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Phytochemical Evaluation, FT-IR and GC-MS Analysis of Leaf Extracts of *Pergularia daemia*

M. Maheshwari† and P. Vijayarengan
Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India
†Corresponding author: M. Maheshwari; maheswarim1994m@gmail.com

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ABSTRACT

Pergularia daemia is traditionally used to treat various ailments like anthelmintic, antipyretic and expectorant and to treat infantile diarrhoea, malarial intermittent fever, asthma, mental disorder, toothache and cold. In the present study leaf extracts of *P. daemia* was subjected to qualitative phytochemicals, GC-MS and FT-IR analysis. The quantitative analysis of the leaves showed the presence of flavonoids, steroids, alkaloids, terpenoids, saponins, phenols, carbohydrates, amino acids, tannins and cardiac glycosides. The GC-MS study of methanol extract revealed 16 compounds. Some major compounds identified are 9-Octadecenoic Acid (E), Cis- Vaccenic Acid, N-Hexadecanoic Acid, 1- Dimethyl (Butyl), Silyl Oxy Butane along with other minor constituents. FT-IR analysis revealed the presence of 12 functional groups such as amines, alkanes, carbon dioxide and alkynes. The results suggested that *P. daemia* contains significant photo components and can be used as a source for many pharmacological studies and a curative for various ailments.

INTRODUCTION

All the natural products are a source of many traditional medicines and even some synthetic herbal medicines. In some parts of the world, herbal medicines are generally used to treat various diseases. In India, the medical systems using medicinal plants are Ayurveda, Siddha, Homeopathy, etc. to treat various ailments (Pushpangadan & Atal 1984). According to the World Health Organization (WHO), more than 80% of the world's population in poor and underdeveloped countries depends on traditional plant-based medicines for their primary healthcare needs (WHO 1993). The search for an alternative system of medicine having potential anti-inflammatory, antibiotic and other activities has gained importance considering the harmful side effects of modern synthetic medicines (Akharayi et al. 2012). India is very much rich with many species of plants having medicinal value. These plants are broadly used by the society as herbal medicines or as pharmaceutical preparation of modern medicine. Since herbal medicines are prepared from materials of plant origin, they are prone to contamination, deterioration and variation in composition.

Pergularia daemia Forsk is a perennial twinning plant belonging to the family Asclepiadaceae. This plant is widely distributed in the roadside of India and also in the tropical and subtropical regions. The entire plant possesses high medicinal value and traditionally used in treating various ailments for human beings. Some people used this plant to treat jaundice. This plant is used as laxative, anthelmintic, antipyretic, expectorant and also in infantile diarrhoea. The extract of the leaves of *P. daemia* is applied to treat rheumatic swelling and also used in the preparation of purgative medicinal oil given for amenorrhoea, rheumatism and dysmenorrhoea. The bark of the root is also used as a purgative in rheumatic cases (Wealth of India 1966). The root of this plant is used to treat anaemia, mental disorders, leprosy, piles, uterine and menstrual disorders (Yoganarasimhan 2000). The whole plant is employed for pulmonary afflictions, biliousness, asthma, piles, cough, leprosy and syphilis, and leaves are used in infantile diarrhoea, and as an expectorant, uterine tonic and emetic (Mohammed et al. 2004). Therefore, the present study aimed to document the phytochemicals using GC-MS and FT-IR in the leaf extract of *P. daemia* to explore its chemical resource.

MATERIALS AND METHODS

Collection of Plant Materials

Pergularia daemia leaves were collected from Mayiladuthurai, Tamil Nadu during March 2018.

Preparation of the Extracts

The collected leaves were washed thoroughly in tap water and rinsed with distilled water to remove dust particles. Then it was surface sterilized with 10% sodium hypochlorite solution

and rinsed with sterile distilled water. After that, it was shade-dried at room temperature for 15 days and then the leaves were packed in brown cover and kept in an oven at 60°C for an hour to make grinding easy. After an hour, the leaves were ground using an electrical blender. The powdered plant materials were then packed in a ziplock pouch. One hundred gram of powder was extracted with different organic solvents like hexane, acetone, ethyl acetate and methanol for 8 hours using the Soxhlet apparatus and solvent was evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried powder was stored at 4°C for further use.

Phytochemical Analysis

The different extracts of *P. daemia* were used for qualitative phytochemical analysis for alkaloids, flavonoids, terpenoids, steroids, phenols, saponins, cardiac glycosides, tannin, carbohydrates and amino acids (Trease & Evans 1989, Harborne 1973).

