Acta Scientific Pharmacology

Volume 2 Issue 11 November 2021

Research Article

Antimicrobial Activity and Physicochemical Properties of Sudanese Medicinal Plants *Balanites aegyptiaca*

A Gobara[1]*, Halima Elfadel Alebead Ali[1], Madena komi[1] and Awad Salim Ibrahim Holy[1,2]

[1]*Department of Chemistry, Faculty of Education, Dalanj University, Dalanj, Sudan*

[2]*Department of Chemistry, Faculty of Education, West Kordufan University, Sudan*

***Corresponding Author:** A Gobara, Department of Chemistry, Faculty of Education, Dalanj University, Dalanj, Sudan.

Received: June 14, 2021

Published: July 20, 2021

Abstract

The main objective of this study is to assess the antibacterial and antifungal activities of *Balanites aegyptiaca* kernel seeds and characterized the physicochemical properties. In the current study the *Balanites aegyptiaca* kernel seeds, bark was collected, dried, grinded then nonvolatile oils were extracted separately with n-Hexane using a Soxhlet extractor. The percentage yields of extract of the plants were found to be 25.64%. The Oil have been tested against (*P. aeruginosa* (G-), *E. coli* (G-), *B. subtilis* (G+), *St. aureus* (G+) and against (*Candida albicans*) to assess their antimicrobial properties. All n-Hexane extracts possessed strong antimicrobial activity. However *Balanites aegyptiaca* kernel seeds extract had the highest activity. Therefore, the latter plant physicochemical studies were done. The fatty acids profile of *Balanites aegyptiaca* oil was analyzed by gas chromatography-mass spectrometry, it contained a total of five fatty acids including saturated linoleic and Oleic acids, and unsaturated Palmetic and steric acids also had unique antioxidant (compound). Phenol 2,6bis (1,1- dimetylethyl) 4-methyl ester. The physiochemical results showed that the oil contained kinetic viscosity (57cp), density (0.917g/cm^3), refractive index (1.472), acid value is (49.96 mg/kg), saponification value is (248.75mg/g), ester number (234.79mg/kg) and pyroxide number (0.02mg/kg). Through the physiochemical analysis, it was found that oil can be used for human consumption due to the percentage yield of unsaturated acids (81%). The results of the antioxidant activity of oil showed that the oil had moderate antioxidant activity.

Keywords: Antimicrobial Activity; Medicinal Plants; *Balanites aegyptiaca*; Oils

Introduction

Balanites aegyptica (L.) Delile, was the first to name it. aegyptiaca (L.) Del was signifying by (N.P) after being the Arabic name 'Heglig' in 1592 by Prosper Alpinio Data Center as shown below [1]. Ximenia aegyptiaca was named by Linnaeus in 1753, whereas, Delile replaced the name Agihalid with Balanites in 181, which was meant the fruit by the Greek language [1,2]. The genus of Balanites was discussible for a long time. Originally was *Zygophyllaceae* then changed to *Olacaceae Simaroubaceae* in end to *Balanitaceae* [3].

Upholding the recognition of a separate family *Balanitaceae* depends on its unique ovule and seed characters. The floral anatomy, embryology, and taxonomy, and pollen morphology uphold the retention of the genus under *Zygophyllaceae* [4].

Thus, with strenuous effort and comprehensive review, it was recognized as an independent separate family of Balanita Ecae [5,6]. The study was revealed that genus of Balanites consist of nine species and eleven intraspecific taxa [7].

Africa and some developing countries, *Balanites aegyptiaca* utilized in food preparations as well as herbal medicine, It is also called desert date (English), adua (Hausa, Nigeria), tanni (Fulfulde, Nigeria), and heglig (Arabic). *B. aegyptiaca*. The plant attains it can grow in various habitat soil types climatic conditions. All parts of plants are used, while the pulp of the fruit is eaten fresh. Protein content in this fruit is large in compared with guava, banana, and mango and papaya, the percentage of carbohydrates are about 64 - 72%, addition to crud protein steroidal saponins vitamin A, vitamin C. as well as, it has various electrolytes or minerals such as Ca(II), Mg(II), Fe(II), Zn(II), Cu(II), K^+ and P(III) ions.

