

SCREENING OF PHYTOCHEMICAL CONSTITUENTS IN SOLVENT EXTRACTS OF *ACORUS CALAMUS*

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ABSTRACT

Plants have the ability to synthesize mixtures of structurally diverse bio-active compound, with multiple and mutually potential therapeutic effects. *Acorus calamus* a medicinal harp is known to be rich in β asarone in its composition. The rhizome of this plant appears to have traditional usage for the treatment of insomnia, melancholia, remittent fever, delirium, neurosis, cough etc. The solvent extracts of the drug yielding plants were subjected for preliminary phyto-chemical screening, before evaluation of their biological activity. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The successive extracts of root of *Acorus calamus* revealed the presence of tannins, flavonoids, steroids, glycosides, alkaloids, proteins and phenols. The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Keywords: *Acorus calamus*, Phytochemical constituents.

1. INTRODUCTION

Phyto-chemicals are the individual chemicals; the plant comprises of, and has the capacity of manufacturing the secondary products. The phyto-chemicals with antioxidant properties, tend to be brightly colored because they contain chromospheres, a series of alternating single-bonded carbons. Isoprene is often the building block of such units, and abundantly found in the chlorophyll and the required antioxidants. Hundreds of phyto-chemicals are currently being studied and are believed to have major positive impact on human health. Important plant secondary metabolites have been isolated over a period of time from natural sources. The phyto-chemicals belong to the following categories such as terpenoids, phenolic compounds, alkaloids, glycosides, carbohydrates, lipids, proteins, nucleic acids, etc. (Raaman, 2006).

Binomially named *Acorus calamus* (Sweet flag) (Figure 1) is a seasoning and medicinal herb used in both Indian medicine (Ayurveda) and traditional Chinese medicine for its cognitive properties. *Acorus calamus* is a tall perennial wet land monocot of the Acoraceae family of the genus *Acorus calamus*. The scanted rhizomes (Figure 2) have traditionally been used medicinally and to make fragrances and the dried powdered rhizome (Figure 3) has been used as a substitute for ginger, cinnamon and nutmeg (Raja *et al.*, 2009).

Sweet flag has a very long history of medicinal use in Chinese and Indian herbal traditions. The leaves, stems, and roots are used in

various *Siddha* and *Ayurvedic* medicines (Avadhini and Mythili, 2013). It is widely employed in modern herbal medicine, as it has sedative, laxative, diuretic and carminative properties (Balakumbahan *et al.*, 2010; Simonetti, 1990). It is used in *Ayurveda* to counter the side effects of all hallucinogens. Sweet flag, known as "Rat Root" is one of the most widely and frequently used herbal medicines amongst the Chipewyan people (Johnson *et al.*, 1995).



Figure 1 *ACORUS CALAMUS*



Figure 2 Rhizomes OF *ACORUS CALAMUS*



Figure 3 POWDER OF *ACORUS CALAMUS*

Both triploid and tetraploid *Acorus calamus* contains alpha-Asarone (Simonetti, 1990) and the other Phyto-chemical includes.

Phyto-chemical screening for the presence of tannin, phlobatannin, saponin, flavonoids, steroids, glycosides, alkaloids, carbohydrates, proteins and phenols, in different solvent systems

was carried out to standardize, the suitable solvent for extraction and the detection of the compounds that is eluted with the solvent. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

2. MATERIALS AND METHODS

2.1. Preparation of extracts

For aqueous extraction, 10g of air-dried powder was added to distilled water and boiled on slow heat for 2h. It was then filtered through 8 layers of muslin cloth and centrifuged for 10 minutes. The supernatant was collected, this procedure was repeated twice. After 6h, the supernatant collected at an interval of every 2h, was pooled together and concentrated to make the final volume one-fourth of the original volume (Parekh *et al.*, 2005). It was then autoclaved at 121°C temperature and at 15 lbs pressure and stored at 4°C. For solvent extraction, 10g of air-dried powder was taken in 100ml of organic solvent (methanol or ethanol) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24h. After 24h the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Parekh *et al.*, 2005) and stored at 4°C in airtight bottles.

2.2. Phyto-chemical screening

Chemical tests were carried out on the aqueous extracts of *Acorus calamus* and on the powdered specimens using standard procedures to identify the constituents eluted in the solvent extracts (Harborne *et al.*, 1998).

2.2.1. Test for tannins

About 0.5g of the dried powdered sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green (or) a blue – black coloration.

2.2.2. Test for phlobatannins

Deposition of a red precipitate when an aqueous extracts of each plant sample was boiled with aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.2.3. Test for saponins

About 0.5g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3

drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.2.4. Test for flavonoids

Following methods were used to determine the presence of flavonoids in the plant sample. 0.5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of H₂SO₄ (Harborne *et al.*, 1998). A yellow coloration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration observed indicate a positive test for flavonoids.

2.2.4.1. Ferric chloride test

To a small quantity of the alcoholic solution of the extracts a few drops of neutral ferric chloride solution was added. A green colour produced indicates the phenol nucleus.

