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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF METHANOLIC EXTRACT OF NEEDLES OF *PINUS WALLICHIANA*

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ABSTRACT

Objective: To investigate the phytoconstituents of methanolic extracts of a conifer *Pinus wallichiana* using gas chromatography-mass spectroscopy (GC-MS), which is famous for its timber quality, resins and oils.

Methods: The present investigation was carried out to determine the possible chemical constituents present in the methanolic extracts in the needles of *P. wallichiana.*

Results: The result of the GC-MS confirmed the presence of 38 compounds. Momeinositol (43.27%), 9, 12-octadecanoic acid, 2 (12.52%) pentadecanoic acid (4.12%) and dibutyl phthalate (3.34%) were the major compound found through the GC-MS analysis.

Conclusion: The presence of various compounds in the methanolic extract of *P. wallichiana* confirms its relevance not only for pharmaceutical purpose but for various industrial purposes too.

Keywords: *Pinus wallichiana*, Gas chromatography-mass spectroscopy (GC-MS), Gas chromatography-mass spectroscopy of plant extract, Gas chromatography-mass spectroscopy of conifer, Gas chromatography-mass spectroscopy profiling.

INTRODUCTION

Since ancient time, people have been using various natural compounds in the forms of medicines for the treatment of various diseases. Many of recent therapies are based on this ancient system of medicine. Documentation of plants for their ethnopharmacological properties is reported from the 1000 years. Around 50% of modern drugs are derived from the plant and the natural sources [1,2].

Pinus wallichiana (Common names: *Kail, Biar*) is a famous conifer for its timber quality. The plant is useful for its gum, resin and fuel wood purposes and also suitable for various medicinal purposes [3]. *P. wallichiana* is habituate to grow in the altitude range of 1800-4300 m above sea level. Plants grow naturally in Himalayan range, Karakoram and Hindukush Mountains and India, Pakistan, Nepal, China, Afghanistan and Bhutan [4].

Bark and needles extract of plant has shown the presence of a good amount of phenolics and flavonoids along with antioxidant properties [5,6]. HPLC analysis of the needles has shown the presence of quercetin, rhamnetin, isorhamnetin, and kaempferol [7]. We extracted the phytoconstituents of needle of *P. wallichiana* with methanol using accelerated solvent extractor (ASE) at room temperature and high pressure to increase the yield and diversity of phenolics. In upcoming years, gas chromatography-mass spectroscopy (GC-MS) has emerged as a promising method for the analysis of bioactive constituents. It has been employed to identify the various bioactive constituents e.g., non-polar compounds, long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds with volatile oil, fatty acids and lipids [8,9]. GC-MS spectrum is based on mass to charge ration (m/z) of the compounds. Each compound has a unique mass spectrum (molecular fingerprint) on the basis of which compounds are identified [9,10].

In this study, we analyzed the methanolic extract of *P. wallichiana* needles by the GC-MS for the extensive analysis of its phytoconstituents.

METHODS

Collection of plant

Needles of *P. wallichiana* were collected from Barkot region of Uttarkashi district of Uttarakhand India. The species was identified and authenticated by the expert Dr. Rakesh Mohan Painuli, Department of Botany, H.N.B. Garhwal University, Uttarakhand, India. The specimen sample is submitted in the herbarium vide specimen, voucher number is GUH20744.

Preparation of extract

Needles of the plant were dried and crushed to get fine powder of them. The extraction was done in accelerated solvent extraction system equipped with a solvent controller unit (ASE350, DIONEX, and Corporation Sunnyvale, CA, USA) [11]. 50 g of powdered sample is loaded into cell with 50 g silica powder which helps in the homogenous lysis of plant cells to enhance the release of the phytoconstituents. Solvents used for extraction was 100% methanol. Cell is filled with solvent and a pressure of 1500 psi is applied. For the extraction, five cycles each of 5 minutes were performed. The solvent was evaporated in rotavapor (Rota Vapor124, Bucchi, and Flawil, Switzerland). Afterward, plant extract was lyophilized and stored at 4°C until further use. Different dilutions of the lyophilized herbal preparations were made in appropriate solvents for further use in different assays.

GC-MS analysis

GC-MS analysis was performed at University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi using a GC-MS QP2010 ultra system interfaced to a GC-MS equipped with a Elite-5MS {5% diphenyl/95% dimethyl polysiloxane) fused to capillary column 30 × 0.25 µm ID (inner diameter of column) × 0.25 µm df (film thickness)}. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (a split patterns). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST(National Institute of Standards and Technology, U.S.A.) database.

