**International Journal in Management and Social Science**
Volume 07 Issue 07, July 2019 ISSN: 2321-1784 Impact Factor: 6.319
Journal Homepage: http://ijmr.net.in, Email: irjmss@gmail.com
Reviewed Refereed Open Access International Journal
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# ANTIASTHMATIC ACTIVE COMPOUND FROM THE ROOTS OF ACORUS CALAMUS (LINN.)

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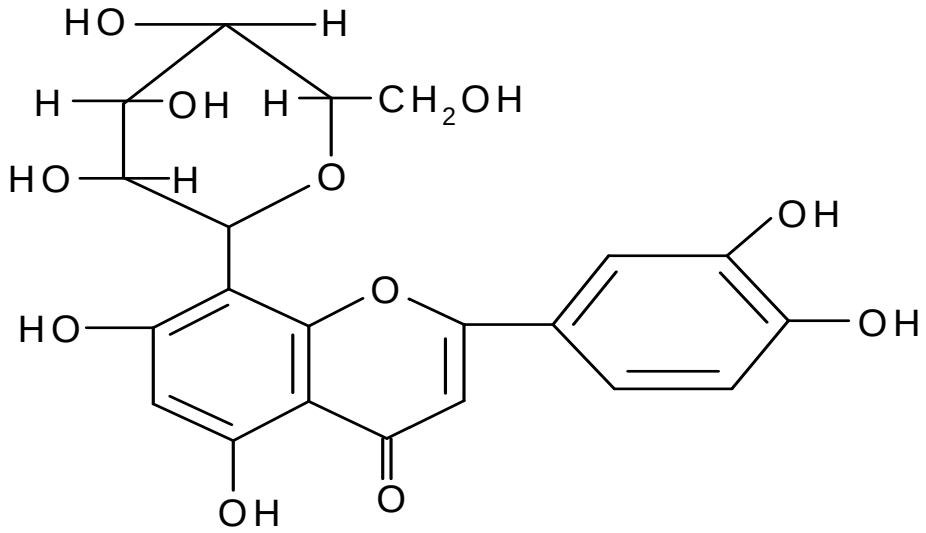
**Abstract**

The isolation of Antiasthmatic compound (Luteolin–8–C–β–D–Glucopyranoside) from ethyl acetate extract of the roots of *Acorus Calamus (linn.)* are characterized by different spectroscopic methods IR, $^{1}H$ NMR, $^{13}C$ NMR and FABMAS . The ethanolic extracts of the plants produced a dose dependent increase in respiratory capacity in the histamine-induced bronchospasm in guinea pigs. The compound showed the results at 8.42%, 5.59 % and16.39 % at doses 50,100, 200 mg/kg in which the significant protection was found at 16.39 % which was found to be more close to the reference compound salbutamol which was 63.25 %.




**Key words:** *Acorus Calamus (linn.),* Araecea, C-flavonoid, Luteolin, guinea pigs.

## Introduction

*Acorus calamus* Linn.[1-5] is commonly known as 'Bach' in Hindi. It is a water loving plant. It has been found to possess important medicinal value. Its rhizome is used in emetic, stomache, dysentery, colic, bronchitis and in snake-bites. The alcoholic extract of this plant has sedative and analgesic food effects, moderate depression in blood pressure and respiration. This plant also possesses potent anti-asthmatic and spasmolytic properties etc. Its narrow leaves and an aromatic rootstock. It is similar to the iris in appearance and can be found in moist habitats such as the banks of ponds or streams and swamps in North America, Europe, and Asia. Traditional medicine includes use of the rhizome, and the herb's main traditional uses include therapy for colic, dyspepsia, and flatulence. In Ayurveda there is major use of calamus for diseases of the kidney and liver, eczema, rheumatism, and enhancement of memory. Currently, traditional uses lack substantiation in the available medical literature. Vomiting was the primary toxicity reported following use of the root for assumed production of euphoria.