2.2.4.2. Zinc-hydrochloride acid reduction test

To the alcoholic solution of the extracts a pinch of zinc dust was added and few drops of concentrated hydrochloric acid were added. Magenta color would be produced after a few minutes. Flavanones and dihydro flavanols at the presence of HCl produce bright red color, isoflavonoids and flavones develop yellow, sometimes red colour, and flavonoles do intensive red colour.

2.2.5. Test for steroids

2ml of acetic anhydride was added to 0.5g ethanolic extracts of each sample with 2ml H₂SO₄. The color change from violet to blue or green in same samples indicates the presence of steroids.

2.2.5.1. Salkowski reaction

To the 0.5ml of chloroform extract in a test tube 0.1ml of concentrated sulphuric acid was added from the sides of the test tube. A reddish brown colour observed in chloroform layer indicate the presence of steroids

2.2.5.2. Libermann Buchardt test

To 1ml of petroleum ether extracts in chloroform, 1ml of concentrated sulphuric acid was added followed by the addition of 2ml of acetic anhydride solution and mixed well. A greenish colour developed and turned indicate the presence of steroids.

2.2.6. Test for cardiac glycosides (Keller- Killani test)

5ml of each extracts was treated with 2ml of glacial acetic containing one drop of ferric chloride solution. This was treated with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Harborne, 1973).

2.2.7. Test for alkaloids

2.2.7.1. Mayer's test

The various extracts were distilled in chloroform. The chloroform was acidified and added few drops of Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicates the presence of alkaloids.

2.2.7.2. Dragendroff's test

The various extracts were dissolved in chloroform. The chloroform was evaporated and the residue was acidified and added few drops of Dragendroff's reagent (potassium bismuth iodide) appearance for orange red precipitate was observed.

2.2.8. Test for carbohydrates

2.2.8.1. Fehling's test

The extracts with heated with Fehling's A and B solution formation of an orange red precipitate show the presence of reducing sugar.

2.2.8.2. Molisch's test

The extracts with Molisch's reagent and added concentrated sulphuric acid along the sides to form layers. A reddish violet ring at the interference show the presence of carbohydrate. Monosaccharide's give a rapid positive test. Disaccharides and polysaccharides react slower.

2.2.9. Test for proteins

2.2.9.1. Biuret test

The Biuret test is often used to determine the presence of peptide bonds in protein. When a protein reacts with copper (II) sulfate (blue), the positive test is the formation of a violet coloured complex.

2.2.9.2. Ninhydrin test

The Ninhydrin test is a test for amino acids and proteins with a free -NH₂ group. When such an -NH₂ group reacts with ninhydrin, a purple-blue complex is formed.

2.2.10. Test for Phenols

2.2.10.1. Reaction with FeCl₃

Phenol gives violet colouration with neutral ferric chloride solution due to the formation of a coloured iron complex, which is a characteristic to the existence of keto-enol tautomerism in phenols (predominantly enolic form).

3. RESULTS

In the present investigation, various solvent extracts of *Acorus calamus* was analyzed for the elution of the significant primary and secondary metabolites to assess the suitability of the solvents and to know the interfering substances present in the particular solvents extract. The results are summarized in (Table 1).

The aqueous and methanolic extracts were tested for flavonoids, tannin, protein, glycosides, phenol, steroids, alkaloids and their presence was confirmed. The tests for carbohydrate, saponin showed the absence of the compounds. (+) is presence of compounds, (-) is absence of compounds in phyto-chemical analysis.

4. DISCUSSION

The investigated plants did not show strong antibacterial activity; however, negative results do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor *et al.*, 2001). Lack of activity can thus only be proven by using large doses (Farnsworth, 1993). Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents (Jager *et al.*, 1996).

Preliminary phyto-chemical analysis of *Acorus calamus* revealed the presence of alkaloids and flavonoids, though the latter was in lesser amount. The other secondary metabolites like tannins, phenols, steroids, cardiac glycosides, etc were present in trace amounts in some of the extracts, which could contribute to the differences in the activities of extracts, due to phyto-chemical properties and differences among species.

The tests for carbohydrate, and saponin showed the absence of the compounds. Kaushik *et al.*, (2012) reported the presence of carbohydrate, tannin, terpenoids, glycoside, saponin, flavonoid, and the absence of amino acid, alkaloid and steroid. Ashok *et al.*, (2012) reported the presence of

carbohydrate, flavonoid, steroids, glycosides, alkaloids, tannin, and the absence of saponin. Savitha *et al.*, (2010) reported the presence of alkaloid, carbohydrate, protein, glycosides, phenol, tannin, and the absence of amino acid, steroid, saponin and terpenoids, where as in the present studies the extracts showed the presence of alkaloid, flavonoid, tannin, protein, glycosides, phenols, steroids, and the absence of carbohydrate and saponin.

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Table 1. Phyto-chemical analysis of the solvent extracts

S. No	Analysis	Compounds confirmed
1	Tannins	+
2	Saponins	-
3	Flavonoids	+
4	Steroids	+
5	Glycosides	+
6	Alkaloids	+
7	Carbohydrates	-
8	Proteins	+
9	Phenols	+