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GC-MS Analysis of Bioactive Constituents of *Pinus roxburghii* Sarg. (Pinaceae) from Northern India

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ABSTRACT

The Chir Pine, *Pinus roxburghii*, named after William Roxburgh, is a pine native to the Himalaya. *Pinus roxburghii* Sarg. (Pinaceae) is traditionally used for several medicinal purposes in India. The GC-MS analysis revealed the presence of steroidal moiety 2,2 dibromocholestanone. However presence of intact lactone ring was not revealed, some ester group containing moiety like trimethy ester, 4-ethoxy-ethyl ester, isobutyl octadecyl ester were also observed. Other constituent found to be present are antracene, phthalic acid, 2-chloropropionyl chloride, benzoic acid, boric acid and dibutyl phthalate. The constituent found to be present in *Pinus roxburghii* are reported to have very significant medicinal property like anticancer, chemopreventive, anthelmintic, antiproliferative. The same activity needs explanation and elucidation in various column fraction and extract of *Pinus roxburghii*.

Key words: *Pinus roxburghii* Sarg., chemopreventive, GC-MS analysis

INTRODUCTION

Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional practice such as Ayurveda, Unani and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections like dysentery to skin diseases, asthma, malaria, etc (Kumar *et al*., 2006).

The Chir Pine (*Pinus roxburghii* Sarg.) named after William Roxburgh, is a pine native to the Himalaya. The range extends from northern Pakistan (North-West Frontier Province, Azad Kashmir), across northern India (Jammu and Kashmir, Punjab, Himachal Pradesh, Uttrakhand, Sikkim) and Nepal to Bhutan (Uniyal *et al*., 2006). The chief chemical constituents extracted from resin of plants are α-pinene (18.1%), longifolene (13.8%) and carene (51.8%) (Makhaik *et al*., 2005). The plant is used in Indian traditional system of medicine to treat a number of disorders namely bronchial infection, chronic rheumatism, skin disease, convulsion, ulcers etc (Nadkarni and Nadkarni, 1995). Various pharmacological activities like that of analgesics, anti-inflammatory (Kaushik *et al*., 2012a) and anti convulsant (Kaushik *et al*., 2012b) have been ascribed to the plant *Pinus roxburghii*.

The preliminary phytochemical investigation for presence of various natural compounds revealed the presence of cardiac glycosides in n-butanol fraction of alcoholic extract. As there is increasing demand of phytotherapeutic compounds from the plants (Capasso *et al*., 2000). Keeping all the above facts in mind it was decided to identify the chemical constituents of *Pinus roxburghii* using GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material: The stems bark of *Pinus roxburghii* Sarg. were collected from the hilly region of Morni, District Panchkula, Haryana, in the month of August 2013 and was authenticated by FRI, Dehradun, Uttarakhand, India, where a voucher specimen no. 129 FHH was deposited for future reference.

Preparation of extract: Shade dried coarse powdered bark of *Pinus roxburghii* Sarg. in a quantity suficient as per the volume of extractor was packed in thimble (made of filter paper sheet), defatted with petroleum ether and then extracted with ethanol in a Soxhlet extractor. This extraction process was continued for about 48 h or until alcohol coming down the siphoning tube became colourless. The extract was concentrated by distilled off under reduced pressure using rotatory vacuum evaporator (Heidolph Laborota 4011, digital). The extracts thus obtained was weighted, transferred to separating flask, treated with n-butanol. The n-butanol fraction was then concentrated to 1 mL by bubbling nitrogen gas in to the solution. The 2 µL sample of the solution prepared in HPLC grade methanol was employed in GC-MS for analysis of different compounds.

GC-MS analysis: The GC-MS analysis was carried out using Agilent Chem station Gas Chromatograph equipped and coupled to a mass detector with a polar column. The instrument was set to an initial temperature of 60°C and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 210°C, at the rate of an increase of 3°C min^{-1} and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 mL min^{-1}. The ionization voltage was 70 eV. The samples were injected in split mode as 1:40 (Fig. 1).

