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Phytochemical Screening and GC-MS Analysis of Leaf Extract of *Pergularia daemia* (Forssk) Chiov

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ABSTRACT

This study was implement to actuate the chemical components of Pergularia daemia leaves using Perkin-Elmer Gas Chromatography–Mass Spectrometry, our results of GCMS compounds in the extract was relevant to the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanolic extract of Pergularia daemia leaves confess the presence Hexadecanoic acid, methyl ester (33.42), Pentadecanoic acid, 14-methyl-methyl ester (36.23, Ethyl 9,12,15-octadecatrienoate (33.12 and 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole (31.24). Qualitative phytochemical screening of the ethanolic extracts of the leaves revealed the presence of many compounds such as flavonoids, tannins, alkaloids, terpenoids, steroids and phenols. This study result will make a way for the production of herbal medicines for various ailments by using Pergularia daemia leaves..

Keywords: Bioactive compounds, Phyto drugs, Herbal medicine

INTRODUCTION

In olden days itself the importance of medicinal plants have been discovered. At that time there was no synthetic medicines, they have been using only the herbal medicines to treat all diseases. From this we can understand that plants are rich in medicinal properties and they are very useful in human health and wellbeing. Biological studies are essential to find more medicinal properties of the plants [1]. But still the many medicinal plants and their medicinal properties are unexplored. Treating of diseases through natural medicine is the most ancient treatment known to mankind [2,3]. In India the medical systems using medicinal plants are Ayurveda, Siddha, Homeopathy, etc., to treat various ailments [4]. Traditional plant based medicines for primary healthcare need is followed in underdeveloped countries of about 80% of world's population (WHO) [5]. Recently many of the research were being carried out in medicinal plants. The main reason was that the synthetic drugs which was now taking up by the human have many side effects that often lead to serious complications. The development of herbal medicine was done by the primary screening of the compounds in the plant extracts.

Comparing to modern medicine the herbal medicine was the lifesaving drug. Among 4,00,000 plant species only 6% of the plants are studied for their biological activity and only few have been phytochemically investigated. This shows that the investigation is needed for many medicinal plants for its activity and pharmacological properties. *Pergularia daemia* (Figure 1) is a plant that was named as "Veliparuthi" in Tamil and "Utranajutuka" in Hindi. *Pergularia daemia* dried leaf was used as an effective agent in treating bronchitis, asthma, rheumatic fever, amenorrhea, dysmenorrheal and wounds [6]. It also has many anti-microbial, anti-inflammatory, anti-rheumatic, anti-arthritic, anti-fungal properties [7,8]. They also serve as an anti-proliferative and a best anti-oxidant [9,10]. All these medicinal properties of *Pergularia daemia* is due to the presence of its phytochemical constituents which is not yet explored thoroughly [11]. In this study the Gas Chromatogram Mass Spectrometric method (GCMS) was carried out in the ethanolic extract of dried leaves for phytochemical analysis followed by qualitative and quantitative determination of the compounds. The non-nutritive plant chemicals are called as phytochemicals which have the properties to protect or prevent diseases.

Plant produces these chemicals to protect themselves but the research have shown that they have the capacity to treat human diseases in an effective way [12]. There are thousands of phytochemicals, each have their pharmacological properties of their own [13]. The plant possesses various medicinal properties; the aim of this study was to identify the phytocompounds in the ethanolic leaf extract of *Pergularia daemia* by qualitative screening of phytochemicals and to identify each specific compound with their concentrations by Gas Chromatography – Mass Spectrum (GC-MS) analysis [14].

MATERIALS AND METHODS

Collection of the plant

The *Pergularia daemia* (Figure 1) plant leaves were collected from the villages of Tirunelvelli district, Tamilnadu on August 17 at 10.00 AM. The collected plant was verified by Dr. V. Nandagoapalan, Associate Professor, PG and Research Department of Botany, National College, Trichy, Tamil Nadu.