Test for flavonoids: To 2 mL of crude extract taken in a test tube, 3-4 drops of 1% sodium hydroxide solution was added. Development of intense yellow colour, which becomes colourless on the addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for steroids: Two mL of extract was dissolved in 2 mL of chloroform and filtered. The filtrate was treated with 2 mL of concentrated sulphuric acid, shaken and allowed to stand. Development of a golden yellow colour indicates the presence of steroids.

Test for alkaloids: Two mL of extract was stirred with 2 mL of 2N hydrochloric acid and Mayer's reagent (1.36 g mercuric chloride and 5 g of potassium iodide) and 100 mL of distilled water was added to it. Development of yellow coloured precipitate indicates the presence of alkaloids.

Test for terpenoids: Two mL of extract was dissolved in 2 mL of distilled water and treated with a few drops of copper acetate solution. Development of green colour indicates the presence of terpenoids.

Test for saponins: Two mL of extract was diluted with distilled water and made up to 20 mL. This was shaken in a graduated cylinder for 20 minutes. Development of 1 cm thick layer of foam indicates the presence of saponins.

Test for phenols: Two mL of extract was treated with a few drops of ferric chloride solution. Development of green colour indicates the presence of phenols.

Test for carbohydrates: Two mL of extract was dissolved in 5 mL of distilled water and filtered. Filtrate was treated with a few drops of alcoholic α-naphthol solution in a test tube. Development of the violet ring at the junction indicates the presence of carbohydrates.

Test for amino acids: 2 mL of extract was treated with a few drops of concentrated nitric acid. The colour change from green to yellow shows the presence of amino acids.

Test for cardiac glycosides: Extract was hydrolysed with dilute HCl and then subjected to test for glycosides. 2 mL of the extract was treated with ferric chloride solution and immersed in a water bath for 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The layer of benzene was separated and treated with ammonia solution. The colour change from green to pink colour in the ammonia layer indicates the presence of cardiac glycosides.

Test for tannins: Two mL of crude extract in 1% gelatin solution containing sodium chloride was added. Development of white colour precipitate indicates the presence of tannins.

Fourier Transform Infra-Red Spectra

IR spectrum was recorded in a spectrophotometer (Thermo Scientific NICOLED-iS5). The active principle was mixed with KBr and pellet technique was adopted to record the spectra.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatography was fitted with DB 5 MS capillary column (30 m × 0.25 mm i.d., film thickness μM). The injection temperature was set at 280°C, and the oven temperature was initially at 45°C then programmed to 300°C at the rate of 10°C/min and finally held at 200°C for 5 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. One microliter of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of the composition of the compound was calculated by the GC peak areas. GC-Mass spectrometry (GC-MS) analysis of compounds was performed using Varian 3800 gas chromatography equipped with Varian 1200 L single quadrupole Mass spectrometer. GC conditions were the same as reported for GC analysis and the same column 1000 amu. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data (Adams 2009).

RESULTS

The preliminary phytochemical screening of the leaf extracts of *P. daemia* shows to contain flavonoids, steroids, alkaloids, terpenoids, saponins, phenols, carbohydrates, amino acids,

tannin and cardiac glycosides in all the extracts (Table 1). The methanol extracts yielded a very high number of secondary metabolites when compared to other extracts. The phytochemicals identified in different extracts were in the order methanol (7 compounds), acetone (5 compounds), ethyl acetate (4 compounds) and hexane (3 compounds). The principal functional groups present in the methanolic leaf extract of *P. daemia* were identified by FT-IR analysis and presented in Table 2. Absorbance and functional groups are interpreted as follows, 3385.04 cm^{-1} indicate N-H stretching, 2922.55, 2854.24 cm^{-1} indicate C-H stretching; 2318.57 cm^{-1} indicate the O=C=O stretching; 2242.64 cm^{-1} indicate C≡C stretching; 1711.78 cm^{-1} indicate C=O stretching; 1631.88 cm^{-1} indicate C=C stretching; 1453.19 cm^{-1} indicate C-H bending; 1370.51 cm^{-1} indicate O-H bending; 1241.95, 1168.56 cm^{-1} indicate C-O stretching and 1030.27 cm^{-1} indicate S=O stretching. Some major compounds were amine, alkane, carbon dioxide, alcohol and alkyl aryl ether. The FT-IR spectrum of *P. daemia* is shown in Fig. 1.

