Studies on Fixed Oil of Seeds of *Sida acuta* Burm

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ABSTRACT

The fixed oil of the seeds of *S. acuta* growing around the University of Saugar campus was studied for its physico-chemical characteristics and fatty acid composition. The percentage of fixed oil was found to be higher than, and its composition differed from, that reported earlier. The saponifiable fraction was found to have oleic, linoleic, palmitic, stearic, myristic and plamitoleic acids, whereas the unsaponifiable fraction contained β-amyrin, β-sitosterol and an unknown waxy nonsteroidal substance.

INTRODUCTION

Sida acuta Burm. (family Malvaceae) is a hardy shrub, 50-90 cm high, with many branches, found throughout the hotter parts of India. A number of medicinal properties have been attributed to this plant, and it is frequently used in the indigenous system of medicine (1). Pande and Tiwari (2) have reported that the seeds yield 9% fixed oil, with oleic, linoleic, palmitic, stearic and arachidic as the constituent fatty acids. They were able to identify only β-sitosterol in the unsaponifiable matter. The present study was undertaken because the fixed oils obtained from the same biological sources are known to differ in composition from region to region.

EXPERIMENTAL PROCEDURES

Extraction of Fixed Oil and Determination of Physico-Chemical Characteristics

Two kilograms of seeds, collected from plants growing around the University of Saugar campus, were crushed to a coarse powder and extracted with petroleum ether (60-80 C) in a Soxhlet apparatus. The extract was concentrated to .25 its original volume and passed through a column of Alumina (S. Merck). The eluate was freed of the solvent to obtain a pale greenish yellow fixed oil in a yield of 12%. The various physico-chemical determinations were made according to the methods recommended by the Pharmacopoeia of India (3).

Chromatography of Fatty Acids

The saturated and unsaturated fatty acids were separated by Hilditch's modification of Twitchwell's method (4) and then subjected to paper, thin layer (TLC) and gas liquid chromatography (GLC).

Paper chromatography: Buchman's method (5) using paraffin oil-impregnated Whatman No. 1 paper strips was followed. One gram each of the saturated fatty acid mixture of the oil and authentic samples of the saturated fatty acids was treated with acetic acid (14 ml) and a mixture (2 ml) of 88% formic acid and 30% hydrogen peroxide (1:1). The mixture was allowed to stand overnight, diluted with water (15 ml), and extracted with chloroform (5 ml). The chloroform extract obtained was used for spotting, whereas the unsaturated fatty acids were spotted as such.

TLC: The method adopted by Malins and Mangold (6), using silicone-impregnated chromatoplates for the resolution of methyl esters of mixed fatty acids, was followed. One gram each of the mixed fatty acid mixture of the oil as well as the authentic fatty acids was converted into methyl esters by refluxing for 1 hr with methanol (10 ml) in the presence of sulphuric acid (0.02 ml). The methyl esters formed were extracted with ether, and the ethereal solution was washed with dilute solution of potassium carbonate. The ether extract was washed with water and dried over anhydrous sodium sulphate. The esters obtained after removal of the solvent were subjected to TLC.

GLC: The methyl esters of the mixed fatty acids were subjected to GLC using a column of succinic acid polyester of diethylene glycol at 200 C. Hydrogen was used as the carrier gas, and the rate of flow was 30 ml/min. The identity of methyl esters was confirmed by successively injecting authentic samples of the respective methyl esters (7).

Studies of Unsaponifiable Matter

Unsaponifiable matter was isolated (8) and subjected to chromatography through a 50 g column of Alumina grade II, prepared by incorporation of 3% water in Alumina grade I (S. Merck), and successively eluted with petroleum ether (60-80 C), petroleum ether-chloroform 3:1, petroleum ether-chloroform 1:3, and alcohol (95%). Fifteen milliliter fractions were collected, and TLC of the fractions was carried out on Silica Gel G (E. Merck), using benzene-ethyl acetate 9:1 as the solvent system and concentrated sulphuric acid as detecting agent. The last three eluants were successful in eluating one component each. The fractions having the same R_f values were combined and allowed to crystallize after concentration. The products obtained from the various fractions were recrystallized, tested for their chemical nature and identified with the help of their melting points and IR spectra.

RESULTS AND DISCUSSION

A pale greenish yellow fixed oil was obtained from the seeds of *Sida acuta*. The yield (12%) was greater than that reported by Pande and Tiwari (9%). The physico-chemical characteristics of the oil are given in Table I.

The paper chromatography of the fatty acids of the oil

TABLE I

Physico-Chemical Characteristics of Fixed Oil of *S. acuta*

Characteristics	Value
Specific gravity, 28 C	0.9581
Refractive index, 28 C	1.4570
Acid value	13.8
Saponification value	164.4
Iodine value	105.6
Acetyl value	10.7
Unsaponifiable matter	1.4%

TABLE II

Paper Chromatography of Fatty Acids of Fixed Oil of *S. acuta*

Fatty acid	R_f value	
	Fixed oil	Authentic sample
Saturated		
Myristic acid	0.43	0.45
Palmitic acid	0.21	0.21
Stearic acid	0.16	0.15
Unsaturated		
Oleic acid	0.51	0.51
Linoleic acid	0.57	0.59

TABLE III

Thin Layer Chromatography of Fatty Acid Esters of of Fixed Oil of *S. acuta*

Fatty acid ester	R_f value	
	Fixed oil	Authentic sample
Saturated		
Methyl myristate	0.89	0.91
Methyl palmitate	0.63	0.63
Methyl stearate	0.84	0.85
Unsaturated		
Methyl oleate	0.33	0.34
Methyl linoleate	0.55	0.56

TABLE IV

Composition of Fatty acids in Fixed oil of *S. acuta* by Gas Liquid Chromatography

Fatty acids	Percentage, w/w
Saturated	
Myristic acid	2.3
Palmitic acid	13.4
Stearic acid	8.4
Unsaturated	
Palmitoleic acid	1.2
Oleic acid	47.9
Linoleic acid	25.2

indicated the presence of three saturated (myristic, palmitic and stearic) and two unsaturated (oleic and linoleic) fatty acids (Table II). These observations were confirmed by TLC of the methyl esters (Table III). GLC of the methyl esters, however, showed the presence of an additional fatty acid (palmitoleic). The fatty acid composition of the oil, based on the area measurement of the various peaks of fatty acid esters, is given in Table IV. Arachidic acid, which was reported by Pande and Tiwari (2), was not found in the present studies. The percentage composition of the various fatty acids also varied; the percentage of linoleic acid was particularly high (25.2%), compared to that reported earlier (12.9%).

The unsaponifiable matter had three components, but only two of them could be obtained in crystalline form. The crystalline components were identified as β-amyrin and β-sitosterol. The third component, which was waxy in nature, gave negative tests for steroids and unsaturation.

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