Preparation of extract

The leaf was shadow dried and it was made as a powder using electrical blender. This powder was treated with 95% ethanol and kept for 48 h. Then it was filtered by using Whatman filter No.1. This extract was used for the phytochemical analysis and GC-MS analysis to find the bioactive components.

Phytochemical analysis

Phytochemical screening of crude extracts of *Pergularia daemia* was carried out according to the methods described by Trease and Evans [15,16]. Phytochemical analysis of crude powder of the samples for the evaluation of phytochemicals such as a tannins, alkaloid, steroid, phenols, glycosides [17,18] and terpenoid, flavonoid, etc. [18,19].

GCMS analysis

GCMS analysis is a common confirmation test. It is best used to make an effective chemical analysis. This analysis will provide a representative spectral output of all the compounds that get separated from the sample. The first step of GCMS was started by injecting the sample to the injected port of the Gas chromatography (GC) device. The GC instrument vaporizes the sample and then separates and analyzes of the various components. Each component was ideally produces a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed between elution and injection is called the "retention time". Differentiate between some compounds was identified using the Retention time. The peak is measured from the base to the tip of the peak. GCMS analysis was done by The South Indian Textile Research Association, Coimbatore.

Identification of bioactive constituents by GCMS

GCMS analysis of the ethanol extract of leaf of the plant was performed using a THERMO Gas Chromatography-TRACE ULTRA VER: 5.0 [20]. The oven temperature is maintained at 220ºC at a rate of 6ºC/min; the carrier gas with a flow rate of 1 ml/min. The split sampling technique was used to inject the sample in the ratio of 1:10. Retention indices (RI) of the compounds were determined by comparing the retention times of a series and identification of each component was confirmed by comparison of its retention index with data in the literature. Interpretation of

Figure 1: *Pergularia daemia*

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 which was stored in the NIST library. The molecular weight, name, chemical structure and molecular formula of the components of the test materials were ascertained. The peak in GCMS of ethanol extract of leaf of *Pergularia daemia* showed the presence of the secondary phytochemical compounds like phenolic and fatty acids and its esters (Figure 2).

RESULTS

The phytochemical components have been analyzed qualitatively in our lab by the common methods using chemicals. Flavonoids, glycosides, tannins, terpenoids, steroids, alkaloids and carbohydrates were found in the leaf extract of this plant (Table 1). GC-MS is the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters, etc. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented. GC/MS analysis of ethanolic extract of *Pergularia daemia* leaves revealed the existence of Hexadecanoic acid, methyl ester (33.42), Pentadecanoic acid, 14-methyl-methyl ester (36.23, Ethyl 9,12,15-octadecatrienoate (33.12 and4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole (31.24) (Table 2). From the GC-MS analysis of *Pergularia daemia* leaves the presence of seventeen compounds (phytochemical constituents) were revealed the medicinal quality of the plant (Table 3).

DISCUSSION

Metabolite profiling in plant species was done by gas chromatography mass-spectrometry method for last few years.

