Phytochemical Screening And GC-MS Analysis of Pedalium Murex Leaf Extract

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Abstract- *Pedalium murex Linn, commonly called Gokhru a member of family Pedaliaceae. The fresh leaves were collected shade dried and ground using mechanical motor. The powdered materials were sequentially extracted with respective solvents viz., Petroleum ether, Chloroform, Ethyl acetate and Methanol. The extract was stored and used for Phytochemical and GC-MS analysis. Methanol extracts showed the presence of strong phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids and tannins and moderate amount of saponins and steroids. The GC-MS characterization of methanol extracts of leaves of P.murex showed the presence of five major compounds such as Cyclopentaneundeconic acid, methyl ester(1), 15-Octadecadienoic acid,methyl ester (2), 1Cyclobutanedicarboxamide,2-phenyl-N,N'-bis(1-phenylehtyl) (3), Corynan-17-ol,18,19-didedro-10-mehtoxy-,acetae (ester) (4)and 2,3,16,17-Octadecanetetraone tetraoxime (5). This study forms a basis for the biological characterization and importance of the compounds identified.*

Keywords- GC-MS, Phytochemical, Extract, Saponins

I. INTRODUCTION

Medicinal plants grow naturally around us. Over centuries, cultures around the world have learned how to use plants to fight illness and maintain health. These readily available and culturally important traditional medicines form the basis of an accessible and affordable health care regime and are an important source of livelihood for indigenous and rural population (Rajalakshmi *et al.,* 2013). Moreover, many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic deriva-tives of natural products and used in the traditional systems of medicine (Sukanya *et al.*, 2009). In China, India and many other countries people by natural products especially from plant sources, including species designed the basis of stylish traditional medicine practices that have been investigated for their characteristics and health effects used for thousands of years (Sneader, 2005). Plant chemicals are regarded as secondary metabolites because of the plants that manufacture them may have little need for them. They are synthesized in all parts of the plant body bark, leaves, stem, root, flower, fruits and seeds. The any part of the plant body may contain active components (Ugochukwu *et al*., 2013). This chemicals work with nutrients and fibres to form an integrated part of defence system against various diseases and stress conditions (Thilagavathi *et al*., 2015). These chemical substances are called secondary metabolites. The most important of these bioactive groups of plants are alkaloids, terpenoids, tannins, saponins and phenolic compounds (Edeoga *et al*., 2005). Gas Chromotography-Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of phyto compounds (Sampathkumar and Ramakrishnan, 2011; Johnson *et al*., 2011).

Pedalium murex Linn, commonly called Gokhru a member of family Pedaliaceae. It is commonly found in Deccan and in some parts of Ceylon and Gujarat and in the coastal areas of southern India (Nadkarni, 1982). The leaf decoction is used to control white discharge due to excessive body heat. Root decoction is used as an anti bilious agent, while the juice of the fruit is used as an emmenagogue and to promote lochial discharge (Satyavathi *et al*., 1987). The decoction of the seeds and glycosides obtained from it showed mild diuretic activity and the alcoholic extract of the fruits reduced blood pressure in dog and rat (Harvey, 1996). The fruits are rich in flavonoids, saponin soluble proteins (Mukherjee, 2002). An infusion extract prepared using cold water from the leaves, stems and fruits of *Pedalium murex* is demulcent, diuretic and also found to be useful in the treatment of disorders of urinary systems such as gonorrhea, dysuria, incontinence of urine, etc., (Shukla and Khanuja, 2004). *Pedalium murex* is an important medicinal plant that contains several alkaloids like pedalitin, Diosmetin, Dinatin, Pedalin dinatin-7-glucuronide (Subramanian and Nair, 1972).

II. MATERIALS AND METHODS

Pedalium murex L. Leaves were collected from Alapakkam, Cuddalore District, Tamilnadu, India. The fresh leaves were shade dried and ground using mechanical motor in the Research department of Botany, Annamalai University.

The powder materials (100g) were transferred into a soxhlet apparatus containing 200ml of respective solvents (Petroleum ether, Chloroform, Ethyl acetate, Methanol). The extract was concentrated to dryness under vacuum dessicator. The extract was stored and used for Phytochemical and GC-MS analysis.

Phytochemical analysis

The different extracts of *Pedalium murex* was used for qualitative phytochemical studies such as alkaloids, flavonoids, cardiac glycosides, phenolic compounds, terpenoids, steroids, saponins and steroids (Trease and Evans, 1989; Harborne, 1973).

Detection of alkaloids

Mayer's test: A fraction of the different extracts were treated with Mayer's reagent (1.36 g mercuric chloride and 5 g of potassium iodide in 100 mL of distilled water) and observed for the formation of cream colored precipitate.

Detection of flavonoids

Aqueous sodium hydroxide test: A fraction of the different extracts were treated with 1 N aqueous NaOH solution and observed for the formation of yellow-orange coloration.

Detection of cardiac glycosides

Keller-Killani test: 5 mL of each different plant extracts was mixed with 2 mL of glacial acetic acid containing one drops of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 mL concentrated sulphuric acid. The brown ring formed at the junction of two liquids indicated the presence of cardiac glycosides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Detection of phenolic compounds

Ferric chloride test: A fraction of the different extracts were treated with 5 per cent Ferric chloride reagent and observed for the formation of deep blue- black colour.

