ORIGINAL ARTICLE

FATTY ACID COMPOSITION OF *ABIES PINDROW* (*West Himalayan fir*)

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ABSTRACT

The leaves of *Abies pindrow*, collected from Murree Hills, Punjab (Pakistan) revealed the presence of eleven fatty acids including eight saturated and three unsaturated fatty acids. They ranged from C_{14} to C_{24} and were detected as methyl esters by GC-MS technique. The saturated fatty acids were present in much greater proportion than unsaturated ones. Isopalmatic acid was found to be major saturated fatty acid and the oleic acid as predominant unsaturated acid. (+)-14-Methyl palmatic acid and (+)-Isosteric acid were the next higher saturated and unsaturated fatty acids respectively.

Keywords: *Abies pindrow*, *Pinaceae*, fatty acids, GC-MS analysis.

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INTRODUCTION

Abies pindrow (family *Pinaceae*) commonly known as West Himalayan Fir, occurs in North Western Himalayas at an altitude of 3000-4500 m. Trees up to 30 m tall or more, with a narrow pyramidal shape, bark fissured, light grey to brown. Leaves spiral, 1-4 cm long, upper surface grooved, dark green and shiny. Male cones 1-2 cm long, axillary, ellipsoid, reddish-green; microsporophyll with two linear sporangia; microspores winged. Female cones 8-12 cm long, solitary or in pairs, narrowly oblong, violet-purple; megasporophyll obovate, 2 cm long. Seeds 1-1.2 cm long; wing twice as long as the seed.

The ethanol extract of leaves of the plant which was collected from Murree region was got positively tested (Hussain *et al.,* 2004) for its hypoglycemic activity. The bioactivity test was done at Institute of Pharmaceutical Chemistry, Westfalische Withelms-Universitat, Munster, Germany.

Abies pindrow is reported (Rahman and Zaman, 1989) to be used as a folk medicine for its antidiabetic activity. The literature survey (Manral *et al.,* 1987; Singh *et al.,* 2000; Singh *et al.,* 2001; Tiwari and Minocha, 1980; Tripathi *et al.,* 1996a and Tripathi *et al.,* 1996b) reveals that apart from being hypoglycemic active, its uses in indigenous medicine are enormous. The leaves of plant are considered useful in the cases of cough, phthisis (Rahman *et al*; Khan *et al.,* 1979 and Kirtikar and Basu, 1918) and used as carminative, stomachic, astringent, expectorant (Rahman *et al*; Khan *et al.,* 1979; Kirtikar and Basu, 1918 and Chopra *et al.,*1956), tonic (Rahman *et al*; Khan *et al.,* 1979; Kirtikar and Basu, 1918; Chopra *et al.,*1956 and Said, 1969), and antispasmodic (Rahman *et al*; Khan *et al.,* 1979; Kirtikar and Basu, 1918; Chopra *et al.,*1956 and Said, 1969), valued in catarrh of bladder (Rahman *et al*; Kirtikar and Basu., 1918 and Nadkaml's, 1976) and pulmonary affections (Rahman *et al* and Nadkaml's, 1976), used in asthma (Rahman *et al*; Chopra *et al.,*1956; Nadkaml's, 1976 and Said, 1969), bronchitis (Rahman *et al*; Chopra *et al.,*1956; Nadkaml's, 1976; and Said, 1969), juice of leaves as regarded as antiperiodic (Rahman *et al*; Kirtikar and Basu, 1918 and Chopra *et al.,*1956), juice of fresh leaves is administered in fever of infants during dentition and affections of the chest (Rahman *et al*; Nadkaml's, 1976 and Said, 1969), given as tonic in cases of parturition (Rahman *et al*; Kirtikar and Basu, 1918 and Nadkaml's, 1976) the powdered leaves with juice of *Adhatoda vasica* relieves cough, asthma and haemoptysis (Rahman *et al*; Kirtikar and Basu, 1918 and Nadkaml's, 1976), used as remedy in fever (Rahman *et al*; Kirtikar and Basu, 1918) a confection called "Talisadadya Churna" is used (Rahman *et al* and Nadkaml's, 1976) in cough, asthma and haemoptysis, increases appetite, aids digestion, stops vomiting and diarrhea, alloys cough, dyspepsia and corrects flatulence, also given in enlarged spleen, normalizes temperature in cases of infants bronchopneumonia, prescribed in colds, consumption, hoarseness, hemorrhage and lung complaints, gum is used externally for headache, neuralgia and produces intoxification if mixed with oil of roses and taken internally (Rahman *et al* and Chopra *et al.,*1956), used as tonic for stomach and intestine and considered as aphrodisiac (Rahman *et al* and Said, 1969).

