Research Article

Preliminary Phytochemical Screening and GC-MS Analysis of Methanolic Extract of Roots of *Pandanus fascicularis*

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Submission Date: 12-10-2021; Revision Date: 19-11-2021; Accepted Date: 03-12-2021

ABSTRACT

Pandanus fascicularis, a small branched tree or shrub belonging to the family *Pandanaceae* with fragrant flowers, is found in India, Burma, and Andaman. *Pandanus fascicularis* is used in traditional systems of medicine to treat varying conditions like rheumatism, fever, headache, earache and also used as antispasmodic. It is a small, slender, branching tree with a flexuous trunk supported by brace roots. With rosettes of long-pointed, stiffly leathery, spiny, bluish-green, fragrant leaves. It bears very fragrant flowers in summer. They are used to produce dyes and in the production of traditional medicines. Roots are used as a decoction for the treatment of skin diseases, ulcers, dyspepsia, diabetes, fever, and leprosy. The GC-MS study of the *Pandanus fascicularis* root extract displayed about 20 peaks. At the retention time of 22, there was a presence of only one major peak was identified. The peak indicates the existence of decanoic acid with a molecular weight of 826. Subsequently, at the retention period of 4.50 to 22.97, there is the presence of 7 minor peaks and 3 moderate peaks at 19.22, 20.44, and 22.97 period due to the presence of Hematoporphyrin (MW: 598), 1(Trimethylsilyloxy) cyclopentene (MW: 156) and Ethanedioic acid, bis(trimethylsilyl) ester (MW: 234) compounds, respectively. This work was the preliminary study to identify the phytoconstituents from the root of *Pandanus fascicularis*. As the major constituents from the *Pandanus fascicularis* like hematoporphyrin, decanoic acid, etc have been reported to have antipsychotic, antidepressant, neuroprotective, and anti-inflammatory activities, further studies on isolation and pharmacological activity of the plant are in progress in our lab.

Key words: GC-MS Analysis, Phytoconstistuents, *Pandanus fascicularis*, Methanolic root extract, Tannin content, Phenolic content, Alkaloid content.

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INTRODUCTION

Medicinal plants are thought to be a rich source of bioactive molecules that can be utilised in the development of drugs. Phytochemical analysis is used to extract, isolate phyto-constituents, identify and screen them, and assess the plant's therapeutic potential. Once an active chemical has been identified, the structure-activity relationship can be studied, making it easy to compare its activity to a physiological target.[1] *Pandanus fascicularis* is a branched palm-like shrub, leaves ensiform, spinescent; male flowers in spikes enclosed in large, white, fragrant spathes; female flowers in solitary spadix; syncarpium yellow or red drupes numerous. Commonly found in coastal regions of India and the Andaman Islands. Propagation by seeds and vegetative methods. The chief constituent of kewda oil is methyl ether of phenyl ethyl alcohol to which is due to the characteristic aroma of the spadices. The oil also contains dipentene, d-linalool, phenyl ethyl acetate, citral, phenyl ethyl alcohol, ester of phthalic acids, fatty acid, and stearoptene. The roots are bitter, sweet, acrid, thermogenic, emollient, depurative, procreant, antiseptic, cephalic, aphrodisiac, carminative, stomachic, suppurative, anodyne, deodorant, urinary

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DOI: 10.5530/ajbls.2021.10.83

astringent, vulnerary sudorific, febrifuge, and tonic. They are useful in vitiated conditions of skin diseases, leprosy, cephalalgia, coxalgia, otalgia, wound, ulcers, dyspepsia, flatulence, colic, fever, diabetes, sterility, spontaneous, abortion, and general debility. The leaves are acrid, bitter, alexeteric aphrodisiac, depurative and somniferous. They are useful in tumors, leprosy, smallpox, syphilis, scabies, leukoderma and cardiopathy, and cerebropathy due to insomnia. The flowers are acrid, bitter, aphrodisiac, anodyne, and demulcent and are useful in pruritis otalgia, cephalalgia, leukoderma, and skin eruptions. The oil obtained from bracts is stimulant and antispasmodic and is useful in cephalgia, rheumatoid arthritis, and coxalgia.[2,3] Tannins, flavonoids, steroids, saponins, proteins, terpens, phenols, glycosides, glucose, and alkaloids were discovered in the fruits of *Pandanus fascicularis* by Jitu *et al.* (2017, Jitu *et al.*) The root's phytochemical investigation has yet to be completed, so the phytochemical screening of plant root was carried out In the current study in order to separate potentially effective components GC-MS technique was used to characterise the phytoconstituents found in *Pandanus fascicularis* root extracts.

