

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

<https://doi.org/10.36673/AJPCR.2020.v08.i01.A04>



PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF THE ETHANOLIC AND AQUEOUS EXTRACT ON THE WHOLE PLANT OF *ACOROUS CALAMUS Linn*

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ABSTRACT

Acorous calamus linnis a species of flowering plant, a tall wetland monocot of the family Acoraceae, in the genus *Acorus*. In the present study, an attempt was made to evaluate the antibacterial properties of latex of *Acorous calamus* on certain microorganisms. The ethanolic and water extract of the root was prepared. The extract were tested for their antibacterial activity against pathogenic microorganism like bacillus subtilis and *Klebsiella pneumonia* etc., A number of active constituents and essential oil were identified and characterized from the whole plant of sweet flag.

KEYWORDS

Acorous calamus, Ethanolic extract, Antibacterial properties, Active constituents.

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INTRODUCTION¹⁻¹⁶

Mother earth has bestowed to the mankind and various plants with healing ability for curing the ailments of human being. This unique feature has been identified since pre historic times. The WHO has also estimated that 80% of the world population meets their primary health care needs through traditional medicine only. It is a most valuable plant in the medical science almost throughout the India. In Ayurvedic science the use of sweet flag is effective against wide varieties of illness. This plant showed anti-spasmodic, carminative and anthelmintic properties. The rhizomes of this plant is used in the number of ailments like epilepsy,

mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent, fevers and glandular, abdominal tumors, kidney and livers troubles, rheumatism, sinusitis and eczema.

It is a highly valued herb because it acts as a rejuvenator for the brain and nervous system. In small doses it reduces the acidity in stomach and used in the treatment of anorexia nervosa while large doses causes nausea and vomiting. A number of active constituents form leaves, rhizomes and essential oils of *A. calamus* have been isolated and characterized, of the constituents, alpha and beta-asarone are the predominant bioactive components. Number of plant products with the useful bioactive properties is increasing rapidly as an outcome of several ongoing research programs on investigation of biological activities of a number of plants. Therefore, we report here the anti-bacterial and anti-fungal properties of this fraction which contain beta asarone assay major component according to proton nuclear magnetic resonance spectroscopy (¹H NMR).

Plant Profile:

Acorus is a genus of monocot flowering plants. This genus was once placed within the family Araceae (aroids),

Scientific Classification:

- Kingdom..... Plantae
- Subkingdom.....Tracheobionta
- Super division.....Spermatophyta
- Division.....Magnoliophyta
- Class.....Liliopsida
- Sub class.....Arecales
- Order.....Arales
- Family.....Araceae
- Species.....Calamus
- Genus.....Acorus

Plant Images:



Vernacular Names:

- Tamil:** matali, mattiri, mattirikam.
- Hindi:** gorbacc, gorbach,
- Telugu:** vadaja, vasa, wasa
- Marathi:** Vekhand
calamus, flagroot,
- English:** sweet flag, sweet grass, sweet rush, sweetroot

Description:

Sweet flag or buch plant is a grass-like, rhizome forming, perennial that can grow to 2 meters high, resembling an iris. This plant found in the wet areas like ponds, lakes and ditches. It consists of long creeping roots which spread out just below the surface of the soil. It is mostly common with the cat tail. The leaves are thick, erect and are very similar in appearance to the iris but edges are crimped. The flower is very rarely grown in this plant if grown than it is 3-8cm long, cylindrical in shape greenish brown in color and covered with the multitudes of rounded spikes. The fruits are small and berry-like containing few seeds. Flowers are grown from early to late summer depending on the latitude. The flowers are small, sessile and densely packed and 5-10 cm of spadix on all sides.

The leaves are free, alternate, green and wavy. 1-3 seeded having thin test which is cylindrical in shape and green in color. Flowering and fruiting occurs in July- August; fruiting very rare.

The dried rhizome of *Acorus calamus* contain the yellow aromatic volatile oils having asarone as a main constituent which contains the small quantity of sesquiterpenes and its alcohols; the rhizome also contains the choline, flavone, acoradin, galangin, acolamone, isocolamone and aerial parts of plant contains lutcolin-6,8-c-diglucoside; chemical constituents vary in ecotypes and polyploides. asarone is a genotoxic substance causing genetic mutation and tumors. Phenylpropanoid promotes defense mechanism in herbivores and ultra violet ray's protection.

It is also active in inducing the liver tumors. Other constituents are alpha pinene, cineole, limonene, terpineol, azulene, eugenol, camphene, cadinene, menthol and camphor (Figure No.1). The Asia varieties contain the varying amounts of amounts of beta asarone which causes the more sedate feeling after ingestion while European varieties yield sesquiterpenoids which is psychoactive agent and having good medicinal properties.

