European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 11, 2021

Determination of Bioactive Compounds from the Extracts of Acorus Calamus

R.M. Balaji [1*] and D. Shanthakumari[2]

[1]Department of Biochemistry, Research & Development Centre, Bharathiar University, Coimbatore - 641 046.
[2]Dept. of Biochemistry, Indira Gandhi Jayanthi Women's College, Kilgudalore, Tindivanam-604 307.

[*]Corresponding author: balagbio@gmail.com

Abstract:

Acorus calamus is familiar for therapeutic plant in the traditional healthcare system of India as well as other countries. The current study is focused in the progress of the phyto-chemical properties and investigation of the therapeutically active compounds that are exist within the methanolic extracts that are attained from the rhizomes of the plant Acorus calamus linn. The Preliminary phyto-chemical screening of the samples in this study unveiled the existence of Terpenoids, Flavanoids, Tannins, Steroids, Saponins and Phenol-based substances. The Thin Layer Chromatography (TLC) is a chromatographic method which is widely utilized in the field of flora-based study. It is a modest, rapid and cost-effective method with the capacity to indicate the constituents present in a crude plant extract. The TLC is widely applicable due to several advantage such as the low cost, quicker analysis, multiple detection capacity and specificized derivation within the similar plate. Amongst the diverse extracts obtained, the methanol extract was utilized for TLC and a novel solvent system was established for the best extraction of the phyto-constituents exist in the extract which was composed of the constituents in the proportion of N-Hexane: Ethyl Acetate: Methanol: Formic acid 3: 4: 3: 0.1 ml for methanol extract. This caused in the documentation of four spots that were present in the Methanol extract. The Rf values of the samples were 0.68, 0.71, 0.79 and 0.81. The research will further deliver information for the precise exploration of the crude floral extract of Acorus calamus linn.

Keywords: Acorus calamus linn, Phyto-chemical screening, Extraction, TLC.

Introduction:

In countries like India, china, Burma etc, the significance of traditional Ethno drug in the treatment of diverse diseases and for maintenance of proper health was widely identified by the medical care experts, specifically those based in the domain of Indigenous System of Medicine crosswise the rural portions. Around hundreds of therapeutic plants, which crossed a longer history for their curative assets towards diverse disorders and ailments and the knowledge about them has been passed on to the next generations for several millenia. However, the screening of floras for their potential bioactive property is highly vital and requires an imperative interest to know the influential aspects of a particular plant. The rich idea on the prevalent flora in countries like India in the field therapeutic plants and health care has paved way to the emergence of keen attention in them by the pharmacological companies for their utilization of this data as a supply for the study and growth in the search of exploring new therapeutic drugs. Nevertheless, numerous plants have been utilized for multiple features in the India subcontinent in the form of crude shape in the absence of any scientific evidences for their potential efficacies. At this critical juncture it is of attention to regulate the technical evidence-based analysis of the traditional use of such plants. Many plants that are widely

harvested in the wild regions of India are utilized by the local people as a drug next to their resource of food, shelter and other livelihood requirements.

The emergence in the attention to examine the plants for their bio activeness is mainly due to their therapeutically and pharmacologically significant phytoconstituents. The floral harvests a wide range of organic products, like Alkaloids, Flavonoids, Glycoside, Steroid, Saponin and Resin and these have often been connected with the therapeutic as well as pharmacologic possessions of the floras. The growth of resistant to the prevailing commercialized antibiotics and the emergence of increased public concern over the environmental pollution and toxicity that has ultimately resulted in the generation of a continuing need for a newer antibiotic agent. About 20% of the known plants has been recognized and utilized in the pharmacological studies, thereby affecting the health care system in a wide number of good ways like in tumor treatment and treatment of damaging illnesses [2]. The Plants are capable of producing a larger variety of different biologically active constituents. The Higher concentrations of phyto-chemicals defend towards the free radical damaging and are accumulated in the fruits and vegetables [2]. The floras comprising beneficial phyto-chemicals can be utilized to complement the requirements of the human body through substitute as organic antioxidants. The effectiveness of a particular traditional medicine is now a putative fact owing to their better compatibility with the physiological human body with a much better cultural acceptability in major parts of the world with lesser side effects.

