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Short Communication

GC-MS ANALYSES OF LEAF AND ROOT EXTRACTS OF *DIDYMOCARPUS TOMENTOSA*

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ABSTRACT

Objective: *Didymocarpus tomentosa* Wight., is a traditional medicinal plant used in the treatment of fever and skin allergy and the present study were conducted to identify the phytochemical constituents in leaf and root extracts using gas chromatography and mass spectrometry (GC-MS).

Methods: The methanolic leaf and root extracts were analyzed using Shimadzu GCMS-QP 2010 gas chromatography-mass spectrometer. The mass spectrum of GC-MS was interpreted using the database of the National Institute of Standards and Technology (NIST).

Results: The GC-MS analyses of leaf and root extracts revealed the presence of twenty six and twenty three phytochemical constituents respectively. 5-Hydrxoymethylfurfural (15.1%), *cis*, *cis*, *cis*-7,10,13-Hexadecatrienal (12.5%), Hexadecanoic acid (11.8%), Lupenone (29.1%), γ-Sitosterol (21.3%) and Lupeol (12.0%) were the major constituents.

Conclusion: The leaf and root extracts of *D. tomentosa* possess various phytochemical constituents, which are of high therapeutic values.

Keywords: *Didymocarpus tomentosa*, 5-Hydrxoymethylfurfural, Lupenone, Lupeol.

For thousands of years traditional herbal medicines have cured many diseases due to their special healing efficacy. It has gradually attracted interest and acceptance by the general public as efficient and cost effective medicine besides being locally available and safe. As a result, in recent years, there is an increasing trend to determine phytochemical composition of such traditional plants and GC-MS has become a powerful analytical tool for efficient separation of chemical constituents in the field of herbal medicines [1]. The phytochemical profiling of the extracts using GC-MS provides reliable data for the identification of medicinal useful compounds.

Didymocarpus of *Gesneriaceae*, comprises of 70 species [2]. Only a few species are chemically studied and most of these species are found distributed in India. *Didymocarpus tomentosa* Wight., [Syn. of *Henckelia incana* (Vahl) Spreng.] endemic to the Peninsular India, is traditionally used in the treatment of fever and skin allergy [3, 4]. Their extracts possess antioxidant and anti-inflammatory activities [5]. However, photochemical constituents of their extracts have not been reported till date. Therefore, in the present study, we report for the first time the GC-MS fingerprinting of leaf and root extracts which was carried out so as to establish volatile chemical constituents of *D. tomentosa* and to identify medicinally useful compounds.

Plant materials of *Didymocarpus tomentosa* were collected in the rocky hills of Savandurga, about 50 km west of Bangalore. The plant was identified using the Flora of British India and the Flora of the Tamil Nadu Carnatic [6, 7]. It was further authenticated by the National Ayurveda Dietetics Research Institute, Bangalore. A voucher specimen has been deposited at the above Centre (RRCBI/MCW/09). The collected plant materials were washed with tap water. They were air-dried thoroughly under shade at room temperature for 15-20 days. The shade dried materials were powdered using a mixer and stored in air-tight container.

Leaf and root powder were extracted separately with methanol using hot extraction method. A total of 50 g of each powder was extracted with 500 ml methanol (1:10) in a Soxhlet apparatus for 2 days. The residue was removed by filtration through What Mann No. 1 filter paper and the extracts were concentrated using a Rotary evaporator. The extracts thus obtained were subjected to GC-MS analyses.

GC-MS analysis of leaf and root extracts were carried out using Shimadzu GCMS-QP 2010 gas chromatography-mass spectrometer interfaced with a turbo mass quadrupole mass spectrometer, fitted with an Rtx-5 fused silica capillary column (30 m × 0.25 mm, with 1 µm film thickness); the oven temperature was programmed from 150 °C to 210 °C (10 min) at 2 °C/min and increased to 260 °C (10 min) at 10 °C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min; the injector temperature was 210 °C, injection volume of 1 µl and with a split ratio of 1: 200. The interface and MS ion source were maintained at 280 °C and 250 °C respectively, the mass spectra were taken at 70 eV with a mass scan range of 40-500 amu. Data handling was done using GC-MS solution software. The mass spectrum of GC-MS was interpreted using the database of NIST.

The GC-MS analysis of leaf extract resulted in the identification of twenty six constituents representing 100% of the chromatographical leaf extract. The phytoconstituents of the leaf extracts with their peak number, retention time (RT), name, molecular formula, molecular mass (MW) and area percentage (%) is presented in table 1.

Table 1: The phytoconstituents in the leaf extract of *D. tomentosa*

Peak no.	RT	Name of the phytoconstituents	Mol. formula	MW	%
1	4.6	5-Methylfuran-2-aldehyde	$C_6H_6O_2$	110	0.8
2	4.8	2, 4-Dihydroxy-2, 5-dimethyl-3 (2H)-furan-3-one	$C_6H_8O_4$	144	0.9
3	5.6	5,6-Dimethyl-2,3-dihydro-1,4-dioxin	$C_6H_{10}O_2$	114	0.9
4	5.7	4-methyl-2-prop-1-enyl-1,3-dioxolane	$C_7H_{12}O_2$	128	1.2
5	5.9	2, 5-Dimethyl-4-hydroxy-3 (2H)-furanone	$C_6H_8O_3$	128	0.6
6	6.2	2-Hydroxy-3-methyl-4-pyrone	$C_6H_6O_3$	126	4.0
7	6.7	2, 5-Monomethylene-1-rhamnitol	$C_7H_{14}O_5$	178	0.9
8	7.2	2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one	$C_6H_8O_4$	144	2.3

