Unsaponifiable Lipid Constituents of Ten Indian Seed Oils

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The unsaponifiable lipid constituents, hydrocarbons, triterpene alcohols and sterols of ten seed oils (*Catharanthus roseus, Nymphaea nelumbo, Casuarina equisetifolia, Lagerstroemia therolli, Prosopis juliflora, Mimusops elengi, Mimusops hexandra, Pongamia pinnata, Acrocarpus fraxinifolius,* and *Bauhinia retusa*) were investigated by gas liquid chromatography. Total unsaponifiables ran from 4-14%. Some of the seed oils contained large quantities of β-amyrin, α-amyrin and cycloartenol. *Acrocarpus fraxinifolius* was found to contain 84% of lupeol. Stigmasterol (24-ethyl-22ε-dehydrocholesterol), β-sitosterol (24-ethyl-cholesterol) and campesterol (24-methyl-cholesterol) were the common constituents in all the seed oils. Besides these constituents, tirucallol, taraxerol, Ψ-taraxasterol, fucosterol, isofucosterol, avenasterol and cholesterol also were detected in small quantities.

KEY WORDS: Estimation, gas-liquid chromatography, hydrocarbons, identification, seed oils, sterols, triterpenols, unsaponifiable lipids.

Unsaponfiable lipid constituents of seed oils contain a variety of biologically active materials, including hydrocarbons, tocopherols, sterols and terpene alcohols. Terpene alcohols have shown cytotoxic (1-4), cytostatic (5), antimicrobial (6,7), herbicidal (8,9) and anti-inflammatory activities (10,11). A great variety of sterols with insect molting hormone activity occur in plants (12). Sterols function as precursors of other steroids, as hormones and as membrane components.

Identification and quantitation of unsaponifiable lipid constituents become difficult because individual components within a class are usually present in very small amounts. In recent years (13) many unsaponifiable constituents of vegetable oils have been analyzed precisely by using capillary glass columns in gas liquid chromatography (GLC). Toshihiro Akihisa and co-workers (14-16) have extensively investigated the distribution and occurrence of sterols, triterpene alcohols and other unsaponifiable constituents of many vegetable oils. Although many other reports have been published on the analysis of unsaponifiables of vegetable oils, much remains obscure and needs to be studied further.

The present investigation reports the results of the analysis of unsaponifiable constituents, i.e., hydrocarbons, triterpene alcohols and sterols, of seed oils of ten uncultivated plants.

MATERIALS AND METHODS

The seed samples for the present study were obtained by a staff botanist from various parts of the country or were purchased from commercial seed suppliers.

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TABLE 1

Systematic Names and Codes of Ten Seeds Investigated

Code	Botanical name and families	% Unsap.
CR	*Catharanthus roseus* (L.) G. Don Apocynaceae	14.0
NN	*Nymphaea nelumbo* L. Nymphaeaceae	10.0
CE	*Casuarina equisetifolia* L. Casuarinaceae	8.0
LT	*Lagerstroemia therolli* L. Lythraceae	7.7
PG	*Prosopis juliflora* (Sw.) D.C. Fabaceae	7.0
ME	*Mimusops elengi* L. Sapotaceae	6.4
MH	*Mimusops hexandra* Roxb. Sapotaceae	4.0
PP	*Pongamia pinnata* (L.) Pierre Fabaceae	5.5
AF	*Acrocarpus fraxinifolius* Wight and Arn. Caesalpiniaceae	5.0
BR	*Bauhinia retusa* Roxb. Fabaceae	4.8

Unsaponifiable materials from the seed oils (Table 1) were obtained from the saponified oils for the study of hydrocarbons, triterpene alcohols and sterols. A mixture of *n*-alkenes was used as reference hydrocarbons.

The acetate derivatives of sterols and triterpene alcohols were used as a reference for sterols and triterpene alcohols and are given in Table 2.

