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Research Article

PHYTOCHEMICAL ANALYSIS OF SIDA OVATA SEED OIL: A NEW SOURCE OF CYCLOPROPENOID FATTY ACID

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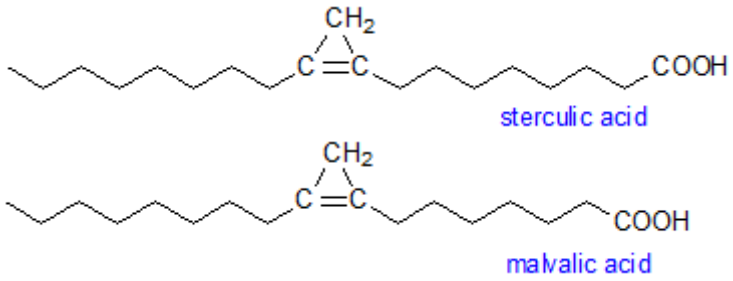
ABSTRACT

The seeds of Sidaovata were collected from the arid zone of Rajasthan and analysed for the characterization of the fatty acids. This paper accounted the presence of Cyclopropenoid fatty acids(Malvalic and Sterculic). The occurrence of CPFA was confirmed by HBr-titration, Halphen test, TLC, NMR and IR spectroscopic methods.

Keywords: Sidaovata (Malvaceae), seed oil characterization, Cyclopropenoid fatty acids.



Structure of Malvalic and Sterculic acids (Cyclopropenoid fatty acids)

INTRODUCTION

The plant of Sidaovata (Malvaceae) is widely spread in the arid parts of tropical land in India. The roots of Sida species are considered excellent adaptogenic and immunomodulator, general nutritive tonic and prolonged life, useful in tuberculosis, heart diseases, cough and respiratory diseases[1]. The plant species belonging to Malvaceae and Sterculeaceae are growing abundantly throughout the Arid zone of Rajasthan and all over India, the fruit of some species Are demulcents, mildly astringent and commonly used in gripping and flalulence[2].

The seed oil of Sidaovata responded positive Halphen test[3] and was selected for the estimation and characterization of CPFA. The naturally occurring CPFA are sterculic (n=7) and malvalic (n=6), found mainly in the seed lipids of four plant families of order malvales which includes (Sterculiaceae, Malvaceae, Bombaceae and Tiliaceae). CPFA occur in plant lipids mainly as glycerides**.** CPFA have been the subject of many investigations due to their Carcinogenic, Co-Carcinogenic activities[4-8]. The presence of CPFA in food is dangerous not only for human healthbut also for animals[9-14]**.** Quantitative estimation of total cyclopropenoid fatty acids content can be achieved most conveniently by HBr titrationto a level of .01%[15].

EXPERIMENTAL

The soxhlet-extraction method was adopted to extract oil from ground seeds, using petroleum ether (40-60^{o}C). The moisture contents, Iodine value, Saponification value of the oil were estimated by standard AOCSmethods[16]. The protein content of the oil was estimated by Kjeldahl method. Thin layer chromatographic techniques were used to resolve different fatty acids components of the oil along with silver ion TLC[17].

The spots were made visible on analytical plates by charring with 20% aquous solution of perchloric acid. The transesterification of the oil was carried out using sodium methoxide. Preparative TLC were used for the separation of large quantity of cyclopropenoid fraction. AmilNucon gas chromatograph model no. 5700 equipped with a flame ionization detector was used for the GLC study of oil samples. The U.V spectra of the oil and methyl esters have been conducted with a Shimadzu UV-1601 spectrophotometer. The NMR spectra were recorded on Bruckerx300 spectrophotometer and oil samples were prepared in $CDCl_3$ containing tetrametylsilane as an internal standard. The IR spectra of oil were obtained from Jasco-made FT-IR spectrophotometer.

RESULTS AND DISCUSSION

The seed oil Sidaovata was evaluated for its physiochemical properties (Tabie-I). The quantitation of total CPFA material was carried out by H-Br titration which revealed the presence of 16% by weight of CPFA[15]. Thin layer liquid chromatography revealed two spots. The methyl ester obtained from the oil sample exhibited the IR band at 1010cm^{-1} and 1852cm^{-1}, supporting the presence of cyclopropene ring. The characterization of unusual acids was achieved by separation using preparative TLC.

