

## Pharmacognostical Evaluation of Leaves of *Acorus calamus* Linn.

\*Bisht AS, Chauhan M, Dimri A, Joshi A, Ali M

Himalayan Institute of Pharmacy and Research, Atak Farm, Rajawala, Dehradun, Pin Code-248008

Available online: 4<sup>th</sup> October, 2013

### ABSTRACT

The present communication attempts to evaluate the physicochemical and preliminary phytochemical studies on the leaf of *Acorus calamus* Linn. (Araceae family). *Acorus calamus* Linn. is a well known medicinal plant in traditional medical systems having various ethanopharmacological uses. Previously leaves of *Acorus calamus* were not regarded as useful part of plant, but now a day there is growing interest in leaves of the plant. The leaves are considered to possess various activities such as an insect repellent, when cut up and stored with dry foods; antihyperlipidemic; antidiabetic; antipsychotic; antiinflammatory and analgesic. As there is no detailed work reported in leaf, therefore pharmacognostical evaluation including physicochemical parameters, preliminary phytochemical standards were determined. The study revealed specific identities for the plant, which will be useful in identification, as a control to abet adulterants and for future standardization work. Physicochemical studies shows, total moisture content(8.06%), total ash(9.4%), acid insoluble ash(0.65%), hexane soluble extractive values(2.5%), alcohol soluble extractive values(3.5%), and water soluble extractive values(30.5%), total starch content(2.46%), total sugar content(0.80%), total tannins(1.09%), total phenolics(0.41%), total flavonoids(0.2%), total flavonols content(0.4%), total proanthocyanidines content(0.65%), total volatile content(2%). Preliminary phytochemical analysis (organic analysis) revealed carbohydrates, glycosides, phenolic compounds, tannins, amino acids, terpenoids and flavonoids are present. TLC analysis shows the presence of Asarone, sitosterol, lupleol and Ursolic acid when matched with marker compounds.

**Keywords:** *Acorus Calamus* Linn., Physicochemical studies, Pharmacognostical evaluation, TLC, Marker compounds.

### INTRODUCTION

*Acorus calamus* Linn. is semi-aquatic herb with creeping rhizomes and sword shaped long leaves, found nearly marshy places, river banks and lake.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3,4</sup> They are also employed for kidney and liver troubles, rheumatism, sinusitis, eczema and anti-cellular activities.<sup>5</sup> Recently roots and rhizomes identified as antibacterial agent against fish pathogen<sup>6</sup> and also shows insulin sensitizing activity.<sup>7</sup> Whereas mature green leaves exhibit various activities including insect repellent, when cut up and stored with dry foods<sup>8</sup>, antihyperlipidemic activity, antidiabetic activity<sup>9</sup>, antipsychotic activity<sup>10</sup>, antimicrobial and analgesic actions.<sup>11</sup>

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.<sup>12</sup> The water extract of leaves of *Acorus calamus* have shown antiinflammatory activity.<sup>13</sup>

### MATERIALS AND METHODS

The leaves were wildy 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. Pharmacognostical and physicochemical evaluation were carried out from shade dried plant powder. Physicochemical standardization methods including determination of moisture content (loss on drying), determination of total ash and acid insoluble ash, extractive values were carried out as per WHO recommendations and authentic procedures mention in Ayurvedic pharmacopoeia of India. Estimation of total Sugar and total starch in plant material was carried out with according to Mont Gomery, 1957 [Spectrophotometric method] taking dextrose and starch (soluble), respectively as a standard solution. Whereas total tannins were determined by using Tannic acid as standard and Gallic acid for the determination of total phenolics. In the determination of total flavonoids and total flavonols Rutin was taken as a standard. Proanthocyanidines were estimated by using Catechin as a standard. In chromatographic processes pre-coated TLC plates were used. Post-derivatization was done with Anisaldehyde Sulphuric acid reagent. Toluene: Ethyl

Table 1 – Phytochemical screening of leaf of *Acorus calamus* Linn.

S. No.	Constituents	Tests	Hexane	chloroform	ethylacetate	methanol	Water
1.	Carbohydrate s	Benedict's test	+	+	+	+	+
		Molisch's test	+	+	+	+	+
		Caramelisation	+	+	+	+	+
2.	Glycosides	Fehling's test	-	+	+	+	+
		Libermann burchard test	+	+	+	-	-
4.	Proteins & Amino acids	Salkowski reaction	+	+	-	-	-
		Biuret test	-	-	-	+	+
		Ninhydrin test	-	-	-	-	+
5.	Saponins	Foam test	-	-	+	+	+
6.	Tannins	FeCl <sub>3</sub> test	+	+	+	+	+
		Alkaline reagent test	-	-	+	+	+
		Vanillin hydrochloride test	-	-	-	+	+
		Libermann burchard test	+	+	+	-	-
8.	Alkaloids	Dragendroff's test	-	-	-	-	-
		Mayer's test	-	-	-	-	-
9.	Resin	Resin	-	-	-	-	-
10.	Flavonoids	Alkaline reagent test	-	+	+	+	+
		Shinoda's test	-	-	+	+	+