All the part of this plant is beneficial even back of root and fruit. *B. aegyptiaca* is utilized to treat many diseases such as diarrhea, laxative, hemorrhoid, stomach aches, jaundice, yellow fever, syphilis even epilepsy [8]. Sometimes, the fruit is used to cure liver disease and sucked by schools children as a confectionary in some developing countries. In addition to the used of tree in different aspect of life, also, the bark 'fruit, and oil of tree have been widely used to treat diseases such as cancer tuberculosis, HIV/AIDS, malaria, diabetes, sleeping sickness wounds, colds, syphilis, liver and spleen disorders jaundice, yellow fever, snakebite, and aches the infusion of a root, bark has been used in diarrhea hemorrhoid and also acts like a fish poison [9].

Experimental Study

Materials and Methods

Materials

The seeds of cultiva *Balanites aegyptiaca*, bark were obtained within December 2019 from the local central market, Khartoum-Sudan.

Instrumentation

GC-MS model (GC/MS-QP2010-Ultra) from Japans, Shimadzu Company, Column Rtx-5MS length (30m), Diameter (0.25 mm), Thickness (0.25m).

Methods

Sample preparation

300g of seeds powder of cultiva *Balanites aegyptiaca* ware extracted with n-Hexane using a solvent extractor. In all extract, the solvent was evaporated.

Preparation of bacterial suspensions

1 ml Aliquots was distributed on nutrient agar slopes and incubated at 37°C for 24 h, then, the bacterial growth was gathering and washed with 100 ml sterile normal saline, to produce a suspension containing about 108- 109 C.F.U/ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [10]. Several dilutions of the stock suspension were carried out in sterile saline solution and 0.02 ml; volumes of the suitable dilution were transmitting by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After the incubation period has passed, the total numbers of developed colonies are count in each drop. An average number of colonies from each drop, multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, is expressed as the number of colonies forming units per ml suspension. The new pending stock has been prepared every time.

Fungi suspension

Take fungal cultures then, maintained on Sabouraud dextrose agar, and incubated at 25°C for four days. Fungal growths were harvested, washed with sterile saline, and finally suspended in 100 ml of sterile saline, and the suspension was has stored in the refrigerator until use [11].

Physicochemical properties

Refractive index: (RI)

A concave mirror was put on the base a retorted stand and a pin were clamped nearly to enable adjustment of its position until it coincided with the image at Co. The distance Co was measured and a sufficient oil sample was poured into the mirror. The position of the pin was adjusted again until it coincided with its image at position C1. The distance C1 was measured [12].

Density of oil

A sample of oil was injected into a density bottle and weighed at 600ºC, and heated for twenty minutes, thus, left to cool. On cooling, the bottle was re-weighed and then, the divergence in weight was registered as the specific gravity of the oil sample.

Determination of acid value (AV)

2g of (*Balanites aegyptiaca*) seeds oil (hexane extract) in conical flask 500 ml, 50ml of diethyl ether and 25ml of absolute ethyl alcohol, a few drops of phenolphthalein was added as indicator onto flask content were titrated against KOH (0.1N), then the volume was registered and the acid value, then, Free fatty acid were counted.

Determination of saponification value (SV)

2g of seeds oil (*Balanites aegyptiaca*) and 25ml of KOH (0.5N) It was heated in a boiling water bath for an hour. After cooling contents then titrated with HCl (0.5N) added phenolphthalein indicator, the volume was recorded, and the saponification number was counted.

Determination of ester value

The following equation shows the determination of the ester value:

Ester Value = Saponification Value - Acid Value

Peroxide value

2g of (*Balanites aegyptiaca*) seeds oil were weighted into a 250ml conical flask and 15ml of glacial acetic acid, 10ml of chloroform, 1ml of potassium iodide solution, 1ml of the starch indicator were added and the flask content placed in a dark room about 30 minutes then titrated against 0.1N Sodium thiosulfate. The volume was recorded; the same process was repeated without a sample. The peroxide value was calculated.

Viscosity of oil

The viscosity of the oil samples were recorded using an Ostwald U-tube viscometer according to Cocks and Van Rede [13].

Antioxidant activity

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of [14]. With some modification. In 96 - wells plate, the test sample was allowed to react with 2.2 Di (4-test-octypheny) -1-picryl -hydrazyl stable free radical (DPPH) for half an hour at 37oC. The concentration of DPPH was kept as (300uM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, a decrease in absorbance was measured at 517nm using a multiple reader spectrophotometer. Percentage radical scavenging Activity by analysis was run in triplicate.

