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STUDIES ON THE FIXED OIL OF JUNIPERUS MACROPODA BOISS SEEDS

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Abstract. The fixed oil (4.5%) of *Juniperus macropoda* has been examined by chemical and physical means. The saturated acids of the oil have been shown to consist of palmitic (9.1%) stearic (11.0%) and behenic (4.3%) acids, while the unsaturated ones are *palmetoleic* (1.6%) oleic (36.45%) and linoleic (23.45%) acids. The unsaponifiable matter consists of β-sito-sterol (14.5%).

Juniperus macropoda, a small to medium sized tree, belongs to the Cuprassacae family. It is found at the head of the Kaghan valley and in the inner Himalayas at 2600-2450 meters, the Quetta Division, the Kurram valley, Gilgit and Chitral. The plant bears a redish brown, 0.3-in-dia fruit in October. The pulpy matter of the fruit contains 4% of an essential oil and 30% sugars and is a successfully used raw material for the production of Gin.[1] The fruit also possesses some medicinal properties.[2,3] The stony portion of the fruit contains 4.5% of a fixed oil and does not appear to have been studied so far. Investigations were, therefore, carried out on the oil for the determination of its fatty acid composition as well as the characterisation of its non-saponifiable matter.

Experimental

The dry Juniper berries (1 kg) were pounded in a wooden pestle mortar and the seeds were hand picked. The seeds were freed from essential oil and resinous matter by washing with alcohol (300 ml and 200 ml) in an electric mincer. The so obtained seeds (300 g) were dried and crushed in an iron pestle mortar. They were extracted with petroleum ether (b.p. 40-60°) in a Soxhlet apparatus for 4 hr. The major portion of the solvent was removed by distillation under reduced pressure and its final traces by blowing a slow stream of nitrogen over the surface of the oil. A thin greenish yellow coloured oil (13.5 g 4.5% yield) with a peculair fatty oil odour was obtained.

Properties of the Oil. The physicochemical properties of the oil were determined by the usual methods[4(a,b)] which are recorded in Table 1.

TABLE 1.

Precentage yield of the oil	=	4.5
Refractive index (Abbe Refractometer)	=	1.46 at 25°
Colour of the cil	=	Yellow = 30, Blue = 2.5
Acid number	=	11.5
Saponification value	=	172.9
Iodine value (wij's 1 hour)	=	76.2
Thiocyanogen value	=	63.56
Maleic anhydride value	=	5.8
Saturated acids	=	24.4%
Unsaturated acids	=	61.5%
Unsaponifiable matter	=	14.05%

Saponification of the Oil. 6g of the oil was refluxed under nitrogen with 120 ml of 0.5 N alcoholic potassium hydroxide for 4 hr. The solvent was removed under reduced pressure and the residual soap was diluted with 150 ml of distilled water. The soap solution was extracted thrice with ether to remove the unsaponifiable matter (0.843 g 14.06% based on the oil). The aqueous phase was acidified with 30 ml of 2 N sulphuric acid and liberated fatty acids extracted with ether. After removal of the solvent a clear oily liquid (5.157 g; 85.95%) was obtained.

Resolution of the Acid Fraction. The iodine value showed the presence of unsaturated acids, which were separated by the Twitchell's lead salt-alcohol method.[5(a,b)] The solid acids (28.3% based on total acids) were obtained.

Preparation of Methyl Esters of the Total Fatty Acids[6]. The total acid fraction (3.0 g.) obtained above was taken in diethyl ether. A solution of diazomethane

in ether was added slowly until the yellow colour persisted. The solution was left for an hour and worked out. The methyl esters thus obtained were examined by IR spectroscopy, the appearance of an intensive peak at 1720-40 cm^{-1} and disappearance at 3600 cm^{-1} (2.78 μ) indicated full esterification of carboxyl group.

Examination of Methyl Esters by Gas Liquid Chromatography. A solution of methyl esters of the total fatty acids in petroleum ether were subjected to vapour phase gas chromatography Column of celite (60-70 mesh) coated with 20% polyethylene glycol succinate, column temperature 196°, carrier gas nitrogen at 30 lb psi. and chart speed 600 mm/hour. In addition to solvent peak, five well defined peaks and a shoulder before the second peak were obtained. The methyl esters of standard acids were run under the same conditions and the individual acids in the sample were identified by comparing their retention times.[7] The various saturated and unsaturated acids as identified by GLC are recorded alongwith their retention times in Table 2.

TABLE 2. GLC OF THE METHYL ESTERS OF JUNIPER BERRIES OIL

Acid	Retention time
1. Palmitic acid	34.0
2. Palmitoleic acid	63.0
3. Stearic acid	70
4. Oleic acid	91
5. Linoleic acid	123
6. Behenic acid	178

Further confirmation was achieved by the co-injection of supposed constituents. The acids on the basis of total fatty acids were estimated as under:

Palmitic acid (10.6%), Palmitoleic acid (1.9%), Stearic acid (12.8%) Oleic acid (42.42%), linoleic acid (27.27%) and Behenic acid (5.0%) from their respective areas.

Unsaponifiable Matter. The unsaponifiable matter gave a positive Liebermann-Burchard test,[8] characteristic of the 3-β-hydroxy steroids. It was recrystallised from methanol to give white leaflets m.p. 139-140°. The mixed m.p. with an authentic sample of β-sitosterol remained unchanged.

Discussion

Fatty acids from the oil of *Juniperus macropoda* Boiss seeds, were obtained by saponification, the low saponification value (207.7) of the oil indicate the presence of fatty acids of long carbon chain. The oil contains a high proportion of unsaturated acids (iodine value 76.2) of non conjugative structure (thiocyanogen value 63.56) however, the maleic anhydride value (5.8) predicts the presence of a low percentage of acids having non conjugative structure also.

The methyl esters of total fatty a cids were subjected to vapour phase chromatography and were found to consist of palmitic, palmitoleic, stearic, oleic, linoleic and behenic acids.

These acids were further confirmed by separating them into saturated and unsaturated acids by Twitchell's lead-salt-alcohol method.[5(a,b)] The saturated acids were examined separately by gas liquid chromatography which confirmed the presence of palmitic, stearic and behenic acids.

The unsaturated acids were hydrogenated by Brown's method[9] in par Fisher hydrogenator using Raney Nickel catalyst. The methyl esters of the hydrogenated acids were then examined by gas liquid chromatography which showed the presence of fatty acids of C_{16} and C_{18} series. The liquid acids were also examined by making their bromoderivatives[10] when only tetrabromo-stearic acid (m.p. 115°) was obtained. The overall composition of the oil was found to be as follows:

	β-sitosterol	= 14.05%
Saturated acids		
	Palmitic acid	= 9.1%
	Stearic acid	= 11.0%
	Behenic acid	= 4.3%
Unsaturated acids		
	Palmitoleic acid	= 1.6%
	Oleic acid	= 36.45%
	Linoleic acid	= 23.45%

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