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Pharmacognostical Evaluation of an Antioxidant Plant - *Acorus calamus* Linn

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Abstract – The rhizome of *Acorus calamus* Linn. is commonly known as "Vacha" in indigenous systems of medicine. It is distributed in marshy tracts of Kashmir, Sirmaur (Himachal Pradesh), Manipur and the Naga hills. It is regularly cultivated in Koratagere Taluk in Karnataka and other parts of India. This study deals with the detailed pharmacognostical evaluation of the dried rhizomes of *Acorus calamus* collected from DehraDun (Uttaranchal), Lucknow (Uttar Pradesh). The commercial sample procured from Delhi market was also evaluated to observe the difference between collected and market samples. Dried rhizome is vertically compressed, pale yellow to dark brown and occasionally orangish brown in colour. Transverse section showed two distinct region with scattered, concentric vascular bundles surrounded by fibrous bundle sheath. Some vascular bundles just beneath the endodermis devoid of bundle sheath. Though the botanical and physico-chemical characters of all the samples were quite similar but some variations were observed in High Performance Thin Layer Chromatography (HPTLC) fingerprint profile, the essential oil content and total percentage of asarone which was found to be highest in Lucknow and lowest in Delhi market sample. These variations may be explained due to some edaphic factors or storage conditions. An attempt was also made to test antioxidant activity *(in vitro)* and it was found to be 88% at 0.2 g/ml concentration.
Keywords – *Acorus calamus*, Asarone, Antioxidant, Pharmacognosy

Introduction

Acorus calamus Linn. (Family-Araceae) is a semi-aquatic, perennial, aromatic herb with creeping rhizomes. It is the official drug for flatulent colic and chronic dyspepsia (Parotta, 2001). The rhizome is aromatic, stimulant, bitter, tonic, carminative, anti-spasmodic, emetic, expectorant, emmenagogue, aphrodisiac, laxative, and diuretic (Kapoor, 2001). The insecticidal activity of the solvent extracts and steam-distilled volatile principle of rhizome against common houseflies is quite marked (Agarwal *et al.*, 1956). α- and β-asarone and 1-allyl-2, 4, 5-trimethoxybenzene from essential oil exhibited spasmolytic action on isolated guinea pig trachea and ileum contracted by acetylcholine, histamine serotonin and barium chloride. Both α and β-asarone showed cardiac depressant activity, moderate hypotensive action in anaesthetized dogs and antiacetylcholine activity (Janssen & Sceffer, 1985). The major chemical constituents of *A. calamus* are α- and β-asarone (Iguchi *et al.*, 1969; Patra & Mitra, 1981), calacone, telekin, isotelekin, calarene, isocalamendiol, calamendiol (Saxena, 1986), shyobunone, epishyobunone, isoshyobunone (Iguchi *et al.*, 1969; Iguchi *et al.*, 1970), acolamone, isoacolamone, acoragermacrone, (+)calamusenone, isocalamusenone, galangin (flavone), acoradin, 2, 4, 5 hydrodxy benzaldehyde, 2, 5 dimethoxy benzoquinone (Patra & Mitra, 1979), calamensesquiterpene (Janssen & Scheffer, 1985), triterpenoid saponins viz. 1β, 2α, 3β, 19α - tetrahydroxy-12-en-28-oic acid-28-O-{β-D-glucopyranosyl (1→2)}-β-D-galactopyranoside, 3β,22α, 24, 29-tetrahydroxyolean-12-en-3-O-{-β-D-arabinosyl(1→3)}-β-D-arbinopyranoside (Rai *et al.*, 1998) and sesquiterpene viz. 2-hydroxyacorenone, 2-acetoxyacorenone, epicorone, 1-hydroxyepiaorane, epiacoronene, acorusnol (Nawamiki & Kuroyanagi, 1996).

As no detailed pharmacognostical evaluation of this plant is on record, hence the present study has been undertaken. The study includes macro and microscopic features, physical and chemical characters of the rhizome of *A. calamus* collected from different regions of the country, isolation of asarone and high performance thin layer chromatography (HPTLC) profile of the extracts. Apart from these, the antioxidant activity by using 1,1 diphenyl 2-picryl hydrazyl (DPPH) scavenging (Govindarajan *et al.*, 2003) of the extract was also studied.

