

Phytochemical Analysis of *Pergularia Daemia* for Its Bioactive Components through Gas Chromatographic Mass Spectrometry (GCMS)

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ABSTRACT: *Pergularia daemia* is a hispid perennial herb that grows along the roadsides of India and other tropical and subtropical regions. This medicinal plant is traditionally used to treat various ailments like infantile diarrhea, malarial fever, vatha, convulsion, asthma, poisoning, mental disorder, anemia, leprosy, piles etc. However, the bioactive components of this plant are not yet established fully. In the present study, the ethanol extract of this plant was subjected for phytochemical qualitative analysis through Gas Chromatographic Mass Spectrometry (GCMS) and the bioactive components were identified. The analysis revealed the presence of flavonoid, tannins, alkaloid, glycosides, terpenoids, steroids and carbohydrates. Five bioactive constituents were identified namely 2-hydroxy- methyl ester with retention time 6.607, 2-Methoxy-4-vinylphenol with retention time 8.215, Phthalic acid di-(1-hexen-5-yl) ester with retention time 12.279, l-(+)-Ascorbic acid 2,6-dihexadecanoate with retention time 16.751 and Methyl (Z)-5,11,14,17-eicosatetraenoate with retention time 18.556. The presence of these bioactive constituents in the plant extract may authenticate the scientific evidences for various pharmacological activities and therapeutic potentiality of the plant.

KEY WORDS: *Pergularia Daemia*, Herbal Medicine, Holistic Treatment, Bioactive Components, Gas Chromatographic Mass Spectrometry

I. INTRODUCTION

Healing through herbal medicine is the most ancient form of treatment known to mankind. The age-old holistic medical systems such as Ayurveda, siddha, homoeopathy etc., are in practice of using the herbal plant drugs or formulations to treat various ailments. According to World Health Organization (WHO), more than 80% of the world's population in poor and underdeveloped countries depend on traditional plant-based medicines for their primary healthcare needs [1]. Its efficacy, safety, almost- nil adverse effects and lesser cost-factor have attracted the attention of developed and economically sound countries also. For the same reasons, even the leading pharmaceutical companies started entering into the field of medicinal plant research. *Pergularia daemia* (*P daemia*) is one such plant, popularly known as “Veliparuthi” in Tamil and “Utranajutuka” in Hindi. It is a hispid perennial herb that grows along the roadsides of India and other tropical and subtropical regions. Earlier reports indicate various beneficial uses of the herb, validating it as a medicinal herb. Traditionally the whole plant is used as anthelmintic, antipyretic, laxative and expectorant and to treat infantile diarrhea and malarial fever. The root of the plant is effective in treating convulsions, asthma, poisoning, mental disorder, anemia, leprosy and piles (2). Dried leaf of this plant is used as an emetic agent and is effective in treating bronchitis [3], asthma [4], rheumatic fever [5], amenorrhea, dysmenorrhea [6, 7] and wounds [8] and to facilitate parturition. Fresh roots and shoots are found to be useful in treating whooping cough [9]. The shoots of the plant are considered to be an effective agent for abortion [10]. Stem bark is used in treating malaria [11] and twig is effective as an antipyretic agent and serves as a good appetizer [12]. All these therapeutic potentiality of *Pdaemia* may be attributed to the presence of its phytochemical constituents which is not yet explored thoroughly. In the present study the ethanol extract of the aerial parts of the plant was subjected for phytochemical analysis followed by quantitative determination of organic compounds by Gas Chromatogram mass spectrometric method (GCMS).

Perusal of literature reveals the presence of certain bioactive substances in *P daemia* such as glucoside, terpenoids, sterols, α -amyirin, α -amyirin acetate, β -amyirin, β -sitosterol, lupeol acetate, hentriacontane, betaine and different polypeptides (13). Fresh shoots and flowers were shown to contain hyperoside (flavonol), flavonoids and saponins (14). Inorganic substances like potassium chloride (KCl) and potassium nitrate (KNO₃) were found in the whole plant (14). Recent studies have shown the presence of a bitter resin, two bitter principles and a glucoside. Two new sterols namely 5 β -stigmast-7-(8)-en-3-3a-ol and 5 β -stigmast-8(14)-en-3a-ol were also been isolated from the plant. Numerous cardenolides were also found in the stalks. Rastogi RP and Mehrotra BN found alkaloid, daemine, uzarogenin, and coroglucigenin in the leaves and roots and calotropin, calactin, corotoxigenin, calotoxin, calotropagenin, uscharin, dihydrocalotropagenin, uacharidin and protouscharin, in the seeds (15). In spite of the identification of these components in *P daemia*, the therapeutic uses of various parts of this plant raised the curiosity regarding the existence of a lacuna in the area of the presence of some more bioactive substances in this plant. Hence this venture is taken up.

