

## PHYTO-CHEMICAL STANDARDISATION OF ACORUS CALAMUS L.

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### Abstract

*Acorus calamus* L. (Araceae) is commonly known as "Bach" in Hindi. Rhizomes have been taken up for standardisation. The crude as well as formulated mother tincture was standardised to lay down standards in Homoeopathic system of medicine. The determined data under phyto-chemical study viz. Extractive value, Ash value, Thin Layer Chromatography and Spectrophotometry are helpful to authenticate the drug.

### Introduction

*Acorus calamus* Linn. is commonly known as "Bach" in Hindi speaking area, Vacha in Sanskrit, Vashambu in Tamil and Sweet flag in English. It is *Acorus calamus* L. in latin and belongs to the family Araceae. The plant is a semi-aquatic, perennial aromatic herb 5-6 feet in height and produces creeping rhizomes similar to ginger. The dried rhizomes are sold in market. Sweet flag is found wild and cultivated throughout the country upto an altitude of 2200 m. The land and field situated near water source is suitable for its cultivation. The drug is in regular cultivation in marshy tracts of Kashmir, Manipur, Sirmour in Himachal Pradesh, Naga Hills and Koratagere Taluk in Karnataka. In Ayurvedic System of Medicine the drug is described as anti-spasmodic, carminative, emetic, anthelmintic, Vata and Kapha pacifying, activating hunger and used in the treatment of epilepsy and other mental ailments, constipation, dropsy, chronic diarrhoea and dysentery, bronchial catarrh, intermittent fevers, glandular and abdominal tumours, kidney and liver troubles, rheumatism and eczema. The powdered sweet flag has insecticidal and antifungal properties against bed-bugs, moths & lice. This action has been found better than synthetic insecticide chemicals as it is biodegradable and hence it does not leave any residue.

The rhizomes, roots and leaves yield a light brown yellow volatile oil called calamus oil which is a viscous liquid having warm, woody, spicy and

pleasant odour. The fresh rhizomes yield 1.8% while the dried material 1.5-3.5% of the oil, Asarone, the main constituent. 82% of calamus oil exists in both  $\alpha$ - $\beta$  forms and rest composed of 33 different chemical compounds which include calamenone 1.9, methyl eugenol 1.0,  $\alpha$ -pinene-camphene 0.2 and traces of butyric, palamitic and heptylic acids, asaraldehyde, calamol, calamone, azulene and several sesquiterpenes, alcohols and hydrocarbons (Anonymous, 1986). Two new triterpenoid saponins have been isolated which are characterised as 1 beta, 2 alba, 3 beta, 19 alba-tetrahydroxyurs-12-en-28-oic acid-28-O- $\beta$ -D-glucopyranosyl (1 to 2)- $\beta$ -D-galactopyranoside 1 and 3 beta, 22 alba, 24, 29-tetrahydroxyolean-12-en-3-O- $\beta$ -D-arabinosyl (1 to 3)- $\beta$ -D-arabinopyranoside 2 [Siddiqui, 1998]. The calamus oil is also useful in variety of medicinal and other purposes. Because of its important medicinal value in Homoeopathy and other system of medicines, the authors have undertaken to standardise phytochemically to lay down standards in the system.

### Material and Methods

The air dried (shade dried) sample [A] of rhizomes of *Acorus calamus* L. was supplied by Survey of Medicinal Plants and Collection Unit, (CCRH) Udhagamandalam (Ooty), Tamil Nadu. Market sample [B] purchased from Lucknow was also studied for comparison purposes. The above samples were comminuted to obtain coarse powder (10/44) which was then used for determination of moisture content [loss on drying at 105°C], total ash content, water soluble ash and extractive values in different solvents (varying polarity). The above parameters have been determined in accordance with the procedure given in Homoeopathic Pharmacopoeia of India (H.P.I.) or Indian Pharmacopoeia (I.P.) Maximum Extractive Value (MEV) of the drug is determined using different

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strengths of alcohol (in order to fix the alcohol content for preparation of mother tincture). Mother tincture was prepared as per HPI (Anon., 1971). In this method 100 gm. of *Acorus calamus* in coarse powder was kept with 500 ml. purified water and 421 ml. of 95% alcohol for 24 hours. After that it was filtered and made upto volume to 1000 ml., using the same solvent ratio. In this way market sample was also prepared using 632 ml. purified water and 368 ml. of 95% alcohol to make 1000 ml. using the same technique.

