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Research Article

Phytochemical and GC-MS Analysis of Bioactive Compounds from *Balanites aegyptiaca*

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Abstract

The aqueous extract of kernel and flesh of *Balanites aegyptiaca* were prepared, both were separately identified using GC-MS analysis. The extract of *Balanites aegyptiaca* kernel indicated the presence of 20 compounds accounting for 99.99%. The major compounds of the kernel including 9-Octadecenamide (29.79%), 3-O-Methyl-d-glucose (21.03%), 13-Docosenamide (20.51%), saturated fatty acids (8.09%) and unsaturated fatty acids (1.49%), while the flesh extract revealed the presence of a total of 22 compounds accounting for 99.99%. The major compounds were 3-O-Methyl-d-glucose (42.56%), 9-Octadecenamide (18.02%), 13-Docosenamide (13.11%), saturated fatty acids (2%) and unsaturated fatty acids (1.05%). The amount of fatty acids in the kernel is higher than that of the flesh.

Keywords: *Balanites aegyptiaca*; Aqueous Extracts; GC-MS; Bioactive Compounds; Fatty Acids

Introduction

The phytochemicals are naturally found in medicinal plants, they possess defense mechanism and protect from several diseases. The preliminary phytochemical screening tests may be useful in the detection of these bioactive principles and successively leads to the drug discovery [1]. GC-MS is considered as one of the best technique for identifying the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids esters and others substances [2].

Medicinal herbs and alternative medicines are safer, effective, available and cheap alternatives in comparison to synthetic therapy [3]. One of these potential herbal medicines which could be included in many developing countries healthcare program is *Balanites aegyptiaca*. The plant is widely used by traditional healers in rural areas of many countries [4-9].

The fruit of *Balanites aegyptiaca* is partly long, narrow drupe, 2.5 to 7 cm tall and 1.5 to 4 cm diameter. The immature fruit is green and tomentose, turning yellow and glabrous when mature. The mature fruit consists of four layers: the outer layer epicarp, the fleshy pulp mesocarp, the wood shield endocarp and the seed kernel [10]. The pulp is bitter-sweet and edible. The kernel is 1.5 to 3 cm long, light brown, fibrous, and very solid. It constitutes up to 60% of the fruit. There are 500 to 1500 seeds/kg [7]. The edible seed kernel is oil-rich part of the fruit and contains 4 main different fatty acids. Two are unsaturated fatty acids named oleic acid and linoleic acid. The other are two saturated fatty acids known as palmitic acid and stearic acid. The percentage of unsaturated fatty acids in the kernel oil to the saturated fatty acids is 1:9 [11]. In order to scientifically corroborate the claim made by traditional healers of the therapeutic success of this plant species, the present study aimed to evaluate some of the biochemical components of *Balanites aegyptiaca* kernel and flesh.

Materials and Methods

Plant material

The plant material used in the present study was selected after an extensive ethnobotanical survey that involved interviewing

traditional healers and literature survey. The fruits of *Balanites aegyptiaca* were obtained from Jeddah, Saudi Arabia. The plant was identified and authenticated by a plant taxonomist at the Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

Preparation of aqueous extracts of kernel and flesh

The mesocarp (Flesh) of the desert date fruits (*B. aegyptiaca*, Lalob) was scraped manually by using sterile sharp surgical blade. The endocarp (woody part) was broken manually by using scissor, and the kernels (seeds) were then collected. Thereafter, the dried kernels were ground into powder using an electric blender and stored in an air tight container.

Briefly, a suitable weight of the crude powder of *Balanites aegyptiaca* (kernel, flesh) was placed in a stoppered container with water. They were separately allowed to stand at room temperature for a period of at least 3 days until the soluble matter has dissolved. The aqueous extracts were decanted into clean dry conical flasks and then filtered through Whatman No.1 filter paper by using a Buchner funnel. Rotary evaporator (Eyela A-10005 China) was employed for the efficient and gentle removal of solvents from samples. Then the mixture was strained, the marc (the damp solid material) was pressed. Finally, the complete dryness of the aqueous extract was carried out by freeze drying (Labconco USA). The dried samples were stored into air-tight containers for subsequent use.

