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STUDIES ON THE FIXED OIL OF THE SEEDS OF *ALBIZIA PROCERA*. *Part III*

MUNIR AHMED, F. M. CHAUDHARY AND M. BASHIR
PCSIR Laboratories Complex , Lahore-54600, Pakistan

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A fixed oil to the extent of 4.9% has been extracted from the seeds of *Albizia procera* which belongs to the family Leguminosae. Its physical properties, neutral lipid fractions, fatty acid composition and vitamin A and B contents have been studied. The oil is found to countain myristic (0.34%), palmitic (20.49%), stearic (10.22%), arachidic (3.29%), behenic (6.81%), lignoceric (1.48%), palmitoleic (2.58%), oleic (22.45%), linoleic (28.13%), linolenic (2.40%), and behenoleic (1.8%) acids. The vitamin A is found to be 24 I.U while vitamin D 2 I.U.

Key words: Fixed oil, Fatty acids, Esterification.

Introduction

We have already reported our studies on the fixed oil of the seeds of *Albizia lebbek* [1] which showed a noticeable difference in the various results published by the previous authors [2-7]. This prompted us to study the other varieties of *Albizia* so that the relevant results of the fixed oil of the seeds of the different varieties of this plant may be rectified and correct information may be brought on the record which is based on modern techniques. Our present studies on the fixed oil of *Albizia procera* have also shown a noticeable difference in the various results published by the previous workers, so it has been considered necessary to publish our findings. We have also dealt with the vitamin A and D contents and fractionation of the oil into lipid classes which are quite important in determining the quality of the oil for edible purposes.

Material and Methods

The course of studies was the same as followed by us previously [1]. The seeds were collected from the pods and crushed to a powdery material. This was extracted with *n*-hexane which yielded a yellow-brown oil (4.9%). The physical constants were determined by the standard procedures [8]. Vitamin A and D contents were determined according to the U.S.P. XX [9]. Fractionation into the lipid classes was carried out as follows:-

Oil 0.8 g was subjected to the thin layer chromatography. Glass plates (20x20 cm) coated with 1 mm thickness kieselgel (60 G Art 7731) were taken as chromatograms and these were developed in a mixed solvent having the volumetric compositions as, hexane: diethylether: acetic acid (80:2:1) [10,11]. A solution (0.2%) of 2',7'-dichlorofluorescein in ethanol was used as a spray for marked visibility under U.V. light. Typical R_f's of the lipid classes were hydrocarbons 0.92, wax esters 0.79, triglyceride 0.53, free fatty acids 0.42, diglyceride 0.32, sterol 0.24, monoglyceride 0.18 and polar lipids 0.02. Lipid classes were identified by comparison of their R_f's with those of the standard under identical conditions. The polar band having R_f (0.02) did not move and remained at the origin of the chromatogram. The identified bands were removed and extracted with chloroform. The contents were isolated under reduced pressure. The results are given in Table 2.

Isolation of fatty acids was carried out by standard methods of saponification [8]. The fatty acids thus obtained were esterified into methyl esters. A comparative study of the infrared spectra of the fatty acids and their esters was carried out. The ester spectrum showed the absence of carboxyl peak at 2.9μ and also the shifting of carbonyl peak from 5.9μ to 5.7μ. This result indicated that all the fatty acids have been esterified. They were then purified by column chromatography using silica gel 60 and *n*-hexane as an eluent. Purity was checked by TLC. The methyl esters were identified on a Pye Unicam 104 series gas chromatograph fitted with an F. I. detector using W COT carbowax 20 meter column. Hydrogen was used as the carrier gas having 14 lbs per sq. inch pressure. The sample size was 0.02 ml. The temperature was programmed as 150° for 5 min. with 10 min. increase to 220°, while detector and injector temperature were 300° and 250°, respectively. The qualitative and quantitative identification of the fatty acids was confirmed by running a standard mixture under identical conditions.

Results and Discussion

The physico-chemical characteristics of the seed oil of *Albizia procera* are given in Table 1. The results of the fractionation of the oil into lipid classes have been reported in Table 2. Neutral lipids are the main constituents (99.1%) while the polar lipids are minor constituents (0.9%). Amongst the neutral lipids the triglycerides are present in abundance (76.8%). A comparative study of the fatty acid composition of the saponifiable matter of the oil is presented in Table 3. This study has been carried out to compare the fatty acids position of the seed oil of other species of *Albizia* [1,4,6,7,12-14] and also with results published by the previous workers on the species under discussion. Interestingly the percentage of the total saturated and unsaturated fatty acids in *A. procera* has not been found to differ noticeably from the one determined

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF THE SEED OIL OF *ALBIZIA PROCERA*

Fixed oil	4.9%
Colour	Yellow-brown
Specific gravity	1.091
Refractive index at 30°	1.465
Saponification value	172.302
Acid value	3.284
Iodine value	108.104
Ester value	169.018
I.N.S. value	64.197
Peroxide value	27.87
Unsaponifiable matter	2.14
Vitamin A	24 I.U. per gm.
Vitamin D	2 I. U. Per gm.

