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Evaluation of the Lipid Content of *Balanites Aegyptiaca* KernelMuhammad, I.U.^{1*}, Mohammad, A.², Alhassan, A.J.³, Idi, A.³, Nasir, A.⁴, Yaradua, A.I.⁴ and Ahmad, S.M.⁵

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Abstract

Balanite aegyptiaca seeds powder have been shown to contain a relatively high amount of protein and lipids and is useful in treatment and management of various ailments such as jaundice, intestinal worm infection, malaria, syphilis, epilepsy, dysentery, constipation, haemorrhoid among others. The aim of this study was to evaluate the lipid contents of *Balanites aegyptiaca* kernel using gas chromatography mass spectroscopy (GC-MS). *Balanites aegyptiaca* kernel oil was extracted via cold and hot extraction technique, it was then subjected to GC-MS analysis to identify the probable component of the oil. The analysis showed the presence of saturated and unsaturated/long chain fatty acids, cyclic and non-cyclic hydrocarbons as well as mono and diacylglycerols. *Balanites aegyptiaca* kernel could therefore be used as raw materials for many industries such as food, pharmaceutical, fuel/lubricant and soap industries.

Keywords: Cold and hot extraction, oil content, GC-MS, *Balanites aegyptiaca*, and Kernel.

Introduction

Balanite aegyptiaca (Linn.) is a genus of flowering plants in the caltrop family, commonly known as desert date. *Balanites aegyptiaca* is an important multipurpose tree found in most African countries (Clement *et al.*, 2011). It is a woody evergreen xylophytic tree with a height of 10m grown in various ecological conditions mainly distributed in semi-arid zones in tropical Africa especially in Senegal, Sudan and also Asian countries such as India (Chothani and

Vaghasiya, 2011). In Arabic, it is known as lalob, hidjihi, inteishit and heglig. In Hausa, it is called Aduwa and in Swahili and Amharic, it is respectively called mchunju and bedena (Guinand and Dechassa, 2009).

Balanite aegyptiaca is used in treatment and management of various ailments such as jaundice, intestinal worm infection, malaria, syphilis, epilepsy, dysentery, constipation, haemorrhoid among others (Daya and Vaghasiya, 2011). The seed is about 1.5-3cm long, light brown, fibrous and hard, making up to 50-60% of the fruit with about 500-1500 dry clean seeds/kg. These seeds were reported to contain cytostatic saponins balanitins (Yadav and Manju, 2010), deltonin and isodeltonin which are used as molluscicidal agents (Gnoulou *et al.*, 2007). In addition, various reports on the nutritional and anti-nutritional profile of *Balanites aegyptiaca* seeds powder have shown the powder to contain a relatively high amount of protein and lipids (Lohlum *et al.*, 2012) and some amount of anti-nutritional factors such as tannins, oxalate and phytic acid (Chothani and Vaghasiya, 2011) as compared to other plant products. Antinutritional factors such as phytic acid, tannins, saponins, oxalic acid, have adverse effect on health through inhibition of protein digestion, growth, iron and zinc absorption (Larsgon *et al.*, 1996).

Due to the presence of high lipids content in the kernels as reported by Lohlum *et al.* (2012), it is therefore imperative to determine the content of this kernel via two extraction protocols. This study could go a long way in proving the lipid content of the kernel obtained in Kano state,

Northwest Nigeria. Thus, providing a scientific basis of the use of this seed as source of food in drought areas, war zones as well as commercialization of *Balanites aegyptiaca* oil which could bring about boosting the economy, encouraging its cultivation and hence solving the problem of malnutrition and food insecurity.

Materials and Methods

Sample Collection and Preparation

Balanites aegyptiaca fruits were obtained from Gwammaja market, Dala Local Government Area, Kano State Nigeria. It was authenticated by the department of plant biology Bayero University Kano, given an accession number of BUKHAN0359. The fruit was processed by soaking in water for few days, de-pulped and hard cracked with hammer to obtain its kernel. The kernels were air-dried for 12 hours and pulverized using mortar and pestle. The pulverized sample was then subjected to both hot and cold extraction.