II. MATERIALS AND METHODS

2. 1. Collection of the plant:

The whole plant was collected from Karuppur, Salem district, Tamil Nadu in India, and the voucher specimen was deposited in the Herbarium and authenticated in CAS Botany Department, University of Madras (Authentication No. MUCASB-H106)

2. 2. Preparation of extract:

The aerial part consisting of the leaves, stem and flowers was separated from the whole plant and was washed in water to remove the dust and the sand. Then it was dried in shade at room temperature and was powdered in an electric blender. This powder was subsequently sieved to obtain a fine powder. Then, the ethanol extract of the fine powder was prepared by treating it successively with 95% ethanol in a soxhlet apparatus by continuous heat exposure for 72 hours. The extract was concentrated in rotary vacuum evaporator at 40°C and reconstituted in minimum amount of dimethyl sulphoxide (DMSO) and dried extract was stored in refrigerator at 4°C for their future use in phytochemical analysis.

2. 3. Phytochemical analysis

Ethanol extract of the plant was tested for the presence of various bioactive compounds.

Test for flavonoid: To 1 ml of the extract, few drops of 1% NH₃ was added in a test tube. An intense yellow colour was produced indicating the presence of flavonoid compounds.

Test for tannins: To 1 ml of extract, 2 ml of 5% FeCl₃ was added. Formation of blue black precipitate indicated the presence of tannins.

Test for alkaloids: 1 ml of the extract was stirred with 2 ml of 2N hydrochloric acid and Mayer's reagent (Potassium mercuric iodide solution) was added to it. A white turbid precipitate was formed showing the presence of alkaloids.

Test for glycosides: To 1 ml of extract, 1 ml concentrated sulphuric acid (H₂SO₄) was added. After hydrolysis of test solution, Fehling's solution was added. A black red precipitate was formed indicating the presence of glycosides.

Test for terpenoids: 2 ml of extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated H₂SO₄ was carefully added to the mixture to form a layer. An interface with reddish brown coloration was formed indicating the presence of terpenoids.

Test for steroids: 2 ml of extract was dissolved in 2 ml of chloroform and 2 ml of concentrated H₂SO₄ was added along the sides of the test tube. The upper layer turned red and H₂SO₄ layer showed yellow colour with green fluorescence indicating the presence of steroids.

Test for carbohydrates: To 1 ml of extract, 5 ml of Benedict's reagent was added and boiled for 5 minutes. Formation of bluish green colour showed the presence of carbohydrates. When this was boiled for few minute more, a reddish pink colour was formed indicating the presence of flavonoid.

Table 1. Phytochemical components in the ethanol extract of aerial parts of *P daemia*

S No	Phytochemical Components	Ethanol extract of <i>P daemia</i>
1	Flavonoid	Present
2	Tannins	Present
3	Alkaloid	Present
4	Glycosides	Present
5	Terpenoids	Present
6.	Steroids	Present
7.	Carbohydrates	Present

Gas Chromatography Mass Spectrometry (GCMS) analysis

GCMS analysis is a common confirmation test. It separates all the components in a sample and provides a representative spectral output. The sample is injected into the injection port of the Gas Chromatography (GC) device. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The peak is measured from the baseline to the tip of the peak.