Sample preparation for GC-MS analysis

2ml of (*Balanites aegyptiaca*) seeds oil was taken into test tube then alcoholic NaOH (prepared by dissolving 2g sodium hydroxide in 100ml methanol), 7ml of alcoholic H_2SO_4 (prepared by mix 1ml conc H_2SO_4 and 99ml methanol) were added, the mixture shacked by vortex for 3min and left overnight, 2ml of supersaturated NaCl and 2ml of normal hexane ware added, shacked for 3min and the hexane layer was collected, from hexane collected 2µL was taken and diluted with 5ml diethyl ether, 1g from sodium sulphate as drying agent was added and filtered through syringe 0.45µm, the filtrate was transferred directly to the GC/MS vial, 1µL was injected directly to the GC/MS. The sample components were identified and recorded.

Results and Discussion

The Extraction of *Balanites aegyptiaca* kernel seeds, the bark were performed using 9% n-Hexane solvent by the soxhlet method.

Extraction of *Balanites aegyptiaca* kernel seeds

Plant name	Extract Weight (mg) yield (%)	Color
*Balanites aegyptiaza*n-Hexane Kernel seeds	76.9025.64	Yellow

Table 1: Shows the weights and percentage extractability of n-Hexane solvent. For L. *Balanites aegyptiaca* kernel seeds.

Antimicrobial activity of *B. aegyptiaca* seeds, bark extracts

Concentration (mg/ml)	Zone of inhibition				
Extract (%)	*E. coli*	*Ps. aeruginosa*	*Bs. subtilis*	*Sa. aureus*	*Ca. albicans*
100	19	22	15		21
5017	20	17	-	20	
25	20	18	16	-	19
12	22	15	18	-	13
6	25	13	20	-	-

Table 2: Shows the antimicrobial activity of *B. aegyptiaca* seeds extract n-Hexane (sox).

Antioxidant activity of *B. aegyptiaca* oil

The antioxidant activity of *B. aegyptiaca* oil result has been shown in table 2.

Sample	Sample	%RSA ± SD (DPPH)
1	101N	54 ± 0.02
Standard	Propyl gallate	90 ± 0.01

Table 3: Antioxidant activity of *B. aegyptiaca* oil.

DPPH radicals are widely used to investigate the scavenging activity of natural compounds. When DPPH radicals encounter a proton donating substance such as an antioxidant, the radicals are scavenged and their absorbance is reduced. The results show, the extract could be an electron donor, and hence can react with free radicals to convert them to a more stable product and terminate the radical chain reaction. According to the above results, *B. aegyptiaca* oil showed moderate antioxidant activity (54%).

Sample Viscosity	Density	Refractive index	Color
57cp	0.9173g/cm^3	1.472	Yellow
Saponification value	**Acid value**	**Ester value**	**Peroxide value**
284.758(mg/g)	49.96 (mg/g)	234.79 (mg/g)	.02 (mg/kg)

Table 4: Some physicochemical properties of *B. aegyptiaca* oil.

Chemical compositions of *B. aegyptiaca* seed essential oil

The chemical composition of n-Hexane extract of *B. aegyptiaca* seeds was analyzed by gas chromatography-mass spectroscopy (GC/MS). The results have shown in table 1, the identified compounds are miscellaneous compounds that gave the highest percentage yield, the major fatty acids present in *B. aegyptiaca* oil were Phenol 2,6 bis (1,1- dimethyl ethyl) 4-methyl ester(antioxidant) unsaturated hydrocarbons fatty acids linoleic acid and oleic acid while saturated compound Palmitic acid, stearic acid [15].

Peak	R. time	Area	Area %	A/H	Name
1	11.440	6156223	2.44	1.76	Butylated hydroxytoluene
2	16.132	28858012	11.45	2.17	Hexadecanoic acid methyl ester
3	17.874	81610323	32.39	2.08	9,12 Octadecadienoic (Z,Z)- methyl ester
4	17.917	110058509	43.69	2.51	9-Octadecenoic (Z)- methyl ester
5	18.143	25245542	10.02	2.39	Methyl stearate
		251928609	100.00		

Table 5: Chemical compositions of *B. aegyptiaca* seeds essential oil.

Peak	IUPAC-Name	Common-Name	M.F.
1	Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$
2	Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$
3	(9Z)-Octadec-9-enoicacid	Oleic acid	$C_{18}H_{34}O_2$
4	9, 12, 15-octadeca triennia acid-(Z, Z, Z).	Linoleic acid	$C_{18}H_{30}O_2$
5	2-6ditertbutyl-3-methyl phenol	Phenol 2,6 bis (1,1- dimethylethyl)-4-methyle	$C_{15}H_{24}O$

Table 6: Fatty acids profile.

