Research Article

Characterization of an Oxygenated Fatty Acid in *Jatropha pandurifolia* Seed oil for Hydroxy Fatty Acids

Swatika Sharma*, Seema Parveen, Abdul Malik and MRK. Sherwani

Department of Chemistry, J.N.V. University, Jodhpur-342001, Rajasthan, India.

ABSTRACT

The seed oil of J.pandurifolia was soxhlet extracted with petroleum ether. The physico-chemical properties of J. pandurifolia were studied which revealed 38.40% oil content. The GLC analysis of methyl ester showed saturated and unsaturated fatty acids in varying proportions along with Hydroxy fatty acid upto 8.6%.

Keywords: Hydroxy fatty acids, Jatropha pandurifolia (Euphorbiaceae).

INTRODUCTION

Jatropha pandurifolia is a shrub belonging to the family Euphorbiaceae and habitat in mainly tropical areas. The species is reported to be used as purgative, stypic and emetic and is also used in treatment of warts, tumor, rheumatism, herpes, pruritis, toothache, scabies, eczema and ringworm[1-4].

MATERIAL AND METHODS

Jatropha pandurifolia seeds were collected from Arid and semi Arid regions of Rajasthan. The seeds were dried and crushed with blender prior to extraction. The Soxhlet technique was employed for oil extraction, petroleum ether (40-60^{0}C) was used as a solvent. The solvent was then evaporated using rotary evaporator. The various physico-chemical properties have been determined using AOCS[5] methods shown in Table-I. The fatty acid composition of J.Pandurifolia seed oil have been shown in Table-II. The ricinoleic acid comprises only 8.6% of the total fatty acid composition. Other fatty acids were Palmitic, stearic, oleic, linoleic, linolenic and Arachdic acid. Abbe's refractometer was used for determining refractive index of the oil and it was found to be 1.477. The analytical TLC of the oil sample was performed with glass plates coated with silica gel G (.025mm or 1m thick). The developing solvents used were hexane-Diethyl ether-Acetic acid (70:30:1 v/v).The UV spectra were recorded with shimadzu made UV spectrophotometer, using methanolic solution or cyclohexane as a solvent. NMR were carried out with BruckerDRX300 (300MHz) spectrometer using $CDCl_3$ as a solvent. The fatty acid composition of J.pandurifolia was determined by GLC (Amil Nucon gas chromatograph model 5799),equipped with FID having a stainless steel packed column (6ft length, 5mm diameter) coated with DMS gum rubber (SE-mesh, 80-100u, wt%). The identification of each acid was done by compairing its retention time with that of a reference standard. Varian 3400 gas chromatograph-Finnigan TSQ7000 mass spectrometer, microwave power source, BP x capillary column 30mx0.32m.m x 0.25um film thickness was used for GC-mass spectral analysis. The fragments resulted after cleavages were identified by GLC after methylation with ethereal diazomethane.

RESULTS AND DISCUSSION

The oil and ester were quantitatively examined for the presence of Hydroxy acid by sulphuric acid turbidity test[6] as well as by UV and IR spectroscopy. The IR spectrum of the oil as well as ester gave band at 3400 cm^{-1}. UV spectra show absence of conjugation and trans-unsaturation. Silver nitrate thin layer chromatography showed spot for saturated, monoenoic, dienoic and trienoic esters in addition to a low R_F value spot corresponding to that of Ricinus communis used as reference standard. The peaks corresponding to different classes were separated and purified by passing through column.

CHARCTERIZATION OF NON-OXYGENATED FRACTION

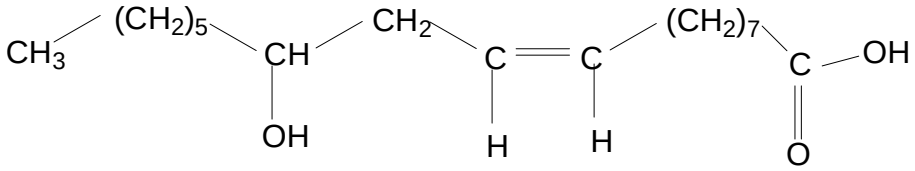
This fraction gave negative Turbidity test[6]. The IR spectrum of this fraction gave no band at 3400cm^{-1} corresponding to the hydroxyl function however the characteristic band of ester carbonyl was observed at 1740cm^{-1}. This fraction also did not respond to the DNP test which ruled out the presence of keto group[7]. Direct TLC showed only non-oxygenated fatty acids. The quantification of fatty acids was done by GLC and their fatty acid composition is given in Table-II.

