Material Science Research India Vol. **6**(1), 105-109 (2009)

Determination of some components of *Balanites aegyptiaca* oil by chromatographic methods

M.J.A. ABUALREISH

Department of Chemistry, Faculty of Science and Technology,
Umdurman Islamic University, Umdurman (Sudan).

(Received: March 16, 2009; Accepted: April 24, 2009)

ABSTRACT

A sample of *Balanites* oil was analyzed by some chromatographic methods. The saponifiable and the unsaponifiable contents of the oil were determined, It was found that the oil contains 84.56% fatty acids and 1.03 % sterols. The percentages of the fatty acids Stearic, Palmitic, Oleic and Linoleic in the oil were found 7.18, 12.50, 30.90, and 34.34% respectively.

Key words: *Balanites aegyptiaca*, analysis and chromatography.

INTRODUCTION

Balanites aegyptiaca is a tree widely distributed in the drier areas of east , west and north-east africa. In Sudan it is known as "Hejlij" tree , and the fruit known as "Lalobe" the trees are predominate in western Sudan[1]. The *Balanites* kernel (10% of the fruit) contain 40–58 % of liquid glyceride oil[2]. Investigation of *Balanites* oil has started by Wellcome research laboratories[3]. They found that the acid value of the oil is 0.39 ; the iodine value 89.0 and the saponification value 189.0 [4]. Balanites aegyptiaca oil is very stable one which could be used for nutritive purposes[5].

MATERIAL AND METHODS

Saponification of oil

An accurately weighed 4.9424 g sample of oil was saponified by boiling under reflux with (1M , 50 mL) ethanolic potassium hydroxide for an hour , 100 mL of water was then added through the condenser . The mixture was transferred into a separtory funnel and extracted three times with ethoxy ethane (100 mL total) . The combined ether extracts were washed with water (40 mL) and (0.5M, 40 mL) potassium hydroxide and the process repeated twice . The ether solution was washed several times with water until washing gave no colour with phenolphthalein . The solution was dried with sodium sulphate and the solvent was evaporated in vaccue. The unsaponifiable residue was weighed and its percentage in oil was evaluated. The Unsaponifable fractions were kept in freezer for further analysis .

Fatty acid extraction

The soap solution obtained after saponification was acidified with concentrated hydrochloric acid (36 % 10 mL). The fatty acids generated were then extracted with petroleum ether three times (100 mL total) . The fatty acids solution was washed several times with distilled water until free from acid (neutral to methyl orange indicator). The ether was removed in vaccue (25-30°C) .

Preparation of fatty acid methyl ester [6]

1.9726 g of the fatty acid generated previously were refluxed with 50 mL methanolic

sulphuric acid (2 ml of 98 % H_2SO_4 in 230 ml methanol) . The methyl esters were then partitioned between petroleum ether (40-60°C) twice (100 ml total) and the solvent was removed . Methyl esters of the sample was then dissolved in n-hexane and the solution thus obtained was introduced into gas liquid chromatography .

Preparation of fatty acids standard

The same above procedure was repeated with 1.0031 g of the standard Oleic acid , 1.0022 g of the standard Stearic acid and 0.9908 g of the standard Palmitic acid .

Thin layer chromatography of the unsaponifiable component

The unsaponifiable fraction was dissolved in n- hexane , 5×20 cm glass plates were coated with silica gel 60PF (in the ratio 2:1) water and silica gel , the temperature to complete dryness was 110 °C for half an hour in drying oven. A presaturated tank was prepared containing the solvent mixture ((mobile phase)) n- hexane : diethyl ether : acetic acid in the ratio 79:20:1 respectively . The unsaponifiable solution was then spotted using a capillary tube on the lower edge (2 cm from the edge), the plates were then inserted cautiously in the presaturated tank and left until the solvent reaches a few cms below the upper edge of the plate , finally removed and visualized by iodine vapour .

RESULTS AND DISCUSSTION

Thin layer chromatography is used for the analyzing of the unsaponifiable component quantitatively and four spots appeared on the plate of silica gel , the retardation factor of each of the four spots was calculated and to be due to demethyl sterol , 4- mono methyl and 4,4 dimethyl sterol.

The unsaponifiable component was found to be 1.03 % of the sample which is near to the standard value of 1.14% .

By comparing the retardation factors of the sample with the standard ones , it found that spot No.1 is due to 4-mono methyl sterol and spot No.3 is due to 4,4 dimethyl sterol .

Table 1: The unsaponifiable component of the sample (Thin layer chromatography T.L.C)

Number of spots	Distancein cm	R_f	Compound	R_{f*}
Mobile phase	15.1	-	-	-
Spot No.1	2.2	0.14	Demethyl sterol	0.13
Spot No.2	2.7	0.18	4-monomethylsterol	0.17
Spot No.3	3.4	0.22	4,4-dimethylsterol	0.20
Spot No.4	4.3	0.28		

Rf - The retardation factors of the unsaponifiable compound of the Balanites oil .

Rf * - The standard retardation factor of the same unsaponifiable compounds .