All chemicals and solvents used were of analytical grade. Silica gel-G of E-Merck was used for thin layer chromatography and work was carried out at room temperature. U.V. spectra was recorded on U.V. Spectrophotometer (Shimadzu, Model 160 A).

The above alcoholic extracts [mother tincture] were studied for their -

- A. Physico-chemical constants
- B. Chromatography
- C. U.V. Absorbance.

### Physico-Chemical Constants

Physico-chemical parameters viz. organoleptic properties, wt. per ml., total solids, alcohol content, pH value were determined as per the procedure laid down in the Homoeopathic Pharmacopoeia of India.

### Chromatography

For thin layer chromatography 25 ml. of alcoholic extract was evaporated on water bath to remove alcohol, the remaining aqueous part was extracted with 25 ml. of chloroform (three times). All the three fractions were combined and concentrated to 2 ml and 15 $\mu$ l was applied on T.L.C. plate and it was developed using Toluene and Ethyl acetate [93.7 v/v] as mobile phase and Vanillin Sulphuric acid used for visualisation.

### U.V. Absorbance

For U.V. absorbance the mother tincture was diluted with 99 parts of menstruum [i.e. Ooty sample in 40% and market sample in 35% alcohol]. The spectrum were recorded at the range of 200-400 nm and 200-500 nm in respect of Ooty samples and Lucknow market sample respectively.

The peaks of maximum absorption are given in Table 5.

## Results and Discussion

*Acorus calamus* L. has been allotted for Drug Standardisation programme at Homoeopathic Drug Research Institute, Lucknow by Central Council for Research in Homoeopathy, New Delhi to lay down standard for raw drug and finished product in Homoeopathic System of Medicine. The raw drug studies show that moisture content, total ash content of powdered drug, water soluble ash, extractive value in different solvents like Acetone, Alcohol, Chloroform, Methanol, Pet. ether (60-80°C), distilled water have been determined to supplement the analytical data for laying down standard for this drug. The above results are presented in Table 1.

Table - 1  
Standardisation of Raw Drug

S.No.	Parameters	Quantitative Values	
		A*	B**
1.	Moisture content (Loss on drying at 105°C)	10.87% w/w	8.6% w/w
2.	Total ash content of powdered drug	7.02% w/w	7.47% w/w
3.	Water soluble ash	4.10% w/w	3.9% w/w
4.	Acid insoluble ash	1.13%w/w	1.27% w/w

### Extractive Values in Different Solvents

S.No.	Solvent	Extractive Values	
		A	B
1.	Acetone	3.75% w/w	3.65% w/w
2.	Absolute alcohol	10.85% w/w	11.0% w/w
3.	Chloroform	4.0% w/w	3.7% w/w
4.	Methanol	10.0% w/w	9.7% w/w
5.	Pet.Ether(60-80°C)	5.0% w/w	3.55% w/w
6.	D. water	9.80% w/w	10.50% w/w

\* A - Ooty sample

\*\* B - Market sample, Lucknow

Formulation of the mother tincture have been done on the basis of Maximum Extractive Value (MEV) determined by using various fraction of alcohol (Table 2) and percolation method has been used for the preparation of the tincture. Physico-chemical standardisation studies of the

mother tincture namely wt. per ml, total solids, alcohol content, pH value have been summarised in Table 3.

**Table - 2**  
**Determination of MEV using different Ratio of Alcohol and Water**

S.No.	Strength of Alcohol% v/v	Mean Extractive Value		Remarks
		A	B	
1.	30	22.30	13.47	A-40% of alcohol used for preparation of mother tincture on the basis of MEV  B - 35% of alcohol used for preparation of mother tincture on the basis of MEV.
2.	35	22.80	14.42	
3.	40	24.60	13.72	
4.	45	21.95	13.12	
5.	50	21.20	12.72	
6.	55	19.60	12.62	
7.	60	17.58	12.50	
8.	65	19.18	12.40	
9.	70	23.00	11.90	
10.	75	20.01	12.00	
11.	80	16.16	12.01	
12.	85	18.50	11.03	
13.	90	18.13	11.05	
14.	95	07.72	09.01	
15.	99.5	10.85	11.00	

The alcohol content of formulated tincture has been fixed in the range of 35-40% v/v depending on MEV determined as above.

