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Phytochemical Investigations, Extraction and Thin Layer Chromatography of Acorus Calamus Linn

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Abstract: *The present study is aimed at the development of phytochemical parameters and to investigate the medicinally active substances present in methanolic extract obtained from rhizomes of Acorus calamus linn. plant. Preliminary phytochemical screening of the extracts revealed the presence of terpenoids, steroids, flavanoids, tannins, saponins and phenolic compounds.TLC is a technique with large applicability in the fields of plant material analysis.TLC is a simple,quick and inexpensive procedure that indicates how many components are in acrude extract. TLC has many advantages such as lower cost, short time analysis, the possibility of multiple detection and specific derivatization on the same plate. Among the different extracts methanolic extract was used for Thin Layer Chromatography. For the TLC new solvent system developed for the best separation of the phytoconstituents present in the extract. The solvent system selected for the best results of TLC was the ratio of N-Hexane: Ethyl Acetate: Methanol: Formic acid 3 : 4 : 3 : 0.1 ml for methanolic extract.TLC resulted in identification of four spots found in the methanolic extract. The Rf values of the extract were 0.68, 0.71, 0.79 and 0.81.The study will provide referential information for the correct identification of the crude plant extract of Acorus calamus linn.*

Keywords: *Acorus calamus linn, Phytochemical screening, Extraction, TLC.*

1. INTRODUCTION

In India, the importance of Ethno medicine in treating various ailments and ill-health has been recognized by health care experts and planners, particularly in the sector of Indigenous System of Medicine across the rural parts of India. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the plant. The rich knowledge base of countries like India in medicinal plants and healthcare has lead to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development in the pursuit of discovering novel drugs. However, several plants are used for various aspects in India in the form of crude form without scientific evidence of efficacy. At this juncture it is of interest to determine the scientific basis for the traditional use of these plants.[1-2] . Many plants harvested in wild in India are used by local people as medicine-next to their source of food, for shelter and various livelihood needs. *Acorus calamus* Linn. (family: Araceae) commonly known as Sweet flag, Sweet Sedge, Myrtle Flag is a semi-aquatic, perennial, aromatic and marshy herb with creeping rhizomes originating in Asia but now widely distributed in Europe, North America and Africa. It is also found indigenously in the marshy tracts of Kashmir, Shirmaur (Himachal Pradesh), Manipur and in Naga Hills of India[3-4]. The rhizome, root and leaf yield a light brown to brownish yellow volatile aromatic oil known as calamus oil. It is a semi aquatic perennial plant of Acoraceae having scented rhizomes and tapered reed-like leaves. [Figure1]. The rhizomes are considered the officinal part of the plant and have been reported to possess tranquilizing, antimicrobial, anti diarrheal, antidyslipidemic, neuroprotective, anti-inflammatory and analgesic activities. The different pharmacological activities of *Acorus calamus* such as low-grade mentally retarded children[5-6]. Anticonvulsant[7]. Rhizomes extracts of *Acorus calamus* linn. possess CNS depressant, tranquilizing, inhibiting the spontaneous motor activity[8] .For the manufacturing of modern drugs advanced chemical intermediates needed are also obtained from plants[9]. This paper focuses on the phytochemical investigation and thin layer chromatography of *Acorus calamus* linn.

Botanical description of acorus calamus: A. Calamus linn. Commonly known as sweet flag, belongs to the family Araceae (Adoraceae). Acorus calamus grows either as wild or cultivated crop throughout India ascending upto 1800 meters in the Himalayas. wild woody liane belongs to the Family Araceae .

1.1. Scientific Classification

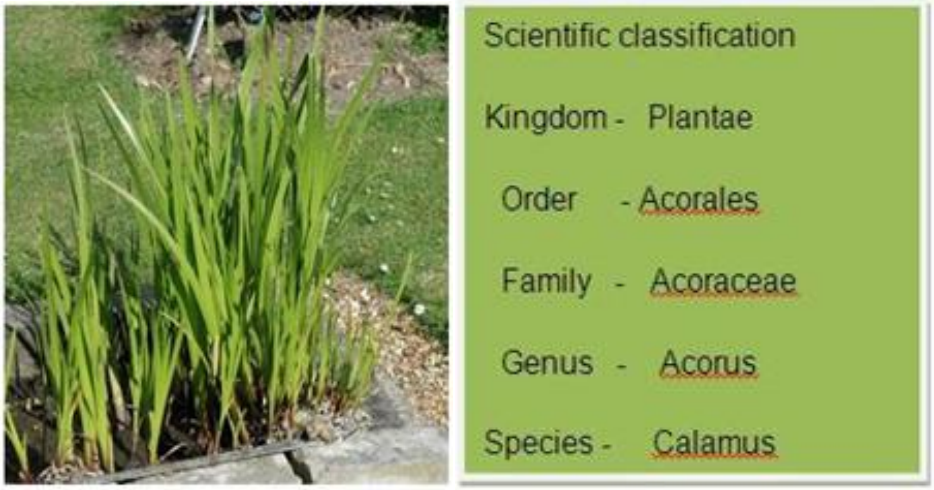


Fig1. *Acorus calamus* linn.