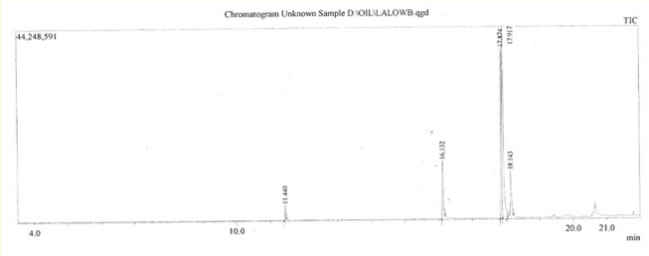

Figure 1: Shows fatty acids profile.

Conclusion

This study concluded that *Balanites aegyptiaca* seeds, *Azadirachta indica* seeds bark had potential sources of oil due to their high percentage oil yield obtained in the present study. The result obtained from GS/MS for the essential oil of *Balanites aegyptiaca* seeds extracted by n-hexane demonstrated promising physicochemical properties especially rich with unsaturated essential fatty acid. The physicochemical characteristics of the Fatty acid profile of *Balanites aegyptiaca* oil made it a potential raw material for cosmetics, soap, and food processing (as edible vegetable oil). The results of the study displayed that *Balanites aegyptiaca* seeds inhibited the growth of various species of gram-negative and gram-positive bacteria and fungus. *Balanites aegyptiaca* seeds had moderate antioxidant activity.

Bibliography

1. Sands MJS. "The desert date and its relatives: a revision of the genus". *Balanites Bulletin* 56.1 (2001): 1-128.

2. Hall JB and DH Walker. "Wales, Balanites aegyptiaca; A monograph". School of Agricultural and Forest Sciences Publication (1991).

3. Saed A Al-Thobaiti and Isam M Abu Zeid. "Medicinal Properties of Desert Date Plants Balanites aegyptiaca) – An Overview". Jadah. Biological Sciences, Faculty of Sciences, King Abdulaziz University 12.1 (2018).

4. Mokhtar Mohamedalamin Mokhtar., *et al*. "Nsecticidal efficacy and chemical composition of Balanites aegyptiaca (L.) Delile seed oils against Tribolium castaneum Herbst (Coleoptera: Tenebrionidae)". *Chilean Journal of Agricultural Research* 81.1 (2021).

5. Sands MJS. "Kew, Flora of tropical East Africa: Balanitaceae". In: Beentje H.J. (ed.) and Ghazanfar S.A. (subed.), Flora of tropical East Africa, Royal Botanic Gardens (2013): 1-17.

6. Singh KK., *et al*. "India, Evaluation of certain feed resources for carbohydrate and protein fractions and in situ digestion characteristics". *Indian Journal of Animal Sciences* 72.9 (2002): 794-797.

7. Orwa C., *et al*. "Kenya. Agroforestree Database: a tree reference and selection guide; version 4.0". *World Agroforestry* (2009): 1-5.

8. Ojo OO., *et al*. "Protection of rats by extracts of some common Nigeriantreeb against acetaminophen inducedhepatotoxicity". *African Journal of Biotechnology* 5 (2006): 755-760.

9. Bhimraj Gawade., *et al*. "Evolution of antioxidant potential and phytochemicals of Balanites aegyptiaca (Linn), Leaf Extract". *Asian Journal of Science and Technology* 11.11 (2020): 11336-11340.

10. Hajir B Abdllha., *et al*. "Evolution of Antimicrobial, Antioxidant Potentials and Phytochemical Studies of Three Solvent Extracts of Five Species from Acacia Used in Sudanese Ethnomedicine". *Advances in Microbiology* 6.9 (2016): 691-698.

11. Abdel Karim M., *et al*. "Isolation, Characterization and Antimicrobial Activity of a Flavanone from Sudanse Albizia amara (Fabaceae) leaves". *International Journal of Advanced Research* (2016).

12. Magu Thomas., *et al*. "Physico-chemical properties and fatty acid composition of Lagneraria siceraria seed oil". *World News of Natural Sciences* 10 (2017): 70-79.

13. Cocks and Van Rede. "Laboratory handbook for oil and fat analysis". London: Academic Press (1966).

14. Shimada K., *et al*. "Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion". *Journal of Agricultural and Food Chemistry* 40 (1992): 945-948.

15. Bioactivity. "Therapeutic utility and toxicological risks of Khaya senegalensis Indian" 1.4 (2001): 123-124.

Volume 2 Issue 8 August 2021