The information in this monograph is intended for informational purposes only, and is meant to help users better understand health concerns. Information is based on review of scientific research data, historical practice patterns, and clinical experience. This information should not be interpreted as specific medical advice. Users should consult with a qualified healthcare provider for specific questions regarding therapies, diagnosis and/or health conditions, prior to making therapeutic decisions.[25]

Saponins and Gemfibrozil were unable to bring the lipids back to baseline value; while the tannins decreased the serum cholesterol and triglyceride levels back to the baseline value. However, Gemfibrozil raised the HDL-cholesterol level significantly as compared to control. Simvastatin on the other hand demonstrated the lipid profile analogous to that of tannins whereby the cholesterol and triglyceride level were brought back to the baseline but serum HDL-cholesterol was not raised back to baseline value. Thus findings indicate the contribution of tannins and saponins of the plant towards hypolipidemic activity. S. A. Mengi.[24]

Hyperlipidemia is recognised as one of the greatest risk factors for coronary artery diseases1. Several experimental, animal and interventional studies have indicated lower morbidity and mortality in coronary heart diseases with reduction of serum total cholesterol and/or improvement in HDL cholesterol[2]. Currently available hypolipidemic drugs have been

associated with number of side-effects[3]. The current scenario sees the emergence of a number of medicinal plants being evaluated for various diseases and disorders. Although a number of medicinal plants like *Commiphora mukul*, *Gymnema sylvestre*, *Pterocarpus marsupium*, *Trigonella foenum-graceum*, *Azadirachta indica*, *Terminalia arjuna* and *Boswellia serrata* have been evaluated for their hypolipidemic activity, only *Commiphora mukul* has been commercially well established.

*Acorus calamus* Linn. belonging to the family *Araceae*, commonly known as Vacha, has been indicated in the Indian system of medicine to be useful in treatment of lipid disorders. The plant is a perennial herb growing throughout India, Europe, Asia and America. The literature survey reveals the usage of the plant as an insecticidal, antibacterial and antifungal agent. Studies have also been conducted with the ethanolic extract of the plant against gastroduodenal induced ulcers and the aqueous and hydroalcoholic extracts have also been evaluated for neuropharmacological activity, which has been reported to be carcinogenic. Preliminary studies with respect to the hypolipidemic activity of the roots and rhizomes of the plant have been conducted by Arora16 and co-workers and since then no further studies have been reported. Previous study dealt with the evaluation of the standardized aqueous and hydroalcoholic extracts prepared from the roots and rhizomes of the plant for hypolipidemic activity by monitoring the serum cholesterol, triglycerides and HDL cholesterol in rats fed with an atherogenic diet. The present study deals with the evaluation of the phytoconstituents of the plant extract for hypolipidemic activity in rats.[24-25]

## Material and Methods

### Plant Materials

The fresh roots of *Acorus Calamus* were collected in village area in Jabalpur.

### Test Animals

Guinea pigs of either sex, weighing 250-500 gm of Pir Bright White strain were used for the biological study. The animals were housed under standard conditions of temperature and humidity for 24 hrs and fasted with fresh leafy vegetables, carrots and with water *ad libitum.*

## Chemicals

Histamine aerosol (histamine diphosphate (0.5%) in saline)(Sigma chemicals Co.), Salbutamol sulphate (Cipla Ltd., Mumbai), All the chemicals used was of analytical grade.

## Equipments

1) Histamine chamber

2) Glass sprayer.

## Preparation of extracts

The rhizomes of *Acorus calamus* were obtained from Kalbadevi market, Mumbai and were authenticated at St. Xavier's Blatter's Herbarium, St. Xavier's College, and Mumbai. The plant material was coarsely powdered and then subjected to extraction with 50% ethanol for 18 hours in a Soxhlet extractor to obtain the hydroalcoholic extract. After completion of extraction, the solvent was removed by vacuum drying at low temperature. The yield of hydroalcoholic extract was found to be 24.6% w/w. The crude extracts were evaluated for absence of asarone by measuring the extinction at 253 and 303 nm.Saponins and tannins were extracted from the hydroalcoholic extract by the method of Harborne18 and a HPTLC fingerprint was obtained. The percentage yield of saponins was 6.543 % and that of tannins was 20.12% of the hydroalcoholic extract. Saponins(10mg/kg and 20mg/kg) and tannins(10mg/kg and 20mg/kg) were evaluated for their hypolipidemic activity in atherogenic rats. Snehalata, et.al. 2003.