Extraction of oils. Cleaned and dried samples of seed were ground in a disentegrator. The powdered seeds were extracted repeatedly with petroleum ether in a Soxhlet apparatus. The extracted oils were dried over anhydrous sodium sulphate. The solution was filtered and the solvent removed under reduced pressure in a rotary film evaporator.

Isolation of unsaponifiable lipids. Oil (10 g) dissolved in 100 mL ethanolic potassium hydroxide (1N) was refluxed for 1 hr under nitrogen. The reaction mixture was then diluted with 200 mL water, and the unsaponifiable material was extracted three times with 100 mL of diethyl ether. The combined diethyl ether extract was first washed with 100 mL aqueous solution of potassium hydroxide (0.5 N), followed by washing with 5 × 100 mL of water and dried over anhydrous sodium sulphate. The solution was filtered and the solvent was removed in a rotary film evaporator as described earlier. The content of unsaponifiables in oil was expressed by weight percent (Table 1).

Preparative thin-layer chromatography (TLC). A chloroform solution (50%) of unsaponifiable material (30 mg/plate) was applied uniformly along a line 1.5 cm from the edge of a 25 cm × 25 cm plate coated with 0.5 mm

TABLE 2

Relative Retention Times[a] of Acetate Derivatives of Triterpene Alcohols and Sterols Used as Reference Compounds in GLC

Code	RRT OV-17	Compound (Acetate)
Triterpene Alcohol		
a	1.30	24-Dihydrolanosterol
b	1.47	Tirucallol
c	1.55	Taraxerol
d	1.63	β-Amyrin
e	1.69	Butyrospermol
f	1.76	24-Methylene-24-dihydrolanosterol
g	1.84	α-Amyrin
h	1.86	Cycloaretenol
i	1.92	Lupeol
j	1.98	24-Methylene-24-dihydroparkeol
k	2.07	24-Methylenecycloartanol
l	2.38	Ψ-Taraxasterol
m	2.48	Taraxasterol
Sterol		
n	1.00	Cholesterol
o	1.14	24-Methyl-22*E*-dehydrocholesterol
p	1.31	24-Methylcholesterol (campesterol)
q	1.35	24-Methylenecholesterol
r	1.43	24-Ethyl-22*E*-dehydrocholesterol (stigmasterol)
s	1.63	24-Ethylcholesterol (β-sitosterol)
t	1.69	24-Ethyl-22*E*-dehydrolathosterol
u	1.72	Fucosterol (24-ethylidencholesterol)
v	1.81	Isofucosterol
w	1.92	24-Ethyllathosterol
x	2.13	Avenasterol

[a]Expressed relative to cholesterol acetate (RRT = 1.00).

layer of silica gel (G) and developed three times with hexane/ethyl acetate (6:1, v/v) as developing solvent. After developing, the plate was sprayed with a solution of rhodamine-6G in ethanol (0.05%) and observed under ultraviolet light (3600 A) for marking the separate zones, containing less polar compounds (n-alkane, R_f 0.9–1.0), triterpene alcohols (4-4'- dimethyl sterol, R_f 0.4–0.5) and sterols (4-dimethyl sterol, R_f 0.02–0.04). Each zone was carefully scraped from the plate and thoroughly extracted with diethyl ether. In all samples, hydrocarbons were minor constituents and triterpene alcohols were present in substantial quantity, while the sterols were the major constituents. The sterol fraction was further purified by repeated preparative TLC for subsequent GLC analysis.

Acetylation. Free sterols and triterpene alcohols were converted to acetates (13) by allowing 10 mg of compound to stand in acetic anhydride (0.5 mL) and pyridine (0.5 mL) in a round bottom flask for 24 hr at room temperature. The reagents were removed in a stream of nitrogen with gentle warming. The residue thus obtained was mixed with water and then extracted with diethyl ether. The ether layer was washed with water and dried over anhydrous sodium sulfate. The solution was filtered and the solvent was removed under reduced pressure in a rotary film evaporator.