MATERIALS AND METHODS

Collection of Plant Material

Pandanus fascicularis roots Figure 1 were collected from the swampy portions of the Kottayam district. To remove siliceous earth matter, fresh plant portions were carefully washed and sanitised under running tap water, and surplus water was drained and shade dried. The dried plant components were crushed into a coarse powder and stored in airtight containers until they were used.

Figure 1: Root of Pandanus fascicularis.

Extraction of Plant Material

Using an accelerated solvent extractor, the extraction was carried out at Kerala Veterinary and Animal Sciences University (KVASU) in Thrissur. A pestle was used to combine the dried powder with diatomaceous earth and a small amount of alumina in a mortar. The extraction was carried out using the solvent methanol with nitrogen gas as an inert environment at a pressure of 200 psi. Using a rotary evaporator, the residual solvent in the extract was evaporated, and the lyophilized product was used for GC-MS analysis.[4]

Phytochemical Screening

Qualitative Analysis

Phytochemical ingredients were subjected to certain chemical tests. To validate the existence of various compounds in the methanolic extracts solely, standard techniques were used to identify the constituents as reported by standard procedure.[5-7]

Quantitative analysis

The plant is found to be rich in phytoconstituents. Therefore the quantitative phytochemical studies were carried out to estimate total phenols, Tannin, flavonoid, alkaloid, and saponin.

Estimation of Phenol

The aliquot of material was pipetted out, and the volume in the tube was increased to 3.0 ml distilled water, which was compared to the blank. Folin-ciocalteau reagent (0.5mL) was added, followed by 2mL of 20% sodium bicarbonate solution; the tubes were then placed in a boiling water bath for one minute. The tubes were cooled, and the absorbance was measured against a reagent blank in a spectrophotometer at 750nm. Standard gallic acid solutions with concentrations ranging from 20 to 100 g were likewise treated in the same way.[8]

Estimation of Tannin

Content of tannins in sample was determined by Folin-ciocalteu method. Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungsto molybdic acid by tannin like compounds in alkaline medium. 1ml of extract and standard solution of tannic acid (20-100 µg) was made up to 7.5mL with distilled water. Then 0.5mL of Folin-ciocalteu reagent and 35% 1mL sodium carbonate solution were added. The volume was made up to 10mL with distilled water and the absorbance was measured at 700nm.[9]

Estimation of Flavonoid

Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1mg/ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 min, 0.3 ml of 10 % aluminium chloride was mixed. After 5 min, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of Quercetin (20, 40, 60, 80 and 100 µg) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavanoid content was expressed as µg/ mg of extract.[10]

Estimation of Alkaloid

The plant extract (1mg) was dissolved in 1 ml dimethyl sulphoxide (DMSO), added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer.[11]

Estimation of Saponin

The vanillin-sulphuric acid assay for determining the total saponin content of plant materials is usually done by incubating 1mg/ml of plant sample extracts, standards or reagent blank with 0.25 mL of 0.8% (w/v) vanillin in ethanol and 2.5 mL of 72% (v/v) sulphuric acid in water for 15 min at 60°C in a shaking water bath, with the standard as diosgenin and the reagent blank made up with the solvent used for extracting the plant samples (extraction solvent). After cooling in water at the ambient temperature for 5 min, the absorbance of the standards and extracts are measured at 544 nm using a UV–VIS spectrophotometer.[12]

Chromatographic Analysis

Methodology

The plant based phytochemical identification of methanolic extracts of *Pandanus fascicularis* was executed on a Gas chromatography-mass spectrometry (GC-MS) technique and it was performed at the Manoothi Veterinary College, Thrissur, Kerala by utilizing a Thermo Scientific TSQ 8000 GC-MS instrument. The observed peaks which are segregated in a GC-MS were determined by National Institute of Standards and Technology (NIST) mass spectra databases. The constituents which exist in the root extract were recognized based on the assessment of their comparative retention time and mass spectras.[13] The component's name, and their molecular weight were also determined. The compound's IUPAC name is listed in Table 3.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemical constituents

Table 1 shows the phytochemical investigation of the methanolic extract of *Pandanus fascicularis* revealed the presence of carbohydrates, tannins, steroids, glycosides, alkaloids, anthraquinone, and terpenoids except for flavonoids and saponins.

Quantitative analysis of methanolic root extract of *Pandanus fascicularis*

The results obtained from the quantitative analysis of root extract of *Pandanus fascicularis* were tabulated. The Table 2 result clearly indicated that the tannins were the most prominent phytoconstituent followed by phenols and alkaloid.

GC-MS analysis of the root extract of *Pandanus fascicularis*

The GC-MS studies of *Pandanus fascicularis* revealed the presence of around 20 compounds which have been tabulated and identified using NIST library.

