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# Antimicrobial activity and physicochemical properties of *Balanites aegyptiaca* seed oil

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ABSTRACT

This study was aimed to assess the antibacterial and antifungal activities of *Balanites aegyptiaca* seed oil and characterize the physicochemical properties. Seeds were collected from the local central market, Khartoum-Sudan (2019). The samples were dried under shade and grinded, then the oil was extracted with a Soxhlet extractor using *n*-hexane. The percentage yield of the extract was found to be 25.64%. The seed oil was tested against *Pseudomonas aeruginosa* (G-), *Escherichia coli* (G-), *Bacillus subtilis* (G+), *Staphylococcus aureus* (G+), and *Candida albicans* to assess their antimicrobial properties. The extract of *B. aegyptiaca* seed oil has antimicrobial activity against most of the organisms tested. The fatty acid profile of the *B. aegyptiaca* seed oil was analyzed by GC/MS. The results revealed that the presence of five fatty acids, including saturated linoleic acid, oleic acid, and unsaturated palmate and stearic acids, also a unique antioxidant compound butylated hydroxytoluene. The physiochemical properties of the seed oil showed that the oil contained kinetic viscosity (57 cp), density (0.917 g/cm$^3$), refractive index (1.472), acid value (49.96 mg/kg), saponification value (248.75 mg/g), ester number (234.79 mg/kg) and peroxide number (0.02 mg/kg). Through physiochemical analysis, it was found that oil can be used for human consumption due to the percentage yield of unsaturated acids (81%). In addition, the results of the antioxidant activity of the seeds oil showed that the seed oil had moderate antioxidant activity.

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## 1. Introduction

*Balanites aegyptiaca* (L.) Delile is an underutilized fruit yielding tree native to Africa and distributed in tropical and subtropical regions of Africa, from Senegal in the west to Somali in the east and Jordan in the north to Zimbabwe in the south. *B. aegyptiaca* is also distributed in India, Myanmar, Iran, Jordan, Oman, Palestine, Saudi Arabia, Syria, and Yemen [1]. *B. aegyptiaca* is used in African and India as a folk medicine. The roots and bark of *B. aegyptiaca* are used as purgative and anthelmintic. The root of *B. aegyptiaca* is also used to treat malaria. Bark is used to deworm cattle, and roots are used to treat edema and stomach pain. Roots are also used as an emetic [2]. The fruit of *B. aegyptiaca* is used to treat jaundice in Sudan. Seed oil of *B. aegyptiaca* is used as a laxative and for the treatment of hemorrhoids, stomach aches, jaundice, yellow fever, syphilis and epilepsy. Bark extracts are used to kill freshwater snails and copepods. The bark of *B. aegyptiaca* has antifungal activity against *Candida albicans* infections, it is also antiviral and antibacterial [3-5]. *B. aegyptiaca* [L.] Delile seed oils has been used against the red flour beetle (Tribolium castaneum Herbst) [6]. Sometimes, the fruit is used to cure liver disease. In addition to the use of tree in different aspects of life, also the bark, fruit, and oil of *B. aegyptiaca* have been widely used to treat diseases such as cancer, tuberculosis, malaria, diabetes, sleeping sickness wounds, colds, syphilis, liver and spleen disorders jaundice, yellow fever and snakebite [7].

## 2. Experimental

### *2.1. Materials and measurements*

The seeds of *B. aegyptiaca* bark cultiva were purchased from the local central market, Khartoum, Sudan, in December 2019. The powder of seeds (300 g) of cultiva *B. aegyptiaca* were extracted with *n*-hexane. The Shimadzu GC/MS-QP2010-Ultra model GC/MS instrument (Column RTX-5MS, length (30 m), diameter (0.25 mm), and thickness (0.25 m)) was used for the oil characterization.



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### 2.2. Preparation of bacterial suspensions

Aliquot (1 mL) was distributed on nutrient agar slopes and incubated at 37 °C for 24 hours, then the bacterial growth was gathering and washed with 100 mL sterile normal saline, to produce a suspension containing about $1\times10^8$-$1\times10^9$ CFU/mL. The suspension was stored in the refrigerator at 4 °C until use. The average number of viable organisms per microliter of stock suspension was determined using the surface viable counting technique [8]. Several dilutions of the stock suspension were carried out in sterile saline solution and 0.02 mL; volumes of the suitable dilution were transmitted by micropipette to 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 number of colonies developed in each drop is counted. 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 that form units per ml of suspension. The new pending stock has been prepared every time.