Identification of Bioactive Constituents by GCMS

GC analysis of the ethanol extract of aerial parts of the plant was performed using a Shimadzu GC 17A – QP5050A gas chromatograph. The oven temperature was programmed to be 290.00°C at a rate of 10°C/min; the carrier gas was helium with a flow rate of 1ml/min. The sample was injected using the split sampling technique in the ratio of 1:10. Retention indices (RI) of the compounds were determined by comparing to the retention times of a series and identification of each component was confirmed by comparison of its retention index either with those of authentic compounds or with data in the literature. Interpretation of Mass-Spectrum was carried out by using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular formula, molecular weight and chemical structure of the components of the test materials were ascertained. The peak in GCMS of ethanol extract of *P daemia* showed the presence of the secondary phytochemical compounds like phenolic and fatty acids and its esters. (Fig.1, Fig 2, Fig 3, Fig 4, Fig 5, Fig 6)

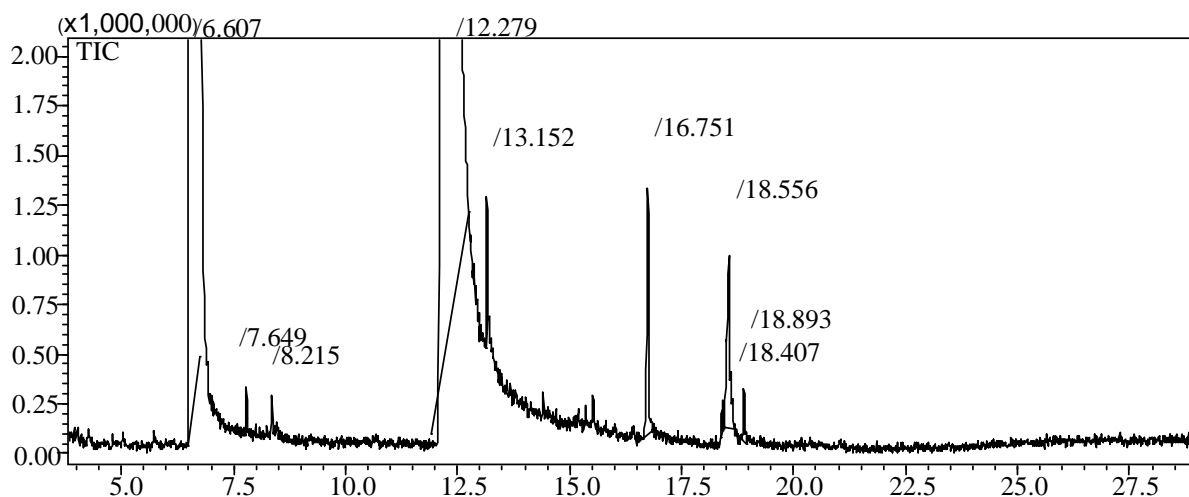
III. RESULTS AND DISCUSSION

The phytochemical characteristics of the *P daemia* extract are summarized in Table 1. The ethanol extract from the aerial parts of this plant contains flavonoid, tannins, alkaloids, glycosides, terbenoids, steroids and carbohydrates. By GCMS method, benzoic acid compound, phenolic compound, organic acid ester, fatty acid and fatty acid ester were isolated by their retention time (Table 2, Fig 1). Mass spectrum of the bioactive compounds with their retention time (RT) is shown in the Figures 2.3.4.5 and 6. GCMS is one of the hyphenated analytical techniques that help to do qualitative and quantitative evaluation of the chemical components in a sample. It involves two different techniques in a single method. It works on the principle of gas chromatography (GS) and mass spectrometry (MS). By GS, the components of a mixture are separated depending on their volatility (ease with which they evaporate into gas) and by MS individual characterization of the components are done depending on their structure. . GCMS is also useful in many fields like detection of

Table 1. Bioactive compounds in *P daemia* identified by Gas Chromatography Mass Spectrometry (GCMS)

S no	RT (min)	Name of the compound	Compound nature	Molecular formula	Molecular weight	Therapeutic use
1	6.607	Benzoic acid, 2-hydroxy-, methyl ester	Benzoic acid compound	C8H8O3	152	Antimicrobial
2	8.215	2-Methoxy-4-vinylphenol	Phenolic compound	C9H10O2	150	Anti-microbial, Analgesic Anti-oxidant, Anti-inflammatory
3	12.30	Phthalic acid, di-(1-hexen-5-yl) ester	Organic acid ester	C20H26O4	330	Antifungal.
4	16.751	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Fatty acid	C38H68O8	652	Anti-oxidant, anti-inflammatory, anti- nociceptive, anti-bacterial, enhances sperm motility
5	18.556	Methyl (Z)-5,11,14,17-eicosatetraenoate	Fatty acid ester	C21H34O2	318	Anti-bacterial, to treat dysentery and diarrhea