MATERIAL AND METHOD

Plant material collection

Plant material was collected from wild population around velimalai, kallakuruchi district and also available in other medicinal plants like *tinospora cordifolia*, *gotukola*, *ashwangandha* etc. Collected plant material was washed in running fresh water and dried under shade at room temperature and whole plant were size reduced to coarse granules.

Requirements

Chemicals - Ethanol and water

Instruments - 1000ml Beaker and stirrer.

Preparation of extract:

The extract were prepared by using maceration method, when the 100gm of powdered crude drug was macerate with alcohol 350ml for one week and finally extracting the crude drug. When the same process for the aqueous extract of the crude drug is prepared. The 100gm of crude powdered drug was soaked in a beaker by using 350ml distilled water for one week day by day shaking and finally prepared the extract. This maceration method is simple, easy to handle and economically chief, extraction process is easy and activity produced quickly.

Phytochemical Screening:

The phytochemical test of whole plant extract of *Acorous calamus* was studied and reported in the following Table No.1.

Pharmacological studies:

- ❖ Methanolic extract of *Acorus calamus* showed analgesic effect on the rat in a study done through the Writhing response and rat caudal immersion method while the anticonvulsant effect was studied through the Pentylenetetrazol induced seizures

method. The studies showed that *Acorus calamus* roots have significant anti-inflammatory and anti-convulsant activity.

- ❖ The anti-spasmodic and anti-diarrhoeal activity of *Acorus calamus* was studied. In the study, jejunum was isolated from the rabbit and further effect of crude extract was evaluated. It was found that plant extract causes the spontaneous inhibition of high K (+) induced contractions which resulted in spasmolytic activity which is mediated through the calcium channel blockade.
- ❖ Peroxidase activity of leaves extract of *Acorus calamus* was evaluated. The leaf extract protein of *Acorus calamus* was purified through the chromatography and peak giving fractions were tested for the anti-fungal activity by gel filtration using Superose 12 10/300 GL column. Through leaves of the plant peroxidase enzyme activity was observed in the lumen of the xylem and vessels.
- ❖ Study of *Acorus calamus* was done for inducing neurotoxicity against acrylamide for increasing the activity of corpus striatum while dopamine receptors decreased. These neurobehavioral changes are occurring by ACR (acrylamide) for the treatment of diseases with the *Acorus calamus* rhizome.
- ❖ Effect of *Acorus calamus* on acetaminophen induced toxicity in rats for anti-hepatotoxic and anti-oxidant activities was studied. It was seen that the effect of ethanol extracted *Acorus calamus* confer the hepatoprotective and anti-oxidant activities by biochemical and pathological observations against acetaminophen induced liver injury in rats.
- ❖ Anti-cancer activity of *Acorus calamus* rhizomes was evaluated. In this study, hydro alcoholic extract of *Terminalia chebula*, rhizome of *Acorus calamus* and root of *Glycyrrhiza glabra* was prepared and further their anti-proliferative activity on anti-cancer cell was studied. Results predict the fact that all of these plant materials have significant anti-proliferative activity.
- ❖ Anti-cellular and immunosuppressive potential of ethanolic extract of *Acorus calamus* was evaluated. The ethanolic extract of *Acorus calamus* rhizome

showed anti-proliferative and immunosuppressive properties. This extract causes the tumor necrosis which inhibits the proliferation of mitogen, antigen stimulated peripheral blood mononuclear cells in humans, nitric oxide and interleukins-2.

- ❖ The methanol and acetone extract of *Acorus calamus* leaves was evaluated for their CNS activity in mice. They showed the spontaneous locomotor activity for immobility by time using through forced timed swim test, diazepam induced sleeping time and motor impairment assessment using Rota rod for CNS depression/ analytic activity of ACME and ACAE in mice.
- ❖ *In vitro* and *in vivo* releasing alpha glycosidase inhibitory activity of ethyl acetate fraction of *Acorus calamus* was studied. The effects of serum glucose were detected in the fasted and amylin challenged normal mice. They used *Acorus calamus* extract for *in vivo* studies and found suppression in blood glucose level after the 2g/kg glucose loading in the normal mice. *Acorus calamus* extract had the hypoglycemic effects and glycosidase inhibition and improves the postprandial hyperglycemia and CVS complications.
- ❖ The reversal neurotoxicity of *Acorus calamus* in mice was studied which is induced with the phenytoin and Phenobarbital. The administration of *Acorus calamus* at ED50 dose of phenytoin markedly potentiated the anti-convulsant activity of phenytoin. The main reason is that the combination of *Acorus calamus* with phenytoin does not show any significant effect on the PTZ (pentylentetrazole) induced convulsions.
- ❖ The effect of acetone extract of *Acorus calamus* in albino rat for their anti-inflammatory activity was studied. Anti-inflammatory activity was evaluated using paw edema model induced by formaldehyde injection in the male rat. The inflammatory effect was completely diminished and the normal status of paw was achieved when 25-75% acetone extract was tested against inflammation in male rat within 30 minutes.
- ❖ One study showed that *Acorus calamus* possesses the ability for preventing the development of FeCl induced epileptogenesis by modulating antioxidant