+: present; -: absent

Fig. 1 *Acorus calamus* Linn. LeafTable 2 – Different physicochemical parameters of leaf of *Acorus calamus* Linn

Parameters	% present
Total starch content	2.46
Total sugar content	0.80
Total tannin content	1.09
Total phenolic content	0.41
Total flavonoidal content	0.2
Total flavonols content	0.4
Proanthocynadines content	0.65
Total volatile content	2

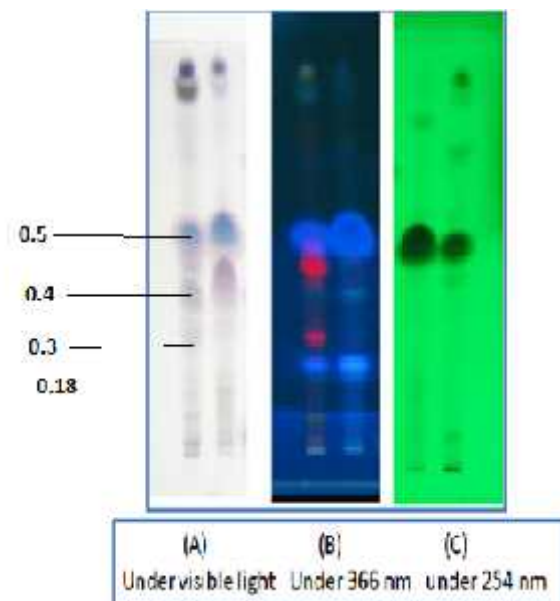


Fig. 2 Hexane extract chromatograms acetate: 9:1 used as a mobile phase for both non- volatile components and Volatile constituents.

## RESULT AND DISCUSSION

The powder studies shows powder to be yellowish green, characteristic odor, bitter. The % of moisture content was 8.2, total ash 9.4, acid insoluble ash 0.65, hexane soluble extractive 2.5, alcohol soluble extractive 3.5 and water soluble extractive 30.5. A known quantity (10 gms) of dried powder was extracted in a Soxhlet with hexane, chloroform, ethyl acetate, methanol and water for 72 hrs successively and tested for phytochemical analysis. The % of successive extractive values were hexane 1.92 ,

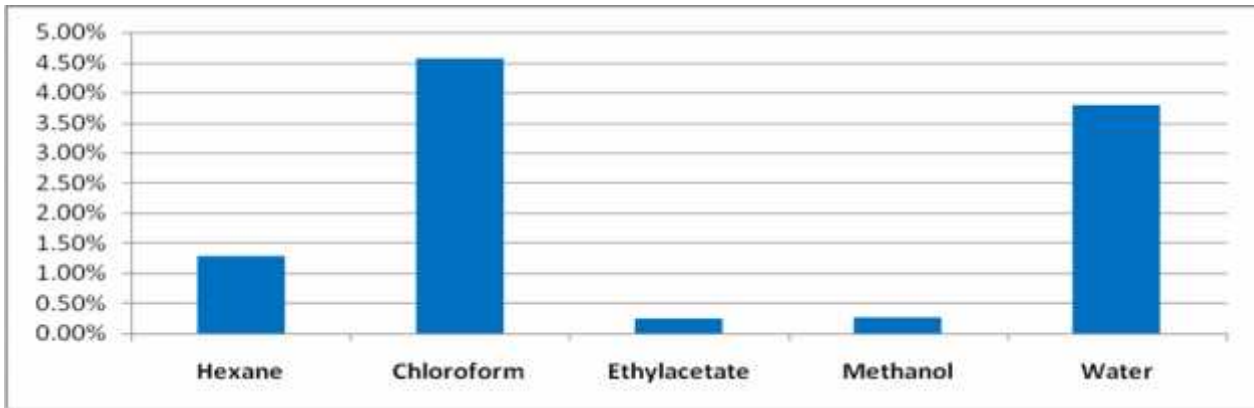


Fig. 3 Graphical presentation of Extract preparation through hot percolation method (Soxhlet Apparatus) in *Acorus calamus* Linn leaf.