RESULT

A total of 38 compounds were identified in the methanolic extract (prepared through ASE) of the plant. The identification of the phytochemical compounds was done on the basis of comparing

mass fragmentation attributes (peak area and retention time) of the unknown compounds with the NIST database. The putative identified compounds, with their RT, molecular formula, molecular weight and peak area in percentage, are presented in Table 1. The range of RT lies between 3.127 and 36.66 minutes (Fig. 1).

DISCUSSION

GC-MS analysis of plant extract shows the presence of total 38 compounds. Out of these, various compounds have got their applications in pharmaceutical and other industries as raw material and solvents. Momeinositol one of the major compounds detected through GC-MS analysis is reported to be used as antialopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic, lipotropic and as a sweetening agent [12].

Pentadecanoic acid is also one of the major compounds identified, which is an edible saturated fatty acid, found very rarely in nature. So far, cow's milk is reported to be a major dietary source of it [13,14].

Previous study has shown the presence of 17 constituents in oil fraction of *P. wallichiana* in which β-pinene and α-pinene were the major compounds detected through GC-MS [15].

As per to our information, this is the first study on the identification of important phytoconstituents in methanolic extract of *P. wallichiana* needles. Plant extract has shown the presence of various compounds of pharmaceutical and industrial importance. Therefore, neddles of *P. wallichiana* could be used for the sourcing of these compounds from the extract prepared through the method described herein.

CONCLUSIONS

Various organic compounds (total: 38) have been identified by the GC-MS in the extract prepared from the needles of *P. wallichiana* through ASE at room temperature and high pressure. These are mainly alkenes, esters and fatty acids. Some of them have got pharmaceutical properties and others are useful in various industries. Neddles of *P. wallichiana* may be used as an alternative source for these useful compounds.

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Table 1: Phytoconstituents identified in the methanolic extract of needles of *P. wallichiana*

Peak	RT	Area %	Name	Molecular formula
1	3.127	1.38	Dl-Glyceraldehyde dimer	$C_6H_{12}O_6$
2	4.275	1.64	1,2,3-Propanetriol (Glycerol)	$C_3H_8O_3$
3	4.676	0.57	Octane, 2,4,6-trimethyl-	$C_{11}H_{24}$
4	7.289	2.07	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	$C_6H_8O_4$
5	7.567	0.59	Benzoic acid	$C_7H_6O_2$
6	8.072	0.94	Dodecane	$C_{12}H_{26}$
7	8.840	0.65	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$
8	11.017	1.44	Tetradecane	$C_{14}H_{30}$
9	13.552	0.82	Hexadecane	$C_{16}H_{34}$
10	13.854	0.95	1,3,4,5-tetrahydroxy-cyclohexanecarboxy (quinic acid)	$C_7H_{12}O_6$
11	13.991	0.83	Ethyl alpha-d-glucopyranoside	$C_8H_{16}O_6$
12	15.012	43.27	Mome inositol	$C_7H_{14}O_6$
13	15.408	0.56	10-methoxy-Nb-alpha-methylcorynantheol	$C_{21}H_{29}N_2O_2$
15	15.936	0.33	(1-butyloctyl) benzene	$C_{18}H_{30}$
16	17.125	0.16	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
17	17.294	0.19	1,4-Dioxacyclohexadecane-5,16-dione	$C_{14}H_{24}O_4$
18	17.403	0.35	Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydro	$C_{24}H_{40}O_3$
19	17.478	4.12	Pentadecanoic acid	$C_{15}H_{30}O_2$
20	17.603	3.34	Dibutyl phthalate	$C_{16}H_{22}O_4$
21	17.826	0.14	Eicosane (Icosane)	$C_{20}H_{42}$
22	18.778	0.22	3,7-Dihydroxy-3-phenyl-4-chromanone	$C_{15}H_{12}O_4$
23	19.143	1.23	9,12-octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$
24	19.217	12.52	9,12-octadecadienoic acid (lionolelaidic acid)	$C_{18}H_{32}O_2$
25	19.372	1.41	Octadecanoic acid (stearic acid)	$C_{18}H_{36}O_2$
26	19.581	5.03	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$
27	19.814	0.82	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$
28	19.941	3.96	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$
29	20.970	0.39	Ricinoleic acid	$C_{18}H_{34}O_3$
31	21.361	2.42	2,4a, 8,8-tetramethyl-decahydro-cycloprop (viridiflorol)	$C_{15}H_{26}O$
32	21.681	1.45	1,4,4-trimethyl-8-methylene-1,5-cycloundecaniene	$C_{15}H_{24}$
33	22.061	0.49	1-phenanthrenecarboxylic acid, 7-ethenyl-1,2,3,4,4a, 4b, 5,6,	$C_{21}H_{32}O_2$
34	22.534	1.44	Dehydroabietic acid	$C_{20}H_{28}O_2$
35	26.459	1.25	Acetate, [6-(acetyloxy)-5,5,8a-trimethyl-2-methyleneperhydro-1-napthalenyl] methyl estetr	$C_{19}H_{30}O_4$
36	27.178	0.81	1-phenanthrenecarboxylic acid, 7-ethenyl (leopimaric acid)	$C_{21}H_{32}O_2$
37	31.518	0.54	10-nonadecanol	$C_{19}H_{40}O$
38	36.663	0.78	Stigmast-5-En-3-Ol, (3 beta)-(B sitosterol)	$C_{29}H_{50}O$
	Total: 100.00			