MATERIALS AND METHODS

Plant material

Abies pindrow leaves collected from the Murree Hills (Punjab) during the month of June 1999 dried under shade at room temperature for two weeks. The specimen was identified and deposited in the herbarium of Department of Biological Sciences, Quaid-i-Azam University, Islamabad.

Extraction

The leaves of the plant were dried under shade and powdered (coarsely). Whole of the amount (2.5 kg approx) was extracted with ethanol by percolation at room temperature for three times. The plant material was soaked for a week's period each time. The combined extract so obtained was evaporated at reduced pressure to afford a gummy residue (600 g).

Partitioning of the extract

Ethanol extract got by the above method was partitioned between ethyl acetate and water. The process was carried out five times. After separating both the layers the former ones were combined and evaporated under reduced pressure to get ethyl acetate extract (205 g).

Isolation of fatty acids

The ethyl acetate extract was subjected to vacuum liquid chromatography, using silica gel (TLC grade), eluting with n-hexane, dichloromethane, methanol. The elution was carried out by increasing gradually the order of polarity. The fractions obtained by eluting the extract with n-hexane-dichloromethane (3:7) were combined and evaporated to get semisolid material (5.86 g). The resulting fraction was further chromatographed by column chromatography, using silica gel 60 (particle size 0.2-0.5 mm, 35-70 mesh ASTM). The elution was carried out with pure hexane, hexane:ethylacetate, (4:1, 1:1 & 3:7), pure ethyl acetate, ethylacetate:methanol (4:1, 1:1 & 3:7) and then pure methanol. The elution with these solvents afforded oily fractions whereas the fractions eluted with hexane:ethylacetate were less viscous than those ethylacetate:methanol and methanol.

Preparation of methyl esters of fatty acids

Procedure Methanolysis of the glycerides in an alkaline medium: Transfered about two drops of oil (Ap5B3) into a 50 ml round-bottomed flask. Added about 20 ml of

methanol, two pellets of potassium hydroxide & boiling chips (fat free), refluxed for 30 minutes at 66-67^{0}C. Cooled the flask under the running water & transferred the contents to a separatory funnel. Rinsed the flask with 10 ml of hexane & mixed to the contents in separatory funnel. Added about 20 ml of water, shaken & allowed to separate. The ester passed into the upper hexane layer. Extracted the aqueous layer again with 20 ml of hexane. Combined the two extracts & washed them with 10 ml portions of water. Separated & dried the ester solution over anhydrous sodium sulphate Filtered through cotton wool into a conical flask & evaporated the solution down to approximately 10 ml over a boiling water bath then analyzed by GC-MS.

Identification

The fatty acids (methyl esters) were finally analyzed and identified by GC-MS. The analysis was performed on JEOL JMS 600H Agilest 68 g ON, equipped with 30 m × 0.32 mm HP-5 column, stationary phase coating 0.25 µm. The column temperature was kept at 50^0 C for 2 min with increased at the rate of 5^0 C per min up to 250^0 C. Injection temperature, 250^0 C, split ratio 1:35, the carrier gas (Nitrogen/Helium) flow rate 1.8 ml/min.

The FTIR spectrum exhibit the diagnostic peaks relating to C=O and C-O absorption at 1738 cm^{-1} and 1171 cm^{-1} respectively. These peaks verify the required data regarding the fatty acids methyl ester. The spectrum was obtained using a ThermoNicolet Avatar 330 FT-IR spectrometer controlled by OMNIC software (ThermoNicolet Analytical instruments, Madison, WI, USA) station with a deuterated triglycine sulfate (DTGS) detector and KBr optics. The sampling station was equipped with overhead ATR accessory (Spectra-Tech, Shelton, CT) comprising of transfer optics with in the chamber through which infrared radiation is directed to a detachable ATR zinc selenide crystal mounted in a shallow trough for sample containment. A single beam spectrum (4000-650 cm-1) of the sample was obtained against air as a background at a resolution of 4 cm^{-1} and a total of 32 scans.

