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Research Article

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Phytochemical Screening, GC-MS Analysis of Methanolic Extract of the Fern Adiantum Caudatum from Southern Western Ghats

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Abstract

The present study was to screen the presence of phytochemicals in the methanol extract of fern Adiantum caudatum species by composed with qualitative and quantitative screening methods. In qualitative analysis, the presence of pharmacologically active phytochemicals such as alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids, saponins, phenols, volatile oils and resins were screened. GC-MS chromatogram analysis of the methanolic extract of Adiantum caudatum showed 12 peaks corresponding to 12 different compounds. The percentage of major bioactive compounds were 2-Hydroxymethyl-9-[Beta.-D-Ribofuranosyl] Hypoxanthine (17.995%), 2-Undecene,5-methyl- (33.497%), Pentadecane,2,6,10,14-Tetramethyl- (14.977%), Nonadecane (7.588%), Dodecane, 2,6,10-trimethyl- (1.421%), 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol (2.825%), Nonadecane (7.855%). The identification of bioactive compounds is based on the retention time, peak area, molecular formula, and probability.

Keywords: Phytochemicals, Qualitative, Adiantum Caudatum, Methanol and GC-MS.

1. Introduction

It is impossible to imagine the survival of human race if the earth had no plants on it. The dependence of human beings on plants dates to the start of the human race. Medicinal plants are common sources of medicine. Solid evidences can be cited in favor of herbs being used for the treatment of diseases and for restoring and fortifying body systems in ancient systems of medicine such as Ayurvedic, Unani and Chinese traditional medicine. The innately desired purpose of the use of herbs was to obtain a positive interaction with body chemistry [01].

Traditional medicinal information is important not only for its potential contribution to the drug development as well as people's healthcare. According to the World Health Organization 80% of the world's population mostly individuals of developing countries depend on plant-derived medicines for their healthcare needs. Most of the aboriginal people are not well identified about the uses of Pteridophytes ever since it's not simply available like flowering plants. Pteridophytes are represented by 144 genera and about 1200 species distributed in India [02]. Pteridophytes are regarded as a facultative wetland plant and which can also adopt well in terrestrial habitat. They have a vital role in the earth's biodiversity [03].

Ferns have measurable indications that may reflect the effects of change in environmental factors. The ferns are not only taxonomic oddities but those are plants with dynamic relationship to their environment [04]. Pteridophytes are of immense economic importance and there is a great need for their exploitation towards the economic utility in daily life [05]. Since prehistoric times, plants have been used as a source of treatment. The use of traditional medicine is increasing day by day due to high cost of the allopathic medicines and their potential side effects [06]. Several plants or their parts have been exploited for this purpose. The value of these plants is attributed to the presence of some chemical substances, which produce a definite physiological action. These chemical constituents. These non-nutritive plant chemicals have protective or disease preventive properties. They are nonessential nutrients, meaning that they are not required by the human body for sustaining life. It is well-known that plant produces these chemicals to protect them, but recent research demonstrates that they can also protect humans against diseases [07].

Considering the rich diversity of Indian medicinal plants including pteridophytes, it is expected that, the screening of plant extract for antibacterial activity may be beneficial for humans and plants diseases. The synergistic interaction among crude extracts or the active compounds may be useful in the preparation of improved herbal or drug formulations. The extensive survey of antibiotic activity among the ferns conducted and reported about a hundred species having such property. Traditionally people

used pteridophytes as medicine and antibacterial agents [08].

Since bioactive compounds occurring in plant material consist of multi-component mixtures, their separations and determination still creates problems. Practically most of them have to be purified by the combinations of several chromatographic techniques and various other purification methods to isolate bioactive compounds. Though, lot of studies have focused on the medicinal properties of Angiosperms, information on the medicinal potentialities of the pteridophytes are limited [09-12]. The present study focuses on the phytochemical screening and GC-MS analysis of the fern Adiantum caudatum L. a small fern belongs to the family Pteridaceae.