The GC-MS analysis of methanol extracts of leaves of *P. daemia* was analysed and represented in Table 3. From the GC-MS study, a total of 16 major compounds were identified. Mass spectrum of the bioactive compounds with their retention time (RT) is shown in the (Fig. 2). Some major compounds were 9-octadecenoic acid (E), cis-vaccenic acid, n-hexadecanoic acid, 1-dimethyl (butyl), silyl oxy butane, etc. The name, molecular weight, molecular formula and chemical structure of the compounds were noted.

DISCUSSION

The medical system deeply depends upon medicinal plants and their products for the production of drugs. The world requires a new source of crude drugs because due to over-exploitation and climatic changes, the currently used source

Table 1: Preliminary phytochemical analysis of leaf extracts of *Pergularia daemia.*

Phytochemical compounds	Hexane	Acetone	Ethyl acetate	Methanol
Flavonoids	+	++	-	+++
Steroids	-	-	+	-
Alkaloids	+	+	-	+
Terpenoids	-	+	-	++
Saponins	-	-	-	+
Phenols	-	-	+	-
Carbohydrates	+	++	-	+++
Amino acids	-	+	-	-
Tannin	-	-	+	++
Cardiac Glycosides	-	-	+	+

(+++) = More strong, (++) = Strong, (+) =Positive (Present), (-) = Negative (Absent)

Table 2: FT-IR absorption and functional group of leaves of *Pergularia daemia*.

Sl.No.	Wave Number	Molecular motion	Functional group	Absorption intensity
1	3385.042	N-H Stretching	Amine	Strong
2	2922.553	C-H Stretching	Alkane	Medium
3	2854.249	C-H Stretching	Alkane	Medium
4	2318.570	O=C=O Stretching	Carbon Dioxide	Strong
5	2242.643	C≡C Stretching	Alkyne	Weak
6	1711.785	C=O Stretching	Carboxylic Acid	Strong
7	1631.884	C=C Stretching	Alkene	Medium
8	1453.198	C-H Bending	Alkane	Medium
9	1370.512	O-H Bending	Alcohol	Medium
10	1241.957	C-O Stretching	Alkyl Aryl Ether	Strong
11	1168.569	C-O Stretching	Ester	Strong
12	1030.271	S=O Stretching	Sulfoxide	Strong

Table 3: GC-MS analysis of methanolic leaf extract of *Pergularia daemia*.

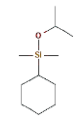
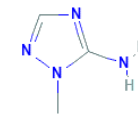
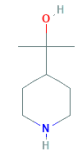
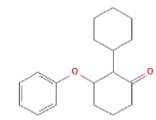
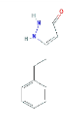
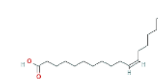
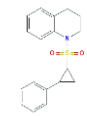
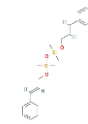
Peak No.	Rt	Chemical compound	Molecular formula	Molecular weight (g/mol)	Peak area %	Chemical structure
1	2.134	Tricyclon [4.2.1.1(2,5)] Decan-9-One Oxime	$C_{10}H_{15}NO$	165.23	3.53	
2	3.983	Cyclohexyldimethyliso-propoxysilane	$C_{11}H_{24}OSi$	200.39	1.11	
3	4.470	1H-1,2,4-Triazol-5-Amine, 1-Methyl-	$C_3H_6N_4$	98.11	3.99	
4	5.491	4-Pyridinemethanol, Hexahydro-. Alpha.,. Alpha. -Dimethyl-	$C_8H_{17}NO$	143.23	1.53	
5	5.595	[1,1'-Bicyclohexy-l]-2-One	$C_{12}H_{20}O$	180.29	0.90	
6	6.077	N-Propyl Nonyl Ether	$C_{12}H_{26}O$	186.33	1.28	
7	9.812	5-Phenethyl-2H-Pyra-zol-3-ol	$C_{11}H_{12}N_2O$	188.23	3.67	
8	9.916	5-Caranol, Trans, Trans-(+)-	$C_{10}H_{18}O$	154.24	1.23	
9	10.285	N-HexadecenoicAcid	$C_{16}H_{32}O_2$	256.42	3.18	
10	11.741	N-HexadecanoicAcid	$C_{16}H_{32}O_2$	256.42	16.90	
11	12.034	HexadecanoicAcid, Ethyl Ester	$C_{18}H_{36}O_2$	284.5	1.69	
12	13.065	9-Octadecenoic Acid, (E)-	$C_{18}H_{34}O_2$	282.46	27.80	
13	13.433	Cis-Vaccenic Acid	$C_{18}H_{34}O_2$	282.5	21.52	

Table Cont....

