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COMPARATIVE STUDY OF HEXANE EXTRACT FOR VOLATILE AND NON VOLATILE COMPONENTS OF LEAVES AND RHIZOMES OF *ACORUS CALAMUS* LINN. USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

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The present communication attempts to evaluate the comparative study of leaves and rhizomes of *Acorus calamus* Linn. (Family; Araceae) using High Performance Thin Layer Chromatography (HPTLC). Since the plant contains various volatile and non-volatile components so paper advocates the quantitative study using hexane extract. *Acorus calamus* Linn. is a well known medicinal plant in traditional medical systems having various ethno-pharmacological uses. As the official source of the plant is roots and rhizomes, but here study had been done comparatively with leaves. Previously leaves of *Acorus calamus* were not regarded as useful part of plant, but recently there is growing interest in leaves of the said plant. The leaves are considered to possess various activities such as an insect repellent, when cut up and kept with grain storage; anti-hyperlipidemic; anti-diabetic; antipsychotic; anti-inflammatory and analgesic. As there is no detailed work reported in leaf constituents of the plant, therefore the study revealed specific quantitative HPTLC data for the plant for future standardization work. HPTLC analysis of both leaves and rhizomes showed the presence of Asarone, β- sitosterol, lupeol and Ursolic acid when matched with marker compounds.

Key words: *Acorus calamus* Linn., HPTLC, standardization, Marker compounds

INTRODUCTION

HPTLC is an advanced versatile chromatographic technique for quantitative analyses with high sample throughout and is complementary to HPLC/GLC. It provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs. The HPTLC technique in standardization is required for quantification of marker components by area under curve, determination of the accurate R_F values for the marker components, determination of the purity of the substance (peak purity) and determination of the absorption maxima of the substance. *Acorus calamus* Linn. is semi-aquatic herb with creeping rhizomes and sword shaped long leaves, found nearly marshy places, river banks and lake.[1] It is up to 6 feet tall, aromatic, sword-shaped leaves bearing small yellow/green flowers and branched rhizome. It is widely distributed throughout India and Ceylon, in marshes, wild or cultivated, ascending the Himalayas up to 6000 feet in Sikkim, marshy tracts of Kashmir and Sirmoor in Manipur and Naga Hills.[2] The roots and rhizomes are used medicinally since ancient times. They possess antispasmodic, carminative and anthelmintic properties and are also used for the treatment of epilepsy, mental ailments, chronic diarrhoea, dysentery, bronchial catarrh, fever and glandular and abdominal tumours.[3,4] They are also employed for kidney and liver troubles, rheumatism, sinusitis, eczema and anti-cellular activities.[5] Recently roots and rhizomes identified as antibacterial agent against fish pathogen[6] and also shows insulin sensitizing activity.[7] Whereas mature green leaves exhibit various activities including insect repellent, when cut up and stored with dry foods[8], anti-hyperlipidemic activity, anti-diabetic activity[9], antipsychotic activity[10], antimicrobial and analgesic actions.[11] Recently methanolic and acetone extracts of *Acorus calamus* leaves have been shown to possess CNS depressant activity, which can be utilized in future for anticonvulsant activity.[12] The water extract of leaves of *Acorus calamus* have shown anti-inflammatory activity.[13]

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MATERIAL AND METHODS

The leaves were wildly collected from catchment of Bhimtal Lake in Uttarakhand located in North India, proclaimed as to have ethanopharmacological

importance. It was preserved in 70% ethyl alcohol for various other studies.

Sample preparation: Samples of leaves and rhizomes of *Acorus calamus* Linn. were prepared by using stock solution of hexane extract, obtained from cold percolation. They had been prepared as a 10 mg/ ml of stock solution.

Stationary phase: Pre-coated silica gel 60 F254 plate (E. Merck) in uniform thickness 0.2 mm

Sample applicator: The CAMAG Linomate-5 applicator for application of sample in the form of narrow bands, particularly analysis of mixture compound like plant extracts. The CAMAG linomate-5 uses the spry-on technique for applying samples on to the chromatogram layer as narrow band this permits the application of larger sample volume than is possible with contact sample transfer. A 500 µl syringe can be used instead of the standard 100 µl dosage syringe; another advantage of the linomate-5 is its self adjusting plate support. It allows the use of layers differing in thickness without re adjusting the spray nozzle. This feature makes attractive for the preparative application.

Sample application: 10 mg/ml of plant hexane extract was prepared 20 µl of this solution was applied on the plate, and 1mg/ml standard marker solution was prepared and different amount like 10 µg, 20 µg, and 30 µg and was applied.