**Table - 3**  
**Physico-chemical Standardisation of Mother Tincture**

S.No.	Parameters	Observations	
		A	B
1.	Organoleptic properties		
	a. Appearance	Non-viscous, dirty yellow	Non-viscous, straw yellow
	b. Colour	Dirty yellowish brown	Straw yellow
	c. Odour	Characteristic	Characteristic
2.	Sediments	Absent	Absent
3.	Wt. per ml.	0.96 g	0.96 g
4.	Total solids	2.995% w/v	2.85% w/v
5.	Alcohol content	39% v/v	35% v/v
6.	pH value	5.6	5.3

It is evident from the TLC studies that the chloroform extract of the mother tincture [A] shows five prominent spots while the sample [B] reveals two spots only. The colour of all the spots (sample A and sample B) are purple. The results are depicted in Table 4.

**Table - 4**  
**Chromatographic Results of Acorus calamus Linn.**

EXTRACT : Chloroform extract of the mother tincture  
 ADSORBENT : Silica Gel-G  
 LAYER THICKNESS : 0.5 mm on wet condition

S. No.	Solvent System	Detecting agent	No. of spots		Rf values		Colour of the spots	
			A	B	A	B	A	B
1.	Toluene : Ethyl- acetate (93:7 v/v)	Vanillin sulphuric acid	5	2	0.96	0.96	Purple	Purple
					0.86	0.65	Purple	Purple
					0.55		Purple	
					0.25		Purple	
					0.10		Purple	

Diluted mother tincture [A] when scanned under U.V. visible spectra in the range of 200-400 nm shows three distinct peaks (maximum absorbance) at 300.8 nm, 258.4 nm and 252.2 nm whereas market sample [B] exhibits two distinct peaks at 299.5 nm and 257.5 nm. The above statistics clearly indicates that the market sample discerned less number of spots and peaks in comparison to Ooty sample under the TLC and U.V. studies.

**Table - 5**  
**U.V. Absorbance of Alcoholic Extract of Acorus calamus L.**

S. No.	Mother tincture	No. of Peaks	U.V. absorbance
1.	Acorus calamus 40% alcohol extract [A]	3	300.8 nm 258.4 nm 252.2 nm
2.	Acorus calamus 35% alcohol extract [B]	2	299.5 nm 257 nm

## Conclusion

It is evident from these physico-chemical constants, chromatography and spectroscopic studies, the drug can be easily identified and differentiated. Hence the observed data can be taken as a standard for the said drug.