TABLE 2

(a). Wt% of Lipid fraction of *Albizia procera* seed oil.	
Neutral lipid	99.1%
Polar lipid	0.9%
(b). Fractions of the neutral lipids of *Albizia procera* seed oils.	
Hydrocarbons	0.8%
Wax esters	2.9%
Triglycerides	74.5%
Diglycerides	10.6%
Monoglycerides	5.5%
Free fatty acids	1.8%
Sterols	3.9%

by the previous workers [11], but an amazing difference has been found in the precentage of the individual fatty acids. For instance, amongst the unsaturated acids, oleic acid has been found to be 22.45% and linoleic acid 28.13% of the total fatty acids, while the previous workers have determined them as 50.89% and 15.14%, respectively. Similary amongst the saturated acids, palmitic acid has been found to be 20.49% and arachidic acid 3.29 while the previous workers have published as 7.23% and 12.21%, respectively. Besides these alarming percentage differences we have also found the presence of behenic, lignoceric, linolenic, palmitoleic and behenoleic acids in this fixed oil.

The cause of such discrepancies in the results could be found either in the methodology or genetic variation within the species as was discussed previously [1]. The riddle of genetic variation within the species was similarly solved by collecting samples from the various areas of Pakistan. We found that all of them gave almost the same results. The difference in climate or soil did bear only a negligible effect on the fatty acids composition of the seed oil. Therefore, differences in methodology are responsible for the variation in results. The previous workers have used only the TLC technique which is not capable of providing accurate results. Our finding are based on the use of high pressure gas liquid chromatography, a modern technique that provides reliable results.

Work is in progress for the identification and structure elucidation of the sterols and wax esters present in the oil

TABLE 3. COMPARATIVE STUDY OF THE FATTY ACIDS IN *ALBIZIA*

Acids	*Albizia lebbek*			*Albizia procera*			*Albizia amara*	*Albizia odoratissima*
	Kafuku and Hata [5] %	D.N. Grindley [6] %	Farooq and Varshney [7] %	Munir & Abid [1] %	Farooq & Siddiqui[14] %	Munir & Bashir present study %	Chandra *et .al* .[13] %	Farooq & Siddiqui[12] %
(i) Saturated	29.0	29.0	27.78	25.58	33.70	42.63	17.57	22.02
Myristic	Small quantities	–	–	0.11	–	0.34	1.62	–
Palmitic	Small quantities	The large quantities	7.26	16.17	7.23	20.49	8.04	14.33
Stearic	–	–	9.63	2.71	14.26	10.22	4.55	6.88
Arachidic	The large quantities	–	10.89	2.01	12.21	3.29	2.30	0.81
Behenic	–	3.19	–	4.57	–	6.81	0.61	–
Lignoceric	–	–	–	–	–	1.48	0.45	–
(ii) Unsaturated	71.0	71.0	72.22	72.46	66.30	57.37	82.43	77.98
Palmitoleic	–	–	–	–	–	2.58	–	–
Oleic	Present	43.0	39.28	18.23	50.89	22.45	33.18	26.56
Linoleic	Present	28.0	32.94	54.06	15.41	28.13	49.25	51.41
Linolenic	–	–	–	0.16	–	2.40	–	–
Behenoleic	–	–	–	1.81	–	–	–	–

which may be useful for various purposes.

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References

1. Munir Ahmed, F.M. Chaudhary and Abid Hussain Shah, Pak. j. sci. ind. res., **32,** 361 (1989).
2. A. Sen Gupta and M.M. Chakrabarty, Ind. J. Appl. Chem. , **21,** 227 (1958).
3. A. Sen. Gupta and M.M. Chakrabarty, Ind. J. Appl. Chem., **27,** 49 (1964).
4. A. R. Chowdhary, R. Benesji, G. Misra and S.K. Nigam, J. Am. Oil chem. Soc., **61,** 1023 (1984).
5. K. Kafuku and C. Hata, J. Chem. Soc. Japan, **55,** 369 (1934).
6. D. N. Grindley, J. Chem., Soc. Ind. , **64,** 152 (1945).
7. M. D. Farooq and I. P. Varshney, Bull. Soc. Chim. France, 729 (1954).
8. J. Devine and P. N. Williams, *The Chemistry and Technology of Edible Oil and Fats* (Pergamon Press, 1961).
9. U.S.P. (United States Pharmaceutical Convention, Inc) Vol. 20, pp. 933.
10. Egon Stahl, *Thin -Layer Chromatography* , (George Allen and Unwin Ltd., London, 1969), 2nd edition., pp. 377.
11. V. P. Skipski, A. F. Smolowe, R. C. Sullivan and M. Barclay, Bio Chem. Bipphys. Acta, **106**, 386 (1965).
12. M. O. Farooq, M. S. Siddiqui, Bull Soc. Chim. France, 741 (1954).
13. I. Chandra, R.P. Sud and K. L. Handa, J. Sci. Ind. res., **15-B** (1956).
14. M. O. Farooq, I. P. Varshney, M. S. Siddiqui and Hameedul Hasan, Bull. Soc. Chim. Biol., **5-6,** 901 (1959).