2. MATERIALS AND METHODS

Collection of plant material: After proper identification and authentication, mature rhizomes of *Acorus calamus* linn. Were collected from the Amarkantak district in Madhya Pradesh, India. The plant was identified and authenticated by Dr. Madhuri Modak Professor Deptt. of Botany Govt. M.V.M. Bhopal,(M.P.) the voucher specimen (Herbarium No. 3050-191.02-X.1) has been deposit in the department herbaria. The rhizomes were cleaned off the roots, attached leaves and washed thoroughly in water to remove the soil adhered and dried in partial shade for a period of four weeks.

2.1. Extraction and Preliminary Phytochemical Screening

Preparation of Crude Methanol Extract: The dried plant rhizomes of *Acorus calamus* linn. was grounded by electrical grinder. Till the fine powder in a mixer grinder and weighed accurately. The powdered material was subjected to solvent extraction with methanol by Soxhlet apparatus at room temperature for 48 hours. The resulting mixture was filtered and evaporated in a shaker water-bath; temperature maintained at 55-65^{0}C the obtained dried crude extract was used for phytochemical analysis.

Phytochemical evaluation: The phytochemical evaluation of the plant is carried out by testing of different class of compounds using standard methods[10] to identify the compound showing in Table 1.

The preliminary phytochemical investigations were carried out with the methanolic extract of *Acorus* calamus linn. Rhizomes of plant for qualitative identification of phytochemical constituents using standard conventional protocol. All the chemicals and reagents used were of analytical grade[11.]

2.2. Test for Terpenoids

Libermann-Burchard Test: Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added the side of test tube, shows brown ring at the junction of two layer and the upper layer turns green which shows the presence of sterols and formation of deep red colour indicate the triterpenoids.

Salkowski's Test: Treat extract in chloroform with few drops of concentrated sulphuric acid, shaken well and allow to stand for some time, red colour appear in the lower layer indicate the presence of sterols and formation of yellow coloured lower layer indicate the presence of triterpenoids[12.]

2.3. Test for Flavonoids

Shinoda Test: (Magnesium hydrochloride reduction test): To the test solution add few fragments of magnesium ribbon and concentrated hydrochloric acid drop wise, pink scarlet, colour appears after few minutes indicating the presence of flavonoids.

Ferric Chloride Test: To the test solution, add few drops of ferric chloride solution, intense green colour was formed to show the presence of flavonoids.

2.4. Test for Carbohydrate

Molisch Test: Treat the 2 ml of test solution with few drops alcoholic α*napthol solution in a test tube and the1 ml of concentrated sulphuric acid was added carefully along with side of the test tube. Formation of vateiolet ring at the junction indicates the presence of carbohydrates[13]

Fehling's Test: Equal volume of Fehling solution A and Fehling solution B are mixed and few drops of sample is added and boiled, a brick red precipitate indicate the presence of reducingsugar[13]

2.5. Test for Tannins

Ferric Chloride Test: Some amount of extract was dissolved in distilled water to this solution 2ml of 5% ferric chloride solution was added. Formation of blue green indicates presence of tannins

Lead Acetate Test: Some amount of extract a few drops of lead acetate solution was added. Formation of precipitate indicates presence of tannins.

2.6. Test for Saponins

Foam test: The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

2.7. Test for Glycoside

Borntrager's Test: To3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red colour inammonical layer indicates presence of anthraquinone glycoside.

Keller-Killiani Test: To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of Cardiacglycosides[14.]