#### Acknowledgements

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#### References

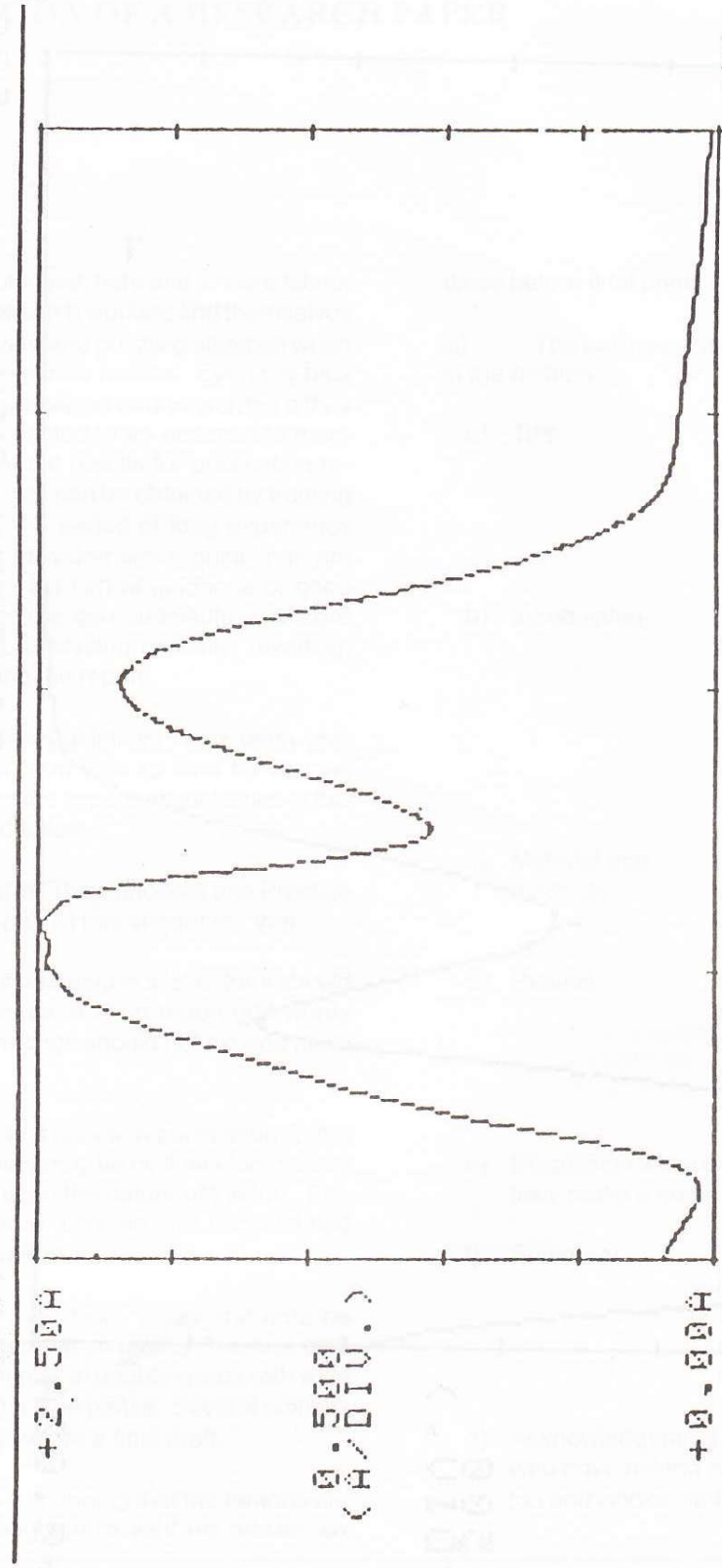
1. Anonymous, 1971. Homoeopathic Pharmacopoeia of India, Vol. I, Controller of Publication, New Delhi.
2. Anonymous, 1985. The Wealth of India. Raw Materials, Vol. I, Council of Scientific & Industrial Research, New Delhi.
3. Anonymous, 1986. The Useful Plants of India. Council of Scientific & Industrial Research, New Delhi.
4. Mishra, Gopal and Nigam, S.K. 1991. Jr. of Sci. Res. in Plants and Medicines, Vol.XII, no. 1-2, p 307.
5. Pathak K.M.L., Bhatnagar C.S.: 1997. Efficacy of herbal ectoparasiticide (AV/EPP/14) against dog tick *Rhipicephalus sanguineus*. Ind Veterinari Med. Jr., Vol.21 (4); p. 326-329.
6. Rai R., Siddiqui I.R., Singh J., 1998. Triterpenoid saponins from *Acorus calamus*, Ind. J. of Chemistry, Vol.37 B(5), p.473-476.
7. Stahl, E. 1969. Thin Layer Chromatography. A Laboratory Hand Book. Springer-Verlag, Berlin.
8. Wagner, H. 1984. Plant Drug Analysis - A TLC Atlas. Springer-Verlag, Berlin.

#### TRUE SCIENCE

"When we begin to base our opinions on medical fact, on inspiration, on more or less vague intuitions about things, we are outside of science and offer an example of that fanciful method which may involve the greatest dangers by surrendering the health and life of the sick to the whims of an inspired ignoramus. True science teaches us to doubt and in ignorance, to refrain".