Chromatography: The plate was eluted with respective mobile phase in CAMAG twin through chambers. The chamber was saturated with respective mobile phase saturation plate (E. Merck) of uniform thickness 0.2 mm was used for all the HPTLC analysis.

Video documentation: the eluted plate was analyzed under CAMAG Reproster-3 for the UV visualization at different λ value like 254 nm, 366 nm

Scanning of tracks: The eluted plate have different tracks of elute which are densitometrically scanned using CAMAG Scanner-3 at the respective wavelengths or at the multi wavelength for the crude extract gives area under curve for respective component present in the extract and the amount of the component would be quantify.

RESULT AND DISCUSSION

Result obtained from current study in HPTLC profile of hexane extract of leaf and rhizomes by CAMAG HPTLC System with wincats-3 programming software; video documentation of plates by CAMAG Reproster-3 video documentation under UV 254 nm, 366 nm, and in visible light after post-derivatization with anisaldehyde sulphuric acid reagent.**TLC plate (1)** using Toluene: ethyl acetate: 9:1 as mobile phase in case of Lupeol, β-sitosterol, and Ursolic acid (which are non- volatile components) were done. Observations are shown below. Another **TLC plate (2)** run in case of asarones (volatile components) with solvent system toluene: ethyl acetate: 8.5:1.5 as mobile phase.

Detection of TLC plate (1)

TLC plate (1) scanning by CAMAG scanner-3 R_f value of the marker compounds which are matched with the sample tracks and identified Lupeol, β-sitosterol and Ursolic acid were present in both tracks. These compounds are generally non-volatile components of the plant which were quantified by the HPTLC method.

Detection of TLC plate (2): Plate (2) scanned by CAMAG scanner-3 and the major volatile compound Asarone was evaluated by densitometric scanning profile of tracks showing below for Leaf and Rhizome of plant *Acorus calamus* Linn. The marker compound compound (α -asarone) were used for quantification.

Markers		**Lupeol**	**β-Sitosterol**	**Ursolic acid**
Sample	R_f	0.45	0.31	0.18
Leaf		+	+	+
Rhizome		+	+	+

Table1: Screening of compounds in plate (1) by HPTLC, (+) shows presence and (−) shows absence

Track	**Vial**	**Sample/ Conc.**
Lf	1	10µl from 10 mg/ml stock solution
Rz	2	10µl from 10 mg/ml stock solution
B	3	10µl from 1mg/ml stock solution
Lu	4	10µl from 1 mg/ml stock solution
Ur	5	10µl from 1mg/ml stock solution

Table 2: Sample application on TLC plate

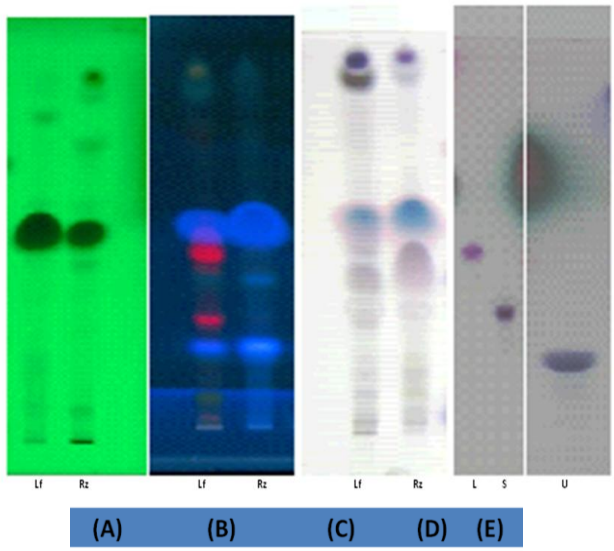
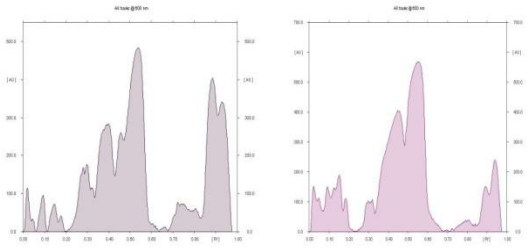
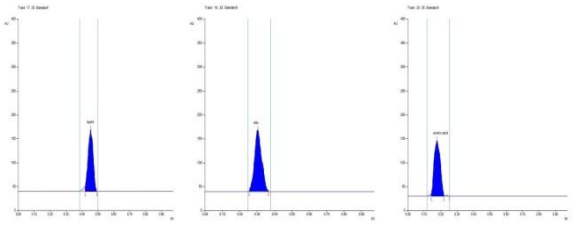


Fig.1: HPTLC profile of Hexane extract of plant *Acorus calamus* Linn sample Leaf extract(Lf) and Rhizome extract (Rz) (A) under 254 nm (B) Under 366 nm (C) Under visible light, (D) Lupeol [L] and β-Sitosterol [S], (E) Ursolic acid [U]