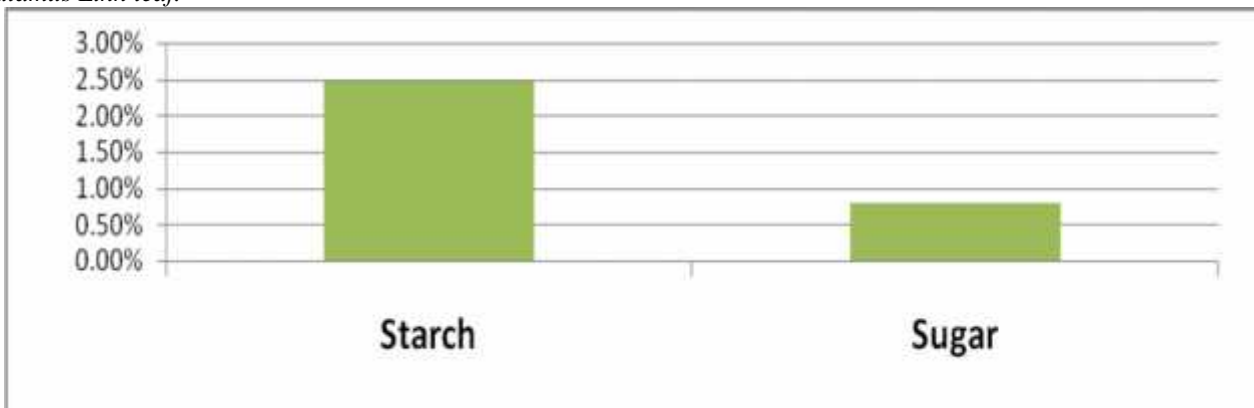


Fig. 4. Graphical presentation of starch and sugar content in *Acorus calamus* Linn. leaf

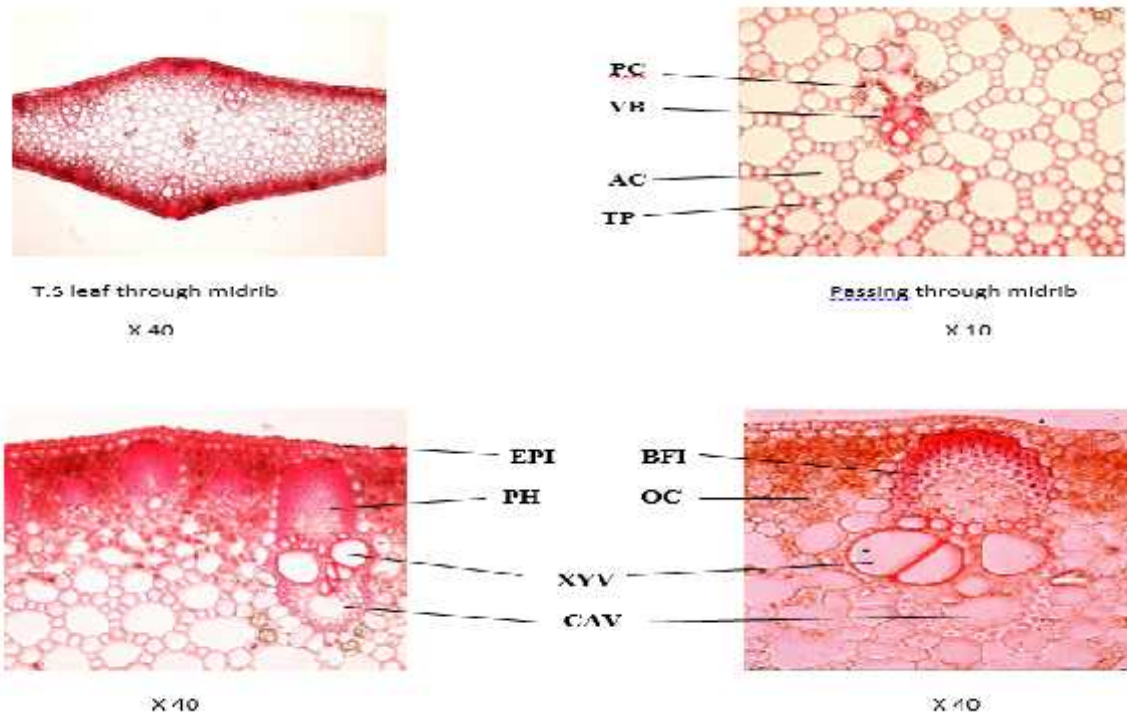


Fig. 5 T.S leaf of *Acorus calamus* linn

Abbreviation: AC-aerenchyma, BFI- bundle fibres, CAV-cavity ,EPI-epidermis,OC-oil containing cell,PH-phloem,TP-thick walled parenchyma,VB-vascular bundle,XYV-xylem vessels

chloroform 4.56 , ethyl acetate 0.24 , methanol 0.26 water 3.8 . The results of phytochemical analysis are provided (Table 1). TLC analysis of hexane extract was done and Rf values were calculated. Lupeol, -sitosterol, Ursolic acid and Asarone have Rf values 0.45, 0.31, 0.18 and 0.5 respectively. The result of total starch content, total sugar content, total tannins, total phenolics, total flavonoids, total flavonols content, total proanthocyanidines content and total volatile content are presented in. (Table 2).