CHARACTERIZATION OF THE OXYGENATED FRACTION

The IR spectrum of this fraction showed a prominent band at 3450cm^{-1} in the oil as well as ester showing the presence of hydroxyl function. The UV spectrum shows no peak at 965cm^{-1} which confirmed the absence of trans-unsaturation. The elemental analysis of the oil supported the molecular formula $C_{19}H_{36}0_2$, indicating a C_{18} skeleton with mono hydroxyl group. On acetylation of oils and its methyl esters there is a loss of H-atom of hydroxyl group by acetyl group confirmed the presence of one hydroxyl functional group. The acetate derivative formed on acetylation showed a strong band at 1235cm^{-1}. This also supported the presence of mono hydroxyl functional group. The IR spectrum of acetate derivative gave no hydroxyl band at 3450cm^{-1}. The NMR spectrum of the methyl ester revealed the signals at 7.25ppm (1H-OH) and 6.7ppm (IH-CH-OH), on D_2O shake the signal at 7.25ppm disappeared thereby confirming –OH proton. Other significant signals obtained were at 4.6ppm m (2H-CH=CH), 6.4ppm s (3H-$COOCH_3$), 7.8ppm (6H, overlapping signals attributed to allylic protons and proton α to the carbonyl), 8.8ppm br s (chain-CH_2-) and 9.12ppm t (3H-terminal –CH_3). The acetate derivative pure hydroxyl ester showed NMR signals at 5.3ppm m (1H-CH-$OCOCH_3$) and 8.05ppm (3H,-$OCOCH_3$). The disappearance of the original signal of the hydroxy group confirmed the original acid as hydroxy acid.

Further, structure was confirmed by mass spectrum of hydroxymethylester as trimethyl derivative. The M^+ ion peak obtained at m/z 294 which was due to M-18. The ion at m/z 198 showed cleavage between C_{11} and C_{12}.The loss of CH_3OH from the carbonyl group gave ion at m/z 166. The ion peak at m/z 73 corresponded to $(CH_3)_3Si^+$ and at m/z 75, a rearranged ion and TMS derivative at m/z 270 in addition m/z 369 (M-15), m/z(M-31) and m/z 37 were also observed. These ion peaks clearly placed the hydroxyl group at C_{12} and double bond at C_9 positions of the chain. The chemical evidence in connection with structure was obtained from catalytic hydrogenation using Pd/C which resulted in the formation of a saturated hydroxyl ester having mp 56-57^{0}C corresponding to molecular formula $C_{19}H_{38}O_3$. IR of this saturated acid gave band at 3450cm^{-1}.The hydrogenation confirmed the presence of only one double bond in the carbon chain[8].Further evidences obtained by reductive deoxygenation using I_2/P and Zn/HCl acid, Von-Rudolff oxidation using Permanganate-periodate and the respective fragments were well identified using GLC and CO-TLC. These evidences strongly confirmed the position of double bond at C_9 and that of hydroxy group at C_{12}.

The chemical analysis and spectroscopic studies showed that the oxygenated fraction of J.pandurifolia seed oil contained 12-Hydroxy-Cis-9-Octadecenoic acid. This acid is commonly known as ricinoleic acid and has been reported upto 80% in castor oil.



12-Hydroxy cis-9-octadecenoic acid

Table 1: Analytical Data of Seed and Oil

	Seed Analysis			Oil Properties		
Name and Family	**Oil %**	**Protein% N X6.25**	**Moisture%**	**I.V Wij's**	**S.V**	**Refractive Index**
Jatropha pandurifolia (Euphorbiaceae)	38.40	21.5	3.17	120.32	189.4	1.4777

I.V=Iodine value
S.V=Saponification value

Table 2: Fatty Acid Composition Determined by GLC (uncorrected weight%)

Name and Family	16:0	18:0	18:1	18:2	18:3	20:0	Hydroxy acid
Jatropha pandurifolia (Euphorbiaceae)	16.3	3.9	23.5	35.6	10.2	1.8	8.6

J. pandurifolia plant

J. pandurifolia seeds

CONCLUSION

The chemical analyses, spectroscopic and chromatographic methods have vary clearly revealed that the seed oil of Jetropha pandurifolia contains 12-Hydroxy-cis-9-octadecinoic acid as a minor component of fatty acids.

ACKNOWLEGEMENT

The authors are thankful to Head Department of Chemistry for providing necessary facilities; Dr. P. Kasera, Botany Department for identification of plants and Director CDRI, Lucknow for spectroscopic analysis and UGC for the financial assistance to Seema Parveen.

REFERENCES

1. Shetty SS, Udupa L, Udupa Al and Vol-lala VR. Saudi Med J. 2006;27(6): 1473.
2. Pertino MG, Schmeda-Hirschmann, Rodriguez JA and Theoduloz C. J Ethnopharmacol. 2007;111:553-9.
3. Mujumdar AM and Misar AX. J. Ethnopharmacol. 2004;90:11-5.
4. Lin JF, Yan L and Tang. F Chem Acta Pharmacol AOCS Sin. 2003;24:241-6.
5. Schneider EL,Loke SP and Hopkins DT. J Amer Oil Chem Soc. 1968;45:585.
6. Lakshminarayana GL. J Amer Oil Chem Sci. 1968;45:523.
7. Davis EN, Wallen LL, Goodwin JC, Rohwedder WK and Rhodes RA. Lipids. 1969;4:357.
8. Barkley KS. Fatty Acids and their chemical properties, production and uses, Part-I. Interscience Newyork, 1960;27.