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Chemical and Antimicrobial Examination of the Fixed Oil from the Seeds of *Adenanthera pavonina* Linn.

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The seeds of *Adenanthera pavonina Linn.* have been found to contain fixed oil (7.5%), crude protein (4.5%), reducing sugars (3.16%) as glucose and unsaponifiable matter (1.36%). The deep-brownish yellow fixed oil has been found to be the glyceride of palmitic (19.06%), stearic (3.66%), arachidic (4.53%) and lignoceric (11.05%) as saturated (38.24%) fatty acids and oleic (24.12%) and linoleic (37.04%) as unsaturated (61.16%) fatty acids. The unsaponifiable matter consists of stigmasterol. The oil has shown feeble activity (comparable to achromycin/ streptomycin) against *Bacillus anthracis*, *Salmonella paratyphi* and *B. mycoides*. It is totally ineffective against the rest of the test gram positive and gram negative plant and/or animal pathogenic bacteria.

Key Words: *Adenanthera pavonina* Linn., Fixed oil, Antimicrobial studies.

INTRODUCTION

Adenanthera pavonina Linn. (N.O. Leguminosae) is a small unarmed tree. It is known as Barigumchi in Hindi, Rakta-kambal in Bengal and Guruvenda in Telugu. The description and medicinal uses of the plant have been reviewed by Chopra *et al.*[1] The powdered seeds make a useful external application hastening suppuration. A decoction is made from the leaves in South India and given as a remedy for chronic rheumatism and gout. Keeping in view the medicinal importance of the plant, it was thought worth while to undertake the chemical analysis of the fixed oil from the seeds of *Adenanthera pavonina* Linn.

EXPERIMENTAL

The dried seeds of *A. pavonina* were collected from Pratap Nursery and Seed Stores, Dehradun (Uttaranchal), India.

Part A : Analysis of the Fixed Oil

The finely powdered seeds (1.5 kg) were extracted with petroleum ether (60–80°C) for 18 h. On distilling off the solvent under reduced pressure, a deep brownish yellow coloured fatty oil was found to have the physico-chemical characteristics as recorded in Table-1.

The separation of mixed fatty acids and unsaponifiable matter has been done by the method of Hilditch[2]. The mixed fatty acids have been resolved into solid and liquid fatty acids by the method of Twitchell[3], as modified by Hilditch[4]. The identification of the fatty acids has been done by paper chromatography[5] and thin layer chromatography[6] by the usual methods comparing the R_f values of unknown fatty acids with those of the authentic specimens under identical conditions.

TABLE-1

Physico-chemical characteristics	*A. pavonia*
Colour	Deep brownish yellow
Yield	7.5%
Specific gravity at 30°C	0.9083
Refractive index at 30°C	1.4673
Acid value	8.00
Saponification value	186.2
Iodine value	94.19
Unsaponifiable matter	1.36%

Model	AIMIL Daul Column Chromatograph GCMK IIA	
Column	Reoplex 400 (15%) supported on Chromosorb-W, stainless steel, internal diameter of the column ¼"	
	Column length	3 ft
	Carrier gas	Nitrogen
	Rate of flow	45 mL/min
	Chart speed	12 mm/min
	Sensitivity	6 K
	Column temperature	190°C
	Detector block temperature	280°C
	Injector block temperature	270°C
	Sample size injected	0.4 µL
	Sample dissolved in the solvent	Acetone
	Detector	TCD

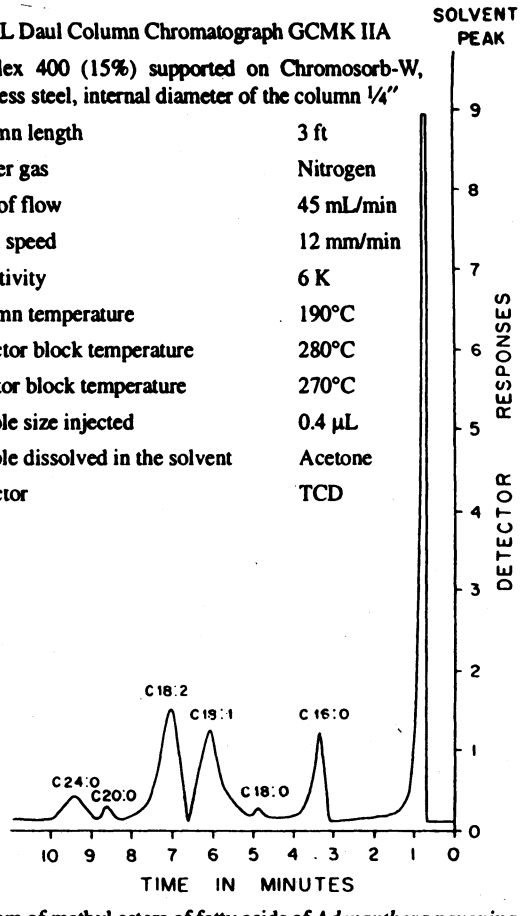

Fig. 1. Gas-liquid chromatogram of methyl esters of fatty acids of *Adenanthera pavonina* Linn.