Gas chromatography–mass spectrometry (GC-MS) method

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1 ml/min at a splitless mode, injection volume of 1 µl and the following temperature program: 60°C for 2 min; rising at 10°C/min to 280°C and held for 10 min. The injector and detector were held at 250 and 300°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 50-550 and solvent delay 3 min. The identification of different constituents of aqueous extracts of *Balanites aegyptiaca* kernel and flesh were determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data and also with published literature.

Results and Discussion

GC-MS Analysis of *Balanites aegyptiaca* Kernel

The GC-MS analysis of the aqueous extract of *Balanites aegyptiaca* kernel revealed the presence of a total of 23 compounds accounting for 99.99%. The major compounds were 9-Octadecenamide (33.21%), 3-O-Methyl-d-glucose (21.03%). and13-Docosenamide (20.51%), saturated fatty acids (8.09%) namely: cyclohexanecarboxylic acid, hexadecanoic acid, methyl ester, heptadecanoic acid, 3-phenylpropyl ester, 16-methyl-, methyl ester, dodecanoic acid, 2,3-bis(acetyloxy) propyl ester, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester and octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester) and unsaturated fatty acids (1.49%) namely 9,12-Octadecadienoic acid (Z,Z)-, methyl ester and 11-Octadecenoic acid, methyl ester) (Table 1).

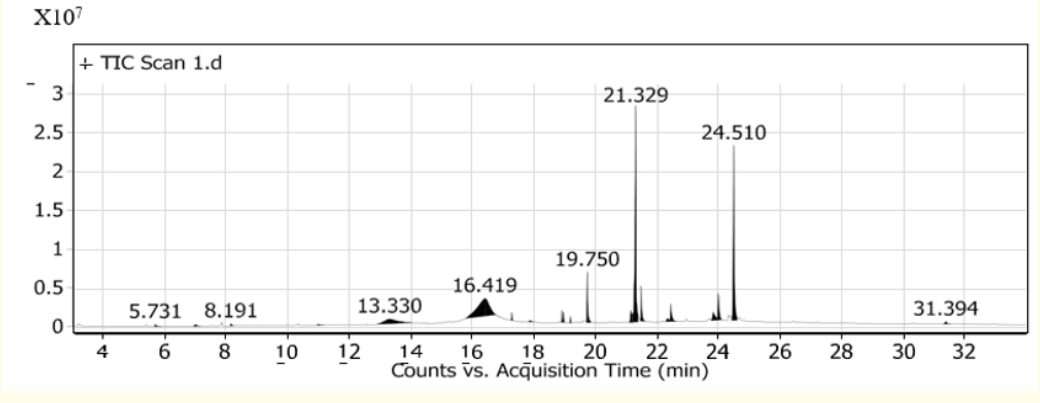

Figure 1: GC chromatogram of the aqueous extract of Balanites aegyptiaca kernel.