Hot extraction of *Balanites aegyptiaca* kernel oil content

The pulverized powder of *Balanites aegyptiaca* kernels was subjected to successive hot continuous extraction using Soxhlet apparatus

with petroleum ether (60-80°C) (Kumar *et al.*, 2014).

Cold extraction of *Balanites aegyptiaca* kernel oil content

The pulverized powder of *Balanites aegyptiaca* kernels was also subjected to successive cold extraction using separating funnel with petroleum ether as described by Roberts and Briggs (1963).








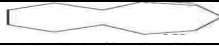



GC-MS analysis of the extracted oil

The extracted oil samples were then subjected to GC-MS analysis using a Shimadzu QP2010 plus gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (Shimadzu, Japan). NIST05 mass spectral library was used for mass spectra comparison (NIST, 2012).

Results

Table 1 shows the possible compounds present in hot extracted oil of *Balanites aegyptiaca* detected by GC-MS analysis. Different organic compounds were speculated including; cyclic aliphatic and non-cyclic aliphatic hydrocarbons, saturated and unsaturated fatty acids with both *cis*- and *trans*- geometry, fatty alcohols and fatty acid derivatives.

Table 1: GC-MS detected compounds in hot extracted oil of *Balanites aegyptiaca* kernel

| Compound Name | Molecular Formula | Retention Index | Structure |
|--------------------------------|------------------------------------|-----------------|---|
| Hydrocarbons | | | |
| Trans-1,4-Dimethylcyclooctane | C ₁₀ H ₂₀ | 1081 |  |
| 1,2,3-trimethylCyclohexane | C ₉ H ₁₈ | 903 |  |
| 1,1,2,3-Tetramethylcyclohexane | C ₁₀ H ₂₀ | 976 |  |
| Vinylcyclohexyl ether | C ₈ H ₁₄ O | 946 |  |
| Propylcyclohexane | C ₉ H ₁₈ | 979 |  |
| 1-Cyclohexyl-1-propene | C ₉ H ₁₆ | 987 |  |
| Allylcyclohexane | C ₉ H ₁₆ | 969 |  |
| 3-Cyclohexyl-1-propanol | C ₉ H ₁₈ O | 1222 |  |
| Cycloundecene | C ₁₁ H ₂₀ | 1301 |  |
| Dodecane (Lauryl chloride) | C ₁₂ H ₂₅ Cl | 1439 |  |
| 1,5-Cyclododecadiene | C ₁₂ H ₂₀ | 1403 |  |



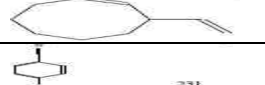


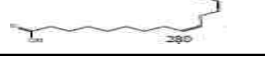
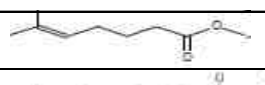
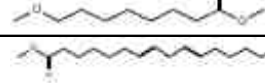
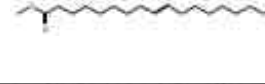
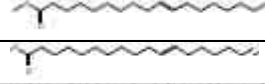
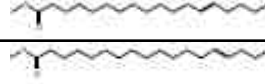
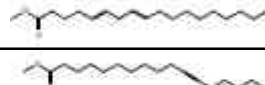
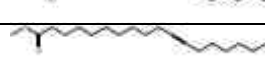
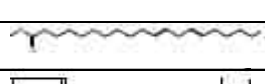
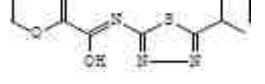
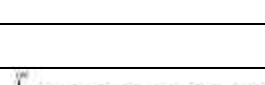