## Experimental Section

Melting points 1PNMR recoded by Varian unity (300 MHz) spectrometer 13C NMR were determine in open capillary tubes. Infrared (IR ) spectra were Recorded FABMS were recorded on VG Auto spec 3000 mass spectroctrometer. Proton Nuclear Magnetic Resonance spectra were recorded on Varian XL (300 MHz).

The roots of *Acorus calamus* were air dried, powdered and were extracted with 95% ethanol. The concentrated extract was partitioned between diethyl ether and water. The aqueous layer on extraction with ethylacetate and on concentration gave brown viscous mass which on TLC[18] examination by using solvent system $CHCl_3$ : MeOH (89:11) and developer as 10% aq. $H_2SO_4$ showed a mixture of three compounds therefore, it was subjected to column chromatography using a eluants as $CHCI_3$ : MeOH in varying proportions and the eluants collected from $CHCI_3$ : MeOH (3:2)

were combined and subsequently solvent was removed to get a yellow amorphous mass which was crystallized from methanol as yellow crystals compound .

Compound: It was obtained as yellow crystals, analysed for m.f. $C_{21}H_{20}O_{11}$, m.p. 264-265°C, $[M^+]$ 448 (FABMS).

IR $\upsilon_{max}^{KBr}$ $cm^{-1}$ 3553.7 (–OH group), 2931.0 (–CH stretching), 1638.2 (unsaturated α, β >C=O group), 1613.0, 1560.0, 1459.6 (aromatic ring system).

FABMS m\z 448 $[M^+]$, 420, 315, 313, 299, 286, 258, 165, 153, 134, 124, 123. **Carbon magnetic resonance spectroscopy(**$^{13}$C-NMR): 163.3 (C-2), 102.0 (C-3), 180.0 (C-4), 159.2 (C-5), 97.3 (C-6), 163.5 (C-7), 103.3 (C-8), 156.6 (C-9), 102.3 (C-10), 121.2 (C-1'), 112.2 (C-2'), 145.5 (C-3'), 148.5 (C-4'), 115.0 (C-5'), 180.0 (C-6'), 74.6 (C-1"), 75.0 (C-2"), 79.0 (C-3"), 70.4 (C-4"), 81.3 (C-5"), 60.1 (C-6").

**Proton nuclear magnetic resonance spectroscopy (**$^1$**H NMR):** δ 6.23 (s, H-3), 13.12 (s, OH-5), 6.26 (d, J=2.5 Hz, H-6), 11.10 (s, OH-7), 7.76 (1H, d, J = 7.5, H-2), 6.92 (1H, d, J=7.5, H-5'), 7.56 (1H, dd, J= 7.6, 1.8 Hz, H-6'), 5.60 (1H, d, J= 9.5, H-1"), 3.40-4.15 (8H, m, glucose protons).

**Acid hydrolysis of compound**

The compound (100mg) on acid hydrolysis with 7% aq. $H_2SO_4$ when refluxed for 7 hrs at 100°C afforded no aglycone and sugar.

**Hydriodic acid fission of the compound**

The compound (2mg) when mixed with 5mg of phenol and 1 ml of HI which on prolonged boiling and gently refluxed for 8 hrs when poured into aq. $NaHSO_3$ gave yellow ppt which after separation gave aglycone.The aglycone analysed for m.p. 324-326°C, m.f. $C_{15}H_{10}O_6$, $[M^+]$ 286 (FABMS), IR $\upsilon_{max}^{KBr}$, 3520.0 (-OH group), 2930.0 (C-H stretching), 1635.2 (α, β-unsaturated >C=O group), 1610.0 1560.0, 1456.0 (Aromatic ring system).

**Oxidation of compound by $FeCl_3$**

Compound (150 mg) when refluxed with 450mg ($FeCl_3$) in 2ml water at 115°C for 8 hrs afforded an oxidized product. The product was then extracted with water and separated by

filtration to afforded yellow coloured mass which when passed through a column of silica gel G using water as eluant. The syrupy product obtained was analysed by Paper Chromatograpy by using solvent system as nBuOH : pyridine : water (6:4:3) and spraying agent as aniline hydrogen, phthalate. Glucose ($R_f$ 0.24) was identified by comparison with an authentic sample.