The Acorus calamus (L.) belongs to the Araceae family is a familiar flora in that has marked its existence in the Indian traditional medicines [2] for several centuries. Throughout the past two decades, both the inorganic and plant-based products from a wide range of sources has been explored and established in this particular esteem. The *Acorus calamus* is one such herb that is requested to have a highly influential antibacterial behavior. It is existing by a wide range of names such as the cinnamon sedge, sweet myrtle, sweet root, flag root, and so on. It is undoubtedly native to India and now originate prevalent across Europe, Northern Asia Minor, Southern Russia, China, etc. It was esteemed as a potential stimulating, bitter herb for craving and it also aids in the process of digestion. In North America, the plant is generally consumed as a decoction for fever, stomach cramps and colic; the rhizome part of the plant is chewed for the curing of toothache and the powder rhizome is generally breathe in for nasal congestion. In Ayurvedic medicinal system the *Calamus* is a crucial herb which is esteemed as a "rejuvenator" for the mind and nervous system, herbal medicine for the digestive diseases.

Vernacular names of the plant include:

English- Sweet Flag Ayurvedic- Vacha Unani- Bacch Hindi- Bajai, Gora-bach, Vasa Bach Marathi- Vekhand Tamil- Vashambu Telugu- Vadaja, Vasa Kannada-Baje Malayalam-Vayambu Sanskrit- Bhutanashini, Jatila

The organic products, like the floral extract, either in the form of pure substances or as a consistent extract deliver limitless chances for the purpose of discovering novel drugs due to their unrivaled accessibility of chemical variety [3]. Owing to its emergence in the increase in the request for chemical assortment pertaining to screening plans for looking healing medicines from the ordinary products, the interest mainly in the indigenous medical flora has increased significantly all over the world. The Botanicals as well as the herbal preparations for the purpose of therapeutic use harbor numerous kinds of biologically active substances.

The extraction of the bioactive compounds present in a particular extract depends upon diverse parameters, like the extraction method, the constituent feedstocks, and the separation solvent which are used [4]. It is possible to further categorize these methods into traditional or non-conventional. Traditional methods include organic solvents, temperature, and agitation to be used. SOXHLET, maceration, and hydro-distillation are typical examples of methodology. New methods, or non-conventional technologies, are green or clean strategies that are good for the environment because of the decreased use of resources and the introduction of an organic solvent [5]. This article concentrates on analytical techniques involving the extraction, molecular characterization phase of active

compounds in extracts and aims to establish phytochemical criteria and investigate the medicinal substances contained in the methanol extract collected from either the rhizomes of the plant Acorus calamus linn.

Materials and Methods:

Collection of floral material:

A. calamus Linn (Fig. 1) is generally called as the sweet flag and is an aromatic therapeutic plant belongs to the *Araceae* family. The pure rhizomes of the plant *Acorus calamus* were reclaimed from Bharathiar University, Coimbatore, Tamil Nadu, India.

Fig. 1*: A. calamus*

Preparation of floral powder:

A portion of the floral substance was washed below running tap H_2O to remove the impurities. The tiny hairs of *A. calamus* were subsequently eliminated and hacked. They were then shade dried at low temperature.

Preparation of Crude Methanolic Extract:

The appropriately dry flora rhizomes of *Acorus calamus* linn. were ground using an electrical grinder for the purpose of homogenization. The process was continued till the formation of finer powder in the mixing grinder and this powder was balanced precisely. The powder element was then exposed to solvent removal with methanolic by utilization of the Soxhlet apparatus at room T for 48 hrs. The resultant mix was then filtered and vanished in a shaker watery bath; T preserved at 55-65^{0}C then the dried crude extract obtained was utilized for further phyto-chemical study.

Phyto-chemical Study:

All the extracts procured are subjected to the preliminary phytochemical analysis by employing the standard procedure for the exploration of diverse phyto-constituents [6]. The Phyto-chemical tests were done for the determination of the class of compounds that are present in the active fractions and can be assumed to be accountable for the characteristic actions of the flora. Accordingly, the presence of Flavonoid, Glycoside,
Saponin, Resin and Steroid were determined by performing the standard protocols. The outcomes were stated as (+) for presence, and (−) for absence.

Test for Terpenoids

Libermann-Burchard Test: The Extract was treated with certain droplets of the acetic anhydride, then boiled and cooled, the concentrated sulfuric acid was introduced to the sides of test the tube, the presence of a brown rings at the junction of the 2 layer and the uppermost layer goes green which dictates the existence of the sterols and creation of a deep red color designates the existence of triterpenoids.

Salkowski's Test: The extract in methanol was treated with fewer drops of concentrated sulphuric acid, shaken well and then allowed to kept for certain time, the red color appearance in the low layer shows the existence of sterols and creation of a yellow-colored low layer indicates the occurrence of triterpenoids [7].