3. SEPARATION OF CHEMICAL CONSTITUENTS

The purity of each eluted sample was tested by using TLC method. It is a technique used to separate wide range of compounds of biochemical interest. It can be utilized to quantitative as well as qualitative and preparatory work [Stahl, 1965]. The petroleum ether extract was subjected to thin layer chromatography about 0.1-0.2 ml of conc. Methanolic extract was loaded on the plate by using capillary tube. During spotted plates were carefully dried and used for elution purpose. Initially various solvents such as benzene, pet ether, chloroform ethanol were tested alone. Later different combinations of solvents were tested depending on polarity basis. The spotting was done at the centre of plate three spots were appeared on the plate. The spotting plate was carefully dried and used for elution purpose. Different solvent systems ranging from lower polarities to higher polarities were tested for the separation of bioactive components. The TLC plates were observed under UV light and the separated spots were marked.[15-16.]

a) Development of chromatogram: The eluted spotted plates were dried at room temperature and they were placed in iodine chambers for the development of chromatogram. The Rf values of cleared sport were calculated & proper solvent system was identified Rf. values determined are shown in table no. 02.

b) The Column Chromatography: 50 ml of concentrated petroleum ether were dissolved in 10 ml of benzene. The activated silica gel H is added slowly to benzene solution and absorbs pet ether extract. The chromatograms are allowed to develop Elution was started after, the formation of

complete bands and it was adjusted to 12-15 drops per mm. Nearly 10 ml of eluted solvent was collected in a clean bottle of 50 ml capacity and was labeled by given number 5.[17]

Table1. *Preliminary Phytochemical screening of Extract of seeds of Acorus calamus linn.*

Sr. No.	Name of Phytoconstituents	Presence/Absence
1	Terpenoides	
2	Flavonoids	+
3	Tanins	+
4	Saponins	+
5	Steroids	+
6	Carbohydrates	+
7	Phenolic compounds	+
8	Reducing sugars	+
9	Oil	+

Key: + = Present and - = Absent

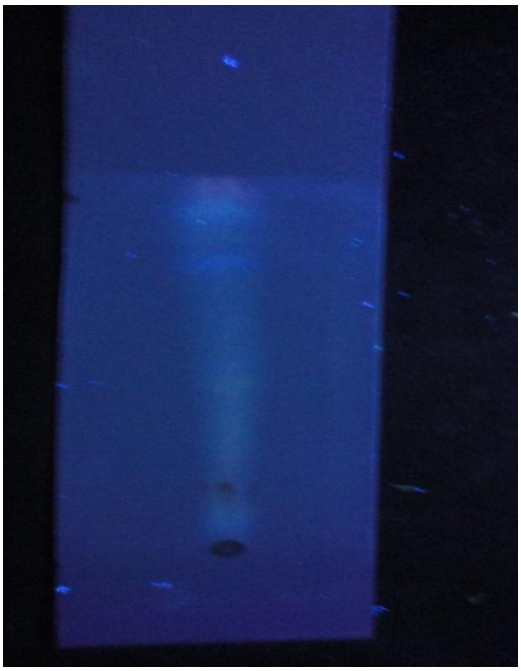
Table2. *Results of TLC of methanolic extract of Acorus calamus linn.*

Solvent system:-	No. of spot	R_f Value of fraction
N-Hexane: Ethyl Acetate: Methanol: Formic acid	3 : 4 : 3 : 0.1 ml	0.68, 0.7, 0.79 and 0.81.

Stationary Phase: Silica gel. 60-120 mesh size (Merk).

4. RESULTS AND DISCUSSIONS

Table No. 01 of the results of preliminary phytochemical screening of petroleum ether extract seeds of C. Paniculata of shows that the seed extract was rich in chemical know as Phytoconstituents, such as terpenoids, tannins, saponins and steroids petroleum ether extract was subjected to TLC in order of separate and identify the bioactive compounds present in the seeds of methanolic extract of *Acorus calamus* linn. the present research work the most suitable TCL system for analysis was shown to be terpenoids, saponins, tannins and steroids with the largest discriminating power TLC plates shown in the fluor escence light under UV at 254-365 nm wavelength and find these active spots in TLC plate with following Rf values (0.68, 0.7, 0.79 and 0.81.) these values indicates the presence of terpenoids .



5. CONCLUSION

The methanolic rhizomes extracts obtained through solvent extraction by Soxhlet apparatus from Celastrus paniculata. of *Acorus calamus* linn. plant have been raw material for the synthesis of many drugs and thus remain an important source of new therapeutic agent. It is found that of *Acorus calamus* linn. has a beneficial effect on the learning and memory process in mentally retarded children. The methanolic extract obtained from *Acorus calamus* linn. Though successive

solvent extraction in order of prove that the ethno pharmacological applications of the plant in Indian folk medicines. Phytochemical screening of *Acorus calamus* linn. Preliminary and important aspect. It is concluded from the data that methanolic extracts of *Acorus calamus* linn.rhizomes exhibited significant role in medicinal chemistry for formulation of life saving drugs.

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