Peak No.	Rt	Chemical compound	Molecular formula	Molecular weight (g/mol)	Peak area %	Chemical structure
14	16.393	Quinoline, 1,2,3,4-Tetrahydro-1-((2-Phenylcyclopropyl) Sulfonyl)-, Trans-	$C_{10}H_{19}No_2S$	147.21	3.49	
15	17.660	1-Dimethyl(Butyl) Silyloxybutane	$C_{10}H_{24}OSi$	188.38	4.61	
16	17.792	Silane, Dimethyl(Dimethyl(3-Phenyl-pro-2-Enyloxy) Silyloxy) (3-Phenyl-pro-2-Enyloxy)-	$C_{22}H_{30}O_3Si_2$	398.6	3.58	

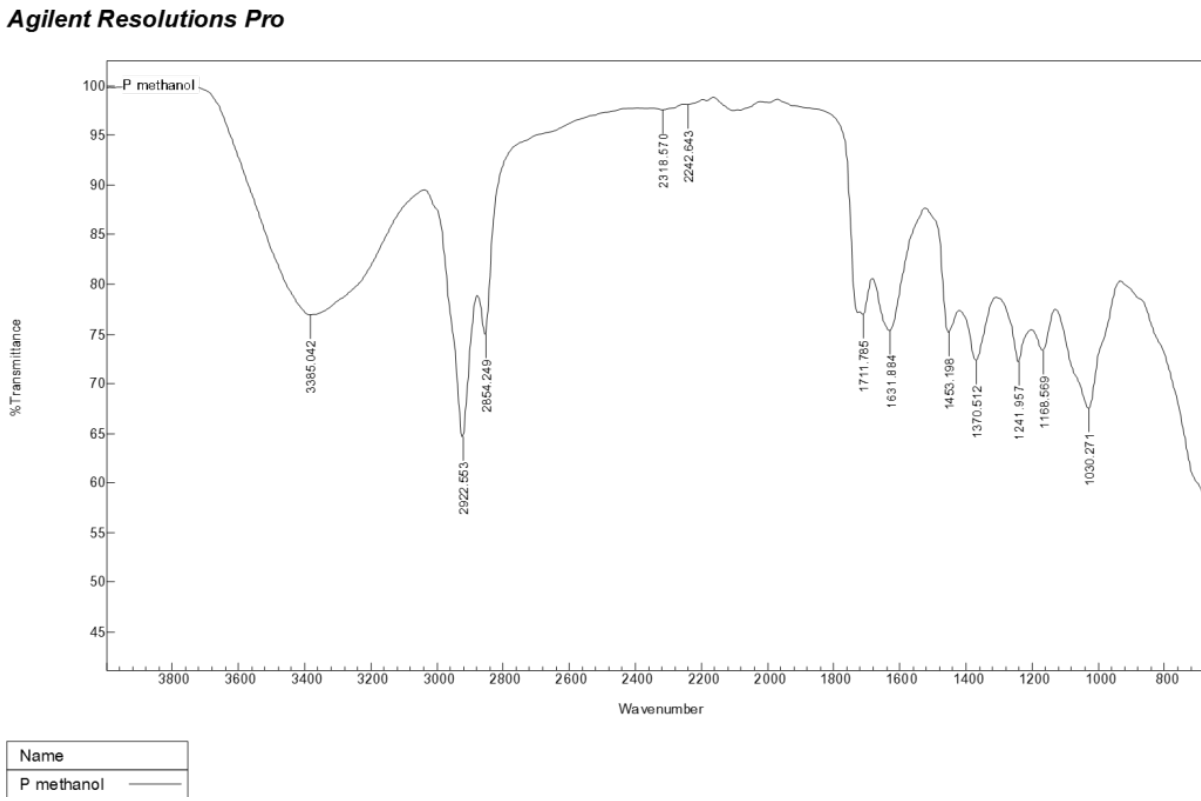


Fig. 1: FT-IR absorption and functional group of leaves of *Pergularia daemia.*

plants become threatened. Phytochemicals such as alkaloids have hypoglycaemic activities (Cherian & Augusti 1995a). The root of *P. daemia* has a high amount of tannins and plays a major role in the treatment of intestinal disorders like diarrhoea and dysentery (Akinpelu & Onakoya 2006). *P. daemia* contains alkaloids, tannins, flavonoids, cardiac glycoside and terpenes. Phytol is one among sixteen compounds of the present study. Phytol is important acyclic diterpene alcohol that is a precursor for vitamins E and K. Similarly, the presence of phytol was observed in the leaves of *Lantana camara* (Mittal et al. 1962) and *Mimosa pudica* (Sridharan et al. 2011). The results of the present study by GC-MS confirmed the presence of bioactive compounds which may be responsible for their medicinal values and physiological activities (Ismaila et al. 2011). The ethanolic extracts of *P. daemia* possess significant hepatoprotective effect in carbon tetrachloride (CCl_4) model of intoxication in rats (Sureshkumar & Mishra 2006). Saponins, terpenoids, flavonoids