Gas liquid chromatography (GLC). GLC was performed with a Shimadzu GC-4CM instrument Shimadzu (Scientific Instruments, Columbia, MI) on a Scot OV-17 glass capillary column (30 m × 0.3 mm i.d.). Flame ionization was used for detection. The acetylated triterpene alcohols and sterols were analyzed on an OV-17 column with the column temperature at 260°C and injection temperature at 280°C. Relative retention time (RRT) values of acetyl derivatives were expressed relative to cholesterol acetate (1.00). Table 2 shows the RRTs in GLC of the authentic sterol and triterpene acetates. Hydrocarbons were analyzed on an OV-17 column at programmed column temperature of 170–260°C (6°/min) and then isothermally at 260°C, with injection temperature at 280°C. Retention times of hydrocarbons were expressed as methylene unit (MU) values (13).

Biological activity. Petroleum ether extracts and unsaponifiable materials of *Casuarina equisetifolia*, *Bauhinia retusa*, *Pongamia pinnata* and *Nymphea nelumbo* were screened for toxicity and repellent activity against *Tribolium castaneum* Herbst. Two- to three-week-old insects (reared in the laboratory) were used for screening. Toxicity tests were performed by stomach poisoning and topical application methods.

Stomach poisoning. Stock solutions of 1% concentration of each compound were prepared in acetone. Three replicates for each compound were run, 0.1 mL solution of the compound was mixed with 100 mg of sterilized wheat flour and kept in a 50 mL beaker. Twenty adults of *Tribolium castaneum* Herbst were released into the beakers. Controls were run simultaneously in which only solvent was used. These beakers were kept in a BOD incubator (Lab Equipment Industries, New Delhi, India) at 28° ± 1°C and 75 ± 5% RH. The insects were observed at 24 hr intervals for 15 days, and mortality, if any, was counted.

Topical application. Stock solutions of same concentration as in stomach poisoning were used for topical application. The compounds were applied on the dorsum of the insects (in the thoracic region) in microliter doses by a microapplicator. Three replicates of 20 insects each were run. The treated insects were released into beakers containing sterilized wheat flour and kept in a BOD incubator (Lab Equipment Industries, New Delhi, India) at 28 ± 1°C and 75 ± 5% RH. The insects were observed after 24 hr intervals for mortality count and were kept for 15 days before being discarded.

Repellency test. Repellency was determined by using Schleicher and Schuell discs, as in our previous paper (17).

RESULTS AND DISCUSSION

The unsaponifiable constituents of the seed lipids (Table 1) were separated into hydrocarbon, triterpene alcohol and phytosterol fractions by means of preparative silica gel TLC.

The hydrocarbon fractions were analyzed directly by GLC on an OV-17 glass capillary column (Table 3). The GLC analysis of hydrocarbon fractions showed mixtures of C_{16-37} *n*-alkanes. The ratio of odd to even numbered alkanes (3:2) is much less than that found in higher plants (18). The composition of hydrocarbon fractions of 10 seed oils is given in Table 3.

The triterpene alcohol fractions were analyzed as acetyl derivatives on an OV-17 column. The identification of individual components was performed by comparing their

TABLE 3

Composition of Hydrocarbon Fractions of Ten Indian Plants[a]