**Methylation of compound**

The compound (400 mg) was refluxed with freshly prepared ethereal solution of diazomethane and allowed to stand for one day. Excess of diazomethane was neutralised by glacial acetic acid. The product was purified and dry over by anhydrous sodium sulphate, which on concentration and crystallization from other solvent gave colourless prisms.

**Periodate oxidation of methylated compound and hydrolysis of the oxidized product**

120 mg of the methylated glycoside was dissolved in 15 mL of MeOH to which 3mg of sodium metaperiodate was added. The mixture was stayed for overnight and resulted into an oxidized product, which on hydrolysis with 1N HCl was filtered. The filtrate when treated with 2,4 – dinitrophenyl hydrazine afforded a yellow precipitate, which on TLC examination displayed two spots ($R_f$ 0.41 & 0.11) which were purified by column chromatography using eluants as benzene: ethylacetate (4:1).

**Periodate oxidation and reduction with $NaBH_4$**

25 mg of solution of methyl ether derivative of compound was mixed with $NaIO_4$ and retained for 6hrs. Then the mixture was treated with $NaBH_4$ and then hydrolysed with 1N HCl at 115° for 15 min. Then sugar was tested on PC by using solvent system as ethyl acetate: pyridine: water (10: 4: 3 v/v) and mixture of $NaIO_4$ and alkaline $KMnO_4$ as developer with Rf. (0.38) for glucose.

**Periodate oxidation and estimation of formic acid**

The methyl ether of the glycoside (150 mg) was dissolved in dioxane (25 ml) and treated with $NaIO_4$ (25 ml) then stayed for whole day at room temperature. The quantity of consumed NaIO4 and liberated formic acid were estimated volumetrically.

**Result and Discussion**

The alcoholic extract from the roots of the plant '*Acorus calamus*' after concentration was partitioned between diethyl ether and water. The aqueous layer was extracted with ethyl acetate and the concentrated ethyl acetate extract afforded a compound, which was crystallized from methanol as yellow crystals. The compound responded to Molisch's test positively, specified for the glycoside as well as various characteristic colour reactions for flavonoids. The compound analyzed for molecular formula $C_{21}H_{20}O_{11}$, m.p. 264-265°C, $[M^+]$ 448 (FABMS).

The IR spectrum of the compound showed a peak at 3553.7 $cm^{-1}$, which indicated the presence of –OH group(s) in it. The compound was found to form an acetyl derivative, $C_{37}H_{36}O_{19}$, m.p. 144-145°C, $[M^+]$ 784 (FABMS). The estimated percentage of acetyl group was found to be (42.80%) as determined by Wiesenberger[16] as described by Belcher and Godbert[17].

The compound on acid hydrolysis and enzymatic hydrolysis did not afforded any sugar. This suggesting a compound to be a flavonoid – C – glycoside[6] which was further supported by the appearance of two peaks in the IR spectrum at $\upsilon_{max}^{KBr}$ $cm^{-1}$ 1047 and 1015.[6,7]

The compound on prolonged boiling with HI and phenol afforded an aglycone, and sugar moiety as D-glucose. The aglycone was analyzed as 5, 7, 3', 4'–tetrahydroxy flavone with m.f. $C_{15}H_{10}O_6$, m.p. 323-324°C, $[M^+]$ 286 (FABMS) which was confirmed by spectral data compared with its literature values.

The oxidation of the compound with $FeCl_3$[8, 9, 10] followed by oxidation of the oxidized product with amyl alcohol when, passed through column of silica gel afforded a sugar moiety which was identified as D-glucose ($R_f$ 0.24).

Methylation[11] of the compound with diazomethane afforded a methylated product, which was identified as O-methyl-ether, m.f. $C_{25}H_{28}O_{11}$, m.p. 273-274°C, $[M^+]$ 504 (FABMS).

Periodate oxidation of methylated compound followed by hydrolysis with 1N HCL and was filtered off. The filterate was treated with 2,4-dinitrophenylhydrazine which on TLC examination displayed two spots. This confirmed the presence of sugar glucose in glycoside.[12, 13]Periodate oxidation of methylated compound with $NaIO_4$ followed by the reduction of the oxidized

product with sodium borohydride and then the product was subjected to hydrolysis with 1N HCl furnished a glycerol, thereby confirming the presence of pyranose form of the sugar glucose [9-10].