### 2.3. Preparation of fungi suspension

The fungal cultures were 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; the suspension was stored in the refrigerator until used [9].

### 2.4. Physicochemical properties

#### 2.4.1. Refractive index (RI)

A concave mirror was placed on the base of a retort stand, and a pin was clamped approximately to allow adjustment of its position until it coincided with the image at $C_0$. The distance $C_0$ 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 $C_1$. The distance $C_1$ was measured [10].

#### 2.4.2. Density of oil

A sample of oil was injected into a density bottle and weighed at 600 °C, heated for 20 minutes, and therefore left to cool. On cooling, the bottle was reweighed and then the divergence in weight was registered as the specific gravity of the oil sample [10,11].

#### 2.4.3. Determination of the acid value (AV)

*B. aegyptiaca* seed oil (2 g) (*n*-hexane extract) was placed in a conical flask (500 mL), later 50 mL of diethyl ether, 25 mL of absolute ethyl alcohol, a few drops of phenolphthalein were added as indicator in flask content. The free fatty acid contents were titrated against KOH (0.1 N) according to reference [12].

#### 2.4.4. Determination of saponification value (SV)

Seed oil of *B. aegyptiaca* (2 g) and 25 mL of KOH (0.5 N), it was heated in a boiling water bath for an hour. After cooling, contents were then titrated with HCl (0.5 N) using pH indicator, volume and the saponification number was obtained according to reference [12].

### 2.5. Determination of ester value

Equation (1) shows the determination of the ester value:

Ester value = Saponification value - Acid value (1)

#### 2.5.1. Peroxide value

*B. aegyptiaca* seed oil (2 g) was weighted in a 250 mL conical flask, later 15 mL of glacial acetic acid, 10 mL of chloroform, 1 mL of potassium iodide solution, 1 mL of starch indicator were added and the flask content was placed in a dark room for about 30 minutes and then it was titrated against 0.1 N sodium thiosulfate. The peroxide value was calculated according to the reference [13].

#### 2.5.2. Viscosity of oil

The viscosity of the oil samples was recorded using an Ostwald U-tube viscometer according to Cocks and Van Rede [14].

### 2.6. Antioxidant activity

DPPH radical scavenging was determined according to the method of Shimada *et al*. with some modifications [15]. In 96-wells plate, the test sample was allowed to react with 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl, free radical (DPPH) for half an hour at 37 °C. The DPPH concentration was kept at 300 µM. The test samples were dissolved in dimethylsulfoxide (DMSO), while DPPH was prepared in ethanol. After incubation, a decrease in absorbance was measured at 517 nm using a multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group and all analysis were run in triplicate [15].

### 2.7. Sample preparation for GC/MS analysis

*B. aegyptiaca* seed oil sample was analyzed using a Gas Chromatography (GC, Shimadzu GCMS-QP2010-Ultra). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.98 mL/min, the temperature program was started from 60 °C with rate 10 to 300 °C as the final temperature degree with 5 minutes hold time, the injection port temperature was 250 °C, the ion source temperature was 200 °C and the interface temperature was 250 °C. The sample was analyzed using scan mode in the range of *m/z* 40-500 charges to ratio, and the total run time was 27 min. The identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, Regional Forensic Laboratories Directorate, Sudan.

### 2.8. Antimicrobial activity

#### 2.8.1. Antibacterial screening

The antibacterial activity of the *B. aegyptiaca* seeds extract *n*-hexane were tested by using agar disk diffusion method against four bacteria; *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The extract concentrations are between 100 to 6 µg/mL. Twenty-five milliliter nutrient agar media was poured in Petri-plates. After solidification, 0.1 mL of bacteria was spread over the medium using a spreader. Diffusion discs of approximately 5 mm diameter were prepared from Whatman No. 1 filter papers and placed at four equidistant places at a distance of 2 cm from the center. Petri plates were incubated at 37 °C for 26 h. The zone of inhibition was calculated and compared with that of DMSO to evaluate the zone of inhibition due to the tested compounds [16].