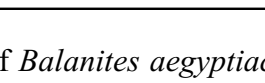
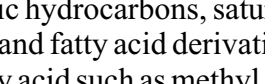
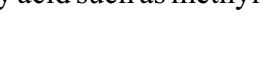


| | | | |
|--|-----------------------|------|---|
| Tetradecane (Myristic chloride) | $C_{14}H_{29}Cl$ | 1638 |  |
| Hexadecane (Palmityl chloride) | $C_{16}H_{33}Cl$ | 1837 |  |
| 3-Vinyl-1-cyclooctene | $C_{10}H_{16}$ | 1092 |  |
| p-Octyloxybenzoxonitrile | $C_{15}H_{21}NO$ | 1843 |  |
| Saturated Fatty Acids | | | |
| Nonadecanoic acid | $C_{19}H_{38}O_2$ | 2266 |  |
| Unsaturated Fatty Acids | | | |
| 9,12-Octadecadienoic acid (Linoleic acid) | $C_{18}H_{32}O_2$ | 2183 |  |
| Fatty Acids Derivatives | | | |
| 5-Heptenoic acid | $C_9H_{16}O_2$ | 1068 |  |
| 8-Methoxyoctanoic acid | $C_{10}H_{20}O_3$ | 1258 |  |
| 7,10-Hexadecadienoic acid | $C_{17}H_{30}O_2$ | 1894 |  |
| 9-Octadecenoic acid (Elaidic acid) | $C_{19}H_{36}O_2$ | 2085 |  |
| 10-Octadecenoic acid | $C_{19}H_{36}O_2$ | 2085 |  |
| 11-Octadecenoic acid | $C_{19}H_{36}O_2$ | 2085 |  |
| 13-Octadecenoic acid | $C_{19}H_{36}O_2$ | 2085 |  |
| 14-Octadecenoic acid, | $C_{19}H_{36}O_2$ | 2085 |  |
| 5,8-Octadecadienoic acid | $C_{19}H_{34}O_2$ | 2093 |  |
| 11-Octadecynoic acid | $C_{19}H_{34}O_2$ | 2095 |  |
| 9-Octadecynoic acid (Stearolic acid) | $C_{18}H_{32}O_2$ | 2184 |  |
| 11,14-Eicosadienoic acid | $C_{21}H_{38}O_2$ | 2292 |  |
| N-(5-Isopropyl-1,3,4-thiadiazol-2-yl)-2-furimidic acid | $C_{10}H_{11}N_3O_2S$ | 1949 |  |
| Glycerols | | | |
| 2-Hexadecanoyl glycerol | $C_{19}H_{38}O_4$ | 2498 |  |
| Eicosanoic acid | $C_{23}H_{46}O_4$ | 2896 |  |
| 1,2-Dipalmitoylglycerol | $C_{35}H_{68}O_5$ | 4013 |  |
| 1,3-Dipalmitoylglycerin | $C_{35}H_{68}O_5$ | 3997 |  |

Table 2 shows the possible compounds present in cold extracted oil of *Balanites aegyptiaca* kernel detected by the GC-MS analysis. Cyclic aliphatic and non-cyclic aliphatic hydrocarbons, saturated and unsaturated fatty acids with both *cis*- and *trans*- geometry, fatty alcohols and fatty acid derivatives were speculated. Presence of unusual fatty acids (valeric acid), substituted fatty acid such as methyl and ethyl esters of fatty acids and monoacyl glycerol were also observed.

Table 2: GC-MS detected compounds in cold extracted oil of *Balanites aegyptiaca* kernel



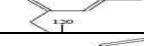




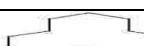
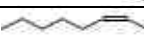














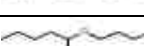

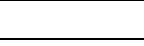
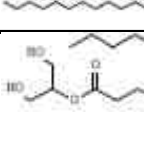
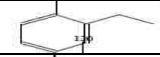