Test for Steroids:

Salkowski test: 2ml of the extract and 1ml of the concentrated H_2SO_4 acid was introduced carefully along the edges of the test tubes and the creation of a red color shows the occurrence of steroids.

Assessment for Flavonoids

Shinoda Test: (Magnesium hydrochloride reduction test): To the testing solvent fewer fragments of magnesium ribbons were added and concentrated HCl was added in a drop wise manner, the appearance of scarlet pink color after some minutes indicate the incidence of flavonoids.

Ferric Chloride Assessment: To the testing solution, fewer droplets of ferric chloride solution were added, the formation of a strong green color indicates the presence of flavonoids.

Assessment for Tannins:

Ferric Chloride Test: small quantity of the extract was thawed in distilled H_2O and to this solution 2 ml of 5% $FeCl_3$ solution was added. The development of blue green designates the occurrence of tannins.

Lead Acetate Assessment: To a small quantity of extract fewer droplets of lead acetate solution was introduced. The creation of precipitate specifies the existence of tannins.

Assessment for Saponins

Foam test: The solution was subjected to dilution with distilled H_2O and then agitated in progressed cylinder for about 15 mins. The creation of layering of foam directs the incidence of saponins.

Assessment for Phenolic content

Utilizing the Folin ciocal teau model previously defined by Gao et al., the overall phenolics present in a sample were evaluated, with little modification [8]. 1 ml of the rhizome sample was dissolved and nurtured at room T for 5 mins with 2 ml of Folin's ciocalteau (1:1) reagent. 1 ml of 20 percent sodium carbonate was applied to the solution and nurtured at room T for 1 hour. The absorption of the mix utilizing a UV spectrophotometry was calculated at 765 nm. To evaluate the calibration curves, gallic acid (GAE) was used. The tests were conducted in triplicates and the findings were reported as GAE equivalent mg/g.

Extraction of Chemical Elements:

By utilizing the TLC process, the purity of every other eluted specimen was checked. It is a commonly used method for extracting a wide variety of biochemically interesting compounds. It can be used both quantitatively and

qualitatively and as preparatory work [9]. The petroleum ether solution was concentrated to thin layer chromatograph with a concentration of around 0.1-0.2 ml. That used a capillary tube, the methanol extract was packed onto the tray. At the middle of the plate, the pointing was conducted in such a manner that three positions emerged on the plate. The spotted plate has been carefully dry and used for purposes of elution. For the purposes of eluent, the spotted surfaces were properly dry and then used. For the purposes of elution, the marked plates were thoroughly dried and then used. At first and, different amounts were evaluated alone, like benzene, pet ether, chloroform ethanol, and later, based on their polarity level, various solvents formulations were examined. For the isolation of bioactive molecules, various solvent systems varying from smaller polarities to upper polarities were utilized. Within UV light, TLC plates were examined and the segregated spots were labelled [10-11].

a) Development of chromatogram: The eluted dotted plates were dry at the room T and it was kept in the iodine channels for the determination of growth of chromatogram. The Rf numbers of clean spot was then evaluated & a proper solvents were recognized Rf. The values examined are depicted in the table 2.

b) The Column Chromatography: 50 ml of the concerted petroleum ether were dissolved in 10 ml of benzene. The initiated silica gel H was introduced mildly into the benzene solvent and it absorbed the pet ether solution. The chromatogram was allowable to progress and the elution was ongoing after the creation of whole bands and it was used to to 12-15 droplets per mm. Nearly 10 ml of eluting solvent was gathered in a cleaner bottle of 50 ml capacity and was categorized by given number [12].

Results:

The results displayed the existence of phyto-constituents like terpenoids, steroids, flavanoids, tannins, saponins and phenolic compounds as depicted in the table 1. The existence of phyto-constituents was assumed to be responsible for the exhibition of known medicinal as well as physiological activities. The rhizome extract was enriched in chemical Phytoconstituents, such as the terpenoids, tannins, saponins and steroids. The methanolic extract was exposed to TLC in order to isolated and recognize the biologically active substances exist in the rhizome of the methanol extract of *Acorus calamus* linn. The current work developed the most appropriate TCL system for examination of terpenoids, saponins, steroids and Phenolic with the major discerning power TLC plates (Fig.1) revealed under the fluorescence light below UV at 254-365 nm wavelength and active regions in TLC plate was observed as Rf values (0.68, 0.71, 0.79 and 0.81.)

Table 1. Phytochemical screening of Extract of *Acorus calamus* linn.