2. Materials and Methods

Collection and preparation of plant materials: The fern, Adiantum caudatum for the proposed study was collected from different areas in Valparai (latitude: 10°22'12"N and longitude: 76°58'12"E, 1050 ma.s.l.), Coimbatore district, Tamil Nadu, India. The fronds of selected fern used for phytochemical screening were washed multiple times with tap water and further with distilled water to remove fine impurities. Leaves were shade-dried for 30 days to remove all the moisture content and to preserve maximum of the bioactive compounds. The dried fronds were cut down into small pieces of size up to 1-2 cm. The cut down parts were crushed using a laboratory blender and then sieved through a mesh size of 3 mm in order to remove the course materials. The fine powder was then packed in an airtight container, labelled and stored for further studies.

2.1 Extract Preparation: Organic solvent, Methanol was used to extract the powder sample of Adiantum caudatum according to the method described by [13]. The sample was extracted using a soxhlet apparatus at a temperature (40-50°C) and was subjected to detect the presence of different phytoconstituents.

2.2 Qualitative estimation of methanol extract: The tests performed for the phytochemical screening were alkaloids, glycosides, phenols, tannins, flavonoids, saponins, volatile oils, terpenoids, resins, steroids, anthraquinones.

• Tests for alkaloids

Mayer's test: To 1ml of extract, 2ml of Mayer's reagent is added. Formation of dull white precipitate indicates the presence of alkaloids [14].

• Tests for glycosides

Borntrager's test: To 1ml of extract, 1ml of benzene and 0.5ml of dilute ammonia solution were added. A reddish pink color indicates the presence of glycosides [13].

• Test for Phenols

To 1 ml of the extract, 2 ml of distilled water followed by 0.5 ml of sodium carbonate and 0.5 ml of Folin Ciocalteau's reagent were added. Formation of blue / green colour indicates the presence of phenols [15].

• Test for Tannin

To 4 ml of extract, 4 ml of FeCl3 was added. Formation of green colour indicates the presence of tannin [16].

• Testsforflavonoids

To 3 ml of the extract, 4 ml of 1N NaOH was mixed in a test tube. Formation of dark yellow colour was observed which indicates the presence of flavonoids [15].

• Tests for saponins

Foam test: 1ml of extract was shaken vigorously with 20ml of distilled water for 5- 10 minutes in graduated cylinders. Formation of one-centimeter layer of foam indicates the presence of saponins

• Test for volatile oils

NaOH-HCl test: 2ml of extract solution was shaken with 0.1ml of dilute sodium hydroxide and a small quantity of dilute HCl. A white precipitate was formed with volatile oils, which confirms the presence of volatile oil.

• Tests for terpenoids

Trichloroacetic acid test: To 1ml of extract, 2ml of trichloroaceticacid was added. Formation of green coloured precipitate showed the presence of terpenoids.

• Tests for resins

Turbidity test: To 2 ml of extract 5 ml of distilled water was added. The occurrence of turbidity showed the presence of resins [14].

• Test for steroid

1ml extract was dissolved in 10 ml of chloroform & equal volume of concentrated H2SO4 was added from the side of test tube. The upper layer turns red and H2SO4 layer showed yellow with green fluorescence. This indicates the presence of steroid.

• Test for Anthraquinones

To 2 ml of extract, 3ml benzene was treated with 10% NH3. Formation of violet colour indicates the presence of anthraquinones [16].

GC-MS Analysis of the Methanolic Extract of Adiantum Caudatum

Gas Chromatography (GC) analysis was carried at Vellore Institute of Technology (VIT), Chennai. It is one of the key techniques generally used for the screening/ identified of many groups of the plant phytochemicals. The high attainable separation power in combination with wide range of the detectors employing various detection principles to which it can be coupled makes GC an important, often irreplaceable tool in the analysis at trace level of plant phytochemical compounds. Gas Chromatographical study includes the important optimization process such as introduction of the sample extract onto the GC column, Separation of its components on an analytical column and detection of target analysis by using Mass Spectrometric (MS) detector.