Gas Liquid Chromatography

The methyl esters of the mixed fatty acids were subjected to gas-liquid chromatography and the methyl esters of authentic specimens were also injected to the GLC under the same conditions. Six predominant peaks of methyl esters were identified by comparing their retention times with the methyl esters of authentic specimens under identical conditions. The percentage composition of different esters was calculated by triangulation method. Thus the presence of the methyl esters of palmitic (19.06%), stearic (3.66%), arachidic (4.53%) and lignoceric (11.05%), oleic (24.12%) and linoleic (37.04%) acids has been established (Fig. 1).

Unsaponifiable Matter

The unsaponifiable matter on TLC over silica gel G developed with benzene and ethyl acetate (9 : 1) and sprayed with concentrated sulphuric acid gave one clear spot and a small diffused spot near the solvent front. Another TLC plate, when sprayed with a steroidal reagent[7], gave a violet spot showing the steroidal nature of the fraction.

The waxy matter was dissolved in benzene and chromatographed[8] over a column of alumina (Grade II, 1 : 50) eluting successively with benzene and ether. The ether fraction (1.07 g) on recrystallization from methanol formed colourless needles, m.p. 166°C. The fraction responded to Libermann-Buchard colour reaction. It was identified as stigmasterol by mixed m.p., Co-TLC and superimposable IR spectrum with that of authentic specimen. It was further confirmed by the preparation of its acetate derivative m.p. and mixed m.p. 141°C. The chemical composition of the fixed oil has been summarized in Table-2.

TABLE-2

	Composition	%
(I)	Saturated acids	38.84
	Palmitic	19.06
	Stearic	3.66
	Arachidic	4.53
	Linoceric	11.05
(II)	Unsaturated acids	61.16
	Oleic	24.12
	Linoleic	37.04
(III)	Unsaponifiable matter, Stigmasterol	1.36

Part B: Investigations on the Antimicrobial Activity of the Fixed Oil.

The oil has been tested for antimicrobial activity against ten gram positive and gram negative plants and/or animal pathogenic bacteria using paper disc plate method of Loo *et al.*[9] Spores were obtained by the method of Abbey[10]. In the same way controls have been run with 1000 ppm solutions of achromycin and streptomycin. The results recorded in Table-3 are the average of three replicates.

TABLE-3
INHIBITION EFFECT OF THE OIL OF *A. PAVONINA* AGAINST TEN PATHOGENIC BACTERIA

Organism	Inhibition zone (mm*)	Control (Achromycin/ Streptomycin, 1000 ppm)
Bacillus anthracis (+)	9.0	30.0 (A)
B. mycoides (+)	7.5	22.0 (A)
B. pumilus (+)	8.0	28.0 (A)
Escherichia coli (–)	—	23.0 (S)
Micrococcus glutomicus (+)	—	25.0 (A)
Sarcina lutea (+)	—	22.0 (A)
Salmonella paratyphi (–)	9.0	20.0 (S)
Staphylococcus albus (+)	7.0	22.0 (A)
Xanthomonas campestris (–)	—	18.0 (S)
X. malvacearum (–)	—	18.0 (S)

* Including the diameter of filter paper disc (6 mm).
(+) Gram positive (–) Gram negative — No activity (A) Achromycin (S) Streptomycin

RESULTS AND DISCUSSION

The oil has shown feeble activity (comparable to achromycin/streptomycin) again st *Bacillus anthracis*, *Salmonella paratyphi* and *B. mycoides*. However, the oil is found to be totally ineffective against the rest of the test gram positive and gram negative plant and/or animal pathogenic bacteria.

The feeble activity exhibited by the fixed oil against the test organisms may be due to the smaller diffusibility of the fixed oil into the agar medium. It is a well known fact that the micro-organisms produce lipase enzyme which hydrolyses the oil and fats and converts them into a suitable substrate for nutrition. Therefore fixed oil may serve as a nutrient for micro-organisms.

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