanol	Dark Green	Dark Green
7	Water	Light Green	Light Green

Fluorescence Analysis of Drug Powder after 48 Hours. Table-6

S.No	Treatment	Day Light	UV Light
1	Drug Powder	Greenish yellow	Yellowish green
2	Drug Powder+Aq.1 N NaoH	Dark Brown	Dark Brown
3	Drug Powder+Alc 1N NaoH	Pale Green	Pale green

4	Drug Powder+1N HCl	Light Brown	Greenish Brown
5	Drug Powder+50% H ₂ SO ₄	Dark Brown	Dark Brown

Fluorescence Analysis of Extracts after 48 Hours. Table-7

S.No	Name of Extracts	Day Light	UV Light
1	Hexane	Pale Green	Pale Green
2	Benzene	Greenish Brown	Dark Green
3	Chloroform	Greenish Brown	Dark Brown
4	Ethyl acetate	Dark Green	Dark Green
5	Acetone	Dark Green	Dark Green
6	Ethanol	Dark Green	Dark Green
7	Water	Light Green	Light Green

Chemical constituents of the selected drug detected through GCMS analysis were recorded in Table -8

S.No.	Components	Retention time	Molecular formula	Molecular weight	% Peak Area
1	Glycerin	7.26	C ₃ H ₈ O ₃	92	1.8108
2	Carbamic acid, phenyl ester	7.41	C ₇ H ₇ NO ₂	137	1.1641
3	Cyclohexanamine, N-3-butenyl-N-methyl-	9.31	C ₁₁ H ₂₁ N	167	0.7405
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	10.69	C ₆ H ₈ O ₄	144	0.9772
5	1,2,4-Triazine-3,5(2H,4H)-dione	10.78	C ₃ H ₃ N ₃ O ₂	113	0.5636
6	Tetradecane	11.51	C ₁₄ H ₃₀	198	2.7550
7	3-Oxo-4-phenylbutyronitrile	12.43	C ₁₀ H ₉ NO	159	1.3229
8	Cycloisolongifolene	14.17	C ₁₅ H ₂₄	204	0.4420
9	ς-Muurolene	15.36	C ₁₅ H ₂₄	204	0.5905
10	Caryophyllene	15.67	C ₁₅ H ₂₄	204	4.6759

11	à-Caryophyllene	16.26	C ₁₅ H ₂₄	204	0.7903
12	á-Farnesene	16.59	C ₁₅ H ₂₄	204	0.8503
13	n-Cetane	18.10	C ₁₆ H ₃₄	226	7.6635
14	Caryophyllene oxide	18.28	C ₁₅ H ₂₄ O	220	2.9532
15	Ethyl à-d-glucopyranoside	18.58	C ₈ H ₁₆ O ₆	208	2.5318
16	3-O-Methyl-d-glucose	19.09	C ₇ H ₁₄ O ₆	194	20.1014
17	Nonadecane	20.90	C ₁₉ H ₄₀	268	5.4711
18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	21.40	C ₂₀ H ₄₀ O	296	26.7788
19	Hexadecanoic acid, ethyl ester	23.37	C ₁₈ H ₃₆ O ₂	284	2.5617
20	Phytol	24.81	C ₂₀ H ₄₀ O	295	8.0405
21	Squalene	33.15	C ₃₀ H ₅₀	410	7.2149

