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Research Article

BALANITES ROXBURGHII: PHYSICO-CHEMICAL PROPERTIES AND COMPOSITION OF FATTY ACID FROM ARID ZONE OF RAJASTHAN

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ABSTRACT
The traditional uses of *Balanites roxburghii* (family: zygophyllaceae) as medicinal plant in healthcare practices are providing clues to new areas of research, however information on the uses of indigenous plants as bio fuel source is not well studied from arid zone of Rajasthan. The aim of present study is to evaluate the physico-chemical properties and the fatty acid composition of *Balanites roxburghii* seed oil from the arid zone of Rajasthan. American Oil Chemists' Society (AOCS) method has been used for determination of physico-chemical properties. The oil content range varied from 40.0 to 42.1% and protein content were found to be 26.4% in kernel. High Performance Liquid Chromatography (HPLC) analysis of fatty acid phenacyl esters showed the presence of palmitic, stearic, oleic and linoleic acids as major fatty acids. The findings of this study evaluate structural elucidation of bio-diesel formation from *Balanites roxburghii* seed oil of arid zone of Rajasthan.

Key Words*: Balanites roxburghii, Physico-Chemical Properties, HPLC*

INTRODUCTION

Natural recourses had always satisfied our energy needs in a renewable manner, until the rapid industrial revolution pushed us to start exploiting the fossil reserves: coal, fossil oil and natural gas. These energy reserves, although abundantly available, are not unlimited and will become exhausted in a few decades. The current circumstances bring forever renewable energy sources back under the focus. Therefore, it is imperative to explore plant-based fuels and plant oils and fats as alternative fuel (Martini *et al.,* 1998). The most common that is being developed and used at present is biodiesel, which is fatty acid methyl esters (FAMEs) of seed oils and fats and have already been found suitable for use as blend fuel in diesel engine(Harrington, 1986). Balanites (Greek word: acorn-shaped) is an important genus of thorny shrubs or trees distributed in dry regions of India, Africa, Syria and Arabia. *Balanites roxburghii* tree is a member of the family *zygophyllaceae* commonly found in north-west Rajasthan (Amalraj *et al.,* 1998). It is locally known as hindota/hiran, is a small to large evergreen scraggy shrub or small thorny tree, deciduous with erect, short trunk and ascending branches, about 8 meters of height. Leaves are alternate, coriaceous, flowers are regular, bisexual, drupe fleshy, ovoid and one seeded (Ghanim, 1991). Traditionally, it is used in the treatment of malaria, syphilis, jaundice, liver, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, spleen problems, epilepsy and yellow fever (Nadkarni *et al.,* 1976). The plant also exhibits insecticidal, antihelminthic, antifeedant, molluscicidal and contraceptive activities (Kirtikar *et al.,* 1933; Chopra *et al.,* 1956). The oil exhibited anticancer activity against lung, liver and brain of human carcinoma cell lines. *Balanites roxburghii* find it place form the ancient Ayurvedic pharmacopoeia of India to modern pharmacologic use but there are not sufficient studies carried out regarding the fatty acid characterization and composition of *Balanites roxburghii* seed oil. Therefore the aim of this study is to analyse the fatty acid composition of *Balanites roxburghii* seed oil in arid zone of Rajasthan using standard Thin Layer Chromatography (TLC) and HPLC methods. The purpose of present paper is to evaluate the prospective of bio-diesel formation from the seed oil of *Balanites roxburghii* from the arid zone of Rajasthan.

MATERIALS AND METHODS

Seed materials and pretreatments

The healthy seeds of *Balanites roxburghii* were collected from Jodhpur, Churu and Jhunjhunu district of north-western Rajasthan were soaked in large bowl of clean water to remove the glycoside pulp from seed coats. The epicarp and pulp of the fruits were removed by hand and the nuts were washed with clean water and air dried to a constant weight for 4 to 5 days in summer season of Rajasthan.

Table 1: Morphological/physical parameters of *Balanites roxburghii*

S. No.	Parameter	Value
1	Fruit weight (g)	33.5 ± 3.5
2	Fruit length (cm)	6.7 ± 0.7
3	Fruit diameter (cm)	4.1 ± 0.5
4	Nut weight (g)	24.1 ± 0.2
5	Nut length (cm)	5.6 ± 0.6
6	Nut diameter (cm)	2.6 ± 0. 3

Extraction of oil and content

The seed kernels of the dried *Balanites roxburghii* were macerated to a fine powder using a mechanical grinder. It is extracted with petroleum ether (at a boiling point of 40-60^{o} C) in a soxhlet apparatus for 6-7 hours. The solvent was removed under reduced pressure in a rotary evaporator. The final extracted oil was weighed and the percentage of oil content was calculated using Equation (1):

$$\% \text{ oil content} = \frac{Weig\,ht\ of\ extracted\ \ oil\ (g)}{Weig\,ht\ of\ sample\ \ (g)} \times 100 \qquad (1)$$

Sufficient quantity of oil is obtained by repeating the extraction procedure. The fatty oil is stored under nitrogen atmosphere in a refrigerator until further use.