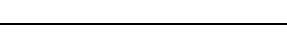






| Compound Name | Molecular Formula | Retention Index | Structure |
|---|--|-----------------|---|
| Hydrocarbons | | | |
| 1-Methyl-2,4-divinylcyclohexane | C ₁₁ H ₁₈ | 1082 |  |
| 3-Methyl-1-cyclooctene | C ₉ H ₁₆ | 1003 |  |
| m-Ethyltoluene | C ₉ H ₁₆ | 1006 |  |
| p-Ethyltoluene | C ₉ H ₁₂ | 1006 |  |
| 1-Fluorodecane | C ₁₀ H ₂₁ F | 988 |  |
| 10-Undecyn-1-ol | C ₁₁ H ₂₀ O | 1355 |  |
| 9,11-Dodecadien-1-ol | C ₁₂ H ₂₂ O | 1455 |  |
| 1,12-Tridecadiene | C ₁₃ H ₂₄ | 1294 |  |
| Bicyclo[10.1.0]tridec-1-ene | C ₁₃ H ₂₂ | 1472 |  |
| 2,4,7-Tridecatrienal | C ₁₃ H ₂₀ O | 1526 |  |
| 1,9-Tetradecadiene | C ₁₄ H ₂₆ | 1411 |  |
| 9,12-Tetradecadien-1-ol | C ₁₄ H ₂₆ O | 1672 |  |
| 9-Tetradecen-1-ol | C ₁₄ H ₂₈ O | 1664 |  |
| Z-10-Pentadecen-1-ol | C ₁₅ H ₃₀ O | 1763 |  |
| 9-Hexadecen-1-ol | C ₁₆ H ₃₂ O | 1862 |  |
| 2-Octadecadecen-1-ol | C ₁₈ H ₃₆ O | 2061 |  |
| 9,17-Octadecadienal | C ₁₈ H ₃₂ O | 1997 |  |
| Saturated Fatty Acids | | | |
| Tetradecanoic acid (Myristic acid) | C ₁₄ H ₂₈ O ₂ | 1769 |  |
| Unsaturated Fatty Acids | | | |
| 9-Hexadecenoic acid | C ₁₆ H ₃₀ O ₂ | 1976 |  |
| cis-Oleic acid | C ₁₈ H ₃₄ O ₂ | 2175 |  |
| trans-13-Docosenoic acid (Brassicic acid) | C ₂₂ H ₄₂ O ₂ | 2572 |  |
| cis-13-Docosenoic acid (Erucic acid) | C ₂₂ H ₄₂ O ₂ | 2572 |  |
| Fatty Acids Derivatives | | | |
| Stearyl vinyl ether | C ₂₀ H ₄₀ O | 2075 |  |
| Pentanoic acid (Valeric acid) | C ₁₆ H ₃₀ O ₂ | 1769 |  |
| Isopropyl linoleate | C ₂₁ H ₃₈ O ₂ | 2228 |  |
| Glycerols | | | |
| Glycerol-1-monopalmitate | C ₁₉ H ₃₈ O ₄ | 2482 |  |
| 2-hydroxy-1-(hydroxymethyl)ethyl ester Linolein | C ₂₁ H ₃₈ O ₄ | 2713 |  |

Table 3 presents the common organic compounds detected in both hot and cold extracted *Balanites aegyptiaca* kernel oil. It indicates the presence of straight chain aliphatic and aromatic benzenoid hydrocarbons. It also shows the presence of saturated fatty acids and fatty acid derivative (Linolelaidic acid).

Table 3: Common compounds in both hot and cold extracted oils of *Balanites aegyptiaca* kernel