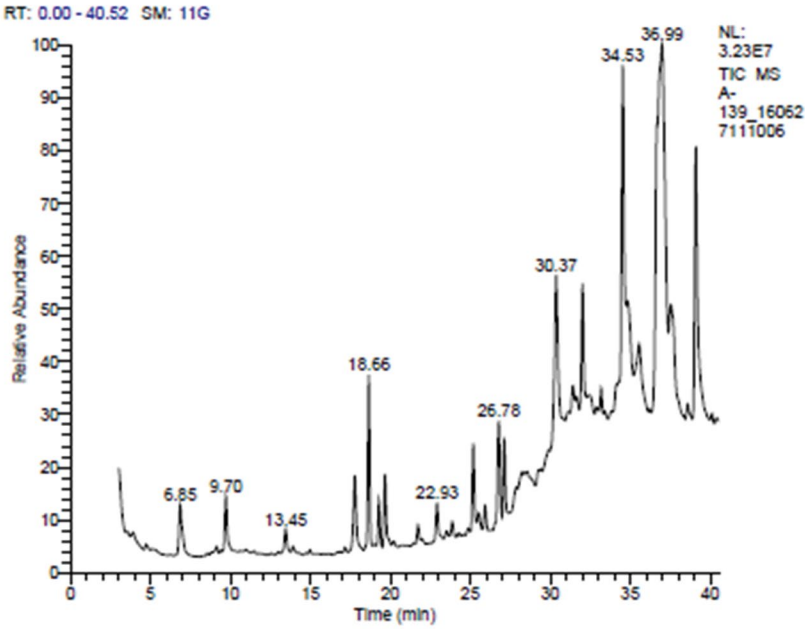
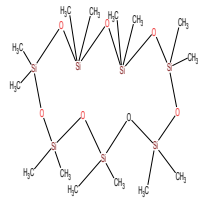
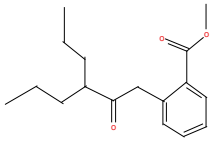
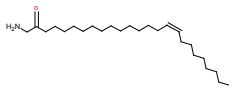
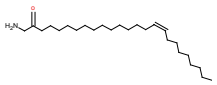
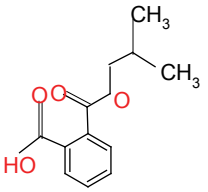
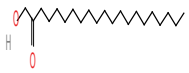
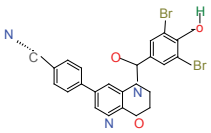
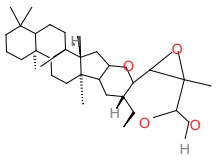
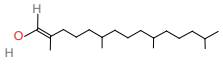


Figure 2: GCMS spectral chromatogram

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

S. No.	Phytochemical Components	Ethanolic extract of *P. daemia*
1	Flavonoid	+
2	Tannins	+
3	Alkaloids	+
4	Glycosides	+
5	Terpenoids	+
6	Steroids	+
7	Phenols	+

Table 2: GC–MS analysis of *Pergularia daemia*

S. No.	Retention Time	Peak area %	Name of the compound	Molecular formula	Molecular weight	Chemical structure
1	88.92	0.25	Tetradecamethylcyclohept-asiloxane	C14H42O7Si7	518	
2	83.59	0.45	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8 -dione	C17H24O3	276	
3	77.94	13.41	13-Docosenamide, (Z)-	C22H43NO	337	
4	73.41	8.64	13-Docosenamide, (Z)-	C22H43NO	337	
5	66.28	2.49	1,2-Benzenedicarboxylic acid, diethyl ester (CAS)	C12H14O4	222	
6	65.37	1.19	Hexadecanoic acid, ethyl ester (CAS)	C18H36O2	284	
7	60.01	0.44	Ethyl 2-dibromomethyl-6-cyano-7-ethoxy-5-phenyl-1,8-n aphthyridine-3-carboxylate	C21H17Br2N3O3	517	
8	52.89	0.39	SYNAPTOGENIN B	C30H46O4	476	
9	59.10	2.91	Phytol	C20H40O	296	

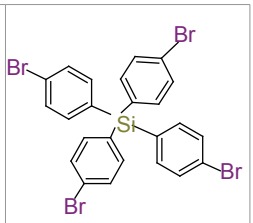
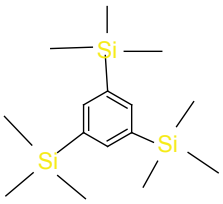
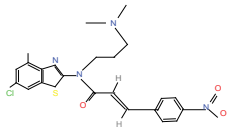
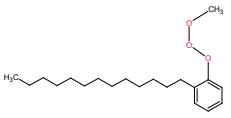
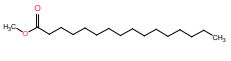
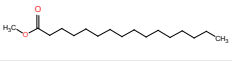
10	43.04	2.10	Tetrakis (Dimethylsilylcarbodiimide)	C12H24N8Si4	392	
11	49.94	28.29	Tris(trimethylsilyl), methyl ester derivative of 3,3,4,4-[2H4]-prostaglandin F2à	C30H56D4O5Si3	584	
12	31.24	2.10	4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole	C22H23ClN4O3S	458	
13	33.12	2.00	Ethyl 9,12,15-octadecatrienoate	C20H34O2	306	
14	36.23	0.77	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	C17H34O2	270	
15	33.42	0.77	Hexadecanoic acid, methyl ester (CAS)	C17H34O2	270	