Detection of saponins

Sodium bicarbonate test: In a test tube, about 5 mL of extracts were taken and a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. The formation of a honey comb like froth showed the presence of saponins.

Detection of steroids

The different extract fractions of each plant (0.5 g) was mixed with 2 mL of acetic anhydride followed by 2 mL of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.

Detection of tannins

Ferric chloride test: 1 drop of Ferric chloride was added to 2 mL of the extracts, the appearance of bluish or greenish black coloration indicate the presence of Pyrogallol or Catechol tannins.

Detection of terpenoids

Salkowski's test: A small amount of samples were dissolved with 2 mL of chloroform in a test tube. Equal volume of concentrated Sulphuric acid was added and the tube was shaken gently. The presence of terpenoids was confirmed by the upper layer of chloroform turning red and lower layer showing yellow green fluorescence.

Gas Chromatography–Mass Spectrometry (GC/MS) analysis:

GC/MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 μMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 μl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0

III. RESULT AND DISCUSSION

Phytochemical screening

In the present study methanolic leaf extract of *P. murex* showed more phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, tannins and steroids when compared to other solvent extracts. Methanolic leaf extracts showed the presence of strong phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids and tannins and moderate amount of saponins and steroids. The ethyl acetate extracts showed strong terpenoids and moderate amount of alkaloids, phenolic compounds, saponins, tannins and steroids. In the chloroform extracts, alkaloids, flavonoids, cardiac glycosides and saponins were present moderately and fewer amounts of steroids were present. In petroleum ether extract, alkaloids, flavonoids, terpenoids, and steroids were present (Table -1)
Phytochemical constituents such as tannins, flavonoids and several other aromatic compounds secondary metabolites of plants serve as defence mechanism against several micro organisms. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins and steroids (Britto and Sebastian, 2012). The presence of saponins, flavonoids ,phenols and terpenoids in the leaf extract are very important and are used in analgesic, anti plasmodic and bactericidal activities (Stary, 1998). Thus the preliminary sreening test may be used in detection of the bioactive principles and subsequently may lead to the drug discovery and development.

GC-MS Analysis

The compound present in the methanolic leaf extract of *P.murex* were identified by GC-MS analysis and presented in table-2.Totally, five major compounds were identified, such as Cyclopentaneundeconic acid, methyl ester(1), 15-Octadecadienoic acid ,methyl ester (2), 1-Cyclobutanedicarboxamide,2-phenyl-N,N'-bis(1-phenylehtyl)-(3),Corynan-17-ol,18,19-didedro-10 mehtoxy-,acetae (ester) (4) and 2,3,16,17-Octadecanetetraone tetraoxime (5) along with other minor constituents.

Similar results were also observed in previous studies. Among the identified phytochemicals of *Pedalium murex* alcoholic extract, oleic acid constituted the major part and propanoic acid, 1-methyl propyl ester was in the least part (Anandanayaki and Uma, 2014).There are 23 compounds identified and only five compounds were selected based on high peek in GC-MS. Among these, two compounds octadecenoic acid and 9, 12 octadecadenoicacid have the antiviral property (Muthu and Nirmala, 2016). The presence of above mentioned bioactive secondary metabolites of *Pedalium murex* reveals the medicinal value of the plant and its significance in the treatment of various diseases. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

IV. CONCLUSION

Pedalium murex is a valuable source of medicinally useful compounds that have been used traditionally for various ailments. Leaves extracts of this plant showed good source for inorganic solvents exhibited the presence of many bioactive compounds whose presence were proved that they could be used for making antimicrobial drugs.

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Table 1. Preliminary phytochemical analysis of leaf extract of *Pedalium murex L.*

I. S. No.	II. Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	+	++	++	+++
2	Flavonoids	+	++	+	+++
3	Cardiac glycosides	-	++	-	-
4	Phenolic compounds	-	-	++	+++
5	Terpenoids	+	-	+++	+++
6	Saponins	-	++	++	++
7	Tannins	-	-	++	+++
8	Steroids	+	+	++	++

(+++) =Strong**;(**++) = moderately present; **(+)** = Positive ;**(-)** =Negative.

Table 2. GC-MS of methanolic leaf extract of *Pedalium murex*

S. No.	I. RT	Name of the compounds	Molecular formula	Molecular weight	Peak area (%)
1	16.17	Cyclopentaneundeconic acid, methyl ester	$C_{17}H_{32}O_2$	268	5%
2	17.85	9,15-Octadecadienoic acid ,methyl ester	$C_{19}H_{34}O_2$	294	13%
3	19.34	1,1-Cyclobutanedicarboxamide,2-phenyl-N,N'-bis(1-phenylehyl)-	$C_{28}H_{30}N_2O_2$	426	19%
4	20.62	Corynan-17-ol,18,19-didedro-10-mehtoxy-,acetae (ester)	$C_{22}H_{28}N_2O_3$	368	23%
5	22.18	2,3,16,17-Octadecanetetraone tetraoxime	$C_{18}H_{34}N_4O_4$	370	32%