Table 1: Details of Phytoconstituents in root extract of *Pandanus fascicularis*.

Sl No	Phytochemical constituents	Methanol extract
1	Alkaloid	+++++
2	Carbohydrate	++
3	Protein	+
4	Tannin	+
5	Aminoacid	++
6	Saponin	-
7	Phenol	+++
8	Flavanoid	-
9	Glycosides	+
10	Anthraquinone	+
11	Steroid	++
12	Triterpenoid	++
13	Amino acid	++

The GC-MS study of the *Pandanus fascicularis* root extract displayed about 20 peaks as shown in Figure 1. At the retention time of 22 only one major peak was identified, which indicates the existence of Decanoic acid with a molecular weight of 826. Subsequently, at the retention time period of 4.50 to 22.97 seven minor peaks were also observed. Three moderate peaks at 19.22, 20.44 and 22.97 period due to the presence of Hematoporphyrin (MW: 598), 1(Trimethylsilyloxy) cyclopentene (MW: 156) and Ethanedioic acid, bis(trimethylsilyl) ester (MW: 234) compounds, respectively. Table 3 clearly depicts molecular weight and percentage of top peak area of the identified compounds from the extract of *Pandanus fascicularis*.

The other peaks identified from the spectra include , Oxazolidin-2-one (MW-87) at 6.77 with an area % 0.45 which has prominent antibacterial activity,[26] 1-Formyl-2,6-dimethoxy-10-methyl-anthracene (MW-280) at R*t* 21.65 with an area percentage of 0.40 also shows Antibacterial, antidiabetic activity.[27,28] R*t* of 21.82, a compound named 3-acetoxy- 7,8-Epoxylanostan-11-ol, (MW-502) with an area percentage 3.57, (Alcoholic compound) shows Antimicrobial, anti-inflammatory activity,[29] 2,4,6-Decatrienoic acid in (MW594) at R*t* 21.93 has area percentage of 6.33 with antibacterial and anticancer activity.(30), 3,4-didehydro-1,2,7',8'-tetrahydro-1-methoxy-2-oxo-Carotene, R*t* on 23.97 with 0.68 area % has Antioxidant, Cytotoxic, and Antimicrobial Activities(21), 1,4-dimethyl-2-octadecyl-Cyclohexane, at R*t* 6.26 with a percentage area 0.34, has antidiabetic activity.[30]

We also discovered isosteres of components of *Pandanus fascicularis* like, silanediol is the functional group of a novel bioisostere of the hydrated amide carbonyl. It's neutral, cell-permeable, and excellent hydrogen bonding allows for good interactions with aspartic proteases' active sites.[31] In enzyme and cell

Table 2: Quantitative analysis of the methanol root extracts of *Pandanus fascicularis*.

Sl no	Phytoconstituent	Optical density	Standard	Concentration µg/ mg
1	Phenol	0.302	Gallic acid	28.7
2	Tannin	0.367	Tannic Acid	60.5
3	Flavanoid	0.022	quercetin	-
4	Alkalod	0.067	atropine	23.5
5	Saponin	0.605	Diosgenin	-

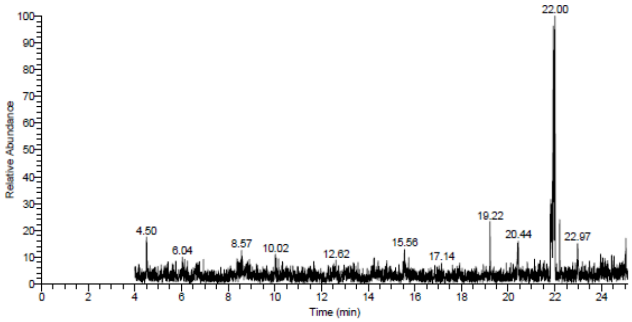

Figure 2: Chromatogram attained from the GC-MS with the root extract of Pandanus fascicularis.

Table 3: Phyto-compounds present in Methanolic extract of *Pandanus fascicularis* using GC-MS Profiling.