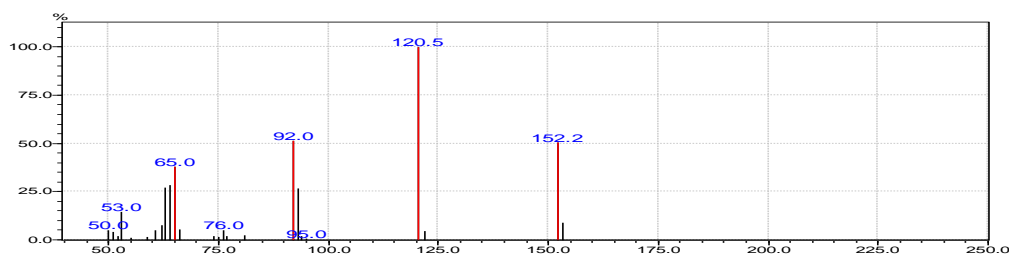
Fig.1. Identification of Bioactive Constituents by GCMS



environmental pollution, in criminal forensics, in law enforcement, in sports for detecting doping, in explosive detection, food analysis, astrochemistry and in medical field [16, 17]

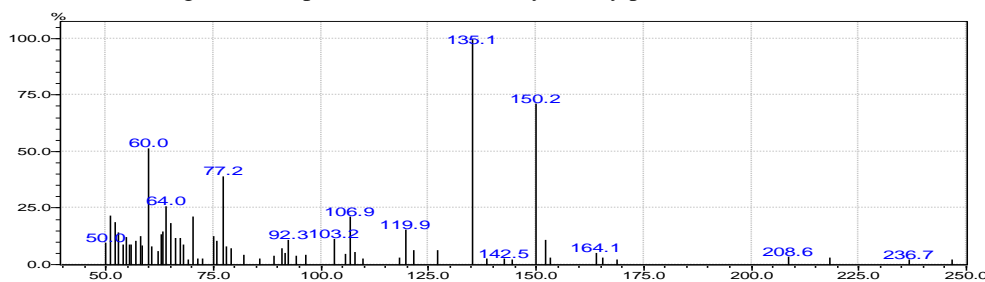
The results of the present study by GCMS confirm the presence of bioactive components which may be responsible for their medicinal values and physiological activities [18]. The phytochemicals like tannins, alkaloid, terpenoids, steroids and saponins were already shown to be therapeutically effective. Mukeshwar et al stated that alkaloids in plants are used as anesthetic agents in medicine [19]. Saponins in some Chinese and Japanese medical herbs were reported to be effective as tonic and stimulating agents [20]. The presence of some of these compounds has also been confirmed to have antimicrobial activity [21].

Fig 2. Mass spectrum of Benzoic acid, 2-hydroxy-, ethyl ester (RT:6.607)



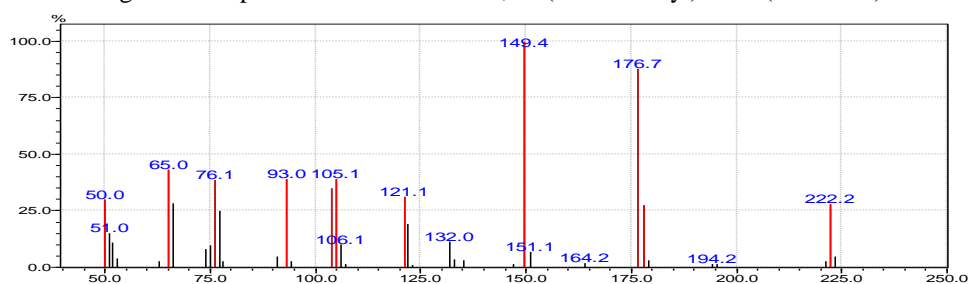
Hit#: 1 Entry:22339 Library:NIST05.LIB
 SI:78 Formula:C₉H₁₀O₃ CAS:118-61-6 MolWeight:166 RetIndex:1380
 Compound name: Benzoic acid, 2-hydroxy-, ethyl ester \$\$ Salicylic acid, ethyl ester \$\$ Ethyl o-hydroxybenzoate \$\$ Ethyl salicylate \$\$

Fig 3. Mass spectrum of 2-Methoxy-4-vinylphenol (RT:8.215)



Hit#: 1 Entry:14775 Library:NIST05.LIB
 SI:74 Formula:C₉H₁₀O₂ CAS:7786-61-0 MolWeight:150 RetIndex:1293
 Compound Name:2-Methoxy-4-vinylphenol \$\$ Phenol, 4-ethenyl-2-methoxy- \$\$ Phenol, 2-methoxy-4-vinyl-phenol.