9	7.9	1, 2-Benzenediol	$C_6H_6O_2$	110	2.3
10	8.4	5-Hydrxoymethylfurfural	$C_6H_6O_3$	126	15.1
11	8.6	1, 2, 3-Propanetriol monoacetate	$C_5H_{10}O_4$	134	1.3
12	9.6	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150	3.8
13	11.2	2-Hydroxy-6-methylbenzaldehyde	$C_8H_8O_2$	136	4.6
14	14.0	2-Amino-3-(3,4-dihydroxy-phenyl)-propionic acid	$C_9H_{11}NO_4$	197	4.3
15	14.2	(E)-13-Docosenoic acid	$C_{22}H_{42}O_2$	338	0.7
16	15.8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.1
17	16.6	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	1.0
18	17.0	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.8
19	18.6	Methyl 3-hydroxyoctadecanoate	$C_{19}H_{38}O_3$	314	1.0
20	18.8	*cis*,*cis*,*cis*-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234	12.5
21	20.4	Octadecanal	$C_{18}H_{36}O$	268	4.3
22	21.9	Palmitic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	5.3
23	23.3	Propylene glycol monoleate	$C_{21}H_{40}O_3$	340	5.2
24	23.4	Methyl (Z)-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318	4.8
25	27.4	Stigmasta-5, 22-dien-3-ol	$C_{29}H_{48}O$	412	0.8
26	27.8	γ-Sitosterol	$C_{29}H_{50}O$	414	8.6

The GC-MS analysis of root extract revealed the presence of twenty three constituents representing 100% of the chromatographical root extract. The phytoconstituents of the root extract is presented in table 2.

Table 2: The phytoconstituents in the root extract of *D. tomentosa*

Peak no.	RT	Name of the phytoconstituents	Mol. formula	MW	%
1	6.2	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126	0.5
2	7.2	2,3-Dihydro-3,5-dihydroxy-6-methyl-4 (H)-pyran-4-one	$C_6H_8O_4$	144	0.4
3	8.3	5-Hydrxoymethylfurfural	$C_6H_6O_3$	126	0.6
4	9.6	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150	0.4
5	11.2	2-Hydroxy-6-methylbenzaldehyde	$C_8H_8O_2$	136	0.9
6	16.6	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	0.8
7	17.0	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.0
8	17.7	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	0.3
9	18.3	*Cis*, *cis*-octadeca-9,12-dienoic acid methyl ester	$C_{19}H_{34}O_2$	294	3.2
10	22.1	4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dimethoxy-2-phenyl-	$C_{17}H_{16}O_4$	284	2.8
11	22.6	(2S)-5-Methoxy-6-methyl-2-phenyl-7-chromanol	$C_{17}H_{18}O_3$	270	0.4
12	22.9	4-Phosphalotricyclo[6.1.1.0(2,6)]dec-2-ene, 4,9,9-trimethyl-	$C_{12}H_{19}P$	194	3.0
13	23.1	1-Benzylidene-2-(diphenylmethylene) hydrazine	$C_{20}H_{16}N_2$	284	3.4
14	23.8	4-Nitrophenylsuccinic acid	$C_{10}H_9NO_6$	239	3.8
15	24.2	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	$C_{30}H_{50}$	410	0.6
16	25.9	2,7,8-Trimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-6-chromanol	$C_{28}H_{48}O_2$	416	0.4
17	26.2	Cholesta-4, 6-dien-3-ol	$C_{27}H_{44}O$	384	0.4
18	27.2	Ergost-5-en-3β-ol	$C_{28}H_{48}O$	400	0.9
19	27.4	Stigmasta-5,22-dien-3-ol	$C_{29}H_{48}O$	412	2.3
20	27.8	γ-Sitosterol	$C_{29}H_{50}O$	414	21.3
21	28.3	Lupenone	$C_{30}H_{48}O$	424	29.1
22	28.4	Lupeol	$C_{30}H_{50}O$	426	12.0
23	28.7	4-Stigmasten-3-one	$C_{29}H_{48}O$	412	8.6

The majority of the phytoconstituents identified in the extracts is attributed with various biological activities. For example, Hydroxymethylfurfural (5-Hydroxymethylfurfural), a known cytotoxic phytochemical may prevent cancer-related processes in breast, prostate and endometrial cancer cells [8]. It also possesses nematicidal activity [9], antioxidant and anti-inflammatory activities [10] and antiproliferative activities [11]. Another major constituent present in both the extracts was γ-Sitosterol, which is known for lowering the cholesterol in human beings [12]. Recently, it has also been reported as an antidiabetic agent [13]. Hexadecanoic acid a very common saturated fatty acid is known as an anti-inflammatory phytoconstituent as it is a phospholipase inhibitor [14] and it's also known for its antibacterial activity [15]. Recently, Lupenone and Lupeol have been reported to inhibit protein tyrosine phosphatase 1B (PTP 1B) a possible alternative to the type 2 diabetes and obesity drug development [16]. In addition, Lupeol is attributed with anticancer activity [17, 18], anti-inflammatory activity [19], antiarthritic action [20, 21] and antifungal properties [22]. Lupenone has more recently been used in the treatment for hypopigmentation diseases [23]. Therefore the presence of various bioactive compounds in the leaf and root extracts of *D. tomentosa* justifies the use of the plant in the treatment of different ailments by the traditional practitioners and it further holds promise for the production of novel pharmaceuticals.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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