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Research Article

PHYTOCHEMICAL ANALYSIS OF *RUELLIA PATULA* USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: *Ruellia patula* (Jacq.) a medicinally important plant belongs to the family Acanthaceae. Traditionally, the leaves are used in the treatment of many diseases such as insect bites, itches, eye diseases, and skin problems. In the present study, the qualitative and quantitative analysis of bioactive compounds by gas chromatography (GC) and mass spectroscopy (MS) techniques.

Methods: The chemical compositions of the ethanol extract of *R. patula* leaves were investigated using Perkin–Elmer GC-MS.

Result: In the GC-MS analysis of ethanol extract of *R. patula* L. reported the presence of 15 compounds. The major compound constituents are 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (28.75%), and α-sitosterol (14.35%).

Conclusion: Identification of these compounds can help in the development of new drugs.

Keywords: *Ruellia patula*, Ethanol extract, Phytochemical compounds, gas chromatography-mass spectroscopy analysis, Biological activity of phyto-components.

INTRODUCTION

Plant-derived substances have traditionally played an important role in the components of health care system. They continue to provide primary health care to more than three-quarters of the world's population. Ancient traditional medicinal practices, such as Indian Ayurveda, Arabic Unani, and Chinese systems, are mainly based on a large diversity of medicinal plants. Besides medicinal uses, plant-derived products are also employed in the preparation of personal care products, phyto-cosmetics, natural health products, and phytopharmaceuticals [1]. During the last few decades, there has been an increasing focus in the study of medicinal plants and their traditional uses in different parts of the world [2].

Three main groups of secondary metabolites (phytochemicals)-alkaloids, phenolic compounds, and terpenoids are present in plants. These biocompounds are the most important plant materials for the development and production of drugs [3,4]. Indeed, the dazzling variety of amazingly diverse chemicals produced by plants may well yield a significant number of novels, more efficacious drugs against human diseases. According to conservative estimates, there are as many as 400,000 secondary plant metabolites on the earth, of which only about 10,000 have been chemically characterized [5,6]. Thus, one can easily imagine the amount of naturally available chemical wealth that lies awaiting in the vegetal pharmacies of our globe.

In this context, a systematic evaluation of medicinal plants is of utmost importance and also a major concern for not only a better understanding of plant resources but also in developing safe, better, and novel therapeutic strategies. Moreover, nowadays, due to the increasing number of adverse effects and other potential limitations of synthetic drugs, natural therapeutic strategies such as plant-based medicines, which are comparatively safe with limited or no adverse effects, are becoming increasingly useful today. So, people are returning to the natural products with the hope of safety and security [7].

Ruellia patula (Jacq.) is a member of the family Acanthaceae, commonly known as Puni chedi in Tamil. It is a hairy small under-shrub, found in Arabia, Africa, India (especially in Southern India), Sri Lanka, and Myanmar. The plant is commonly distributed on the wastelands in Tamil Nadu, India. Its leaves are used for treating syphilis, insect bites, eye diseases, skin diseases, gonorrhea, tumors, rheumatic complaints renal infection, cough, wounds, scalds, toothache, stomachache, and kidney stones problems [8,9]. *R. patula* and another species, *R. brittoniana,* were found to possess good cardiotonic properties [10]. *Dipteracanthus patulus* (Jacq.) Nees. (Syn. *R. patula* Jacq) has significant antimicrobial and anti-inflammatory activities [11]. *D. patulus* uses as a single drug remedy for against the deadly poison of Kaduva chilanthi (Tiger Spider) by Kani tribe of Agasthiarmalai, India [12]. *Ruellia tuberose* has saponins, tannins, and flavonoids, which are responsible for antiulcer activity [13]. Based on these beneficial effects of *R. patula*, the objective of the present study was to determine the phytochemical constituents, and potent bioactive compounds from the ethanol extract of *R. patula* leaves with the aid of GC-MS analysis.

METHODS

Plant materials

Fresh leaves of *R. patula* were collected from Thanjavur (Tamil Nadu, India) in January 2011 and authenticated by a botanist at the Botanical Survey of India, Coimbatore. A voucher specimen has been deposited at the museum of our college.

Preparation of leaf extract

The fresh, undamaged, and disease-free leaves were selected and washed thoroughly with sterile double distilled water, shade-dried and then coarsely powdered in a blender. The coarse powder was successively solvent extracted in a soxhlet extractor using ethanol solvent. The extracts so obtained were further dried in vacuum desiccators. The residue obtained from the extract was used for further studies by preserving it in a refrigerator.

Identification of compounds using gas chromatography and mass spectrometry (GC-MS) analysis

GC and MS make an effective combination for chemical analysis [14].

Table 1: Phyto-components detected in the ethanol extract of the leaves of *R. patula* by GC-MS

Serial number	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1	2.85	Propane, 1,1,3-triethoxy	$C_9H_{20}O_3$	176	8.21
2	11.71	3,7,11,15-tetramethyl-2hexadecen-l-ol	$C_{20}H_{40}O$	296	28.75
3	12.20	3,7,11,15-tetramethyl-2hexadecen-l-ol	$C_{20}H_{40}O$	296	7.58
4	13.17	1,2-Benzenedicarboxylic acid diheptyl ester	$C_{22}H_{34}O_4$	362	1.71
5	13.56	Dodecanoic acid ethyl ester	$C_{14}H_{28}O_2$	228	1.71
6	15.06	Phytol	$C_{20}H_{40}O$	296	8.15
7	15.84	9,12,15-octadecatrienal	$C_{18}H_{30}O$	262	2.39
8	17.45	Pentanal, 2-methyl	$C_6H_{12}O$	100	0.74
9	19.96	Pentanal, 2,4-dimethyl	$C_7H_{14}O$	114	0.63
10	20.99	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	0.97
11	24.84	Squalene	$C_{30}H_{50}$	410	5.55
12	28.01	γ-tocopherol	$C_{28}H_{48}O_2$	416	2.87
13	31.35	9,12-octadecodienoic acid [z, z], phenylmethyl ester	$C_{25}H_{38}O_2$	370	10.27
14	32.64	α-sitosterol	$C_{29}H_{50}O$	414	14.35
15	33.46	α-amyrin	$C_{30}H_{50}O$	426	6.11