Figure -2 gives the details of the chromatogram of the selected drug

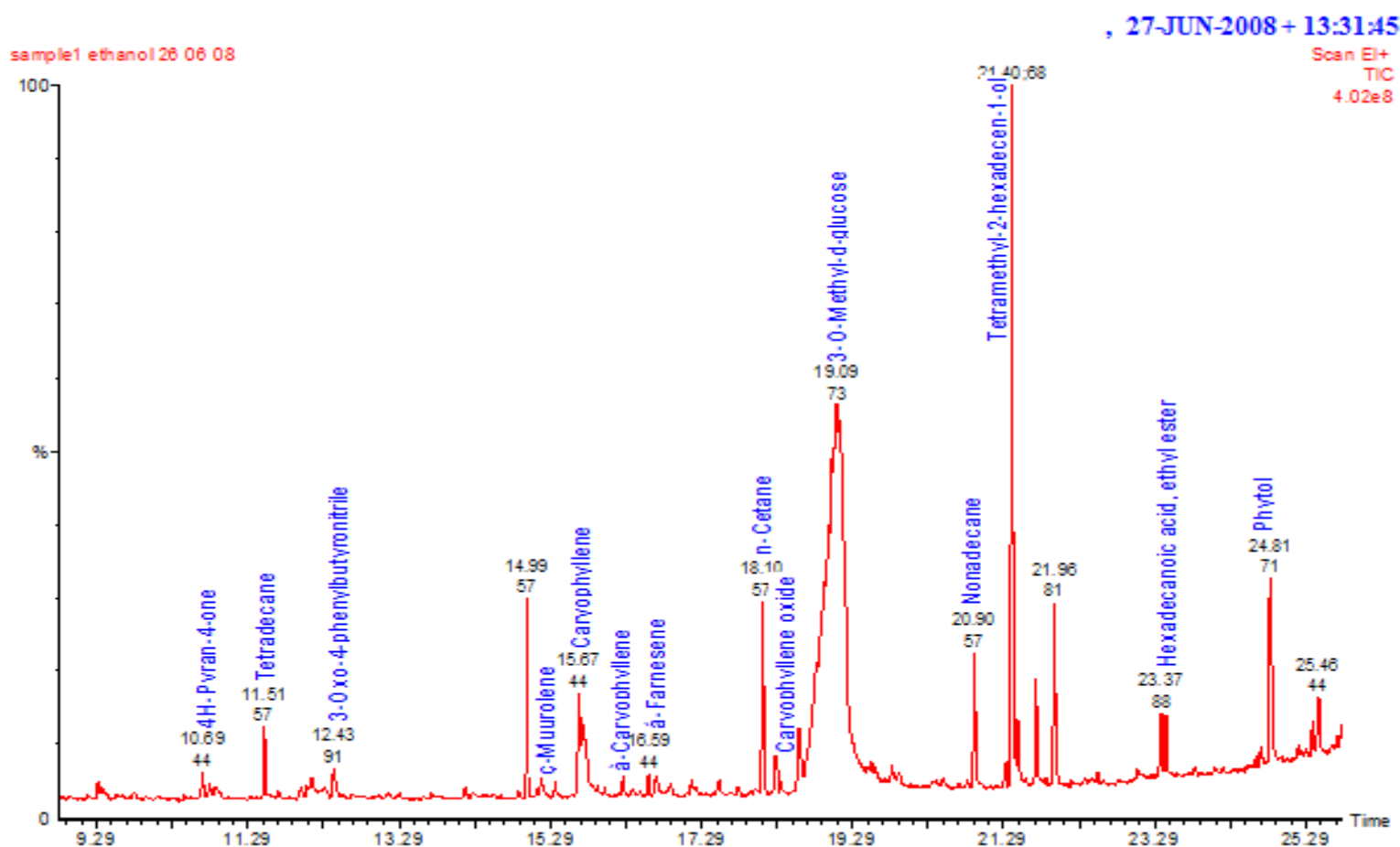
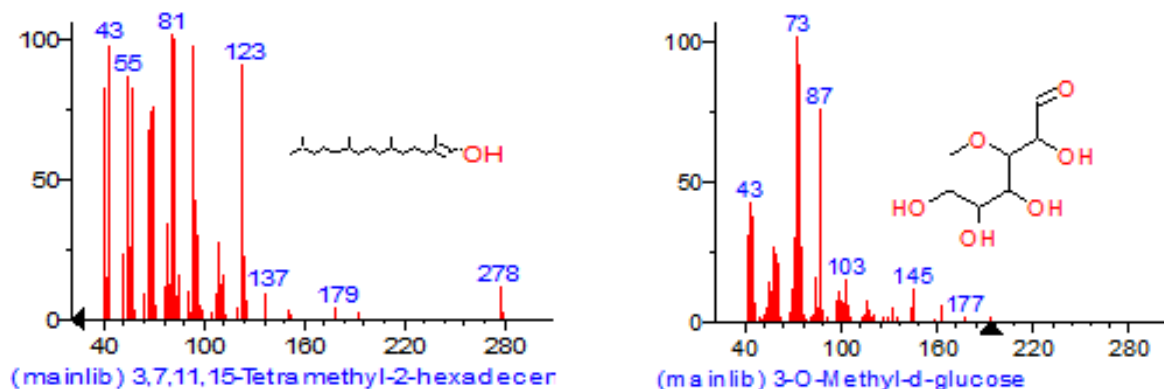


Figure-3 depicts the possible chemical structure present in the drug with highest Peak area.