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Materials and Methods

The rhizomes of the *A. calamus* were collected from Lucknow and DehraDun, the voucher specimen number 221320 was authenticated by Dr. A.K.S. Rawat and deposited in the departmental herbarium of National Botanical Research Institute. The market sample was procured from Delhi herbal drug market. For microscopic studies, the transverse section (T.S.) were cut with the help of microtome and stained with safranin and fast green (Johanson, 1940). Histo-chemical studies were performed for the presence of lignin, suberin, mucilage, oil cells, starch grains and type of crystals present (Kokate, 1986). The reaction of the powdered drug were studied with different chemical reagents as per method described by Chase and Pratt (1949) and Kokoski *et al.*, (1958). The quantitative analysis viz. the total ash, acid insoluble ash, total alcohol and water-soluble extractives were assayed by Indian Pharmacopoeial methods (Anonymous, 1966). Sugar and starch percentage were also estimated according to the method described by Montgomery (1957).

For the isolation of asarone (Patra & Mitra, 1981), 200 g of the dried rhizomes of *A. calamus* were powdered and then extracted exhaustively with 1 L of 95% ethyl alcohol. The alcoholic extract was concentrated at a temperature of 45°C under reduced pressure and then lyophilised. The completely dried extract was then treated with 10% sodium bicarbonate solution and the soluble portion was run on a precoated thin layer chromatography plate (TLC) in solvent system Toluene: Ethyl acetate (90:10) and asarone was isolated by preparative TLC technique by scrapping the silica plate material at Rf 0.63. Asarone thus isolated was characterized with the help of the HPTLC. The HPTLC of the extract from the samples obtained from various sources was performed in HPTLC Silica gel F254 plates in the solvent system Toluene: Ethyl acetate (90:10) and then scanned through Camag TLC scanner 3. The spots were applied with the help of Linomat IV. The finger print profile of HPTLC were obtained with Desaga Video Documentation unit III.

Antioxidant activity of the crude extract was carried out using DPPH free radical scavenging method. Three different concentrations (0.2, 0.1, 0.01 g/ml) of the crude extract of the *A. calamus* was prepared in methanol. 50 µl of methanolic test solutions were taken with 2.95 ml of 1,1-diphenyl 2-picryl hydrazyl (100 µM in methanol) and the optical density (O.D.) was measured at regular intervals (30 seconds for a period of 5 min and then 5 min for the period of 30 min) at 517 nm. DPPH solution without the test solution was used as the control. Percentage activity was calculated using the following formula

$$\% \text{ Activity} = \frac{\text{Control Test}}{\text{Control}} \times 100$$

Results

Macroscopic characters (Fig. 1) – The rhizome is horizontal, jointed, somewhat vertically compressed, spongy within, pale to dark brown or occasionally orangish brown in colour. Cut surface of rhizome is creamy in colour. It possesses pungent and mucilaginous taste; peculiar pleasant smell, fracture hard and texture rough.

Microscopic Characters (Fig. 2) – Transverse section (T.S.) of rhizome exhibit an outer epidermis followed by cork. Broad cortex is separated out from a central cylinder or stele by thin walled endodermis. Outer cortex is collenchymatous while inner cortex and pith are parenchymatous with large air spaces. Vascular bundles are concentric with fibrous bundle sheath. Some vascular bundles without bundle sheath are scattered throughout the stele and occur more numerously just beneath the endodermis. Occasionally root trace bundles are also discernible. Phloem is well developed and consists of sieve tubes, companion cells and phloem parenchyma. Xylem vessels are arranged in the form of a circle and showing reticulate, scalariform or rarely spiral secondary wall thickenings. Mostly parenchymatous cells are filled with spheroidal starch grains which are mostly single, rarely in 2-3 groups 2-10 µ in diameter. Spheroidal oil cells with suberized walls and yellowish orange oil contents are also observed.

Study of powder – The powder of the rhizome is brown in colour with a peculiar pleasant smell and pungent taste. On microscopical examination it shows numerous patches of parenchymatous cells with large intercellular spaces and