Table 2: Composition of Balanites oil (Gas liquid chromatography G.L.C)

Element	Weight in gram	Experimental percentage	Theoretical percentage
Sample	4.9424	100%	-
Saponifiable compounds	4.1794	84.56%	-
Unsaponifiable compounds	0.0510	1.03%	1.14%
Others	0.7120	14.4%	-

Table 3: Retention times of the fatty acids in the sample and the standard ones

Methylated fatty acids	Retention times of fatty acids in the sample	Retention times of standards fatty acids
Methyl palmitate	6.4	6.4
Methyl stearate	11.6	11.2
Methyl oleate	13.8	13.4

Table 4: The percentage of methylated fatty acids of the samples

Methylated fatty acids	Peak high in cm	1/2 Peak base	Area under the peak cm^2	Experimental percentage
Methyl palmitate	19.9	0.25	4.98	14.80%
Methyl stearate	5.50	0.55	2.71	8.50%
Methyl oleate	20.90	0.60	12.32	36.60%
Methyl linoleate	19.50	0.70	13.65	40.55%

Table 5: The experimental and theoretical percentages of fatty acids in Balanites oil

Fatty acid	Weight of each fatty acid = % X weight of saponifiable component	Percentage of fatty acid = weight of fatty acid % Weight of the sample	Theoretical percentage
Palmitic acid	0.619	12.50%	14.50%
Stearic acid	0.355	7.18%	12.90%
Oleic acid	1.529	30.90%	34.70%
Linoleic acid	1.6947	34.34%	37.90%

By comparing the retention times of the methylated sample with that of the standard ones , its appears that there is Palmitic, Stearic and Oleic acids, the fourth peak is due to Linoleic acid .

Four fatty acids namely , Palmitic, Stearic, Oleic and Linoleic were detected in the sample of the oil by gas liquid chromatography and their percentages were calculated . The fatty acids in the sample were converted to methyl esters because they have short time and hence short analysis time[7].

The identification of each fatty acid in the sample of the oil was carried out by comparing their retention times with that of the standard ones .

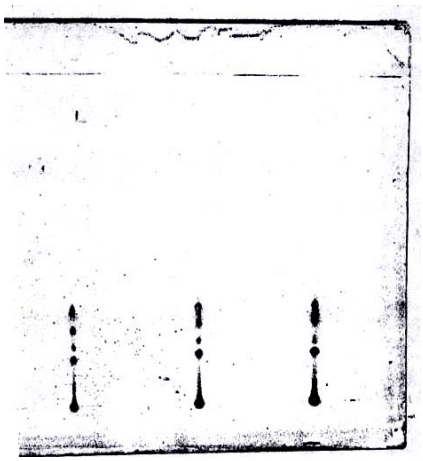

Fig. 1: Thin layer chromatogram of the unsaponifiable components of Balanites oil

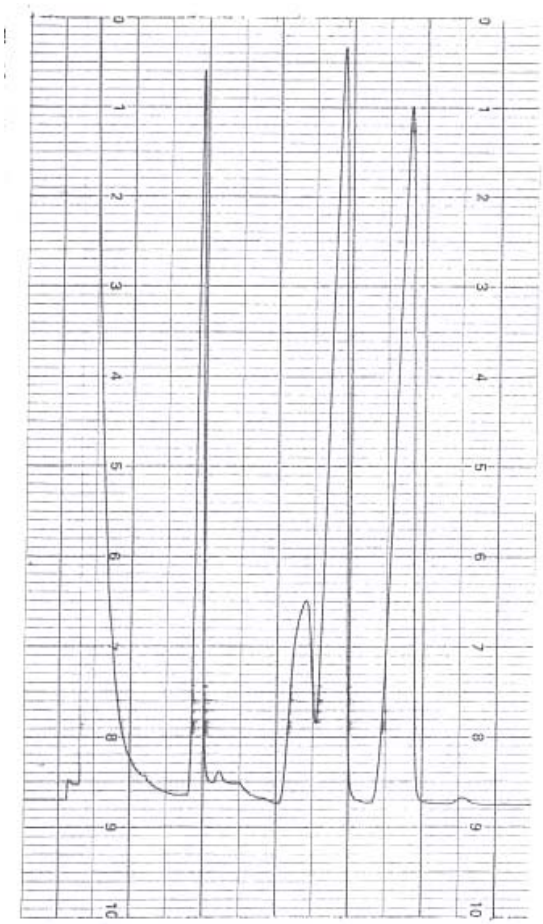

Fig. 2: Chromatogram of methylated fatty acid of the Balanites oil

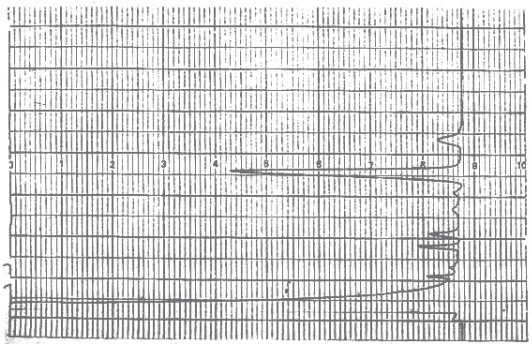

Fig. 4: Chromatogram of standard methylated oleic acid

Fig. 3: Chromatogram of standard methylated Palmitic acid

Fig. 5: Chromatogram of standard methylated stearic acid

The data in table (5) show that two of the four fatty acids determined namely, Oleic and Linoleic acids constitute the major component of the oil 30.90 and 34.34% respectively , while Palmitic and Stearic acids are present in a minor component of the oil 12.50 and 7.18% respectively

REFERENCES

1. Suliman, A.M, Jackson, J.K, Sudan Silva 9 (1959) .
2. J. of development and industry, industrial research and consultancy center 34 (1987)
3. Hussein, S. A, Dollar, F. G and O'Connor, R.T , *J. Amer. Oil. Soc*, **26**: 730-732 (1949) .
4. The Medicinal and Poisonous Plants of Southern and Eastern Africa 2nd edition .
5. Ivanov, S, Popov, A and Bliznakova, L , Compt. *Rent. Acad. Bulg.Sci* , **23**: 1231 (1970) .
6. Metcalte, L.D, Schmitz, A.A and Pelka, T.R, *J. Anal.Chem.* **38**: 514 (1966).
7. Nomana, P, *J. of Chrom*, **615**: 149-150 (1978).