enzyme; exhibit the potentiality of herb to be developed as an effective anti-epileptic drug. Through the various methods which is used for inducing the experimental epileptic models induces the recurrent seizures and epileptic discharge similar to humans post traumatic epilepsy through generation of free radicals into sensorimotor.

- ❖ The effect of *Acorus calamus* on bronchial dilation which is mediated through multiple pathways was studied. Crude extract of *Acorus calamus* in the guinea pig tracheal segments was more effective in the carbachol response with the blockage of calcium channels. In the crude extract of *Acorus calamus* the calcium channel blockers and phosphodiesterase in the hexane fraction has a novel combination which shows anti-cholinergic and anti-depressant effect that provides the pharmacological basis for various uses of *Acorus calamus* in airways disorders.

RESULTS

Test microorganisms and methods:

Antibacterial activity study report:

Name of the organisms used for the study:

- *Bacillus subtilis* - Gram positive
- *Klebsiella pneumonia* - Gram negative

Method

The antibacterial activity of test sample was carried out by disc diffusion method. The target microorganism were cultured in Nutrient broth and incubated for 24 hrs. The Petri dishes containing Nutrient agar (NA) medium were cultured with diluted bacterial strain. The prepared discs were placed on the culture medium. Test sample (100, 150, 200, 250, 300, 350 µg) was injected to the sterile disc. Standard drug Streptomycin (20µg) was used as a positive reference standard to determine the sensitivity of microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimetres as its antibacterial activity.

DISCUSSION

Preliminary phytochemical screening of the extracts of *Acorus calamus* showed that presence of Flavonoids, Glycosides, and Tannins in ethanolic and aqueous extract. Flavonoids, steroids are present in ethanolic extract only, and protein, Amino acids present only in aqueous extract.

The antibacterial studies shows, the ethanolic extract of *Acorus calamus* at 2000µg concentration exhibit mild activity against gram negative bacteria *K.pneumonia* same the 1000µg concentration exhibit the gram positive bacteria *Bacillus subtilis* and the aqueous extract of *Acorus calamus* does not show the any activity against gram positive and gram negative bacterial strains.

Table No.1: Phytochemical screening of whole plant extract of *Acorous calamus*

S.No	Phytochemical test	Whole plant extract of <i>Acorous calamus</i>	
		Ethanol	Water
1	Alkaloids	+	+
2	Flavonoids	+	-
3	Glycosides	+	+
4	Steroids	+	-
5	Saponins	+	-
6	Tannins	+	+
7	Proteins	+	+
8	Amino acids	+	+

(+) indicate presence (-) indicate absence

Table No.2: Various Pharmacological properties of *Acorus calamus* and its active constituents were studied by many researchers given in the following

S.No	Activity	Active compound or extract	Reference
1	Antifungal <i>Candida albicans, Cryptococcus neoformans Epidermophyton floccosum, Microsporum gypseum, Trichophyton mentagrophytes and T. rubrum, Ascospaera apis, Aspergillus oryzae, A. nidulans, A. fumigates, Penicillium aculactum, Phomopsis destuctum.</i>	Essential oil, beta-asarone Essential oil Essential oil	Rajput and Karuppayil (2013), Thirach et al.(2003) Jatisatiener and Jatisatiener (1999) Chantawannakul et al. (2005)
2	Antibacterial <i>Aeromonas hydrophila</i>	Essential oil, alpha_- asarone, beta_- asarone	Bhuvaneshwari and Balasundaram (2006)
3	Anti-inflammatory/immunomodulatory Anti-inflammatory effect in human HaCaT cells Immunomodulatory activity in human PBMCs	Leaf extract Ethanolic extract	Kim et al. (2009) Mehrotra et al. (2003)
4	Antioxidative/protective effect Antioxidant and nephroprotective effect in male albino rats	Ethanolic extract	Palani et al. (2010)
5	Antioxidant activity Neuroprotective effect in ischemic rats	Ethyl acetate extract, Hydroalcoholic extract, _- and _- asarone	Acuna et al. (2002) Shukla et al. (2006)