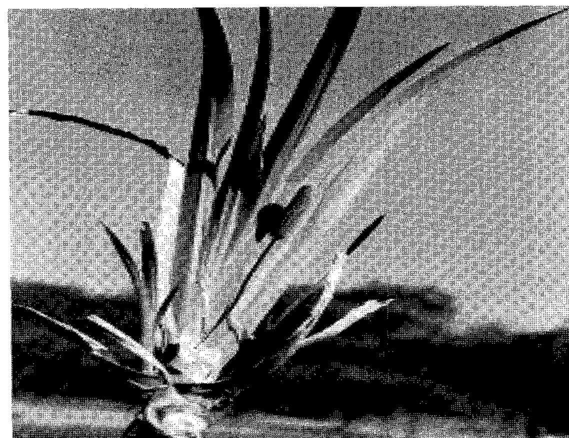

Fig. 1. *Acorus calamus.*

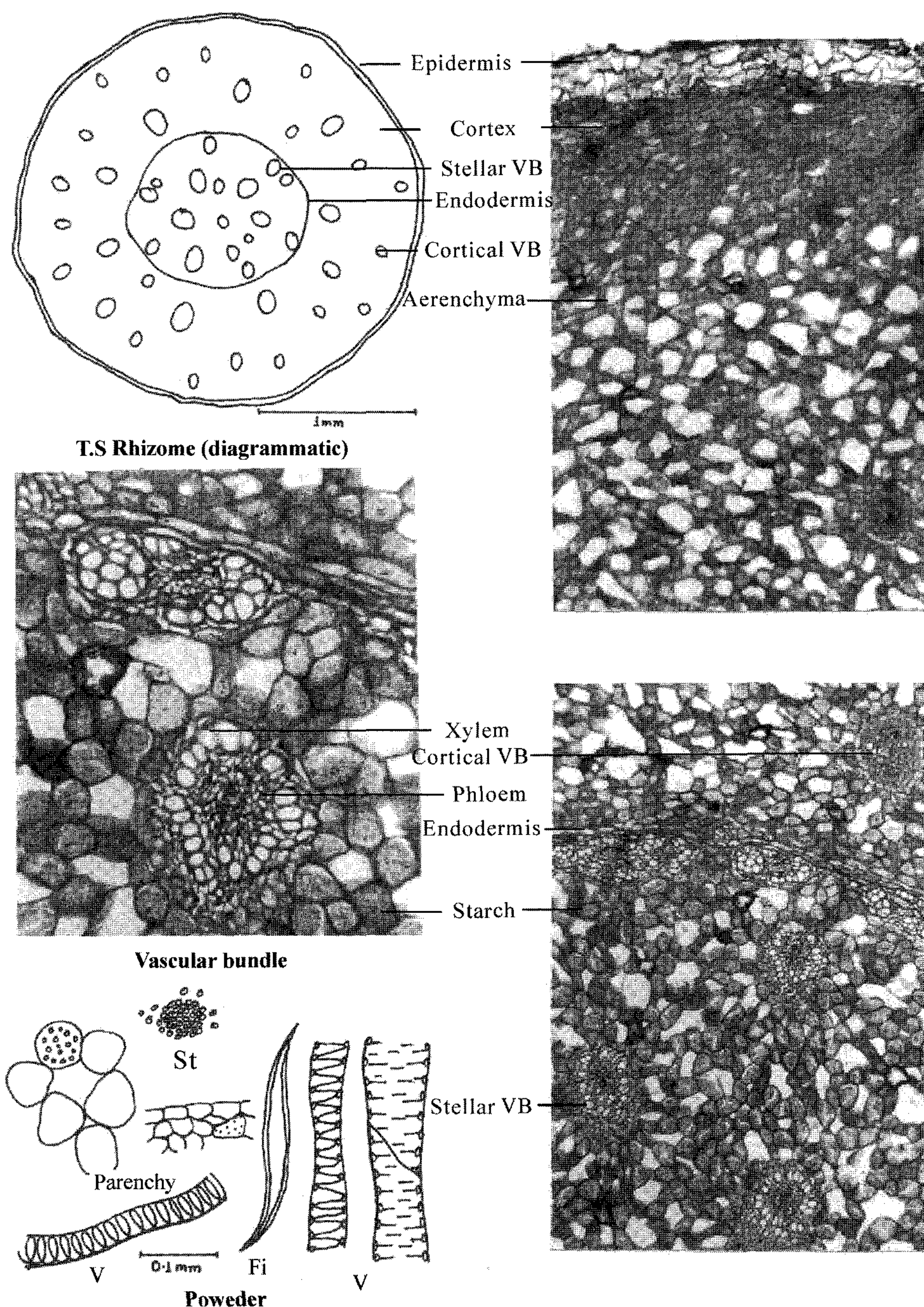

Fig. 2. Microscopy of *Acorus calamus* rhizome.

filled with starch grains, groups of suberized cork cells, oil globules, oil cells, fibres and different types of vessel elements with spiral, scalariform and reticulate secondary wall thickenings. When treated with 50% nitric acid, the power becomes red in day light and fluorescent green under UV 254 nm

Phytochemical studies – The percentage of total ash, acid insoluble ash, alcohol soluble and water soluble extractives, sugar and starch, essential oil and asarone were calculated and the results are tabulated in Table 1.