Sl.No.	R_t (min)	% Conc.	MW	MF	Name	Applications
1	4.50	1.11	92	$C_2H_8O_2Si$	Silanediol, dimethyl-	Bactericidal, Antimicrobial, HIV protease inhibitor,[14–16]
2	6.04	0.73	116	$C_6H_{12}O_2$	2-Pentanone, 4-hydroxy-4-methyl-	Antimicrobial, anti-proliferative, Antioxidant[17,18]
3	8.57	1.28	104	$C_2H_{12}S$	2-Butanethiol, 3-methyl-	
4	10.02	0.51	598	$C_{34}H_{38}N_4O_6$	Hematoporphyrin	Anticancer[19]
5	12.62	0.69	329	$C_{19}H_{23}NO_4$	1-Hydroxy-4-(p-toluidino) anthraquinone	
6	15.56	0.98	284	$C_{18}H_{36}O_2$	1,3-Dioxolane, 2-pentadecyl-	Antioxidant, Cytotoxic, and Antimicrobial Activities, antibacterial, antifungal[20,21]
7	20.44	1.38	156	$C_5H_7OSi(CH_3)_3$	1(Trimethylsilyloxy) cyclopentene	
8	22	5.99	826	$C_{10}H_{20}O_2$	Decanoic acid	Antibacterial, anticancer, Antioxidant[22,23]
9	22.97	1.07	234	$C_8H_{18}O_4Si_2$	Ethanedioic acid, bis(trimethylsilyl) ester	Acidifier, acidulant, antimicrobial, antioxidant and anticancer properties[24,25]

protection studies, silanediol peptidomimetics were found to be nearly as effective as currently available pharmacological medicines. As a result, these neutral, cell-permeable transitionstate analogues provide a unique platform for the development of therapeutic medicines.[16] 2-Pentanone, 4-hydroxy-4-methyl-(at R_t 6.04) commonly known as, Diacetone Alcohol, is a type of anti-hypertensive medication. In cosmetic compositions, this chemical is used as perfumary and a solvent.[32] Hematoporphyrin (Hematoporphyrin IX), (at R_t 10.02) a photosensitizer, is a substrate for heme-binding protein affinity chromatography. When exposed to red light, hematoporphyrin can trigger apoptosis in U87 glioma cells and reduce tumour growth *in vivo*.[33,34] 1-Hydroxy-4-(p-toluidino) anthraquinone (at R_t 12.62) is an anthraquinone color that functions as a colorant in cosmetic and hair dye formulations.[35] Hatice Başpnar Küçük *et al*. synthesised novel 2-pentadecyl 1,3-dioxolane derivatives from chiral and racemic 1,3-dioxolanes. All of the compounds were put through their paces as potential antibacterial and antifungal agents. It's worth noting that the new chiral and racemic 1, 3-dioxolanes have demonstrated outstanding antibacterial and antifungal properties. In the pharmaceutical sector, these substances could be assessed as bioactive agents.[20] Decanoic acid (at R*t* 10:29) is a kind of fatty acid. Known as MCTs (medium chain triglycerides). MCTs are commonly utilised for parenteral nutrition in those who need extra nourishment, and they're becoming more common in meals, medications, and cosmetics. The MCT diet appears to be effective in treating children who have seizures that are uncontrollable by medicines. According to Janine Mett *et al*. findings, decanoic acid and probably MCFAs in general reduce oxidative stress levels which may benefit brain health.[36]

CONCLUSION

Pandanus fascicularis is used in traditional systems of medicine to treat varying conditions like rheumatism, fever, headache, earache and also used as antispasmodic. GC-MS analysis of tap root of *Pandanus fascicularis* methanolic extract showed the presence of about 20 compounds which include carbohydrates, proteins, amino acids, saponins, tannins, phenolic compounds, alkaloids and flavonoids. These compounds exhibit antihypertensive, anticancer, antibacterial, antifungal, antiepileptic, and neuroprotective properties. The presence of bioisosteres such as Silanediol, diacetone alcohol, hematoporphyrin, anthraquinone and decanoic acid were isolated among the compounds from GC-MS analysis has opened avenue for new research.

ACKNOWLEDGEMENT

Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, India, provided GC-MS facilities to the authors. Dr V Ramanth, Professor and Head (KVASU), is also thanked by the corresponding author for his assistance in structural characterization of plant extract components.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Pandanus fascicularis is a little branched tree or shrub in the Pandanaceae family with fragrant blooms. Pandanus fascicularis is used in traditional medicine to treat a variety of ailments. The GC-MS analysis of Pandanus fascicularis root extract revealed approximately 20 peaks. Because significant elements of Pandanus fascicularis, such as hematoporphyrin and decanoic acid, have been shown to have antipsychotic, depressive, neuroprotective, and anti-inflammatory properties, our team is conducting additional research on the plant's isolation and pharmacological activity. The discovery of bioisosteres such as Silanediol, diacetone alcohol, hematoporphyrin, anthraquinone, and decanoic acid among the molecules identified by GC-MS analysis has paved the way for further research.

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Cite this article: Sheeja MTL, Jacob J, Jayakar B, Gomathi V. Preliminary Phytochemical Screening and GC-MS Analysis of Methanolic Extract of Roots of *Pandanus fascicularis*. Asian J Biol Life Sci. 2021;10(3):626-31.