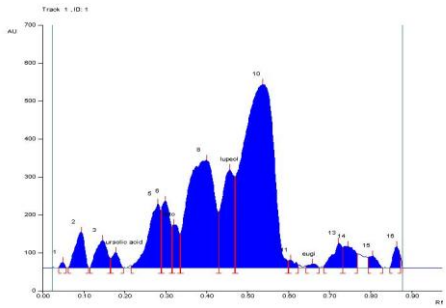
(a) (b)

Fig.2: HPTLC scan profile AUC *vs* R_f for plant sample *Acorus calamus* Linn, (a) Leaf profile (b) Rhizome profile at 600 nm

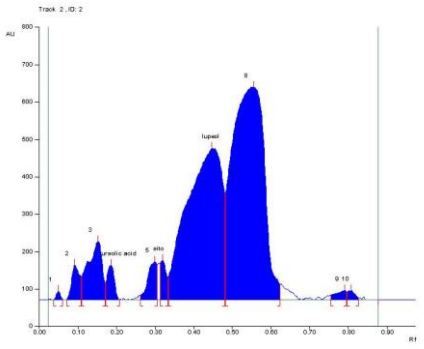


(A) (B) (C)

Fig.3: HPTLC scan profile of Plate (1) showing AUC *vs* R_f for marker compound, (A) Lupeol, (B) β-Sitosterol, (C) Ursolic acid.



(a)

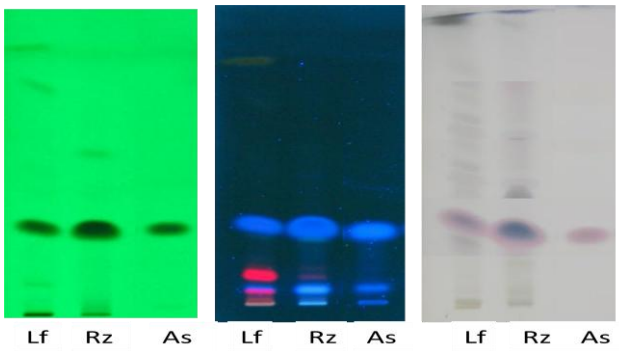


(b)

Fig.4: HPTLC densitometric scan profile for plant sample *Acorus calamus* Linn, (a) Leaf profile (b) Rhizome profile at 600 nm.

Track	Vial	Sample/ Conc.
Lf	1	10µl from 10 mg/ml stock solution
Rz	2	10µl from 10 mg/ml stock solution
Asarone	3	10µl from 1mg/ml stock solution

Table 3: Sample application on TLC plate (2)



(a) **(b)** **(c)**

Fig.5: Plate (2) HPTLC Profiles of plant *Acorus calamus* Linn (a) under UV 254 nm (b) under UV 366 nm (c) under UV Visible light after spray

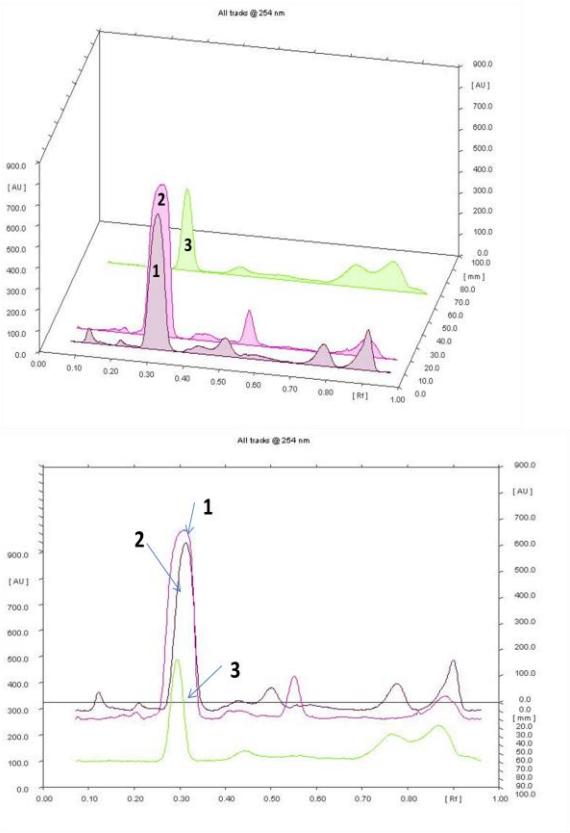
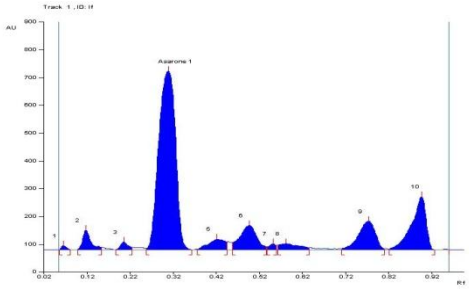
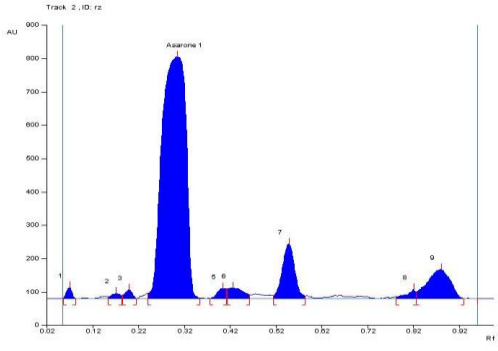