The isolated asarone showed peak at R_f 0.63. The densitometric scan of the methanolic extracts of all the three samples are well depicted in Figure 3. The TLC scanning profile of all the extracts were compared with the isolated asarone and it was found that the asarone at R_f 0.63 present in all the samples with the additional components at R_f 0.19, 0.25, 0.31, 0.67 and 0.81 (Table 2). The asarone percentage varies from 0.54 1.16% in different

Table 1. Quantitative standards for *A. calamus*

Sample source	Replicates	Total ash %	Acid insoluble ash%	Alcohol soluble extractive %	Water soluble extractive %	Essential oil %	Starch %	Sugar %	Asarone %
DehraDun	1.	6.78	1.52	19.85	27.75	0.50	32.7205	14.756	1.15
	2.	6.73	1.51	20.10	25.55	0.48	32.1395	15.097	
	3.	6.81	1.50	18.75	25.95	0.49	36.6885	14.415	
	Mean±SD	6.77± 0.040	1.51± 0.010	19.56±0.718	26.42± 1.171	0.49± 0.010	33.516± 2.475	14.756± .341	
Delhi	1.	6.71	1.63	19.20	25.80	0.39	33.1545	11.873	0.54
	2.	6.90	1.60	18.90	25.75	0.38	32.147	13.531	
	3.	6.05	1.50	19.50	25.55	0.39	32.5345	14.539	
	Mean±SD	6.57±0.446	1.57±0.068	19.38±0.300	25.70±0.132	0.38±0.005	32.612± 0.508	13.314± .346	
Lucknow	1.	6.76	1.62	18.85	26.00	0.39	34.0845	14.085	1.16
	2.	6.70	1.60	18.75	26.23	0.30	36.1925	15.298	
	3.	7.00	1.59	18.65	26.34	0.35	33.6815	14.508	
	Mean±SD	6.82±0.158	1.60±0.015	18.75±0.100	26.19±0.173	0.35±0.045	34.6528± 1.348	14.632± 0.616	