**Table 1.** Antibacterial activity of *B. aegyptiaca* oil.

| Oil concentration (µg/mL) | Diameter of inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | Gram-positive bacteria | | Gram-negative bacteria | | Fungus |
| | *B. subtilis* | *S. aureus* | *E. coli* | *P. aeruginosa* | *C. albicans* |
| 100 | 15 | 18 | 19 | 8 | 21 |
| 50 | 13 | 20 | 20 | 10 | 16 |
| 25 | 16 | 9 | 20 | 11 | 19 |
| 12 | 18 | - | 22 | 14 | 13 |
| 6 | 20 | - | - | 11 | - |

**Table 2.** Some physicochemical properties of *B. aegyptiaca* oil.

| Viscosity | Refractive index | Color | Density |
|---|---|---|---|
| 57 cp | 1.472 | Yellow | 0.9173 g/cm$^3$ |
| **Saponification value** | **Acid value** | **Ester value** | **Peroxide value** |
| 248.75 mg/g | 49.96 mg/g | 234.79 mg/g | 0.210 mg/g |

**Table 3.** Chemical composition of *B. aegyptiaca* seed oil.

| No | Retention time | Area % | Name | Type |
|---|---|---|---|---|
| 1 | 11.440 | 2.44 | Butylated hydroxytoluene | Antioxidant compound |
| 2 | 16.132 | 11.45 | Hexadecanoic acid methyl ester | Methyl palmitate |
| 3 | 17.874 | 32.39 | 9,12 Octadecadienoic(*Z,Z*)-methyl ester | Linoleic acid |
| 4 | 17.917 | 43.69 | 9-Octadecenoic(*Z*)-methyl ester | Oleic acid |
| 5 | 18.143 | 10.02 | Methyl stearate | Stearic acid (40.0-60.0%) |

### *2.8.2. Antifungal screening*

The antifungal activities of the *B. aegyptiaca* seed extract were tested against a fungus, *Candida albicans*. The extract having concentrations of 100 and 6 µg/mL were poured into Petri plates and a similar experiment was repeated and the inhibition zones formed were measured and compared with DMSO to evaluate the inhibition zone [16].

## 3. Results and discussion

The extraction of *B. aegyptiaca* seeds was carried out using a 9% *n*-hexane solvent by the Soxhlet method. The percentage yield of *B. aegyptiaca* is 25.64%, which is comparable to that of various seed oils in other works [17,18]. However, this obtained yield was less than the study reported by reference [17] (36.5%) and higher than cottonseed (18-28%), soya been (11-25%) and rubber (21-25%). The yellow color of the seed oil is in agreement with that reported by reference [18].

The antimicrobial activities of the extracts of *B. aegyptiaca* against five standard microorganisms are given in Table 1. The results were interpreted in terms of commonly used terms (less than 9 mm: inactive; 9-12 mm: partially active; 13-18 mm: active; more than 18 mm: very active). The evaluation was performed using the adopted disc diffusion method. The results obtained were measured with the inhibition zone. Some concentrations possess higher antimicrobial activity against most of the organisms tested. Some extracts also showed high antifungal activity against *C. albicans* (100, 25 µg/mL). This result is in agreement with previous reported studies [6,19,20].

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 that 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 obtained results, *B. aegyptiaca* oil showed moderate antioxidant activity (54±0.02%) (%RSA±SD (DPPH) for propyl gallate: 90±0.01%).

The results show that the oil content of the extracted oil of *B. aegyptiaca* was high, which shows that the processing of the oil for industrial or edible use would be economical and feasible. The viscosity of the oil at room temperature is 57 cp (Table 2). From the results, it can be seen that the viscosity is on the high side when compared to the ASTM standard value. The viscosity of *B. aegyptiaca* seed oil was the highest for the seed oils within reported by reference [21]. The refractive index for the investigated extract was found to be 1.472, which is similar to the value in the reference [22] who reported 1.4784.

The acid value was determined to quantify the fatty acid found in the oil. The acid value was found as 49.96 mg/g. This shows that the oil sample investigated was stable. This result is in agreement with previous reported studies [23,24]. The peroxide value is used as an indication of the quality and stability of fats and oils. The peroxide value determined for the seed oil of *B. aegyptiaca* was found in 0.210 mg/g is within the FAO/WHO standard less than 10 mEq/Kg and the lower peroxide value the more suitable is the oil for long storage, implying longer shelf life [22,25]. The density of the seed oil studied was found to be 0.9173 g/cm$^3$, which is consistent with that reported by reference [22], and slightly higher than the study reported by reference [18] who reported 0.88. Meanwhile, the finding of this study is within the FAO/WHO standard of 0.909. The saponification value was determined to quantify the amount of triglyceride. Saponification value was found to be 248.75 mg/g. The saponification value of oil is a significant quality in determining the suitability of the oil for soap making. Thus, the value of saponification indicates that the oil is useful in the production of soap [13].