| Compound | Molecular formula | Retention Index | Structure |
|---|--|-----------------|---|
| Hydrocarbons | | | |
| o-Ethyltoluene | C ₉ H ₁₆ | 1006 |  |
| Isopropylbenzene (cumene) | C ₉ H ₁₂ | 928 |  |
| Decane | C ₁₀ H ₂₂ | 1015 |  |
| Undecane | C ₁₁ H ₂₄ | 1115 |  |
| Dodecane | C ₁₂ H ₂₆ | 1214 |  |
| Tridecane | C ₁₃ H ₂₈ | 1313 |  |
| 13-Tetradecene-1-ol | C ₁₄ H ₂₄ O | 1663 |  |
| 6,9-Pentadecadiene-1-ol | C ₁₅ H ₂₈ O | 1771 |  |
| Hexadecane | C ₁₆ H ₃₄ | 1612 |  |
| 9,12-Octadecadiene-1-ol | C ₁₈ H ₃₄ O | 2069 |  |
| 2-Octylcyclopropene-1-heptanol | C ₁₈ H ₃₄ O | 2056 |  |
| Saturated Fatty Acids | | | |
| Pentadecanoic acid | C ₁₅ H ₃₀ O ₂ | 1869 |  |
| Hexadecanoic acid (Palmitic acid) | C ₁₆ H ₃₂ O ₂ | 1968 |  |
| Heptadecanoic acid (Margarinic acid) | C ₁₇ H ₃₄ O ₂ | 2067 |  |
| Octadecanoic acid (Stearic acid) | C ₁₈ H ₃₆ O ₂ | 2167 |  |
| Fatty Acids Derivatives | | | |
| 9,12-Octadecadienoic acid (Linolelaidic acid) | C ₁₉ H ₃₄ O ₂ | 2713 |  |
| Linoleic acid chloride | C ₁₈ H ₃₁ ClO | 2131 |  |

Discussion

GC-MS analysis of hot extracted oil shows the presence of saturated and unsaturated acyclic and cyclic aliphatic hydrocarbons. The hot extracted oil also contains specifically saturated nona-decanoic and unsaturated linoleic fatty acids. Linoleic acid is an essential fatty acid and a

precursor of long-chain Arachidonic acid and hence eicosanoids which are involved in a variety of biological processes including lowering of blood pressure, diuresis, modulating inflammation, platelet aggregation, immune response, cell growth, proliferation, contraction and dilation of smooth muscle cells in different

organs. Upon incorporation into structural lipids they can modify membrane fluidity, membrane thickness and alter specific interactions with membrane proteins (Carrillo-Tripp and Feller, 2005).

Furthermore, linoleic acid is an n-6 Poly unsaturated fatty acid (PUFA), known to repress transcription of the leptin gene. Leptin; an adipose derived hormone that regulates appetite, body weight and adiposity. Increased plasma leptin levels have been correlated with increased adiposity, while weight loss results in decreased plasma leptin levels. Substitution of PUFA for saturated fatty acids in the diet causes a decrease in plasma leptin levels (Reseland *et al.*, 2001). Hence the cause of satiety when *Balanites aegyptiaca* kernel is consumed which could be used for weight-loss therapy. In addition, some fatty acids derivatives were also detected from the oil, one of which is unusual *trans* form of 11, 14-Eicosadienoic acid unlike the common *cis* form. Both monoacyl and diacyl glycerols were also detected, these are important intermediates in some metabolic reactions and may be components of other lipid classes used as emulsifying agents in processed foods. Diacylglycerols are used in place of Triacylglycerol in some vegetable oils resulting in less storage as body fat (Lee and Hwang, 2008).

GC-MS analysis of cold extracted oil from the kernel shows the presence of different hydrocarbons. The saturated, long chain myristic fatty acid was found to be peculiar to the cold extracted oil of the kernel. Also detected in this cold extracted oil are unsaturated long chain fatty acid, palmitoleic, oleic acid and both *cis* and *trans* forms of docosenoic fatty acids (Erucic and Brassidic acids respectively). Unsaturated forms of fatty alcohols and monoacyl glycerols were also detected. The presence of an unusual fatty acid; valeric acid which is colorless, oily liquid with an unpleasant odor mainly used as a chemical intermediate for many applications including ester type lubricants used in aviation turbine oils, fire-resistant hydraulic fluids, refrigerator oils, flavors and perfumes, plasticizers, vinyl stabilizers, specialty chemicals, food additive for synthetic flavor and

as an odorant in pesticide formulations for use on crops at concentrations of not more than 2% was also detected (Campbell, 2005). *Balanites aegyptiaca* kernel could therefore serve as a good source of this oil for industrial application. It was established that valeric acid is not likely accumulated in the food chain (bioconcentration potential is low) due to its rapid degradation in the environment from ultimate (complete) aerobic degradation estimated in days (Bizzari *et al.*, 2006).