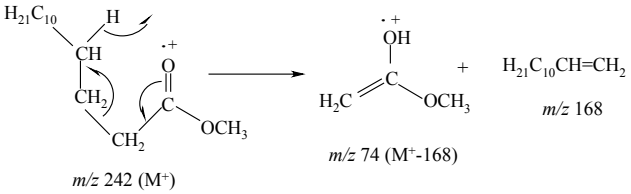
RESULTS AND DISCUSSION

The ethanol extract of leaves of *Abies pindrow* has been fractionated by partitioning into ethyl acetate and water. Ethyl acetate extract was further worked up by vacuum liquid chromatography as described in "Isolation of fatty acid". The resulting fraction was further chromatographed, leading to the identification of eleven fatty acids analyzed through GC-MS. It revealed the presence of methyl esters of *n*-Tetradecanoic acid, *n*-Pentadecanoic acid, 14-methyl-Pentadecanoic acid, Cyclopentaneundecenoic acid, methyl ester, 14-Methyl-hexadecanoic acid, methyl ester, 16-Methyl-heptadecanoic acid, methyl ester, *cis*-9-Octadecenoic acid methyl ester, 5, 9-Octadecadienoic acid, methyl ester, 17-Methyl-octadecanoic acid, methyl ester, Docosanoic acid methyl ester, & Tetracosoic acid, methyl ester. There are various methods for identifying the fatty acid composition of plants. Among them GC-MS is one of the most commonly used techniques to determine the composition of the volatile oil (Yayli *et al*., 2001).

The GC-MS analysis of leaves of *Abies pindrow* shows the presence of total twenty components, eleven of them have been identified as fatty acids methyl ester and nine components are unidentified. The GC-MS of the methylated fatty acid mixture showed the presence of eight saturated and three unsaturated fatty acid methyl esters. The amount of fatty acids methyl esters was found to be 56% in oily fraction separated by column chromatography of the extract of leaves of *Abies pindrow*. The amount of methyl Isopalmitate (16.33%) and methyl Oleate (14.46%) was higher in these fractions than other fatty acids. The minor components methyl Behenate and methyl Lignocerate were present as 0.66% and 0.575% respectively.

The identity of these common compounds of triglycerides was made by comparison of these peaks with the standards by gas chromatography and confirmed by the fragmentation pattern with those of standard mass spectra. The data indicated that amongst these fatty acids no any aromatic ring is present.

The appearance of fragment ion peaks at *m/z* 211, 59, and 74 is the common feature of fatty acids. For example the fragmentation of myristic acid methyl ester gives fragment ion $C_{13}H_{27}C{=}O^+$ at *m/z* 211 (M^+-31) by the loss of CH_3O^+. Another fragment ion at *m/z* 59 (M^+-183) result from the loss of $O{=}CO^+$ CH3 form the molecular ion. The base peak ion observed at *m/z* 74 (M^+-168) in the mass spectrum is formed by the *β*-cleavage by the McLafferty rearrangement as shown below.



The cleavage of C-C bonds gives the alkyl ions and oxygen containing ions. Thus there are hydrocarbon clusters at an interval of fourteen mass units; in each cluster there are prominent peak at *m/z* $C_nH_{2n-1}O_2$ (199, 157, 143, 129, 101, 87). The next abundant peak at *m/z* 87 (M^+-$C_{11}H_{23}$) represent the ion $(CH_2CH_2COOCH_3)^+$. The diagnostic bands for C=O and C-O groups may also be uncarpeted in the results and discussion.

The relative percentage and occurrence of these fatty acid methyl esters is indicated in table 1. The significant ions in the mass spectra of these esters are given below:

Table: Fatty acids of *Abies pindrow* analyzed as methyl esters.