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5 % biphenyl 95 % dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60°c (2min); followed by 300°C at the rate of 10°C min-1; and 300°C, where it was held for 6m in. The mass detector conditions were: transfer line temperature 240°C; ion

source temperature 240°C; and ionization mode electron impact at 70 eV, a scant time 0.2 sec and scan interval of 0.1 sec. The fragments size ranges from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

The chemical composition of the Adiantum caudatum was analysed by GC/MS. Analysis of the Adiantum caudatum extract was carried out using therom GC-turbomass version 5.4.2. couple with therom MS DSQ11 instrument. The clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m×0.25mm ID×250µm df) and the components were separated using the helium as carrier gas at a constant flow of 1ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µl of extract sample injected into the instrument the oven temperature was as follows: 60/°C(2min); followed by 300°C at the rate of 10°C min–1; and 300° C, where it was held for 6 min. The mass detector conditions were transfer line temperature 240°C; ionisation mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST Library.

3. Results and Discussion

Qualitative estimation of methanol extract: The study was undertaken to evaluate the phytochemical constituents of the selected leaf extract to confirm the presence of bioactive compounds. The polar solvent methanol exhibited the presence of major phytochemicals such as alkaloid, resin, saponin, steroid, phenol, tannin and flavonoids. The preliminary phytochemicals of Adiantum caudatum was recorded and tabulated

Phytochemicals	*Adiantum caudatum*
Alkaloids	+
Volatile oils	-
Glycoside	-
Phenol	+
Tannin	+
Saponin	+
Terpenoids	-
Resins	+
Flavonoids	+
Steroids	+
Anthroquinones	-

Table 1.1 Qualitative Estimation of Adiantum Caudatum using the Methanolic Extract

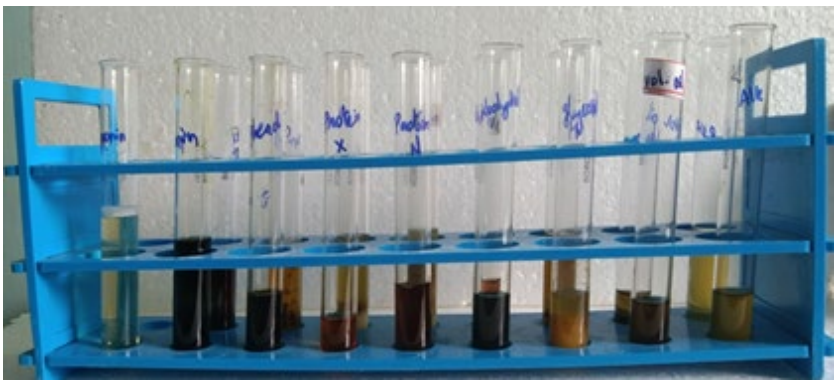

Figure 4.1: Qualitative estimation of Adiantum Caudatum

GC-MS Analysis of Methanolic leaf Extract of Adiantum Caudatum

GC-MS chromatogram analysis of the methanolic extract of Adiantum caudatum showed 12 peaks corresponding to 10 different compounds given in fig.4.2. Different compounds were identified by considering the retention time, molecular weight and formula, peak area percentage obtained in the chromatogram were tabulated and shown [17, 18].