Comparison between the hot and cold extracted oil shows that both contain aromatic benzenoid, saturated and unsaturated acyclic hydrocarbons which were mostly hydroxylated. The common saturated fatty acids were odd numbered carbon fatty acids such as pentadecanoic and heptadecanoic acids and even numbered carbon fatty acids such as palmitic and stearic fatty acids. Unsaturated fatty acids were not common in both extraction indicating that the kernel oil is rich in saturated fatty acids. Also, the fatty acids derivative common to both extracted oils were Linolelaidic acid, an omega-6 *trans* fatty acid (TFA) and a geometric isomer of linoleic acid. It is a colourless viscous liquid found in partially hydrogenated vegetable oils which is known to cause heart diseases (Park, 2009).

From all indications, the effect of heat on hot extracted oil was found to trigger halogenation of the hydrocarbon chains of organic compounds. Both the hot and cold extracted oil were shown to contain some quantities of monoacyl and diacyl glycerols showing that *Balanites aegyptiaca* kernel is possibly rich in triacylglycerol and lipase enzymes which breakdown triacylglycerol releasing the fatty acids attached to the glycerol moiety and form some of the straight chain hydrocarbons, fatty alcohols, fatty acids derivatives, monoacyl and diacyl glycerols among others. Lipases are among the most important classes of industrial enzymes. In plants, lipase activity has been identified in various tissues but relatively high concentration is found in seeds (Gadge *et al.*, 2011). Seed lipases have been the focus of much attention as biocatalysts (Barros *et al.*, 2010). Lipase may be used to produce fatty acids (Linder *et al.*, 2002), aroma and flavor compounds (Athawale *et al.*,

2003), lubricant and solvent esters and bio-modified fats (Neklyudov *et al.*, 2002). With proper processing, *Balanites aegyptiaca* kernel could therefore be used as a source of this enzyme for industrial, food, pharmaceutical and biofuel industries.

Furthermore, it was found that the cold extracted oil of *Balanites aegyptiaca* kernel has 50% each of both *cis*- and *trans*- geometry of unsaturated fatty acids while hot extracted oil of *Balanites aegyptiaca* kernel had 100% *cis*- geometry of unsaturated fatty acid. *Trans* fatty acids act as inhibitors of Δ^5 -desaturase and Δ^6 -desaturase which are necessary for the initiation of transformation of polyunsaturated fatty acids of the n-3 and n-6 families into eicosanoids (physiologically active prostaglandins, thromboxanes, and leucotrienes) (De Schrijver and Privett, 1982). Inhibition of desaturase activity (particularly Δ^6 -desaturase) hence increases the body's requirements for essential fatty acids and contributes to an increase in blood cholesterol level raising the risk of atherosclerosis and ischemic heart disease (Kummerow *et al.*, 2004). Studies in rats have demonstrated that essential fatty acid deficiency resulting from a diet rich in *trans* fatty acids can effectively be prevented by increasing the share of essential fatty acid in everyday nutrition. Therefore, using the hot extraction method could go a long way to reduce presence of *trans* fatty acids and increase availability of essential fatty acids in *Balanites aegyptiaca* kernel oil.

Conclusion

The study revealed that *Balanites aegyptiaca* kernel oil is rich in both *cis* and *trans* fatty acids, mono and diacylglycerols as well as seed lipases which are usually used as industrial enzymes. Therefore, its nutritional information could be of great use to nutritionists, industrialists and researchers.

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