S. No.	Phytochemical test	Presence/Absence
1.	Terpenoids	+
2.	Steroids	+
3.	Flavanoids	+
4.	Tannins	-
5.	Saponins	+
6.	Phenolic	+

Key: + = Present and - = Absent

Table 2. TLC of methanolic extract of *Acorus calamus* linn.

Solvent system:-	No. of spot	Rf Value of fraction
N-Hexane: Ethyl Acetate: Methanol: Formic acid	3 : 4 : 3 : 0.1 ml	0.68, 0.71, 0.79 and 0.81

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

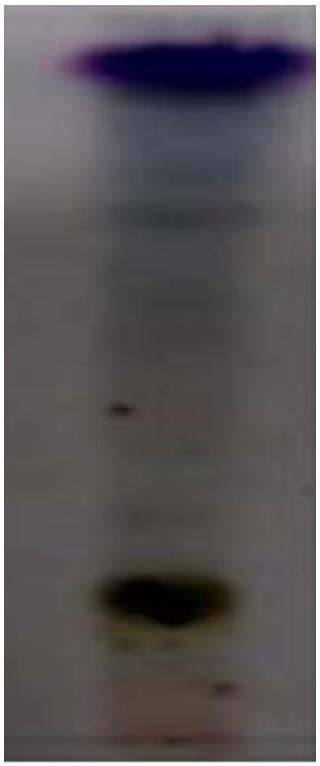

Fig.1: TLC profile of methanolic extract of *Acorus calamus* linn.

Discussion:

The *Acorus calamus* is a prominent perennial plant often employed in the traditional healthcare system and has long been familiar for its therapeutic value. It is a semi-aquatic and smelly flora which is present in both the temperate as well as sub temperate zones. In the timeline of evolution, the Microorganisms have developed resistance to many antimicrobial agents giving rise to the battle of humans versus the drug resistant infections thereby creating an immense clinical problem in the treatment strategy for infectious diseases. This resistance has significantly increased due to the excessive utilization of commercially available antimicrobial drugs. The therapeutic plants show a ironic reservoir of antibacterial substances and these are utilized in traditional medicine systems in different countries and can serve as alternatives for potent and powerful drugs. The scientific interest in plants for therapeutically and pharmacologically significant active constituents is on the rise at a rapid rate. The therapeutic floras are those plants whose 2^{o} metabolites are possible resources of curative medicines. *Acorus calamus* is one such plant with potential efficacies.

The previous researches have shown that the major constituents of A. calamus are the monoterpenes, sesquiterpenes, phenyl-pro-panoids, flavonoids and quinine. The Acorenone was the major constituent in the case

of rhizomes, whereas β-asarone was dominant in the leaves [13]. Besides monoterpene hydrocarbons, choline, flavone, acoradin, galangin, acolamone, and isocolamone were also identified [14] and this is in correlation with our study as most of the above mentioned Phytoconstituents were observed.

Few researches have employed IR spectra of extracts for the revealing of structural information about the major and minor constituents. This information first can be used for the identification (authentication) of crude extract and standardization too however the disparity in our study was that we did not perform IR spectra. The reported chemicals compounds can be used for the confirming of the [15-17]. Our study despite employing only chromatography detected similar phytoconstituents.

In another study by Veena Sharma et al [18] TLC profiling of 5 extracts gave a remarkable outcome that directed against the existence of number of phyto-chemicals. Different phyto-chemicals provide various Rf values in diverse solvent system. This difference in Rf values of the phyto-chemicals shows a very significant evidence in understanding the polarity of the compounds and also aids in collection of suitable solvent system for extraction of pure substances by column chromatography. Combination of solvents with variable polarity in diverse proportion can be utilized for extraction of pure substance from floral extract. The suitable solvent system chosen for a specific floral extract may only be accomplished by analyzing the Rf values of substances in various solvent system.[19] Various Rf values of the substance also replicate an information about their polarity. This is in correlation with our study as the methodology we followed was similar for developing a proper solvent system for better analysis This data will aid in choosing suitable solvent system for further extraction of substance from these floral extracts.

Conclusion:

Acorus calamus is a popular therapeutic plant in the traditional healthcare system of India as well as other countries. Extracts of methanolic rhizomes collected by extraction process from Soxhlet apparatus from Acorus calamus linn. For the production of many medicines, plants were the feedstock and thus remained an impressive sight therapeutic agent. Acorus calamus linn has been found. In children with disabilities, it has a positive effect on the development of memory and learning. Methanolic extract of Acorus calamus linn. While successive distillation process in order to show that the plant's ethno-pharmacological uses in Indian folk medicines are demonstrated. Phytochemical testing of Acorus calamus linn preliminary and significant portion. It is inferred from the evidence that Acorus calamus linn methanolic samples are used. Rhizomes have played an important role in the development of life-saving medicines in medicinal chemistry.

