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ORIGINAL ARTICLE **OPEN ACCESS**

GC-MS analysis of phytoconstituents in aqueous and ethanolic extracts of *Acorus calamus* Linn. leaves

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ABSTRACT

Traditional medicines are considered to be the most important part of developing countries' primary health care systems. The therapeutic efficacy of a traditional plant is determined by the secondary metabolites found in the plant parts. The plant Acorus calamus Linn, also known as Vasambu, belongs to the Acoraceae family. The presence of diverse secondary metabolites has been identified with different extracts of A. calamus. However, no reports were found on phytochemical characterization of aqueous and ethanolic extracts of A. calamus leaves.Hence, the present study was aimed at determining the qualitative phytochemical screening and GC-MS characterization of aqueous and ethanolic extracts of A. calamus leaves. The qualitative analysis of phytochemicals showed the presence of alkaloids, proteins, saponins, tannins, phenols, flavonoids, terpenoids, carbohydrates, and cardiac glycosides. Moreover, the possible bioactive compounds were determined from the aqueous and ethanolic extracts of A. calamus leaves by GC-MS analysis. The GC-MS analysis showed the presence of different phytochemical compounds including 4-vinylphenol, 1,3-Benzenediol, Mome inositol, Hexadecanoic acid, Silicone oil, (-)-Loliolide, Phytol, Octadecanoic acid, 13-Docosenamide, (Z)-, etc. From the results, it is evident that A. calamus could be a potential source of novel therapeutic agents such as antimicrobial, antioxidant, and antitumor drugs.

Keywords: *Acorus calamus; Phytomedicine; GC-MS analysis; Antimicrobial; Antioxidant; Antitumor.*

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INTRODUCTION

Ethnomedicine is a term on pre-scientific medicinal frameworks that have been passed down from one generation to another generation. Practices of ethnomedicine used as a traditional therapeutic system in different countries including Indian Ayurveda, Traditional Chinese Medicine, Traditional medicine of Africa, America, Egypt, and the Mediterranean, Arabic Unani and other forms of different original drugs [1]. From ancient times onwards the usage of plants, animals and other material parts has been described as sacramental objects for several healing practices in the folk medicine system [2]. Herbal plants bioactive extract substances preparation and administration in several methods, for example, grinding or drying to preserve or increase the concentration of bioactive compounds. Preparation and administrations are an important role in the extraction of bioactive substances from herbal plants and delivering to the human body. The methods, maceration, infusion, and decoction are the easiest and simplest methods for herbal extraction. The extraction process aims to isolate and quantify the secondary metabolites from the cellular residues [3].

Most of the traditional medicines used in the healthcare industries are obtained from plants. Many of the currently available drugs were derived either directly or indirectly from medicinal plants. Over the past years, herbal medicines or therapeutics are gaining the momentum because of the inadequacy of the modern medicine and its inevitable side-effects. Furthermore, the herbal formulations contain many active ingredients as opposed to single substance based modern drugs, hence they can offer a spectrum of benefits along with minimal or no secondary effects [4]. Medicinal plants have similar therapeutic values as like conventional synthetic drugs and it contain range of phytochemicals that are usually present in their barks, seeds, fruits, flowers, leaves, branch, stemand roots. The phytochemicals are involved in plant defense against insects, microbes and herbivorous mammals and also they have a

Such collective active ingredients are used to cleanse or detoxify the human body by regulating the digestion and the digestive juices it can stimulate the lipid breakdown, stimulate the metabolism and metabolic waste or toxic removal by urine excretion. Due to the said benefits, it is recommended to consume medicinal plants or their active ingredients to improve the wholeness of human health [5].

Phytocompounds such as amino acids, minerals, phenolic compounds, and vitamins are beneficial ingredients in the plant materials, which demonstrate the significant remedy for different biological activities including prevention and treatment of cancer, cardiovascular disease and various chronic diseases, and they exhibitted distinguished analgesic and antioxidant activities[6]. The commonly employed methods for obtaining phytochemicals from herbal plants include extraction, grinding, milling, and homogenization. Out of these methods, the extraction method is the most recommended method for recovering and isolating the phytoconstituents from herbal materials. The yield of phytoconstituents obtained via extractions depends on the polarity of the solvent, duration of extraction, sample compositions, pH and temperature [7]. Solvents used for the extraction process found to influence the nature and the number of secondary metabolites extracted from herbs. For example, polar solvents are used to extract phenolic compounds, glycosides, saponins, and non-polar solvents are used for the extraction of fatty acids and steroids [8].