	Plant species[c]									
MU[b]	NN	MH	ME	VR	LT	PG	CE	AF	BR	PP
16		2.9	3.4						1.0	
17	1.1	4.8	7.3	2.2		1.2			5.3	
18	3.4	5.6	7.1	5.4	5.4	3.0	0.3	1.7	4.4	0.5
19	7.4	5.8	5.9	4.0	5.9	3.2	1.7	2.3	12.3	5.0
20	7.1	5.3	5.3	6.0	5.3	3.2	2.6	2.2	4.7	5.3
21	6.3	4.9	4.5	3.0	4.8	2.5	1.9	2.1	6.7	5.0
21.9	5.4			2.4		2.2				
22	26.2	4.5	4.2	4.4	4.2	4.2	1.8	1.9	4.4	5.0
22.1		2.2							1.9	
22.8			1.5						1.0	
23	6.3	4.6	3.8	1.8	4.3	2.0	1.5	1.8	5.0	4.6
24	3.4	4.6	4.0	1.4	4.4	2.2	1.4	1.9	4.2	6.1
25	2.8	4.7	4.0	2.6	6.0	2.9	1.5	3.0	5.5	6.7
26	2.6	5.5	4.4	1.6	5.1	3.7	2.4	2.2	5.9	7.8
26.7		2.2				2.0	1.8			
27	2.0	6.2	5.4	5.4	12.4	4.4	1.7	5.6	5.8	9.1
27.6			0.5							
27.7			1.8	5.2	0.4	31.0		1.3	3.1	
28	25.1	6.6	5.9	2.2	5.9	4.6	4.3	2.6	6.7	9.1
28.1			0.8							
29	0.9	6.7	6.3	9.9	12.2	5.9	2.0	12.8	6.7	8.7
29.1				25.2						
29.7					2.4					
30		5.3	4.8	1.6	4.6	3.9	9.8	2.3	4.7	6.1
31		5.0	5.6	6.4	11.3	5.8	1.8	13.2	4.0	4.9
31.6				0.8				10.0		
32		4.2	3.7	1.0	2.5	2.7	15.3	1.7	2.9	3.6
32.2				2.8						
32.7							11.8			
33		4.3	4.5	2.8	4.3	3.0	1.8	3.8	1.5	2.9
34		2.5	1.8	tr.	0.3	2.7	3.6	3.1	0.5	1.1
34.3		1.6			0.9		0.7			
34.5				tr.						
34.8							1.0			
35			0.8		0.6	0.7	4.0	0.9	0.2	0.9
35.1								8.4		
35.3									4.9	
36						1.0	4.4	1.1	0.1	
37							8.0		0.2	
Others			2.7	1.4		0.3	14.7	2.9	0.3	7.6

[a]Determined on OV-17 glass capillary column; tr. = trace.

[b]MU, methylene unit values.

[c]Abbreviations explained in Table 1. Total in each column = 100%.

RRT with authentic samples of acetyl derivatives of triterpene alcohols (Table 2). The triterpene alcohols (Table 4) β-amyrin, α-amyrin, cycloartenol, lupeol, 24-methylene cycloartanol and 24-methylene-24-dihydroparkeol were found in most of the seed oils. β-Amyrin was found at 45% and 41% in *C. equisetifolia* and *M. hexandra*, respectively. The RRTs of α-amyrine and cycloartenol are very close; therefore, they appear together and constitute 53% in *C. roseus* and 31% in *L. therolli*. *Acrocarpus fraxinifolius* contains 84% of lupeol. *Pongamia pinnata*, *B. retusa* and *N. nelumbo* were found to contain 57%, 53% and 32% of 24-methylene cycloartanol, respectively. Taraxerol, tirucallol and Ψ-taraxasterol also were found as minor components in all samples.

The sterol fraction was the major constituent of all unsaponifiable fractions. Sterols were analyzed as their acetyl derivatives on an OV-17 column and identified by comparing their RRT values with authentic samples (Table 2). Table 5 shows sterol compositions of the ten seed oils. Stigmasterol, campesterol and β-sitosterol were the common constituents, among which β-sitosterol was the most predominant in all samples (24-81%). Small amounts of cholesterol also were detected in all samples. The composition of the 4-methyl sterol fraction was not tabulated here due to its low concentration and contamination by sterols even after repeated purification by TLC.

Results of the biological activity tests show that the extracts and unsaponifiable material of all four seeds are active as insect repellents. Unsaponifiable materials showed more repellent activity than the oil. The oil and unsaponifiable material of *P. pinnata* showed maximum repellent activity, i.e., 66% and 79%, respectively. The