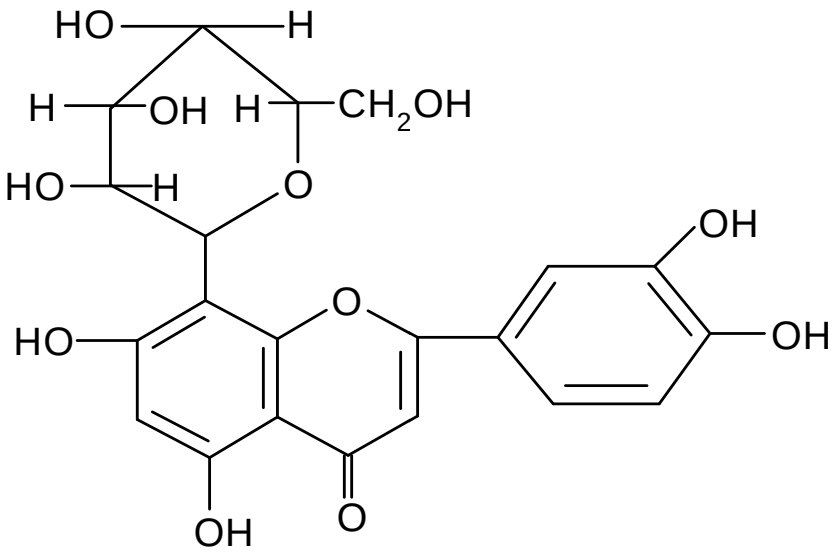
Sodiummetaperiodate oxidation[14] of the methylated glycoside consumed 2.1 moles of periodate and liberated 1.06 moles of formic acid, which indicated that both the sugar and aglycone are present in equimolar ratio.

Potassium permangnate oxidation of the methylated glycoside afforded a veratric acid which indicated that the ring-B was devoid of glycosyl unit. The position C-8 was involved in glycosylation. This was furthered assisted by the fact that $^1$NMR, showed the chemical shift at δ 6.26 (d, J= 2.5, H-6) for the aromatic proton at C-6 position in ring A this showed that only position C-8 was involved in glycosylation[15].

The $^1$NMR signal at δ 5.60 (1H, d, J=9.5, H-1'') for the anomeric proton which confirmed the presence of β-linkage between the aglycone and glucose moiety[16].

The IR spectrum of the aglycone showed a peak at 3523.0 cm$^{-1}$ which indicated the presence of –OH group(s) in it. It form an acetyl derivative which analysed for m.f. $C_{23}H_{18}O_{10}$ m.p. 221-222°C, [M$^+$] 454 (FABMS). Estimation of acetyl group (36.91%) by Wiesenberger[16] method as described by Belcher and Godbert[17] indicated the presence of four –OH group.

On the basis of all the above facts the compound was identified as luteolin-8-C-β-D-glucopyranoside.



**Study of the compound for anti-asthmatic activity**

The Ethanolic extracts of the plants produced a dose dependent increase in respiratory capacity in the histamine-induced bronchospasm in guinea pigs. The results of the present investigation are summarized in table 1

TABLE-1

| Treatment | Dose mg/kg i.p. | Pre-treatment exposition time in seconds ± S.E | Post-treatment exposition time in seconds ± S.E | % protection |
|---|---|---|---|---|
| PS-II | 50 | 117.4±20.02 | 128.2±24.15 | 8.42% |
| PS-II | 100 | 118.2±23.15 | 125.2±22.03 | 5.59% |
| PS-II | 200 | 192.75±15.31 | 230.55±30.11 | 16.30 % |
| Salbutamol | 0.65 | 147.42±5.10 | 389.43±15.17 | 63.25 % |

The compound showed the results at 8.42%, 5.59 % and16.39 % at doses 50,100, 200 mg/kg in which the significant protection was found at 16.39 % which was found to be more close to the reference compound salbutamol which was 63.25 % (Table 1).

**Acknowledgements**

**I give to special thanks to CDRI, Lucknow, (U.P.) and University of Mumbai.**

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