Recent interest in natural therapies and alternative medicines has made researchers pay attention to traditional herbal medicine. In the past few decades, attention has been focused on the scientific evaluation of traditional drugs for the treatment of various diseases. Due to their high effectiveness, along with minimal side effects relatively low costs make the usage of herbal drugs preferable, even though their biologically active constituents are not fully identified [9]. About 50% of all the drugs in clinical use in the world are derived from natural products, of which higher plants contribute 25% of the total. In developing countries, approximately 80% of people use traditional medicines because of its affordability and cultural acceptability [10]. The added advantage of herbal medicines is their easiness of administration. The plant derived medicines are either consumed directly or as crude extracts, hence the administration does not require invasive methods such as syringe based injection. These methods are painless as well as safe [11].The aim of ethnopharmacological research is mainly focused on detailed identification and to understand of the ethnomedical products and their experimental assessment. The categories of ethnopharmacological fields such as studies and classifications of various ethnomedicines help to understand the overview of the herbal medicines and its therapeutical diversity [12].

In recent years, gas chromatography mass spectrometry (GC-MS) has become strongly established as an important technological platform for identifying secondary metabolites in both plant and non-plant species [13]. The plant *Acorus calamus* Linn, also known as Vasambu, belongs to the Acoraceae family. It is a herbaceous perennial that has been used medicinally for a wide range of ailments, including gastrointestinal diseases and pain relief, and its aroma makes calamus essential oil valuable in the perfume industry. The leaves, stems, and roots are used in various Siddha and Ayurvedic medicines [14]. A comprehensive search of the literature on the plant revealed that there have been no reports available on the possible chemical components of "*A. calamus* leaves." As a result, the current study aimed to investigate the potential bioactive compounds by first preparing aqueous and ethanolic extracts of *A. calamus* leavesand then separating and identifying the compounds using GC-MS analysis.

MATERIALS AND METHODS

Collection of plant leaves

The healthy leaves of *A. calamus* were collected from the nearby area of Cuddalore District fields (Tamil Nadu) in August 2022.The plant was identified and herbarium of A*. calamus*was prepared and preservedin Department of Biotechnology, Dhanalakshmi Srinivasan College of Arts and Science for Women, Perambalur, Tamil Nadu, India.

Preparation of extracts

The fresh *A. calamus* plant leaves were cleaned, washed with tap water, shade dried (37 ºC) for one week, and ground into powder. The powdered leaves (30 g) were subjected to successive extraction using aqueous by maceration method for 48 h and ethanol by soxhlet apparatus at 40º C for 48 h[15]. Following extraction, the obtained crude liquids were kept in a water bath (60 ºC) to allow any excess solvents to evaporate, and they were collected and stored in an airtight container for future use.

Screening of phytochemicals

Presence of different phytochemicals such asalkaloids, proteins, saponins, tannins, phenols, flavonoids, terpenoids, carbohydrates, and cardiac glycosidesin aqueous and ethanolic leaves extracts of *A. calamus* was carried out according to the methodology of Prabhavathi et al. [16]. The performed protocols for screening of phytochemicals are presented in Table 1.

GC-MS analysis

The Elite–MS (5% biphenyl 95% dimethylpolysiloxane, 30 m x 0.25 mm ID x 250 μm df) fused silica column (PerkinElmerClarus 680 GC) was used for the GC-MS analysis. Helium was used as a transporter gas for compound separation at a constant flow rate of 1 mL/min. The injector temperature was set to 260 °C during the chromatographic run, and 1.0 μL of the plant extract was injected into the instrument.The oven temperature was kept at 60 °C for 2 min, and then increased to 300 °C at a rate of 10 °Cmin^{-1}; and 300°C for 6 min.The mass detector controls were as follows: ion source temperature of 240°C, transfer line temperature of 240 °C, ionization mode electron impact at 70 eV, 0.2 sec scan time, and 0.1-sec scan time interval. The volatile low-molecular-weight fragments were assessed between 40 to 600 Da.The spectra of the test components in the extracts were compared to the known components stored in the GC-MS NIST (2008) library.

RESULTS AND DISCUSSION

Screening of phytochemicals

After the successful extraction of the leaves of *A. calamus*, the qualitative phytochemical study revealed that aqueous and ethanalic extracts contains alkaloids, proteins, saponins, tannins, phenols, flavonoids, terpenoids, carbohydrates, and cardiac glycosides. Amongst tested two different solvents, ethanolic extract has accounted the presence of higher phytochemical contents than that of aqueous extract (Table 2).Plant-based phytochemicals have previously demonstrated several biomedical applications. The compounds of alkaloids, flavonoids, phenols, and tannins have medicinal properties. An alkaloid compound has been reported to have a lethal effect on colon cancer and breast cancer cells, which can be used for antitumoral, antiviral, antimicrobial, and antiprotozoal biological applications [17]. Flavonoids have been reported to have anti-cancer, anti-diabetic, skin-related problems, anti-viral/bacterial activities, anti-inflammatory, and anti-aging preparations [18]. Previous research on plant studies has shown that phenolic compounds have been reported to have anticancer activity and potential antioxidant properties. They help treat skin burns, wound healing, and HIV infections [19,20]. Tannins have been shown in previous publications to have anticancer, anti-inflammatory, cardiovascular system protection, antimicrobial (bacteria, fungi, and yeasts), antivirus, anti-parasitic, and antioxidant properties [21,22].