Identification of phytoconstituents: Interpretation on Mass-Spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more 62,000

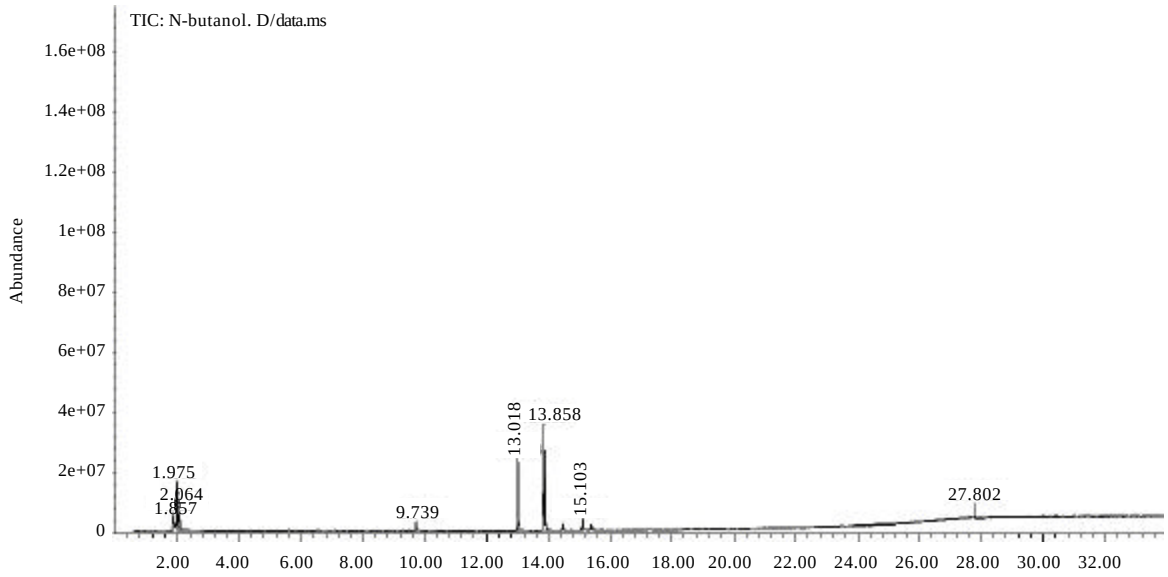

Fig. 1: GC-MS chromatogram of *Pinus roxburghii* Sarg. (Pinaceae)

patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The biological activities of constituents reported in *Pinus roxburghii* (Table 1) are based on data obtained from Pubchem an online database.

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fraction of the n-butanol fraction of ethanolic extract of *Pinus roxburghii* Sarg. these compounds were identified through mass spectrometery attached with GC. The results of the present study were tabulated in Table 2. The results revealed the presence of 2-chloropropionyl chloride, boric acid, trimethyl ester, benzoic acid, 4-ethoxy-, ethyl ester, antracene, phthalic acid, isobutyl octadecyl ester, dibutyl phthalate, 2,2 dibromocholestanone. The spectrum profile of GC-MS confirmed the presence of eight major components with retention time 1.857, 1.977, 2.036, 9.742, 13.021, 13.856, 15.104 and 27.801, respectively (Fig. 2a). The individual fragmentation pattern of the components were illustrated in Fig. 2a-h.

Table 1: Biological activities of identified constituents

Name of compound	RT (min)	Nature of compound	Biological activity
2-chloropropionyl chloride	1.857	Alkyl halide	Not reported
Boric acid, trimethyl ester	1.977	Ester	Not reported
1-chloro butane	2.036	Aliphatic hydrocarbon	Anthelmintic*
Benzoic acid, 4-ethoxy-, ethyl ester	9.742	Aromatic acid ester	Not reported
Anthracene	13.021	Aromatic hydrocarbon	Antiproliferative, cytotoxic*
Phthalic acid, isobutyl octadecyl ester	13.856	Aromatic acid ester	Not reported
Dibutyl phthalate	15.104	Aromatic ester	Plasticizer, antiproliferative, cytotoxic*
2,2-dibromocholestanone	27.801	Steroidal	Not reported

*Data obtained from pubchem

Table 2: GC-MS spectral analysis of ethanolic extract of *Pinus roxburghii*

Name of compound	RT (min)	Molecular formula	Molecular weight	Peak area (%)
2-chloropropionyl chloride	1.857	$C_3H_4Cl_2O$	126.96926	9.26
Boric acid, trimethyl ester	1.977	$C_3H_9BO_3$	103.91276	12.48
1-chloro butane	2.036	C_4H_9Cl	92.56726	9.13
Benzoic acid, 4-ethoxy-, ethyl ester	9.742	$C_{11}H_{14}O_3$	194.22706	2.43
Anthracene	13.021	$C_{14}H_{10}$	178.22920	23.02
Phthalic acid, isobutyl octadecyl ester	13.856	$C_{30}H_{50}O_4$	474.71560	38.69
Dibutyl phtalate	15.104	$C_{16}H_{22}O_4$	278.34348	3.91
2,2-dibromocholestanone	27.801	$C_{27}H_{44}Br_2O$	544.44566	1.08