The analysis of bioactive compounds is the need of the hour to provide a scientific evidence for the efficacies of the plant products. The Thin Layer Chromatography (TLC) is a chromatography method which is widely utilized in the field of plant-based study. It is a modest, rapid and cost-effective method with the capacity to indicate the constituents present in a crude plant extract. The TLC is widely applicable due to several advantages such as the low cost, quicker analysis, multiple detection capacity and specified derivation within the same plate. The initial phyto-chemical screening of samples in this study discovered the existence of various phyto-substances like Terpenoids, Steroids, and so on. The Thin Layer Chromatography (TLC) is a chromatography method which is widely utilized in the field of plant-based analysis. This information can serve as the base for the development of novel therapeutics upon further research **References:**

1. Copping, L. G. (1996). Crop protection agents from nature: natural products and analogues. Royal Society of Chemistry.
2. Pawar, D. P., & Shamkuwar, P. B. (2013). Formulation and evaluation of herbal gel containing lantana camara leaves extract. Asian Journal of Pharmaceutical and Clinical Research, 6(3), 122-124.
3. Cos, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro ‘proof-of-concept’. Journal of ethnopharmacology, 106(3), 290-302.

4. Tiwari, B. K. (2015). Ultrasound: A clean, green extraction technology. TrAC Trends in Analytical Chemistry, 71, 100-109.
5. Soquetta, M. B., Terra, L. D. M., & Bastos, C. P. (2018). Green technologies for the extraction of bioactive compounds in fruits and vegetables. CyTA-Journal of Food, 16 (1), 400-412.
6. Akinmoladun, A. C., Ibukun, E. O., & Dan-Ologe, I. A. (2007). Phytochemical constituents and antioxidant properties of extracts from the leaves of Chromolaena odorata. Scientific research and essays, 2(6), 191-194.
7. Herborne, J. B. (1973). Phytochemical methods. A guide to modern techniques of plant analysis, 5-11.
8. Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation. Journal of agricultural and food chemistry, 48(5), 1485-1490.
9. Stahl, E., & Müller, J. (1980). pH Gradient Layers for the TLC of Fluorescent Dyes. Journal of Liquid Chromatography, 3(6), 775-787.
10. Kokate, C. K. (1994). Practical Pharmacognosy. 4 [sup] th ed. New Delhi: Vallabh Prakashan, 107.
11. Bargali, S. S. (1995). Efficiency of nutrient utilization in an age series of Eucalyptus tereticornis plantations in the tarai belt of Central Himalaya. Journal of Tropical Forest Science, 383-390.
12. Ayurvedic Pharmacopoeia Committee. (2001). Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare. New Delhi, India: Department of AYUSH.
13. Paphonngam, A., Kongdee, A., & Buttaraj, K. (2011). Encapsulation of Acorus calamus Linn. extract by polyurethane microcapsules. Advisory Editorial Board, 12.
14. Muthuraman, A., Singh, N., & Jaggi, A. S. (2011). Protective effect of Acorus calamus L. in rat model of vincristine induced painful neuropathy: an evidence of anti-inflammatory and anti-oxidative activity. Food and chemical toxicology, 49(10), 2557-2563.
15. Mythili, R., Venkatachalam, P., Subramanian, P., & Uma, D. (2013). Characterization of bioresidues for biooil production through pyrolysis. Bioresource Technology, 138, 71-78.
16. Sharopov, F. S., Sulaimonova, V. A., & Setzer, W. N. (2012). Composition of the Essential oil of Artemisia absinthium from Tajikistan. Records of Natural Products, 6(2).
17. Zhang, Y., Liao, C., Li, J., & Liu, X. (2011). A review on resource status, bioactive ingredients, clinical applications and biological progress in Bergenia. Journal of Medicinal Plants Research, 5(18), 4396-4399.
18. Paliwal, R., Sharma, V., & Pracheta, J. (2011). A review on horse radish tree (Moringa oleifera): A multipurpose tree with high economic and commercial importance. Asian J Biotechnol, 3(4), 317-328.
19. Sharma, V., & Paliwal, R. (2013). Preliminary phytochemical investigation and thin layer chromatography profiling of sequential extracts of Moringa oleifera pods. International Journal of Green Pharmacy (IJGP), 7(1).