GC-MS analysis

The GC-MS analysis of the chemical compositions of the *A. calamus* leaves extracts by two different extracts such as aqueous and ethanol werepresented in Fig1 respectively.The presence of the active principles (% of peak area), retention time (RT), molecular formula (MF), and molecular weight (MW) are presented in Table 3.According to the results of the qualitative GC-MS analysis (Table 3) of test sample, the bioactive compounds such as 4-vinylphenol, 1,3-Benzenediol, Mome inositol, Hexadecanoic acid, Silicone oil, 3-(1,3-Dimethyl-1H-Pyrazol-4-yl)-1-(4-Hydroxy-Phenyl)-Propenone, 6,6-Dimethyl-2-(4'-methylphenyl)-(1,2,3)-triazol[4,5-c]azepin-4-one, 13-Docosenamide, (Z)-, and Octasiloxane, 1,1,3,3,5, 5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- were indentified in the aqueous extracts of *A. calamus* leaves. In addition, N-(1-Hydroxy-4-oxo-1-pheny l-decahydro-pyrido[1,2-a]azep in-3-yl)carbamic acid, benzyl ester, Tridecane, 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (-)-Loliolide, 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-, Hexadecanoic acid, Phytol, 6,9,12,15-Docosatetraenoic acid, methyl ester, Octadecanoic acid, Octadecanoic acid, Silicone oil, Lucenin 2, Heptacosane, 13-Docosenamide, (Z)-, Hahnfett, and 5,10-Dihexyl-5,10-diihydroindolo[3,2-b]indole-2,7-dicarbaldehyde were indentified from the ethanolic extracts of *A. calamus*leaves (Table 4). As compared with Table 3 and 4, the following compounds such as Hexadecanoic acid, Silicone oil, and 13-Docosenamide, (Z)- were present in both of the extracts.Amongst, Hexadecanoic acid is a well-known anti-inflammatory saturated fatty acid with antifungal and antibacterial properties **[**23]. Silicone oils have been reported to help maintain the adhesion between the retina and the retinal pigment epithelium. As a result, they are used as surgical instruments in the treatment of many vitreoretinal diseases[24]. Further,13-Docosenamide, (Z)- has significant antioxidant,antiandrogenic, antiulcerogenic, anti-inflammatory, anticancer, antimicrobial, lubricant, nematicide, and hypercholesterolemic potential **[**25].
Moreover, previous research reported that Mome inositol has a wide range of applications, including anticirrhotic, antineuropathic, antialopecic, lipotropic, cholesterolytic, and sweetener properties [15]. Phytol is a strong antimicrobial agent against *Staphylococcus aureus*. It also has antinociceptive and antioxidant properties. According to previous reports, the biofilm formation inhibitory activity of methanolic extracts was due to the presence of a bioactive compound such as lucenin 2 [26]

CONCLUSION

In the present study, the presence of different bioactive compounds was identified after GC-MS analysis using the aqueous and ethanolic extracts of *A. calamus* leaves. The GC-MS analysis showed the presence of Hexadecanoic acid, Silicone oil, 13-Docosenamide, (Z)-, Mome inositol, Phytol, and Lucenin 2 promising

phytochemical compounds which contribute to biological activities like anti-inflammatory, antimicrobial, antiulcerogenic, antioxidant, anticancer, etc. From the results of this study, it could be concluded that aqueous and ethanolic extracts of *A. calamus* leaves contain various bioactive compounds and are recommended as a plant with medicinal value. Further research is needed to develop novel phytopharmaceutical drugs based on bioactive compounds found in *A. calamus*

Table 1: Protocols for phytochemical screening.

Tests	Reagents	Results
Alkaloids	1 mL of extract + 1 mL of marquis reagent + 2 mL of concentrated sulphuric acid + few drops of 40% formaldehyde	Appearance of dark orange or purple colour
Proteins	2 mL of extract + 1 mL of 40% sodium hydroxide + few drops of 1% copper sulphate	Formation of violet colour
Saponins	2 mL of extract + 6 mL of distilled water + shaken vigorously	Formation of bubbles or persistent foam
Tannins	2 mL of extract + 10% of alcoholic ferric chloride	Formation of brownish blue or black colour
Phenols	2 mL of extract + 2 mL of 5% aqueous ferric chloride	Formation of blue colour
Flavonoids	2 mL of extract + few drops of 20% sodium hydroxide (yellow colour observed) + few drops of 70% dilute hydrochloric acid (yellow colour disappeared)	Formation and disappearance of yellow colour
Terpenoids	1 mL of extract + add 0.5 mL of chloroform + few drops of concentrated sulphuric acid	Formation of reddish brown precipitate
Carbohydrates	1 mL of extract + few drops of Molisch's reagent + 1 mL of concentrated sulphuric acid + 2 to 3 min allowed to stand	Formation of red or dull violet colour
Cardiac glycosides	1 mL of extract + 0.5 mL of glacial acetic acid + 3 drops of 1% aqueous ferric chloride solution	Formation of brown ring