Analytical values of the oil and seed were determined using standard AOCS methods (Official Methods and Recommended Practices of the American Oil Chemists' Society) (Nukhet *et al.*, 2001).

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Table 2: Physico-Chemical Properties of seeds and oil of *Balanites roxburghii*

S. No.	Characteristics	Value
1	Moisture Content %	6.7-7.7
2	Oil content (% by w)	40.0-42.1
3	Protein content (% by w)	26.0-26.8
4	Iodine value (g I_2/100g)	96 -106
5	Saponification value (mg/g KOH)	201-203
6	Refractive Index (40^{0}C)	1.472-1.473

Fatty acid phenacyl esters preparation

Sample of free fatty acids are dissolved in methanol and neutral it with KOH solution (with the help of phenolphthalein as an indicator). The mixture is dried under nitrogen on a rotary evaporator under reduced pressure. Then it is mixed with a mixture of 0.1 ml of 2 mM 18-crown-6 in acetonitrile and 0.1 ml 4-bromophenacyl bromide. The mixture is heated at 80^{o}C for 15 minutes while mixing gently several times and then mixture is cooled, diluted with acetonitrile (Christie, 1993).

TLC analysis

Thin Layer Chromatographic (TLC) investigation of the fatty acids present of the oil was done in various solvent systems after converting the acids into their corresponding phenacyl esters.

Analysis of Fatty Acid Phenacyl Esters by HPLC

For the analysis, a modified HPLC method (with gradient elution) was used. The equipment included Gilson HPLC with a degasser, a binary pump and a column (900×6.4 mm) was packed with µBondapack C-18 and was eluted with acetonitrile-water in the proportions 67:33 (by volume) initially and is gradually increased to 74:26 in 10 minutes then gradually increased in another 15 minutes which is also changed to 97:3 in another 15 minutes at flow rate of 2ml/minute and detection was completed with UV/VIS detector. Standard phenacyl esters of caprylic, nonanoic, capric, undecanoic, lauric, myristic, palmitic, stearic, oleic, arachidic and behenic acids (Sigma Chemical Company, USA) were used for identification of the peaks under the same set of conditions and by peak enhancement method. The peak area percentages were taken as weight percentages. The fatty acids present in the lipid under investigation were thus identified by comparison of relative retention time and peak position. The percentage of the acids was computer estimated from the HPLC peaks (Browne *et al.*, 2000). The fatty acid composition is given in Table 3.

Table 3: Fatty acids composition (%) of *Balanites roxburghii* seed oil

S.No.	Fatty Acid	Carbon bond	Obtained % by weight
1	Myristic Acid	C 14:0	0.05
2	Palmitic Acid	C 16:0	17.96
3	Palmitoleic Acid	C 16:1	2.24
4	Stearic Acid	C 18:0	11.45
5	Oleic Acid	C 18:1(n-9)	32.31
6	Oleic Acid	C 18:1(n-8)	1.99
7	Linoleic Acid	C 18:2	28.61
8	Linolenic Acid	C 18:3	2.84
9	Arachidic Acid	C 20:0	0.98
10	Gadoleic Acid	C 20:1	1.12
11	Behenic Acid	C 22:0	0.04
12	Tetracosanoic Acid	C 24:0	0.06

RESULTS AND DISCUSSION

The oil contents and phyico-chemical propeties of *Balanites roxburghii* seed from arid zone are presented in table 2. The oil yield from seed kernel ranged from 40-42.1%. The oil is of pale yellow color and is liquid at room temperature. Fatty acid composition so obtained was tabulated in Table 3. Bulk chemical properties such as acid value (AV), saponification number (SN) and iodine value (IV) give structural stability and quality information about oils and fats. The higher iodine number of *Balanites roxburghii* seed oil indicates a higher degree of unsaturated fatty acid. Fatty acids are the primary components of oils and fats. There are three major fatty acids identified by comparison with the fatty acid phenacyl ester. The saturated fatty acids are identified as palmitic and stearic acids. Palmitic acid and stearic acid are in the proportion of 17.96% & 11.45% respectively. The major unsaturated fatty acids like oleic and linoleic acids were found. Regarding the monounsaturated fatty acid oleic acid (32.31%) was dominant fatty acid in this species.

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