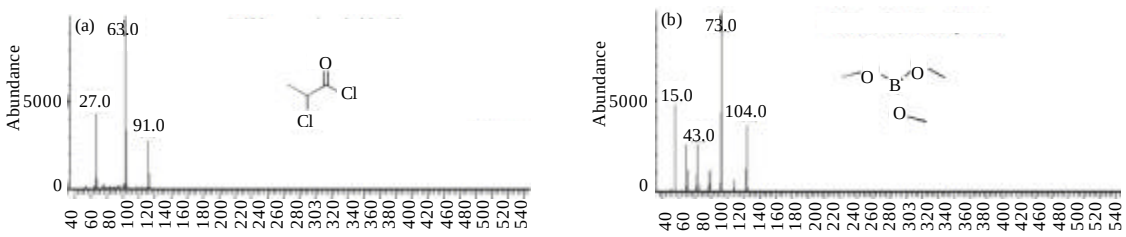

Fig. 2(a-h): Continue

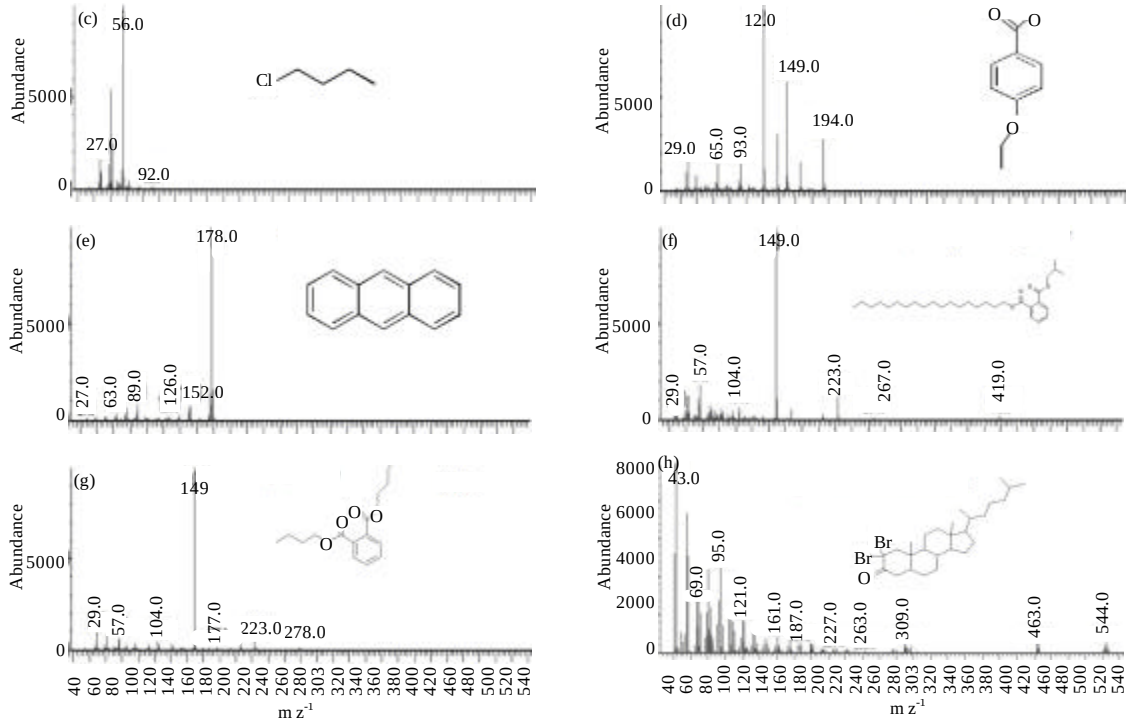

Fig. 2(a-h): Individual fragmentation pattern of the constituents identified from the n-butanol fraction of ethanolic extract of *Pinus roxburghii* Sarg., (a) 2-chloropropionyl chloride, (b) Boric acid, trimethyl ester, (c) 1-chloro butane, (d) Benzoic acid, 4-ethoxy-, ethl ester, (e) Anthracene, (f) Phthalic acid, isobutyl octadecyl ester, (g) Dibutyl phtalate and (h) 2,2-dibromocholestanone

CONCLUSION

The investigation concluded that the n-butanol fraction of ethanol extract of *Pinus roxburghii* Sarg. contains a number of active principles responsible for many biological activities such as chemopreventive, anticancer, anthelmintic, antiproliferative etc. This information can be utilized for development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

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