Table 2: Qualitative phytochemical screening of *A. calamus* extracts

Tests	Solvent extracts	
	Aqueous	Ethanol
Alkaloids	++	++
Proteins	+	++
Saponins	-	+
Tannins	+	-
Phenols	+	++
Flavonoids	++	+
Terpenoids	-	+
Carbohydrates	+	++
Cardiac glycosides	-	+

Note. -. Absent, +. Present, ++. Highly present.

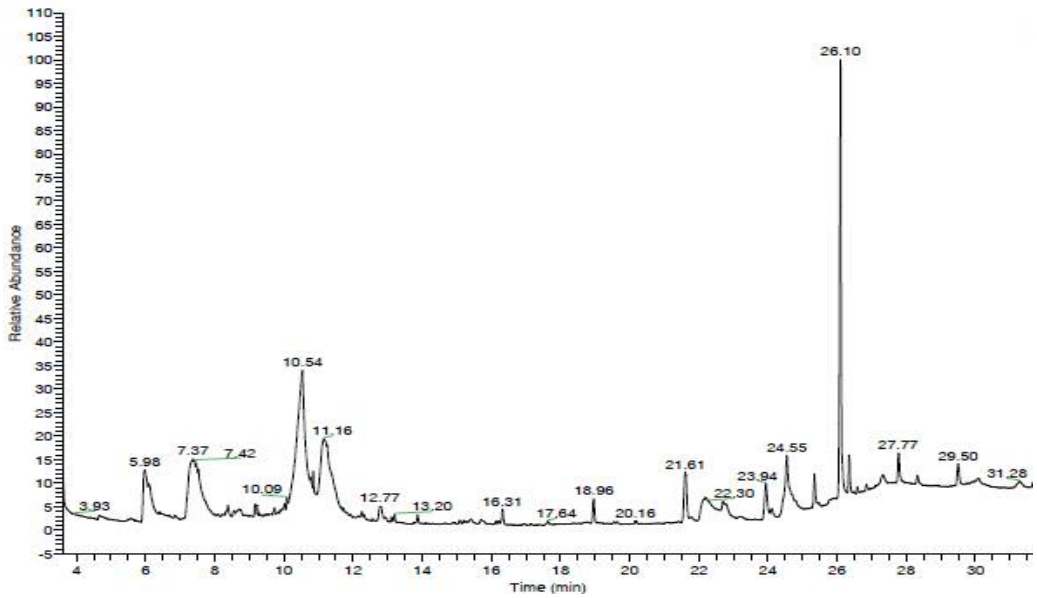

Figure 1.GC-MS chromatographic profile of aquoes extracts of *A. calamus leaves.*

Table 3: Bioactive compounds detected from aqueous extracts of *A. calamus* leaves by GC-MS analysis.

S.No	Name of the compound	% of Peak Area	Retention Time (RT)	Molecular Formula (MF)	Molecular Weight (MW)
1	4-vinylphenol	7.39	5.98	C_8H_8O	120
2	1,3-Benzenediol	11.98	7.37	$C_6H_6O_2$	110
3	Mome inositol	18.32	10.54	$C_7H_{14}O_6$	194
4	Mome inositol	9.90	11.16	$C_7H_{14}O_6$	194
5	Hexadecanoic acid	1.45	12.77	$C_{16}H_{32}O_2$	256
6	Silicone oil	1.08	16.31	NA	0
7	Silicone oil	1.26	18.96	NA	0
8	Silicone oil	3.70	21.61	NA	0
9	3-(1,3-Dimethyl-1H-Pyra zol-4-yl)-1-(4-Hydroxy -Phenyl)-Propenone	3.06	22.30	$C_{14}H_{14}N_2O_2$	242
10	6,6-Dimethyl-2-(4'-methylphe nyl)-(1,2,3)-triazol[4,5-c]azep in-4-one	3.29	23.94	$C_{15}H_{18}N_4O$	270
11	Silicone oil	4.94	24.55	NA	0
12	13-Docosenamide, (Z)-	18.08	26.10	$C_{22}H_{43}NO$	337
13	Silicone oil	1.66	27.77	NA	0
14	Silicone oil	1.32	29.50	NA	0
15	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,1 3,15,15-hexadecamethyl-	0.72	31.28	$C_{16}H_{50}O_7Si_8$	578

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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