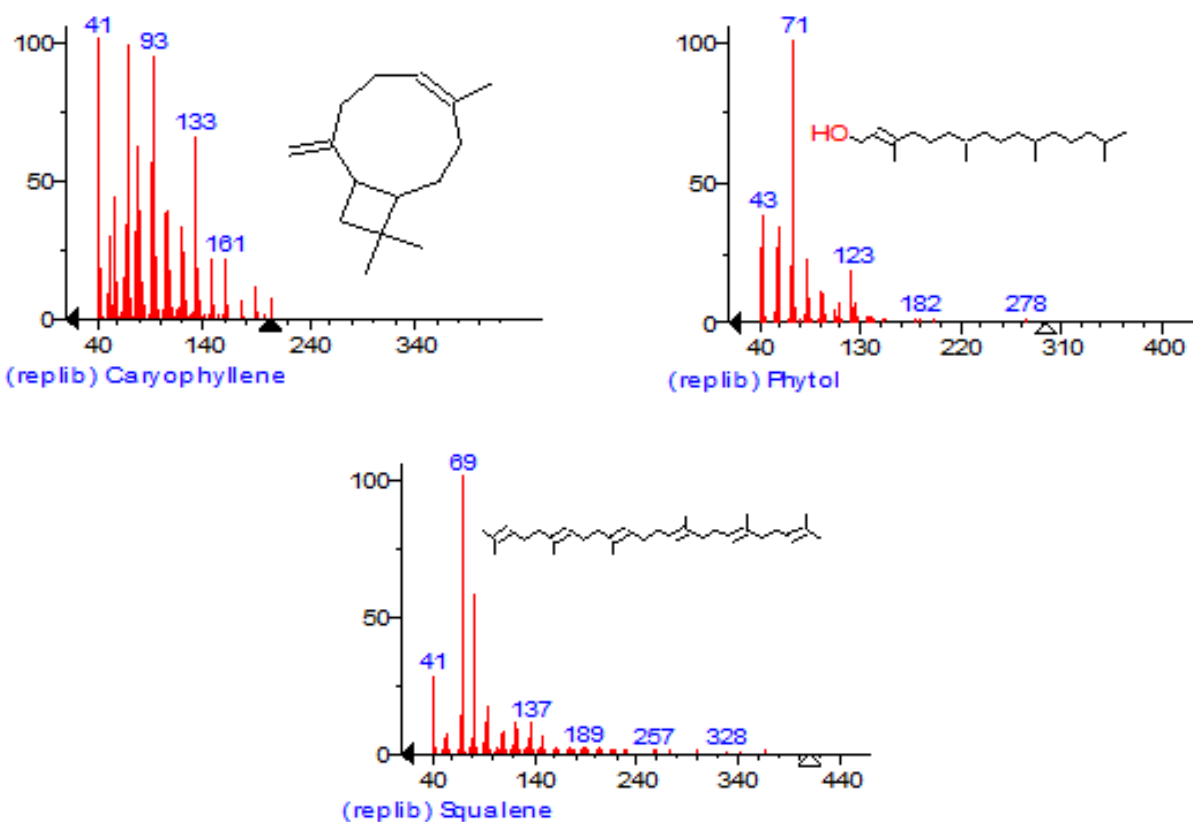


Table 9 reveals the Inorganic standards recorded through AAS method.

S.No	Name of the Parameter	Sample Details(PPm)
1	Cobalt	0.0180
2	Copper	3.2220
3	Iron	3.1940
4	Magnesium	6.7080
5	Manganese	0.5456
6	Nickel	0.2676
7	Zinc	0.8942

FT-IR Spectrum Analysis

FTIR Spectrum showed many peaks which indicated the presence of following groups.