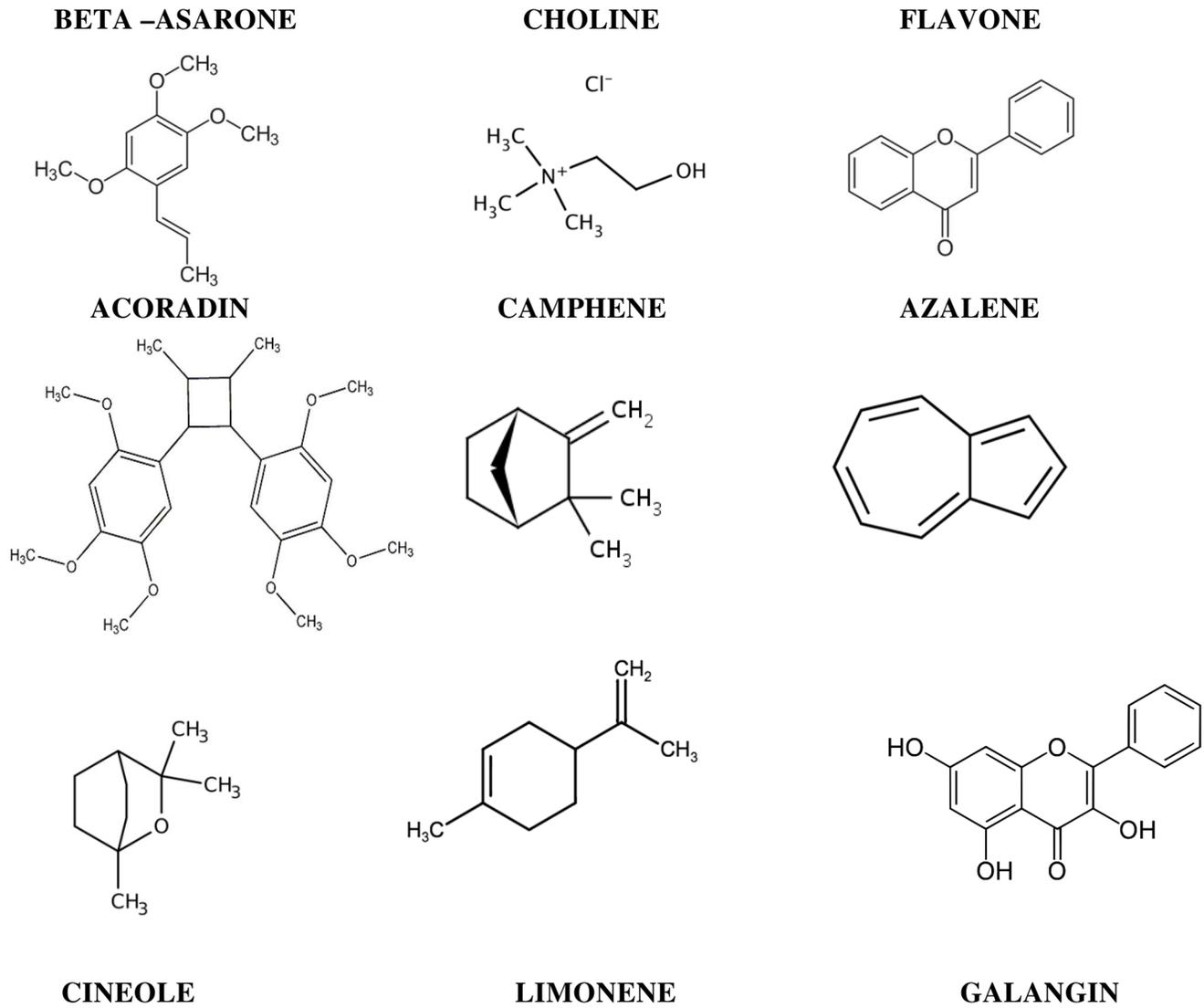
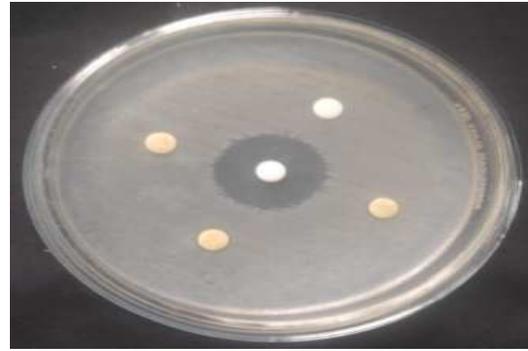
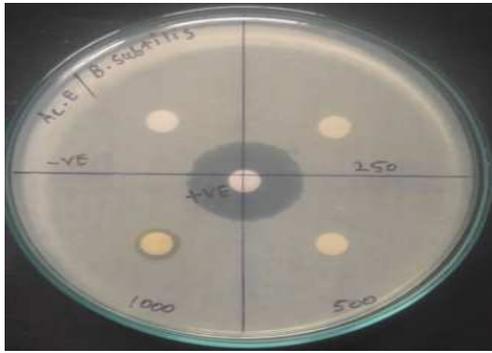
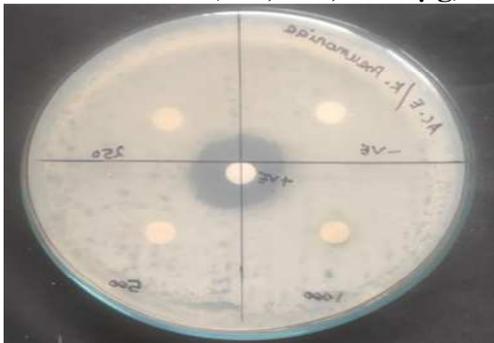