Peak	RT	Name	Formula	Area	Width	Area Sum %
1	5.731	Cyclohexanecarboxylic acid, 3-phenylpropyl ester	C16H22O2	1220596.15	0.24	0.41
2	7.058	Octadecane, 6-methyl-	C19H40	1415741.13	0.235	0.47
3	8.191	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	C12H13N7	943598.28	0.131	0.32
4	11.029	Methyl 2,4-tridecadiynoate	C14H20O2	959825.33	0.246	0.32
5	13.33	Melezitose	C18H32O16	19763513.04	1.144	6.61
6	16.419	3-O-Methyl-d-glucose	C7H14O6	62812087.2	0.997	21.03
7	17.301	Hexadecanoic acid, methyl ester	C17H34O2	1712162.59	0.086	0.57
8	17.884	Octaethylene glycol monododecyl ether	C28H58O9	963705.99	0.145	0.32
9	18.931	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	2306701.69	0.057	0.77
10	18.983	11-Octadecenoic acid, methyl ester	C19H36O2	2160150.95	0.062	0.72
11	19.206	Heptadecanoic acid, 16-methyl-, methyl ester	C19H38O2	1266914.16	0.07	0.42
12	19.75	Hexadecanamide	C16H33NO	13980904.14	0.137	4.68
13	21.329	9-Octadecenamide	C18H35NO	89034043.93	0.292	29.79
14	21.501	Octadecanamide	C18H37NO	10221080.98	0.172	3.42
15	22.376	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	C19H34O6	2199643.02	0.132	0.74
16	22.462	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	7610668.01	0.212	2.55
17	23.847	Glycidyl oleate	C21H38O3	6813489.9	0.2	2.28
18	24.007	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H42O4	10331235.97	0.169	3.46
19	24.51	13-Docosenamide, (Z)-	C22H43NO	61276981.76	0.177	20.51
20	31.394	gamma. Sitosterol	C29H50O	1793583.82	0.267	0.6

Table 1: GC-MS Analysis of Balanites aegyptiaca kernel.

GC-MS Analysis of *Balanites aegyptiaca* Flesh

The analysis of GC-MS of the aqueous extract of *Balanites aegyptiaca* flesh showed the presence of a total of 27 compounds accounting for 99.99%. The major compounds were 3-O-Methyl-d-glucose (42.56%), 9-Octadecenamide (18.02%) and 13-Docosenamide (13.11%), saturated fatty acids (2%) namely hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester and octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester) and unsaturated fatty acids (1.05%) namely 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 11-Octadecenoic acid, methyl ester and Oleic Acid) (Table 2).

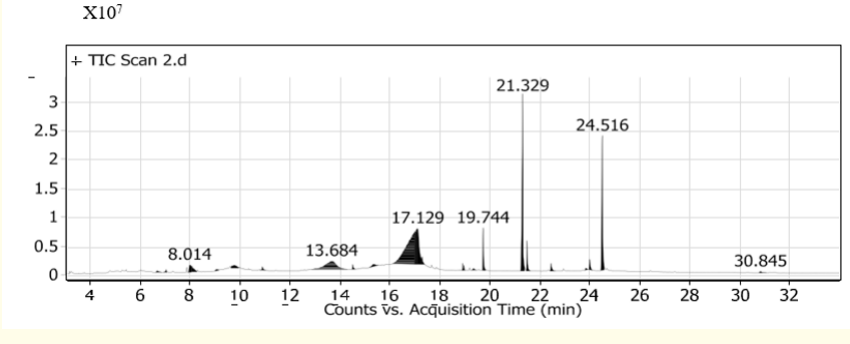

Figure 2: GC chromatogram of the aqueous extract of *Balanites aegyptiaca* flesh.

Peak	RT	Name	Formula	Area	Width	Area Sum %
1	6.704	DL-Arabinose	C5H10O5	1537837.94	0.262	0.33
2	7.07	.beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	C12H22O11	1269228.28	0.137	0.27
3	8.014	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	10447576.28	0.217	2.23
4	8.163	5-Methyl-2-ethylamino-2-thiazoline	C6H12N2S	3033756.27	0.149	0.65
5	9.084	Maltose	C12H22O11	1472444.73	0.17	0.31
6	9.782	Melezitose	C18H32O16	6608475.6	0.32	1.41
7	10.915	.beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	C12H22O11	2255822.09	0.251	0.48
8	13.684	Melezitose	C18H32O16	46406265.91	1.45	9.9
9	14.531	1H-Purin-2-amine, 6-methoxy-N-methyl-	C7H9N5O	2209027.95	0.142	0.47
10	15.35	Desulphosinigrin	C10H17NO6S	4024100.17	0.348	0.86
11	17.129	3-O-Methyl-d-glucose	C7H14O6	199550047.62	1.267	42.56
12	18.932	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	1978238.71	0.069	0.42
13	18.977	11-Octadecenoic acid, methyl ester	C19H36O2	1513598.23	0.069	0.32
14	19.366	Oleic Acid	C18H34O2	1433176.25	0.19	0.31
15	19.744	Hexadecanamide	C16H33NO	15348514.25	0.129	3.27
16	21.329	9-Octadecenamide, (Z)-	C18H35NO	84463884.89	0.166	18.02
17	21.501	Octadecanamide	C18H37NO	11606072.48	0.172	2.48
18	22.462	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	4417638.9	0.217	0.94
19	23.847	4-Hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene	C25H40O2	1313403.89	0.139	0.28
20	24.013	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H42O4	4953260.94	0.16	1.06
21	24.516	13-Docosenamide, (Z)-	C22H43NO	61448882.48	0.142	13.11
22	30.845	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	C27H40O4	1457328.88	0.313	0.31