They are as follows:

- 3540-3200, 1205-0885, 2645-2310, 2310-2000 cm^{-1} indicates the presence of Hydroxyl or Amino group.
- 3465-3200, 2958-2900, 1800-1620, 1510-1460, 1065-1000, 2658-2000, 1650-1620, 1510-1460, 1218-1150, 999-950, 940-900 cm^{-1} indicates the presence

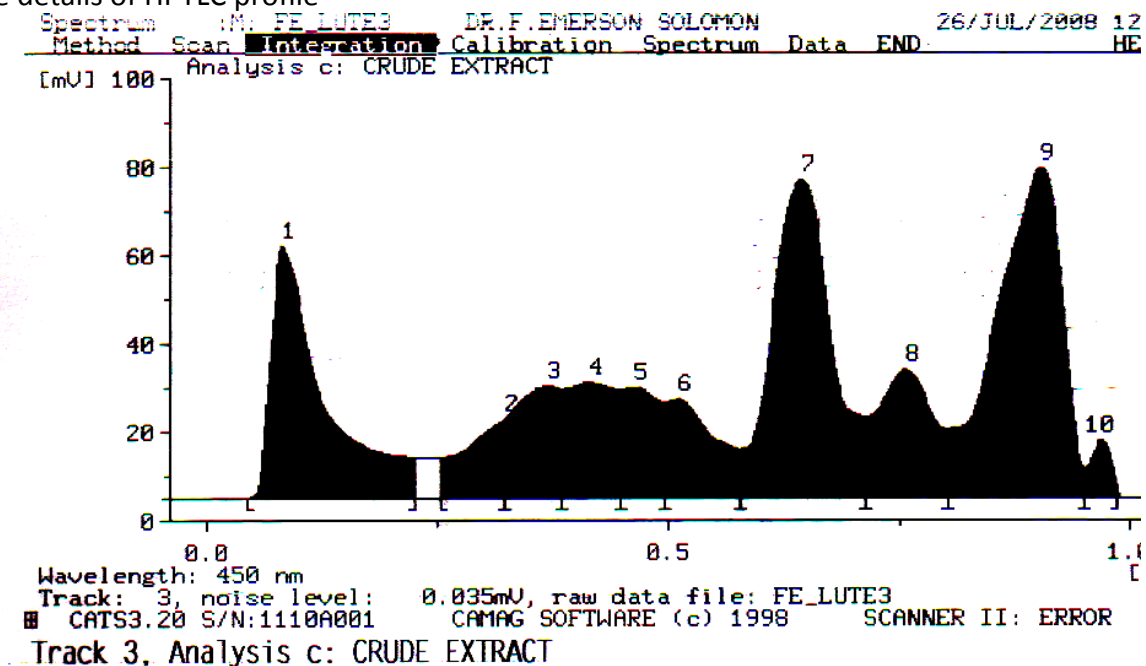
of Hydroxyl carbonyl group (Aliphatic alcohol with Carbonyl substitutes).

- 3030-2855, 1485-1415 cm^{-1} indicates the presence of Hydrocarbons compound (High molecular weight compounds).
- 3000-2850, 1755-1735, 1470-1430, 1380-1350, 1260-1225, 1070-1015, 1620-1485 cm^{-1} indicates the presence of Ester, acetate (Carboxylic acid ester-possible aliphatic acetate).

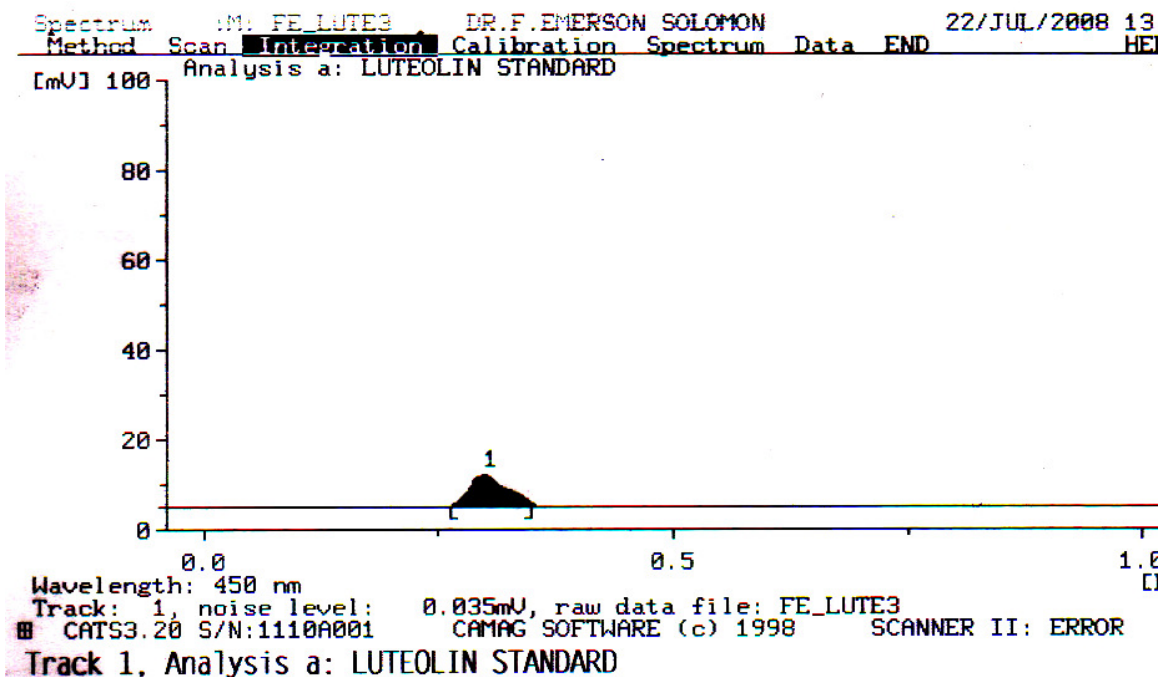
➤ 1750-1731, 1620-1575, 1755-1725 cm^{-1} indicates the presence of Esters (carbonyl and acid compound).