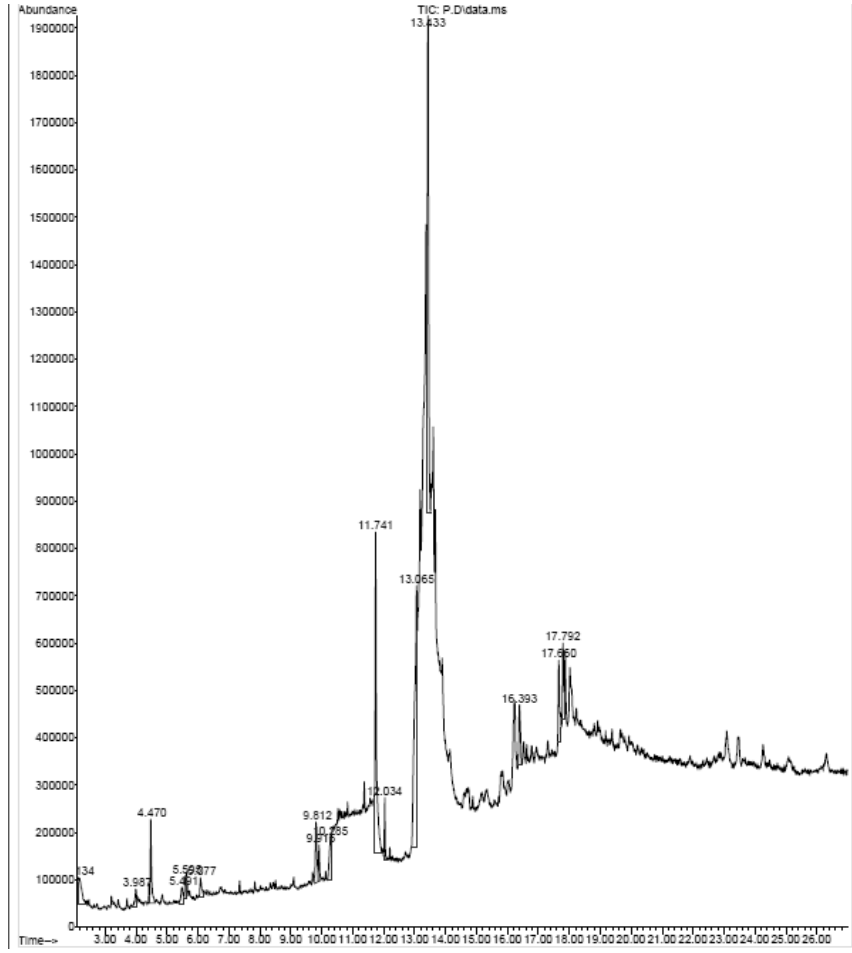


Fig. 2: GC-MS Analysis of Methanolic extract of leaves of *Percularia daemia.*

and alkaloids have anti-inflammatory properties whereas flavonoids, tannin and alkaloids show hypoglycaemic activities (Cherian & Augusti 1995b). Terpenoids are used to strengthen skin, increase the concentration of antioxidants in wounds and restore inflamed tissues by increasing blood supply. Alcohol extract of the leaves of *Kigelia pinnata* has earlier been reported for the presence of hexadecanoic acid compound (Grace et al. 2002) and it is also reported in *Melissa officinalis* (Kumar & Manimegalai 2008, Sharafzadeh et al. 2001). These reports are in accordance with the result of this study. Further investigation is needed to identify the pharmacological importance and phytochemistry of the leaves of *Pergularia daemia*.

CONCLUSION

This study documents the phytochemical constituents of leaf extract of *P. daemia* through quantitative analysis, GC-MS, and FTIR. GC-MS analysis revealed the presence of 16 compounds and FT-IR with 12 major functional groups. Further, this study suggests *P. daemia* can be considered as a potential candidate for extraction pharmacologically active compounds.

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