TABLE 4

Composition of Triterpene Alcohols of Ten Indian Plants

RRT[a]	Plant species[b] NN	MH	ME	CR	LT	PG	CE	AF	BR	PP
1.15	1.8	—	1.2	—	2.7	4.0	6.4	1.7	4.7	1.8
1.20	0.7	—	—	—	—	—	—	—	2.5	—
1.30[a]	2.1	16.8	—	—	—	—	—	—	—	—
1.42	0.2	17.0	0.8	—	—	0.5	0.7	—	—	—
1.47[b]	3.4	—	—	—	0.9	3.1	—	—	—	0.7
1.52	4.9	—	0.4	—	1.7	—	0.9	—	—	—
1.55[c]	—	—	3.2	—	—	5.0	11.5	3.4	—	—
1.63[d]	17.5	41.0	3.9	11.2	13.8	—	45.0	0.6	20.9	36.6
1.65	—	—	18.8	—	—	4.6	—	—	—	—
1.69[e]	2.4	—	7.7	—	—	—	—	—	—	—
1.76[f]	1.9	6.8	1.9	—	—	1.6	—	—	—	—
1.85[g,h]	14.3	—	1.5	53.1	30.7	7.3	—	0.8	3.7	1.9
1.92[i]	8.6	—	9.9	24.3	42.4	26.8	17.3	83.9	5.8	2.4
1.98[j]	3.2	—	—	11.4	—	3.9	12.5	9.7	4.7	—
2.07[k]	32.3	18.4	26.0	—	6.5	32.0	3.6	—	4.7	—
2.15	—	—	14.7	—	—	—	—	—	53.0	56.6
2.20	1.1	—	—	—	—	2.7	—	—	—	—
2.38[l]	0.9	—	2.9	—	—	0.9	—	—	—	—
2.48[m]	0.5	—	1.2	—	—	—	0.7	—	—	—
2.52	—	—	1.2	—	—	—	0.7	—	—	—
Others	4.2	—	4.3	—	—	—	0.7	—	—	—

[a]RRT, relative retention time.

[b]Abbreviation for plant names given in Table 1. Total in each columnh = 100%.

[c–h]Different superscripts represent triterpene alcohols and sterols as given in Table 2.

TABLE 5

Composition of Sterol Fractions of Ten Indian Plants

RRT[a]		Plant species[b] NN	MH	ME	VR	LT	PG	CE	AF	BR	PP
1.00	n	tr	0.9	7.8	tr	1.1	2.2	3.2	0.3	1.5	0.1
1.14	o	—	—	—	1.9	—	—	—	—	—	—
1.31	p	15.1	19.6	15.5	24.2	12.8	21.5	7.6	3.4	4.3	9.9
1.35	q	1.8	3.8	3.1	3.2	—	—	—	—	—	—
1.43	r	—	26.4	20.6	18.8	4.8	16.8	3.8	31.7	9.0	21.9
1.50		—	1.7	tr	3.1	tr	tr	—	0.4	—	—
1.55		—	—	3.1	tr	—	—	—	—	—	—
1.63	s	75.7	43.4	24.2	38.5	81.3	53.7	80.3	53.0	79.8	64.6
1.67		—	—	—	—	—	—	—	—	—	—
1.69	t	—	1.7	16.3	2.3	—	—	—	0.4	2.2	3.4
1.72	u	—	—	—	—	—	—	—	0.2	—	—
1.81	v	5.5	2.5	0.8	4.6	tr	tr	tr	9.2	2.8	—
1.84		—	—	0.8	—	—	—	—	1.6	0.4	—
1.92	w	tr	—	7.0	—	—	—	—	—	—	—
2.13	x	tr	—	0.8	—	—	—	—	—	—	—
Others		0.1	—	—	2.8	—	2.2	5.1	—	—	0.1

[a]RRT, relative retention time.

[b]Abbreviation for plant names given in Table 1. Total in each columnh = 100%.

TABLE 6

Repellent Activity in Percent at 1% Concentration

Name of seed	Unsaponifiable material	Oil
Bauhinia retusa	58.7	40.0
Casuarina equisetifolia	58.00	36.6
Nympheae nelumbo	72.00	57.00
Pongamia pinnata	79.00	66.00

repellent activities of unsaponifiable material and oil are given in Table 6. None of the extracts showed any signs of direct toxicity.

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