## CONCLUSION

Pharmacognostical evaluation on *Acorus calamus* Linn., leaves provides some specific pharmacognostical parameters useful in evaluation and standardization of the leaf drug. The presence of considerable level of polyphenolic compounds including flavonoids, flavonols, proanthocyanidines, etc. suggested that plant part can be considered to show antioxidant activity. TLC analysis predicts that leaf contains asarone, ursolic acid, -sitosterol and leupol in considerable amount.

## ACKNOWLEDGMENT

Authors are thankful to Head, Department of Pharmaceutical sciences, Kumaun University Bhimtal campus, for providing facilities and encouragement.

## REFERENCES

- Rastogi RP, Mehrotra BN. *Acorus calamus*. *Compendium of Indian medicinal plants*. 1980, Vol 3, 11.
- Rao MM, Meena AK, Singh A and Kumari S, Physicochemical and preliminary phytochemical studies on the rhizomes of *Acorus calamus* Linn,
- International Journal of Pharmacy and Pharmaceutical Sciences*, 2 (2) (2010) 130-31.
- Chopra R N, Nayar S L, Chopra I C., *Glossary of Indian medicinal plants*. NISCIR, CSIR, Delhi 2002.
- Nadkarni KM., *Indian Materia Medica*, 3<sup>rd</sup> Edn., Vol. I, Popular Prakashan Pvt. Ltd. Bombay, 1982, 1211-12.
- Agarwal SL, Dandia PC, Singh KP, Arora RB, A note on the preliminary studies of certain pharmacological actions of *Acorus Calamus* L., *J. Am. Pharm. Assoc.*, 45 (1956) 655-56.
- Bhuvanewari R and Balasundaram Chellam, Anti-bacterial activity of *Acorus calamus* and some of its derivatives against fish pathogen *Aeromonas hydrophila*, *Journal of Medicinal Plants Research*, 3(7) (2009) 538-47.
- Hao-Shu Wu, Di-Feng Zhu, Chang-Xin Zhou, Chu-Rui Feng, Yi-Jia Lou, Bo Yang, Qiao-Jun He., Insulin sensitizing activity of ethyl acetate fraction of *Acorus calamus* L. in vitro and in vivo, *Journal of Ethnopharmacology*, 123 (2) (2009) 288-92.
- Chan Park, Soon-Il Kim, Young-Joon Ahn., Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects., *Journal of Stored Products Research*, 39 (2003) 333-42.
- Reshma S, Sushma A., Hypolipidemic activity of *Acorus calamus* L. in rats., *Fitoterapia*, 73 (6) (2002) 451-55.
- Pandy V, Jose N, Subhash N., CNS activity of methanol and acetone extracts of *Acorus calamus* leaves in mice, *J. Pharmacol. Toxicol.*, 4 (2009) 79-86.
- Devi AS, Ganjewala D., Antimicrobial activity of *Acorus calamus* (L.) rhizomes and leaf extract, *Acta Biologica Szegediensis*, 53 (1) (2009) 45-49.
- VengadeshPrabu K, George T, Vinoth KR, Nancy J, Kalaivani M, Vijayapandi P., Neuromodulatory effect of *Acorus calamus* leaves extract on dopaminergic system in mice. *International Journal of PharmTech Research*, 1(4) (2009) 1255-59.
- Hyeri Kim, Tae-Ho Han , Seong-Gene Lee., Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells, *Journal of Ethnopharmacology*, 122 (1) (2009) 149-56.
- AOAC Official methods of analysis of AOAC International, 16<sup>th</sup> edition. AOAC International, Suite 400, 2200 Wilson Boulevard, Arlington, Virginia, USA, 1995.
- R. Oprean, Tamas M, Roman L., Comparison of GC-MS and TLC techniques for asarone isomers determination, *Journal of Pharmaceutical and Biomedical Analysis*, 118 (1) (1998) 227-34.
- Selçuk SS, Bahar M, Mericli AH, The flavonoid compounds from the leaves of *Acorus calamus* growing in Turkey, *Acta Pharmaceutica Scientia*. 51 (1) (2009) 83-85.
- World Health Organization: *Quality control methods for medicinal plant materials*. Published by WHO, Geneva, 1998