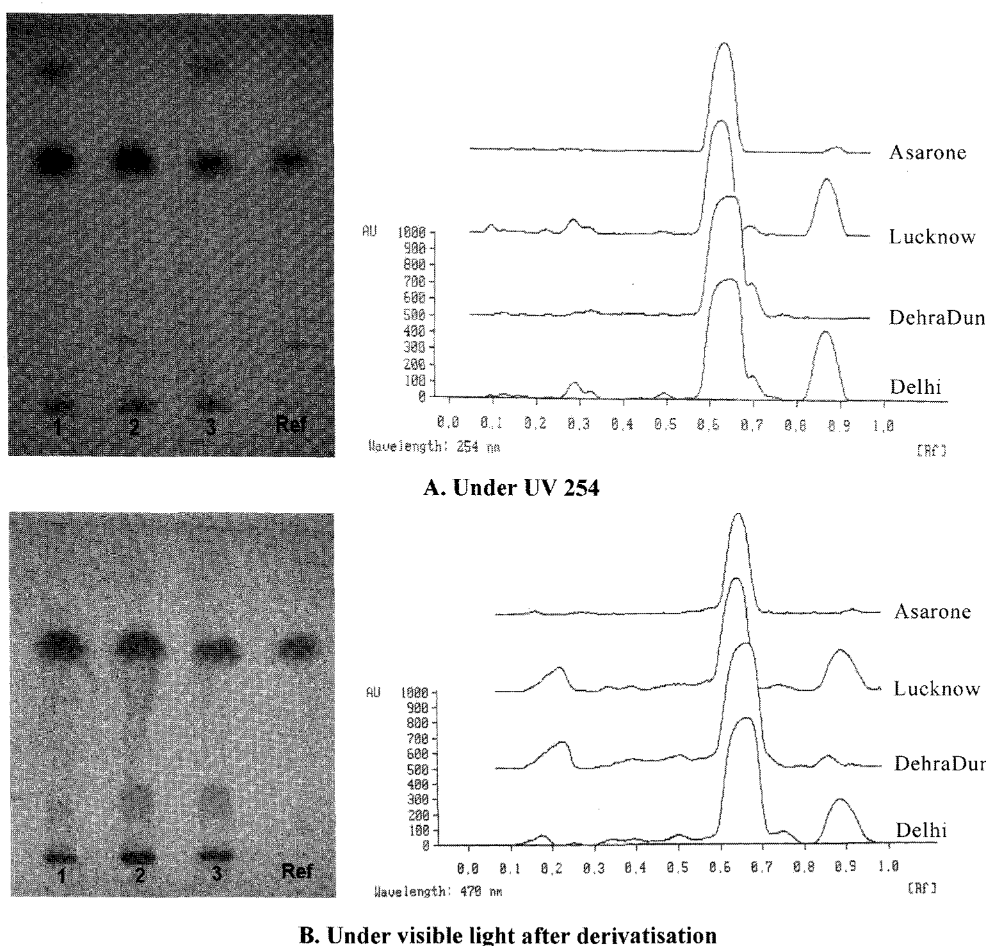

Fig. 3. HPTLC finger print profiles of asarone and 3 samples of *Acorus calamus.*

samples (Table 2).

Antioxidant activity (*in vitro*) by DPPH scavenging method at 3 different concentrations (0.2, 0.1, 0.01 g/ml) showed a maximum activity of 86.43% at 0.2 g/ml and a minimum activity of 45.93% at 0.01 g/ml (Table 3, Fig. 4).

Discussion

From the ongoing studies it was revealed that all the three samples from Delhi, DehraDun and Lucknow possess similar macro and microscopic characters and physicochemical

Table 2. TLC details of *A. calamus*

Scanned under 254 nm		Scanned after derivatization at 470 nm	
R_f.	**Colour**	**R_f.**	**Colour**
0.07	Black	–	–
0.19	Black	0.19	Blue
0.25	Black	–	–
0.31	Black	0.31	Blue
0.56	Black (asarone)	0.56	Reddish brown
0.67	Black	0.67	Yellowish orange
0.81	Black	0.81	Blue

Table 3. Percentage antioxidant activity of *A. calamus*

Time in Seconds	% Scavenging activity of alcoholic extract		
	0.2 g/ml	0.1 g/ml	0.01 g/ml
30	22.68	21.65	9.92
60	37.74	31.46	12.62
90	48.433	37.70	15.57
120	57.75	41.76	17.03
150	63.60	45.59	19.71
180	67.89	48.48	20.83
210	70.955	51.65	23.65
240	73.63	53.30	26.37
270	75.85	55.47	27.35
300	77.31	57.06	28.90
600	78.91	68.45	35.02
900	84.79	74.90	39.27
1200	85.58	78.69	40.90
1500	86.27	81.81	44.15
1800	86.414	84.01	45.93

values. Only slight variations were observed in HPTLC densitometric scan profile, essential oil and asarone percentage. The comparative HPTLC fingerprint showed prominent peaks at R_f 0.75 in Delhi and Lucknow sample and at R_f 0.57 only in DehraDun sample (Fig. 3A). Lucknow and DehraDun sample also showed peaks at R_f 0.22 after derivatization with anisaldehyde sulphuric acid reagent in densitometric scan (Fig. 3B). However the Delhi sample possessed almost 50% less asarone viz. Asarone was found to be 1.15 and 1.16% in DehraDun and Lucknow sample respectively while it was almost half i.e. 0.54% in Delhi sample. On the contrary, the essential oil was more in DehraDun sample i.e. 0.5% as compared to other samples in which it was approximately 0.38%. These variations may be due to the edaphic factors or storage conditions as the Delhi sample is the commercial one. Additionally the presence of the antioxidant activity may give the reason in part for the present plants use in the treatment of many diseases as anti-inflammatory, antidiarrhoeal, anti dysenteric, useful in the treatment of bronchial and chest infection and also for epilepsy which may be caused by the free radicals.

Thus, it can be concluded that the edaphic factors and

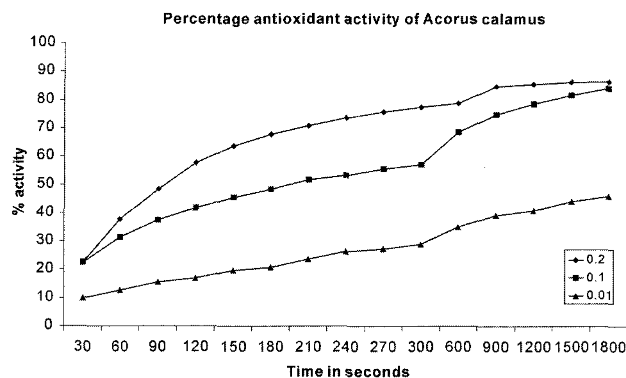

Fig. 4. Comparative percentage antioxidant activity of *A. calamus.*

storage conditions can play an important role on the percentage of secondary metabolites and quality of the drug.

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