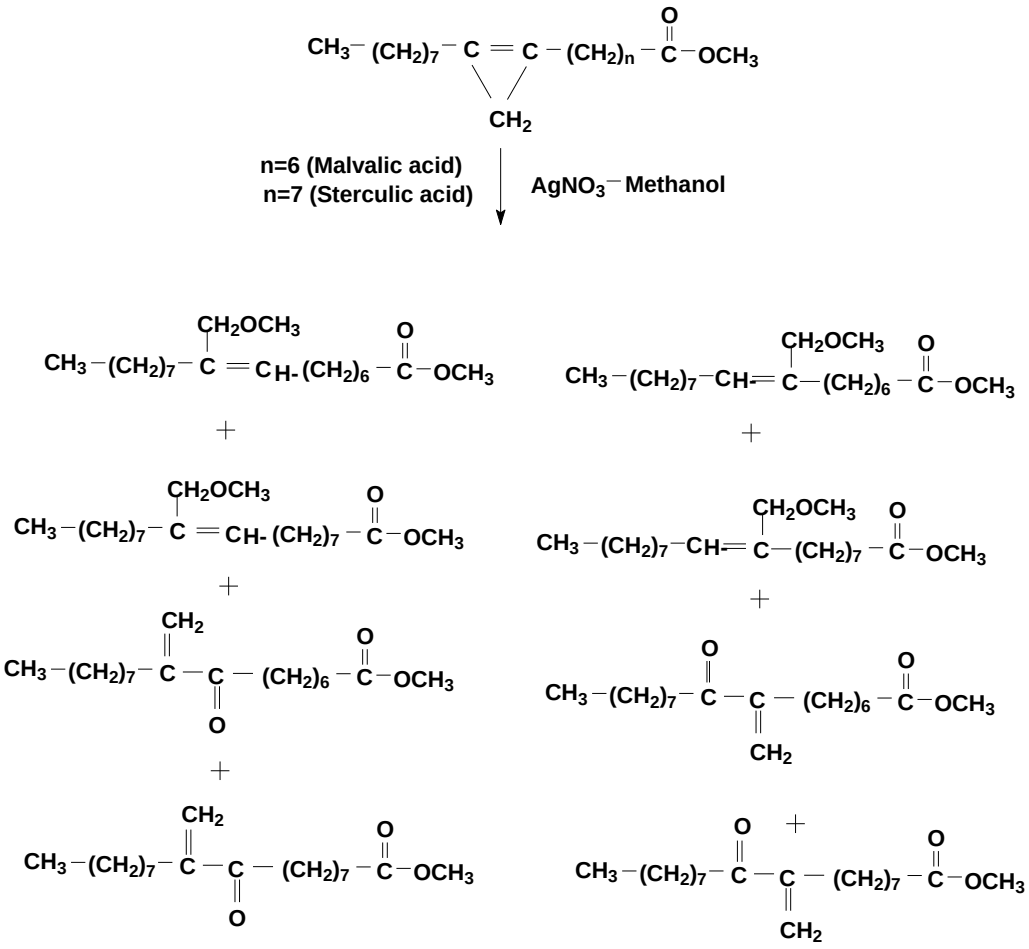
Characterization of fraction-I:

The combined GLC-TLC techniques were used for the analyses of component acids in the CPFA fraction. This fraction have R_F value 0.75 on TLC. The quantitative results of direct, reversed phase and silver ion TLC of this fraction were compared with the esters of Sterculiafoetida used as a reference standard. The spots corresponding to saturated, monoene, diene and triene in this fraction of oil were resolved by A_gNO_3-TLC[17] parallel to those obtained from Linseed esters. The negative Halphen test indicated the absence of CPFA in this fraction. The IR spectrum did not show any peak corresponding to CPFA, however a band at 1710cm^{-1} observed corresponding to ester-carbonyl group.