Claude Bernard  
*An Introduction in the Study of  
Experimental Medicine, Pt.I, Ch2*

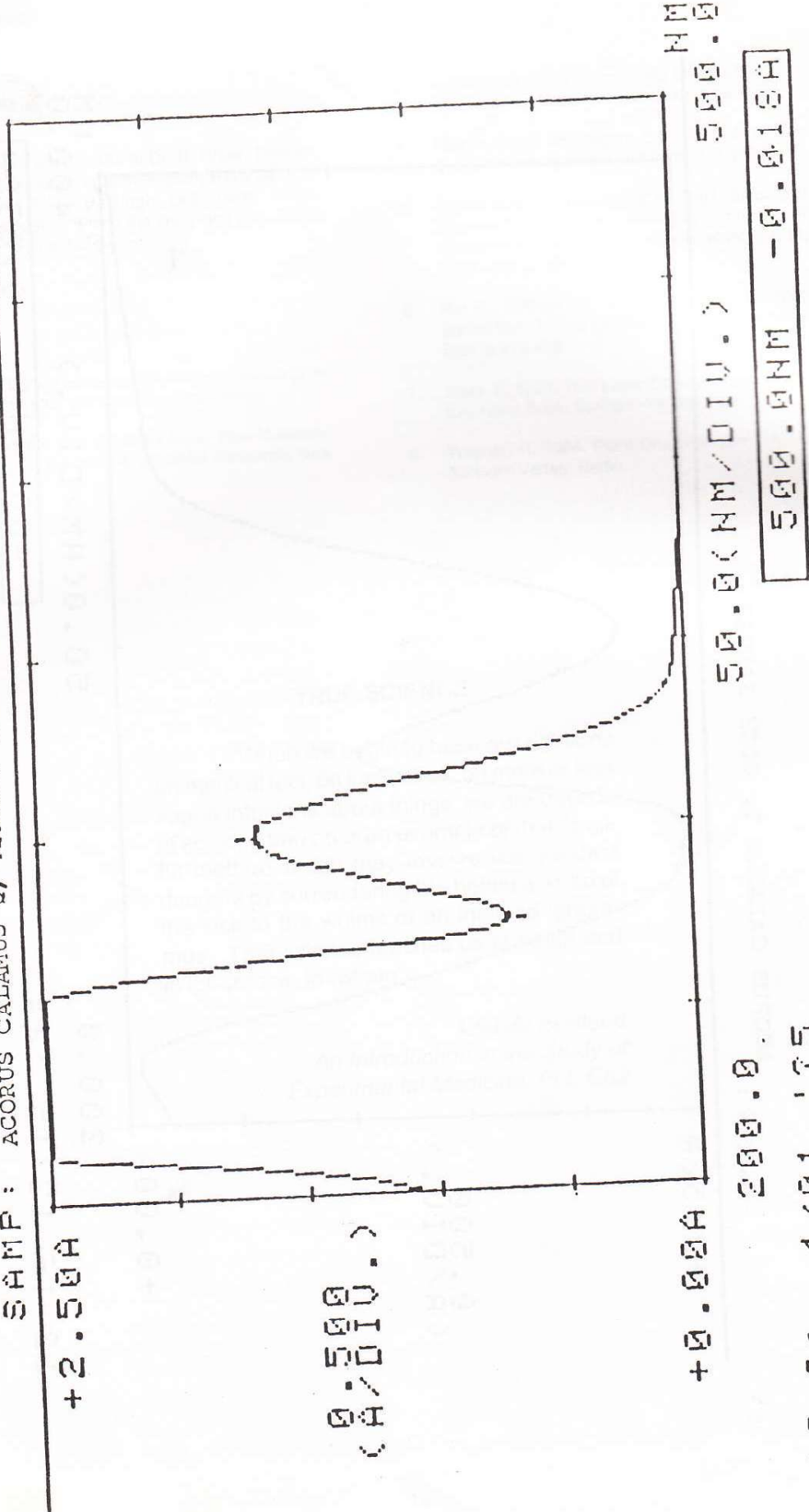
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