Table 3: Biological properties of the phytocompounds

S. No.	Compounds	Biological activity
1	Tetradecamethylcycloheptasiloxa NE	Antioxidant
2	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9 diene-2,8-dione	No activity reported
3	13-Docosenamide, (Z)-	Antimicrobial
4	13-Docosenamide, (Z)-	Antimicrobial
5	1,2-Benzenedicarboxylic acid, diethyl ester (CAS)	Antiasthmatic
6	Hexadecanoic acid, ethyl ester (CAS)	Antimicrobial
7	Ethyl 2-dibromomethyl-6-cyano-7-ethoxy-5 phenyl-1,8-n aphthyridine-3-carboxylate	Antidiabetic
8	SYNAPTOGENIN B	Antimicrobial
9	Phytol	Antimicrobial, Antiasthmatic
10	Tetrakis(Dimethylsilylcarbodiimide)	Antimicrobial
11	Tris(trimethylsilyl),methylester derivative of 3,3,4,4-[2H4]-prostaglandin F2à	Antibacterial, Anti-infective
12	4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole	No activity reported
13	Ethyl 9,12,15-octadecatrienoate	Antimicrobial
14	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	Antibacterial, Antiallergic
15	Hexadecanoic acid, methyl ester (CAS)	Antibacterial, Antiallergic

But only a limited number of plant research laboratories have gas chromatography mass-spectrometry. The identified compounds occupy many biological properties. GC-MS analysis of phytoconstituents in plants gives a clear picture of the pharmaceutical value of that plant. Thus, this type of GC-MS analysis is the first step towards understanding the nature of medicinal properties in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation is needed to identify the pharmacological importance and phytochemistry of *Pergularia daemia*. Phytol is one among fifteen compounds of the present study. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. Similarly presence of phytol was observed in the leaves of *Lantana camara* [17] and in *Mimosa pudica* leaves [21]. Similar result was also observed in the leaves of *Lantana camara* [22]. Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes [23]. Phytol, Phenol, 2, 4-bis (1-phenylethyl) - which are all have medicinal properties. Potential antioxidant and anticancer activities were found in these compounds.

Alcohol extract of the leaves of *Kigelia pinnata* has earlier been reported for the presence of Hexadenoic acid compound [24] and in *Melissa officinalis* [22,25]. 17 compounds with n-Hexadecanoic acid and Octadecanoic acid was identified as the major compounds in the leaves of *Cleistanthus collinus* [26]. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid [27], Hexadecanoic acid, 9, 12 - Octadecadienoic acid, n-hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Squalene and Phytol were identified in the ethanol leaf extract of *Aloe vera* [28]. These reports are in accordance with the result of this study.

The source of many plants (herbs and spices) can be often identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, particular qualitative and quantitative patterns from a GC analysis will show all the compounds in the leaf extract. *Pergularia daemia* have many biological properties which can be used in various purposed to treat many diseases. The compounds identified by the initial qualitative analysis and GCMS analysis have many uses in medical field. Each compounds identified have their unique character to treat various diseases. Further studies needed to reveal its importance in specific field to treat the diseases properly.

CONCLUSION

GCMS analysis of the ethanolic extract of leaves of *Pergularia* daemia reveals the presence of medicinally valued bioactive components like saponins, tannins, alkaloids, terpenoid, steroids and flavonoids. As the medicinal value of similar components in other plant extracts are already proved, no wonder if these components in *Pergularia daemia* may also have equally effective. 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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