➤ 1745-1710, 1300-1000, 2770-2400, 1900-1750, 1705-1660 cm^{-1} indicates the presence of Esters or Ketones (Hydroxy, alkoxy group).

Figure-5 gives the details of HPTLC profile



HPTLC-PROFILE –Standard Luteolin



HPTLC-PROFILE

Ethanol extract values			Standard Luteolin		
Rf	max H	(%)	Rf	max H	(%)
0.08	57.2	15.75	0.29	7.0	100
0.32	17.9	4.92			
0.36	25.3	6.97			
0.41	26.2	7.21			
0.46	25.0	6.90			
0.50	22.3	6.15			
0.64	72.2	19.90			
0.75	29.1	8.01			
0.90	74.8	20.61			
0.96	13.0	3.59			

HPTLC finger print of ethanol extract of *Cardiospermum halicacabum*. Linn.

When compared with authentic Luteolin HPTLC finger print of the selected drug also showed peak almost with similar Rf 0.32. Rf of the pure compound was 0.29. This peak and Rf value confirm the presence of Luteolin in this plant which may be responsible for the biological activity of the plant.

DISCUSSION AND CONCLUSION

Cardiospermum halicacabum. Linn a herbal drug used in traditional system of India was studied from chemical standardization point of view. Chemical standards determined for the selected drug reveals loss on drying-19.1%, Total Ash-5%, Acid insoluble Ash-0.05%. Extractive values for Ethanol-4.18% and for Water-3.86%. Ash value determined proved the purity of the plant drug alcohol extractive values revealed that the plant drug is rich in alcohol soluble phyto-constituents (Table-1). Plant powder and extract when subjected to preliminary phytochemical screening answered positively for the presence of Flavones, Sterol, Terpenoids, Saponin and Alkaloids (Table-3). Various extracts exhibited different shades of green fluorescence, with acids and with alkali emitted brown and green fluorescence. (Table-7). GCMS analysis revealed presence of 21 compounds (Table-8) and FTIR confirmed the presence of various functional groups.

Atomic Absorption Spectroscopy revealed the presence of various elements such as Fe, Cu, Mn, Zn, Ni, Co and Mg (Table-9). Cu, Mg and Fe content was more.

HPTLC finger print showed 10 peaks with Rf at 0.08, 0.32, 0.36, 0.41, 0.46, 0.50, 0.64, 0.75, 0.90, 0.96. Presence of Luteolin a bioactive flavone was also confirmed by comparing the HPTLC finger print with Authentic HPTLC finger print of Luteolin. Luteolin showed a peak with Rf 0.32. In the HPTLC finger print of ethanolic extract also a peak was observed with almost similar Rf. Percentage of luteolin is estimated as 0.001843%.

The chemical standards determined in the present work can further add to the pharmacopoeia of this traditional drug source and can contribute significantly in determining the identify purity and strength of the drug as well provide chemical evidences for the therapeutic action of the drug. Such standardization studies can help not only for International acceptance and recognition of this drug but also in checking the adulteration and substitution of this drug and can promote usage of genuine herbal drugs. This in turn will lead to the development of quality herbal products for the betterment of Human society.