P. wallichiana: Pinus wallichiana, RT: Retention time

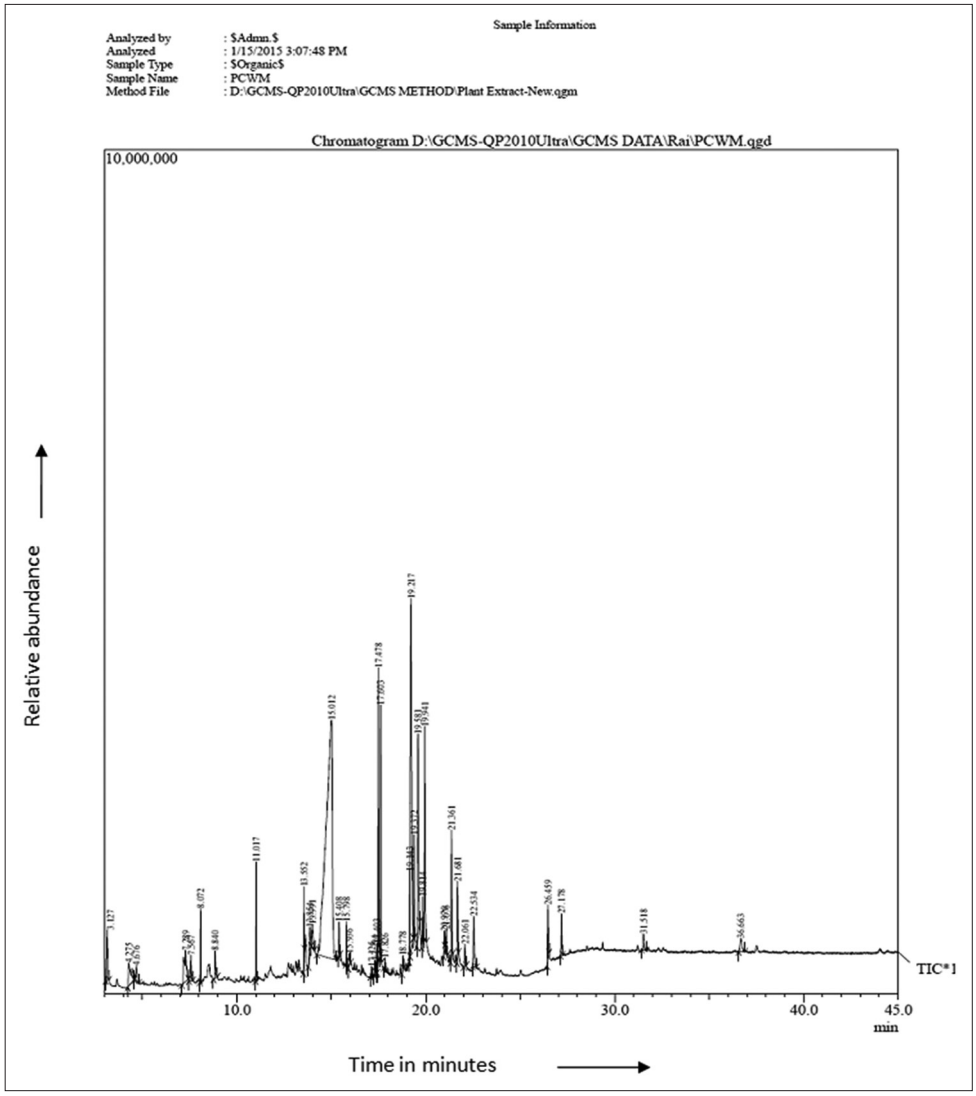

Fig. 1: Total ion chromatogram of methanolic extract of needles of *Pinus wallichiana*

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