RT: Retention time, MW: Molecular weight, *R. patula: Ruellia patula*, GC-MS: Gas chromatography-mass spectroscopy

Table 2: Biological activity of phyto-components identified in the ethanol extracts of the leaves of *R. patula*

Serial number	Molecular formula	Name of the compound	Active biological activity
1	$C_{20}H_{40}O$	3,7,11,15-tetra methyl 1-2-hexadecen-1-01	Cancer preventive, antioxidant
2	$C_{22}H_{34}O_4$	1,2-benzenedi-carboxylic acid, diheptyl ester	Used as softeners, perfumes, textiles as dyestuffs, cosmetic and glass making
3	$C_{14}H_{28}O_2$	Dodecanoic acid, ethyl ester	Anti-inflammatory and anticoronary activity
4	$C_{20}H_{40}O$	Phytol	Cancer preventive
5	$C_{18}H_{30}O$	9,12,15-octadecaterienal	Cancer preventive, hypo-cholesterolemic, anticoronary activity
6	$C_{30}H_{50}$	Squalene	Anticancer, used in cosmetic, immunologic adjuvant in vaccines
7	$C_{28}H_{48}O_2$	C-tocopherol	Antioxidant, fertility factor
8	$C_{25}H_{38}O_2$	9,12, octadecadienoic acid (z, z)-phenylmethyl ester	Anti-inflammatory, Hypo-cholesterolemic cancer preventive, hepato-protective, namaticide, insectifuge, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary
9	$C_{29}H_{50}O$	α-sitosterol	Antirachitic, help to prevent cholesterol uptake in GI tract
10	$C_{30}H_{50}O$	α-amyrin	Antihyperglycemic activity

GI: Gastrointestinal, *R. patula: Ruellia patula*

GC-MS analysis of these extracts was performed with GC Clarus 500 Perkin Elmer system and GC interfaced to an MS equipped with an Elite-1 fused silica capillary column (30 mm × 0.25 mm 1D × 1 µm of the capillary column, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at 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 programed from 110°C (isothermal for 2 minutes) with an increase of 10°C/minutes to 200°C, then 5°C/minutes to 280°C, ending with 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 kDa. Total GC running time was 36 minutes. The plant extract was dissolved in ethanol and filtered with polymeric solid phase extraction column and analyzed in GC-MS for different components. 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 turbomass.

Interpretation on mass spectrum of GC-MS was done using the database of the National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of known components using computer searches on an NIST database (NIST version 2.1). The name, molecular weight (MW), and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC-MS analysis of the ethanol extract of leaves of *R. patula* identified 15 compounds. The active principal components with their RT, molecular formula, MW, and percentages were also determined. The chemical compounds are shown in Table 1, biological activities of the compounds are tabulated in Table 2, and the corresponding chemical shift peaks of the spectrum are shown in Fig. 1.

The qualitative analysis identified the various phytochemicals such as alkaloids, phenols, steroids, glycosides, vitamins, and terpenoids. Interpretation on mass spectrum GC-MS was conducted using the database of NIST data library [8,10]. 3,7,11,15-tetramethyl-2-hexadecen-1-ol (28.75%) was found as the major component followed by α-sitosterol (14.35%), 9,12-octadecodienoic acid [z, z], phenylmethyl ester (10.27%),

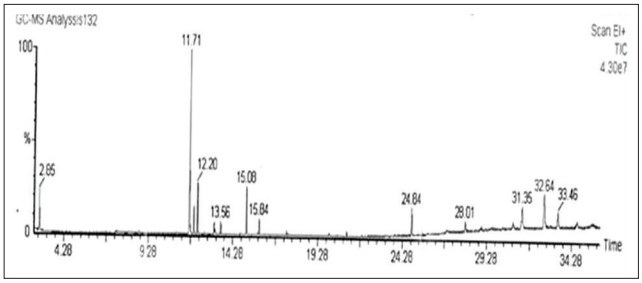

Fig. 1: Gas chromatography-mass spectroscopy chromatogram of the ethanol extracts of leaves of the *Ruellia patula*

Propane, 1,1,3-triethoxy (8.21%), phytol (8.15%), α-amyrin (6.11%), squalene (5.55%), γ-tocopherol (2.87%), 9,12,15-octadecatrienal (2.39%), 1,2-benzenedicarboxylic acid diheptyl ester (1.71%), dodecanoic acid ethyl ester (1.71%), didodecyl phthalate (0.97%), pentanal, 2-methyl (0.74%), pentanal, 2,4-dimethyl (0.63%).

SUMMARY AND CONCLUSIONS

The present study carried out using the ethanol extract of *R. patula* leaves revealed the presence of active medicinal constituents. This study also helped to identify the formulae and structures of biomolecules, which can be used as drugs. Thus, this type of GC-MS analysis is a first step toward understanding the nature of active principal components in these medicinal plants. Hence, this type of study will be helpful for further investigations that may lead to the development of new therapeutic drugs.

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