ACKNOWLEDGEMENT

I place on record my deep sense of gratitude and sincere thanks to our Secretary **Shri.V.S.Narasimham** who was instrumental for our all research activities. I take this opportunity to express my sense of gratitude and sincere thanks to **Dr.K.Prema, Principal**, Srimad Andavan Arts and Science College for giving me this excellent opportunity to carryout this project work. I thank Mrs.J.Radhika, Head of the Department, Srimad Andavan Arts and Science College, for her sincere efforts and support during the course of the work. I take this opportunity to convey my special thanks to all my lecturers who educated me a lot with their excellent way of teaching. Words fail in expressing my sincere gratitude to **my beloved brother** who gave me moral support, love, faith, and encouragement throughout the entire course of my studies and project work.

REFERENCES

1. Brindha, P. & A.Saraswathy. 1999. Phytochemical comparison of *Pentatropis, oldenlandia & Plumeria*. In proceeding of the national seminar on the recent

- trends in natural products chemistry on march 30&31. Bharathidasan university, Trichy.
2. Chopra. R. N., Nayar. S. L. and Chopra. I. C. *Glossary of Indian Medicinal Plants (Including the Supplement)*. Council of Scientific and Industrial Research, New Delhi. 1986.
 3. Chandra Prakash K., Kuppast I.J., Manjunath C, Jawahar N, Jubie S and Swapna B.. Diuretic activity of whole plant extracts of *Cardiospermum halicacabum* (Linn) Phcog Mag. Vol 4, Issue 13 (Suppl), Jan-Mar, 2008.
 4. Ed Perley .Solid Electronic Absorption Spectra Using Potassium Bromide Pellets. <http://www.nfinity.com/~exile/progmenu.htm> March 17, 2001.
 5. Gamble, J.S. 1957. *Flora of Presidency of Madras*. Botanical Survey of India, Calcutta, India.
 6. G. Jayanthi and M. Jagadeesan .Composition of seed oil of *Cardiospermum halicacabum* L. var. *luridum* Blume (Adelb) *J. Sci. Trans. Environ. Technov.* 1(4), 2008. G. Michelsa, W. W"atjena , P. Nieringa, B. Steffanb, Q.-H. Tran Thia, Y. Chovoloua, A. Kampk"ottera, A. Bastc, P. Prokschb, R. Kahla Pro-apoptotic effects of the flavonoid luteolin in rat H4IIE cells. *Toxicology* 206 (2005) 337–348.
 7. Gopalakrishnan C, Dhananjayan R, Kameswaran L. Studies on the pharmacological actions of *Cardiospermum helicacabum*. *Indian J Physiol Pharmacol.* 1976 Oct-Dec;20(4):203-8.
 8. Introduction to Fourier Transform, Infrared Spectrometry Thermo Nicolet Corporation 2001 USA.
 9. Mathew K M. The flora of the Tamilnadu Carnatic. The Ranipat Herbarium, St. Joseph College, Tiruchirapalli. 1983. M. De Palma, F. Fratianni, F. Nazzaro, M. Tucci. Isolation of a flavonoid 3'-hydroxylase sequence putatively involved in luteolin biosynthesis in globe artichoke. *Proceedings of the 50th Italian Society of Agricultural Genetics Annual Congress Ischia, Italy – 10/14 September, 2006.*
 10. Thabrew MI, Munasinghe TM, Senarath S, Yapa RM. Effects of *Cassia auriculata* and *Cardospermum halicacabum* teas on the steady state blood levels of theophylline in rats. *Drug Metabol Drug Interact.* 2004;20(4):263-72.
 11. Thabrew MI, Munasinghe TM, Senarath S, Yapa RM. Effects of *Cassia auriculata* and *Cardospermum halicacabum* teas on the steady state blood levels and toxicity of arbamazepine. *J. Ethnopharmacol.* 2004 Jan ;90(1):145-150.
 12. Thomas Karrasch, Joo-Sung Kim, Byung Ik Jang, Christian Jobin .The Flavonoid Luteolin Worsens Chemical-Induced Colitis in NF-kBEGFP Transgenic Mice through Blockade of NF-kB-Dependent Protective Molecules. *Plos one*, July 2007, Issue 7.