Figure No.1: Chemical Constituents

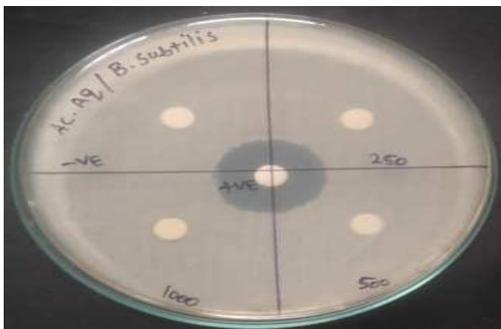
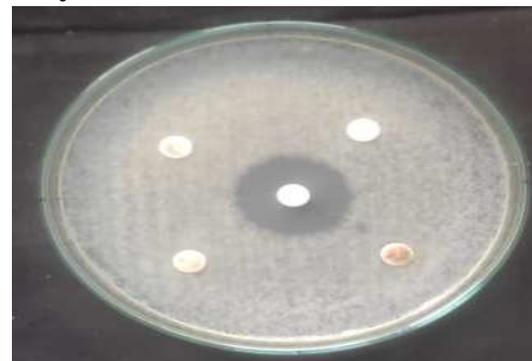
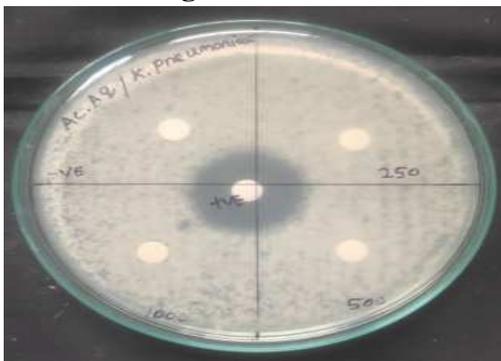


E.B.Subtitis (250, 500, 1000 μ g)



E.K.Pneumonia (200, 350, 1000 μ g)

Figure No.2: Anti-bacterial activity of Ethanollic Extract



Aq.B.subtilis, (500, 1000 μ g)

Aq.K.Pneumonia (250, 500, 1000 μ g)

Figure No.3: Anti-bacterial activity for Aqueous Extract

S.No	Sample	Zone of Inhibition (mm)					
		<i>B. subtilis</i>			<i>K. pneumonia</i>		
		250 µg	500 µg	1000 µg	500 µg	1000 µg	2000 µg
1	A.C ethanol extract	-	-	10	-	-	8
2	A.C aqueous extract	-	-	-	-	-	-
3	Streptomycin (20 µg)	22			24		

CONCLUSION

Acorus calamus Linn., is a traditional plant used to cure many disease. We recommend that further studies are need about this plant to find out the various medicinal properties.

ACKNOWLEDGEMENT

We gratefully acknowledged to our beloved Chairman Dr.K.Varadharajen., Vice chairman Mr.John Ashok Varadharajen and Our Principal, Professors, and others of Thanthai Roever College of Pharmacy who support to carry out this work successful.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Nirmala R *et al.* Phytochemical screening and Antibacterial activity of the ethanolic and aqueous extract on the whole plant of *Acorous calamus* Linn, *Asian Journal of Phytomedicine and Clinical Research*, 8(1), 2020, 39-47.