Seasonal Variations in the Seed Oil of *Thevetia peruviana* (Pers.) K. Schum.

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The fixed oil of *Thevetia peruviana* (Pers.) K. Schum. (*T. neriifolia* Juss) was obtained by solvent extraction, and its yield and composition were studied to reflect the effect of seasonal variations.

Ripe and unripe seeds collected in December and February (the dry season) gave average yields of 72 and 52%, v/w, respectively, and those of May and August (the rainy season) gave 56 and 41%, v/w, respectively.

Sitosterol was isolated and identified from the unsaponifiable fraction. Oleic, linoleic, stearic and palmitic acids were shown to be present in all the oils, while myristic, lauric, capric and caprylic acids were identified only in the oil from unripe seeds collected in the rainy season. The physical and chemical constants of the oils are given also.

KEY WORDS: Capric acid, caprylic acid, lauric acid, seed oil, sitosterol, thevetia.

Thevetia peruviana (Pers.) K. Schum. (*T. neriifolia* Juss, fam. Apocynaceae) is a shrub of 2–6 m high that thrives in both arid and wet climates. It is a widely used ornamental plant that both flowers and bears fruit all year round. The yield of oil and glyceride composition of the ripe seeds have been studied by several researchers (1–6). Miralles (7) showed that the unsaponifiable fraction contained 55% total hydrocarbons, 10% tocopherol, 12% triterpenic alcohols and 20% sterols.

In this work we report the yields and composition of the oils contained from seeds collected in different seasons and at different morphological stages of development. The study has revealed the presence of three fatty acids—lauric, capric and caprylic—in the oil. Tocopherol, reported to be present in the Senegalese varieties by Miralles (7), was shown to be absent in the Nigerian cultivar.

EXPERIMENTAL PROCEDURES

Materials. Seeds were collected from cultivated plants growing on the main campus of the University of Benin, Benin City, Nigeria. Ripe and unripe seeds were collected in February, May, August and December. The kernels were allowed to stand for five days to allow partial drying before removal of the seeds. β-Sitosterol, cholesterol, fatty acid methyl ester and tocopherol were obtained from Sigma Chemical Co. (London, U.K.).

Procedures. Standard methods (8) were used to determine the physical and chemical constants of the oils. Transmethylation of the oil was achieved by refluxing it with 30% H_2SO_4 in methanol for 8 hr at 40°C. Extraction with petroleum spirit (60–80°C) followed by evaporation of the solvent afforded methyl esters of the fatty acids. Esters were analyzed on a Pye Unicam series 304 chromatograph (Pye Unicam, Cambridge, U.K.) with a 1.5 m × 4 mm I.D. glass column packed with 10% diethylene glycol adipate on 100–200 mesh diatomite C-AW treated with H_3PO_4 and maintained at 190°C. Carrier (nitrogen) gas flow rate was 45 mL per min.

The nonsaponifiable fraction (NSF) was obtained after refluxing the oil with methanolic KOH for 2 hr and extracting it with diethyl ether. Thin-layer chromatography (TLC) of the NSF was carried out on activated 0.25 mm layers of silica gel G, and the preparative layer chromatogram (P-TLC) employed 1.00 mm layers of mixed indicator silica gel type Pf 254 + 366. The developing solvent was benzene:methanol (90:1). Compounds present on the TLC plate were visualized by chromogenic sprays: antimony (V) chloride/rhodamine B for tocopherols, and Lieberman-Burchard reagent/vanillin-H_2SO_4 for steroids (9).

The crystalline sterol isolated from the P-TLC band of the NSF was acetylated with acetic anhydride and pyridine, and was then taken up in chloroform. The chloroform extract was analyzed on the chromatograph with a 10% Apiezon L column programmed from 100–200°C at 10°C per min and a nitrogen flow rate of 40 mL per min.

UV spectra of the chloroform extracts of the NSF and of the separated P-TLC bands were determined on a Perkin Elmer 5505 instrument with a scan range of 200-400 nm (Perkin Elmer, Norwalk, CT) by comparison with standard sitosterol and tocopherol.

RESULTS AND DISCUSSION

Previous studies of *T. peruviana* have not considered the effect of seasonal variation on the yield and composition of the seed oil. This cultivar, as shown in Table 1, contains a higher % v/w of oil during the dry season than in the rainy season. The NSF was less than 1%. The physical and chemical constants shown in Table 2 reveal variations when compared with documented values (2,10).

TLC analysis of the NSF indicated the presence of steroids as visualized with Lieberman-Burchard re-

TABLE 1

Seasonal Changes in the Seed-Oil Yield of *T. peruviana*

	Yield of oil (per 110 g of seeds)			
	Ripe		Unripe	
Month	mL	% v/w	mL	% v/w
February	92.20	83.82	61.95	56.32
May	63.80	58.00	46.20	42.00
August	59.95	54.50	43.75	39.77
December	77.20	70.18	53.35	48.50

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TABLE 2

Physicochemical Constants of Oils from Different Seasons

	Month of collection and conditions							
	February		May		August		December	
	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe
Density at 27°C	0.8463	0.8289	0.8769	0.8711	0.8726	0.8799	0.8466	0.8315
Refractive index at 27°C	1.4523	1.4543	1.4543	1.4520	1.4455	1.5510	1.4500	1.4486
Saponification number	182	181	184	185	180	195	183	181
Ester value	180.32	177.07	180.45	180.85	175.29	189.95	181.0	181.0
Acid value	1.68	3.93	3.54	4.05	4.71	5.05	2.00	3.85
Iodine number	86	86	83	84	71	56	84	86

TABLE 3

Fatty Acid Composition of *Thevetia* Oils

	Percentage							
	Caprylate $C_{8:0}$	Caprate $C_{10:0}$	Laurate $C_{12:0}$	Myristate $C_{14:0}$	Palmitate $C_{16:0}$	Stearate $C_{18:0}$	Oleate $C_{18:1}$	Linoleate $C_{18:2}$
February[a]	—	—	—	—	26.40	9.09	44.60	18.01
May[a]	—	—	—	—	27.86	14.18	37.04	19.22
August[a]	—	—	—	—	25.65	16.96	46.01	10.36
December[a]	—	—	—	—	25.95	10.06	44.20	17.90
February[b]	—	—	—	—	30.94	7.95	44.63	17.90
May[b]	—	—	—	—	24.57	13.47	44.08	16.09
August[b]	3.96	1.94	18.63	8.71	15.02	4.14	30.23	16.03
December[b]	—	—	—	—	29.85	7.26	44.10	16.00

[a]Methyl esters of ripe seed-oil.
[b]Methyl esters of unripe seed-oil.

agent. Sitosterol was isolated from the P-TLC and recrystallized in acetone. It was characterized by its melting point (136–137°C), co-TLC and GLC retention times of 7.7 and 8.6 min, equivalent to sitosterol acetate, thus indicating the presence of isomers. Chromogenic sprays, antimony (V) chloride and rhodamine B specific for tocopherols, coupled with uv analysis of the extracts from the P-TLC bands, did not reveal the presence of tocopherols.

The quantitative relationship between the fatty acids from the oils studied is given in Table 3. Oil from unripe seeds collected in August showed the additional saturated fatty acids of lauric, capric and caprylic.

Thevetia peruviana seed oil compares favorably with respect to yield, composition and properties to commonly used pharmaceutical oils. Purification to remove toxic glycosides (11) will afford a suitable substitute for unsaturated fixed oils.

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