Fig 4. Mass spectrum of Phthalic acid, di-(1-hexen-5-yl) ester (RT:12.30)



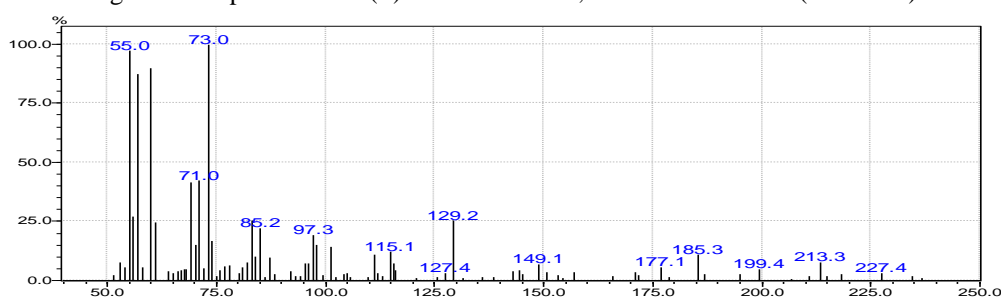
Hit#: 1 Entry: 117551 Library:

SI: ? Formula: C₂₀H₂₆O₄ CAS: 0-0-0 MolWeight: 330 RetIndex: 2286

Compound Name: Phthalic acid, di-(1-hexen-5-yl) ester

Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Fig 5. Mass spectrum of l-(+)-Ascorbic acid 2,6-dihexadecanoate (RT:16.75)

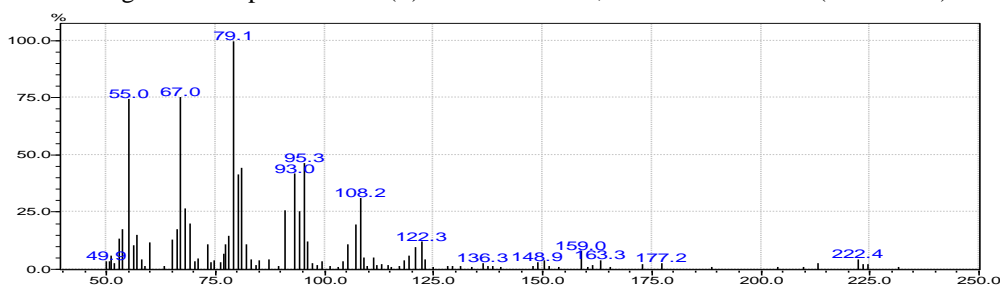


Hit#: 1 Entry: 161860 Library: NIST05.LIB

SI: 82 Formula: C₃₈H₆₈O₈ CAS: 28474-90-0 MolWeight: 652 RetIndex: 4765

Compound Name: l-(+)-Ascorbic acid 2,6-dihexadecanoate

Fig 6. Mass spectrum of l-(+)-Ascorbic acid 2,6-dihexadecanoate (RT:18.556)



Hit#: 1 Entry: 111404 Library: NIST05.LIB

SI: 81 Formula: C₂₁H₃₄O₂ CAS: 59149-1-8 MolWeight: 318 RetIndex: 2308

Compound Name: Methyl (Z)-5,11,14,17-eicosatetraenoate \$\$ Methyl (5Z,11Z,14Z,17Z)-5,11,14,17-icosatetraenoate # \$\$

IV. CONCLUSION

GCMS analysis of the ethanol extract of *P daemia* reveals the presence of medicinally valued bioactive components like tannins, saponin, terpenoid, alkaloids, flavonoids and steroids. As the medicinal value of similar components in other plant extracts are already proved, no wonder if these components in *P daemia* may also have equally effective, if not more, therapeutic values. The work is in progress to ascertain its biological activity and brighten the pharmacological profile of it in the arena of traditional medicine.

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