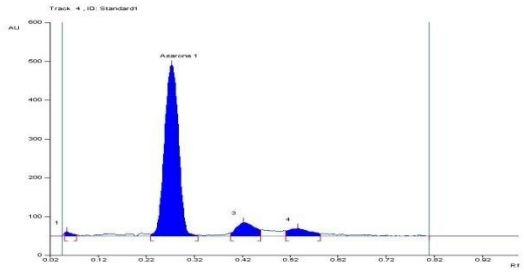
Fig.6: Plate (2) U.V. scans profile both filled and line for plant samples and marker at 254 nm. Here 1= Leaf extract; 2=Rhizome extract; 3=Asarone (marker compound).



(a)

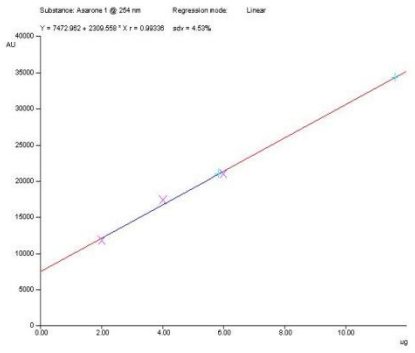


(b)

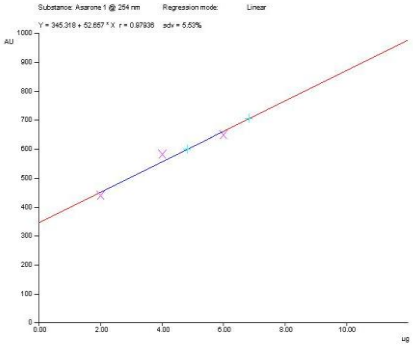


(c)

Fig.7: Plate (2) (a) Densitometric scan profile for Leaf extract (b) Densitometric scan profile for Rhizome extract (c) Densitometric scan profile for Marker compound (Asarone) all at 254 nm



(a)



(b)

Fig.8: Plate (2) graph showing Linearity (a) wrt area of the peak (b) wrt height of the peak; wrt=with respect to

Percent non-volatile content in plant *Acorus calamus* Linn. (Plate 1)

Sample	AMT (µl)	AUC	AP	% CP
Lf	10	1053.30	262.14ng	0.0065
Rz	10	5552.95	1.382 µg	0.138

Table 4: Percent Lupeol present in plant *Acorus calamus* Linn

Sample	AMT (µl)	AUC	AP	% CP
Lf	10	2203.29	434.13 ng	0.011
Rz	10	628.11	123.76 ng	0.0125

Table 5: Percent β- sitosterol presents in plant *Acorus calamus* Linn

Sample	AMT (µl)	AUC	AP	% CP
Lf	10	340.49	82.19 ng	0.011
Rz	10	10008.33	243.41 ng	0.21

Table 6: Percent Ursolic acid present in plant *Acorus calamus* Linn

Percent Volatile content in plant *Acorus calamus* Linn. (Plate 2)

Sample	AMT (µl)	AUC	AP	% CP
Lf	10	20973.73	5.846µg	0.146
Rz	10	34312.61	11.62 µg	1.162

Table 7: Percent Asarone present in plant *Acorus calamus* Linn.

AMT=Amount applied from 10 mg/ml sample solution; AUC= Area under curve; CP=content in plant; AP= Amount present

CONCLUSION

Comparative standardization, through High performance thin layer chromatography (HPTLC), suggested that leaf and rhizomes of Acorus calamus have marked variation in their both volatile and non volatile chemical constituents. The produce figure of the various chemical constituents in both leaf and rhizomes could be further referred for future research work on the same plant. The study revealed specific identities for the plant, which will be utilize in identification, as a control to check adulterants and for future standardization work.

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