Table 2: GC-MS Analysis of *Balanites aegyptiaca* Flesh.

The phytochemical screening tests might be an important tool for the detection of the bioactive principles which subsequently can leads to the drug discovery [1]. GC-MS is still considered as one of the best technique for identifying the constituents of volatile matter, long and branched chain hydrocarbons, alcoholic acids, esters and others organic substances [2].

The results of this study indicated that the GC-MS profile of the phytochemical constituents of the aqueous extracts of *Balanites aegyptiaca* kernel and flesh revealed the presence of 3-0-methyl-D-glucose (3-OMG), 9-Octadecenamide, 13-docosenamide, saturated and unsaturated fatty acids. 3-0-methyl-D-glucose (3-OMG) is a nontoxic, non-metabolizable glucose analogue. It is effective in reducing the toxicity of streptozotocin and protection of the pancreatic beta cell against the toxic action of alloxan [12].

9-octadecenamide is an oleamide, an amide derived from oleic acid biosynthesis [13]. Cheng *et al*. [14] reported both antioxidative and hypolipidemic bioactivity of 9-octadecenamide obtained from methanolic fractionate of mountain celery seed oil. The author's results showed a significant decrease in serum triglyceride, total cholesterol, lipoprotein cholesterol, low-density and hepatic triglyceride. 9-octadecenamide has been studied as a potential medicinal treatment for mood and sleep disorders [15].

On the other hand, 13-docosenamide is an amide of docosenoic acid, also called erucylamide, was reported to be capable of sleep-like induction [16].

Recent studies highlight the properties of bioactive compounds in functional foods, such as polyunsaturated fatty acids (PUFAs) especially omega-3 fatty acids [17]. The edible seed kernel of *Balanites aegyptiaca* is oil-rich part of the fruit and contains 4 main different fatty acids. Two are unsaturated fatty acids named oleic acid and linoleic acid. The other are two saturated fatty acids known as palmitic acid and stearic acid [11,18,19]. PUFAs have been correlated with a reduced risk of diabetes and improved human health [20,21]. Long chain omega-3 fatty acids may reduce mortality in diabetic patient's trough reduction of blood triglycerides, platelet aggregability and improving endothelial functions [22,23].

The beneficial effects of PUFA have been extensively studied [17,20]. These benefits were evidenced by the regulation of metabolic and inflammatory pathways, cardiovascular diseases, as well as in glucose homeostasis and insulin sensitivity among others [24,25]. PUFAs were known to increase some oxidant cleavage pathways and can also act as antioxidants [26]. Experimental studies showed that the PUFAs in cell membrane are prone to attack by free radicals due to the presence of stable multiple bonds [27].

Conclusion

Based on the above results, the *Balanites aegyptiaca* may be a very useful source of bioactive compounds for treating various diseases, therefore, further studies are highly emphasized in order to isolate, identify and elucidate these active components. kernel and flesh of *Balanites aegyptiaca* indicated some similarity in the presence of their chemical compounds such as 3-O-Methyl-d-glucose and 9-Octadecenamide.

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