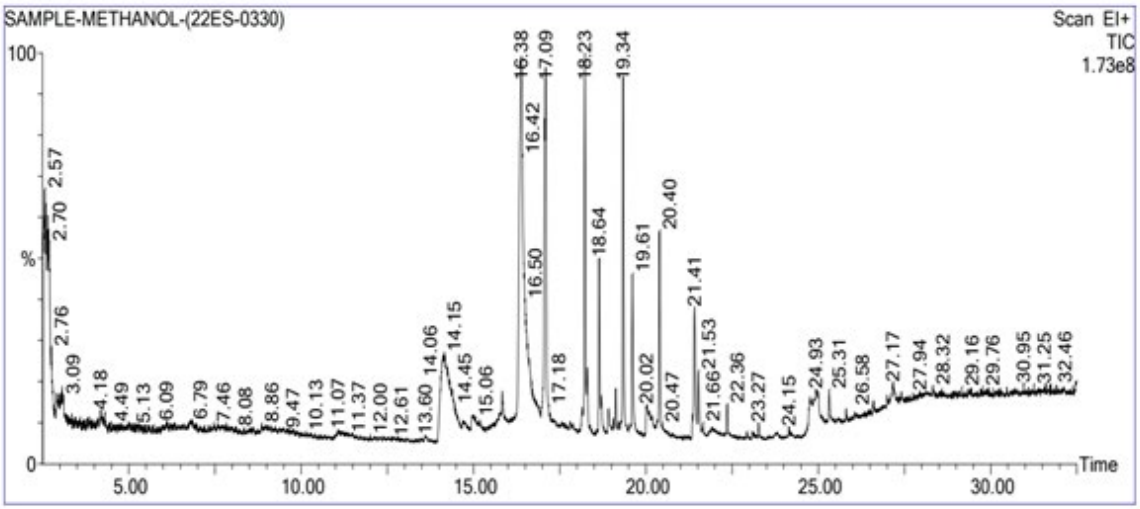

Figure 1.2: GC-MS analysis of methanolic leaf extract of Adiantum caudatum

RT	Compound name	Compound Structure	Molecular formula	Area %	Biological role
14.148	2-Hydroxymethyl-9-[.Beta.-D-Ribofuranosyl] Hypoxanthine	298	$C_{11}H_{14}O_6N_4$	17.995	-
16.389	2-Undecene,5-methyl-	168	$C_{12}H_{24}$	33.497	-
17.094	Pentadecane,2,6,10,14-Tetramethyl-	268	$C_{19}H_{40}$	14.977	Pathogenesis of rheumatoid arthritis and lupus.
18.230	Nonadecane	268	$C_{19}H_{40}$	7.588	Plant metabolite, volatile oil component.
18.305	Dodecane,2,6,10-trimethyl-	212	$C_{15}H_{32}$	1.421	-
18.645	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	296	$C_{20}H_{40}O$	2.825	Antimicrobial, anti inflammatory, anti diuretic, anticancerous.
19.340	Nonadecane	268	$C_{19}H_{40}$	7.855	Plant metabolite, volatile oil component.
19.610	Pentadecanoic acid,14-methyl-,methyl ester	270	$C_{17}H_{34}O_2$	3.556	Biomarker for mammalian metabolism
20.020	1,6;3,4-Dianhydro-2-deoxy-.beta.-d-lyxo-hexopyranose	128	$C_6H_8O_3$	1.541	-
20.395	Nonadecane	268	$C_{19}H_{40}$	3.948	Plant metabolite, volatile oil component.
21.411	7-Hexadecenal,(Z)-	238	$C_{16}H_{30}O$	3.449	Antiviral activity, organic fertilizer
21.526	Phytol	296	$C_{20}H_{40}O$	1.347	Cholinesternse inhibitory activity.
24.867	2-Isopropyl-5-Methylcyclohexyl 3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C	524	$C_{30}H_{33}O_6Cl$		-

Table 1.2: Compounds identified in the GC-MS analysis of methanolic extract of A. caudatum

4. Conclusion

The present study was proposed to investigate the secondary metabolites of Adiantum caudatum, a small sized fern which belongs to the family Pteridaceae. The Fronds of A. caudatum

were analysed for the presence of phytochemicals. Preliminary phytochemical screening reported the presence of alkaloid, resin, saponin, steroid, phenol, tannins and flavonoids in methanolic extract. GC-MS analysis of methanol extract revealed the presence of bioactive compounds such as 2-Hydroxymethyl-9-[. Beta.-D-Ribofuranosyl] Hypoxanthine (17.995%), 2-Undecene,5-methyl- (33.497%), Pentadecane,2,6,10,14-Tetramethyl- (14.977%), Nonadecane (7.588%), Dodecane, 2,6,10-trimethyl- (1.421%), 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol (2.825%), Nonadecane (7.855%). Most of the compounds present showed the antimicrobial properties. Hence, further studies may conduct on the antimicrobial activity against harmful pathogens.

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