FA Type	Systematic name	Common name	Molecular formula	Mol. Wt:	R.R.T	Rel. %age
C14:0	*n*-Tetradecanoic acid, methyl ester	Myristate	$C_{15}H_{30}O_2$	242	1.00	3.063
C15:0	*n*-Pentadecanoic acid, methyl ester	Pentadecylate	$C_{16}H_{32}O_2$	256	1.06	1.87
C16:0	14-methyl-Pentadecanoic acid, methyl ester	Isopalmitate	$C_{17}H_{34}O_2$	270	1.170	16.33
C16:1	Cyclopentaneundecenoic acid, methyl ester	-	$C_{17}H_{32}O_2$	268	1.1025	3.063
C17:0	14-Methyl-hexadecanoic acid, methyl ester	(+)-14-methyl palmitate	$C_{18}H_{36}O_2$	284	1.224	5.7852
C18:0	16-Methyl-heptadecanoic acid, methyl ester	(+)-Isosterate	$C_{19}H_{38}O_2$	298	1.325	5.44
C18:1	*cis*-9-Octadecenoic acid methyl ester	Oleate	$C_{19}H_{36}O_2$	296	1.318	14.46
C18:2	5,9-Octadecadienoic acid, methyl ester	-	$C_{19}H_{34}O_2$	294	1.286	2.55
C19:0	17-Methyl-octadecanoic acid, methyl ester	-	$C_{20}H_{40}O_2$	312	1.516	2.382
C22:0	Docosanoic acid methyl ester	Behenate	$C_{23}H_{46}O_2$	354	1.97	0.66
C24:0	Tetracosoic acid, methyl ester	Lignocerate	$C_{25}H_{50}O_2$	382	2.814	0.575

R.R.T = Relative retention time with respect to the methyl myristate.

Mass Spectral Data

n-Tetradecanoic acid, methyl ester
m/z 242 (M^+, $C_{15}H_{30}O_2$), 211 (M^+-31), 199 (M^+-43), 185, 157,145, 143, 129, 115, 101, 87, 74 (100%).

n-Pentadecanoic acid, methyl ester
m/z 256 (M^+, $C_{16}H_{32}O_2$), 225 (M^+-31), 213 (M^+-43), 199, 177, 157, 143, 129, 123, 111, 97, 87, 74 (100%).

14-methyl-Pentadecanoic acid, methyl ester
m/z 270 (M^+, $C_{17}H_{34}O_2$), 239 (M^+-31), 227 (M^+-43), 213, 199, 185, 171, 143, 129, 115, 97, 87, 74 (100%).

Cyclopentaneundecenoic acid, methyl ester
m/z 268 (M^+, $C_{17}H_{32}O_2$), 250 (M^+-18), 210 (M^+-58), 179, 165, 151, 124, 109, 95, 85, 71, 58 (100%).

14-Methyl-hexadecanoic acid, methyl ester
m/z 284 (M^+, $C_{18}H_{36}O_2$), 241 (M^+-43), 227 (M^+-57),199, 185, 157, 143, 129, 111, 97, 87, 74 (100%).

16-Methyl-heptadecanoic acid, methyl ester
m/z 298 (M^+, $C_{19}H_{38}O_2$), 267 (M^+-31), 255 (M^+-43), 213, 199, 185, 157, 143, 129, 101, 87, 83, 74 (100%).

***cis*-9-Octadecenoic acid methyl ester**
m/z 296 (M^+, $C_{19}H_{36}O_2$), 264 (M^+-32), 253 (M^+-43), 235, 222, 207, 194, 180, 166, 151, 143, 125, 111, 97, 83, 74, 69, 55 (100%).

5,9-Octadecadienoic acid, methyl ester
m/z 294 (M^+, $C_{19}H_{36}O_2$), 263 (M^+-31), 245 (M^+-29), 220, 205, 193, 179, 163, 149, 141, 121, 109, 95, 81 (100%).

17-Methyl-octadecanoic acid, methyl ester
m/z 312 (M^+, $C_{20}H_{40}O_2$), 269 (M^+-43), 255 (M^+-57), 227, 199, 185, 157, 143, 111, 97, 87, 74 (100%).

Docosanoic acid methyl ester
m/z 354 (M^+, $C_{23}H_{46}O_2$), 325 (M^+-19), 311 (M^+-43), 298, 269, 255, 241, 222,199, 185, 163, 143, 129, 111, 97, 87, 74 (100%).

Tetracosoic acid, methyl ester
m/z 382 (M^+, $C_{25}H_{50}O_2$), 351 (M^+-31), 339 (M^+-43), 297, 283, 241, 199, 185, 143, 129, 101, 87, 74 (100%).

CONCLUSION

Total eleven fatty acids including eight saturated and three unsaturated fatty acids ranged from C_{14} to C_{24} were identified by GC-MS from ethanol extract of *Abies pindrow*. The saturated fatty acids were present in much greater proportion (36.06%) than unsaturated ones (20.08%). Isopalmatic acid was found to be major saturated fatty acid (16.33%) and the Oleic acid as predominant unsaturated acid (14.46%). (+)-14-Methyl palmatic acid (5.79%) and (+)-Isosteric acid (5.44%) were the next higher saturated and unsaturated fatty acids respectively.

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