Characterization of fraction-II:

This fraction showed R_F 0.55.The occurrence of CPFA in this fraction was confirmed by the positive Halphentest[3]. The resulting methyl esters obtained from the base catalyzed trans- esterification showed IR band at 1010cm^{-1} and 1852cm^{-1}. The quantitative estimation of CPFA by HBr-titration showed the presence of 16% by wt. of CPFA. The absence of conjugation or trans-unsaturation in this fraction of oil was confirmed by U.V spectrum. The NMR spectra of this fraction showed typical cyclopropenoid group signal at δ 0.71(singlet) other proton signals were also observed at δ 3.6(3H,-$COCH_3$), δ 2.2 (2H, α to carbonyl), δ 1.2 (chain-CH_2-) and δ 0.88 (3H, term—CH_3). The GLC analysis of the A_gNO_3-methanol treated methyl esters confirmed the presence of Malvalic and Sterculic acids by comparison of relative retention time of similar derivatives from Sterculiafoetida esters. The GLC data given in table-II of the CPFA were found in close agreement with those obtained by H-Br titration [15] methods. The Fatty acids composition of the seed oil is shown in Table-II. The Malvalic and Sterculic acids were found at levels of 14.20% and 6.30% respectively as silver nitrate adduct and the products obtained have been depicted in the scheme. The presence of CPFA has been reported for the first time in the seed oil of Sidaovata.

SCHEME



Ethers and Keto derivatives of malvalic and sterculic acids

	Seed Analysis			Oil Properties			
Nameand Family	**Oil %**	**Protein% N X6.25**	**Moisture%**	**I.V Wij's**	**S.V**	**Refractive Index**	**HBr equivalent**
Sidaovate (Malvaceae)	2.85	17.12	2.3	122	185	1.4890	16.0

Table 1: Analytical Data of Seed and Oil

I.V=Iodine value, S.V=Saponification value, HBr = Hydrogen Bromide Equivalent

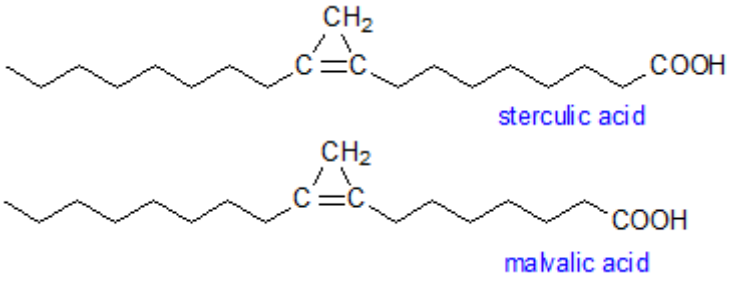
Name and Family	16:0	18:0	18:1	18:2	18:3	20:0	Others
Sida ovate (Malvaceae)	35.2	12.3	15.9	29.6	6.9	_	Traces

Table 2: Fatty Acid Composition Determined by GLC of Fraction-I

Fatty Acids	Sterculiafoetida	Fraction-II
Myristic	-----	2.4
Palmitic	24.8	18.0
Palmitoleic	1.0	------
Stearic	3.4	2.4
Oleic	9.4	14.6
Linoleic	1.8	38.5
Linolenic	1.2	3.5
Malvalic	7.1	14.2
Sterculic	51.2	6.3

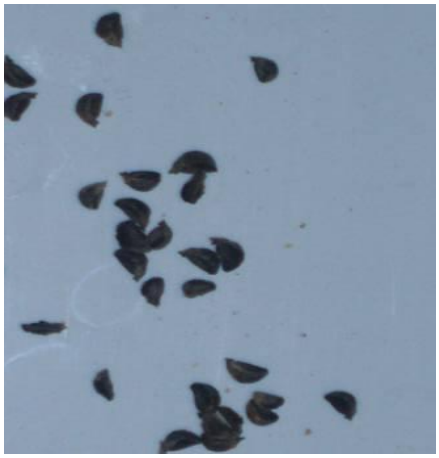
Table 3: GLC Analysis of $AgNO_3$ –Methanol treated Methyl Esters:

Fraction-II (uncorrected wt. %)



Structure of Malvalic and Sterculic acids

Sidaovata plant



Sidaovata seeds

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