### *3.1. Chemical composition of B. aegyptiaca seed oil*

The chemical composition of *n*-hexane extract of *B. aegyptiaca* seed oil was analyzed by gas chromatography-mass spectroscopy (GC/MS). The results have shown in Table 3, the identified compounds are miscellaneous compounds that gave the highest percentage yield. This is in agreement with previously reported studies [6,18].

## 4. Conclusions

This study concludes that the seeds of *B. aegyptiaca* is a potential source of therapeutic oil due to high percentage of oil yield obtained in the present study. The results obtained from GS/MS for the essential oil of *B. aegyptiaca* seeds extracted via *n*-hexane extract solution demonstrated promising physico-chemical properties especially rich with unsaturated essential fatty acid. The physicochemical characteristics of the fatty acid profile of *B. aegyptiaca* oil are potential raw materials for cosmetics, soap, and food processing (as edible vegetable oil). The results of the study showed that B. aegyptiaca seeds inhibited the growth of various species of Gram negative and Gram positive bacteria and fungi. The seeds of *B. aegyptiaca* had

moderate antioxidant activity. Thus, our study can be considered as an approach to future studies about the seed oil of *B. aegyptiaca* to produce antimicrobial.

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Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

**CRediT authorship contribution statement**

Conceptualization: Abdalla Gobara Habieballa; Methodology: Halima Elfadel Elebeed; Software: Abdalla Gobara Habieballa; Validation: Madena Komi Koko; Formal Analysis: Madena Komi Koko; Investigation: Awad Salim Ibrahim; Data Curation: Asha Fadllallah Wady; Writing - Original Draft: Abdalla Gobara Habieballa; Writing - Review and Editing: Abdalla Gobara Habieballa; Visualization: Abdalla Gobara Habieballa; Supervision: Abdalla Gobara Habieballa; Project Administration: Abdalla Gobara Habieballa.

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**References**

[1]. Murthy, H. N.; Yadav, G. G.; Dewir, Y. H.; Ibrahim, A. *Plants* **2020**, *10* (1), 32.
[2]. Agroforestree database: a tree reference and selection guide version 4.0, https://www.worldagroforestry.org/publication/agroforestree-database-tree-reference-and-selection-guide-version-40 (accessed Oct 16, 2021).
[3]. Sarker, S. D.; Bartholomew, B.; Nash, R. J. *Fitoterapia* **2000**, *71* (3), 328–330.
[4]. Iwu, M. M. *Handbook of African Medicinal Plants*, 2nd ed.; CRC Press: Boca Raton, FL, 2014.
[5]. Maregesi, S. M.; Pieters, L.; Ngassapa, O. D.; Apers, S.; Vingerhoets, R.; Cos, P.; Berghe, D. A. V.; Vlietinck, A. J. *J. Ethnopharmacol.* **2008**, *119* (1), 58–66.
[6]. Mokhtar, M. M.; Jianfeng; Du, Z.; Cheng, F. *Chil. J. Agric. Res.* **2021**, *81* (1), 102–108.
[7]. Abdllha, H. B.; Mohamed, A. I.; Almoniem, K. A.; Adam, N. I.; Alhaadi, W.; Elshikh, A. A.; Ali, A. J.; Makuar, I. G.; Elnazeer, A. M.; Elrofaei, N. A.; Abdoelftah, S. F.; Hemidan, M. N. *Adv. Microbiol.* **2016**, *06* (09), 691–698.
[8]. Abdel Karim, M.; Mongid, S. *Int. J. Adv. Res. (Indore)* **2016**, *4* (8), 1799–1805.
[9]. Rodríguez-Tudela, J. L.; Cuenca-Estrella, M.; Díaz-Guerra, T. M.; Mellado, E. *J. Clin. Microbiol.* **2001**, *39* (7), 2513–2517.
[10]. Yadav, J. P.; Panghal, M. *Int. J. Green Pharm.* **2010**, *4* (3), 140–146.
[11]. Al Ashaal, H. A.; Farghaly, A. A.; Abd El Aziz, M. M.; Ali, M. A. *J. Ethnopharmacol.* **2010**, *127* (2), 495–501.
[12]. Mohamed, A. M.; Wolf, W.; Spieb, W. E. L. *Plant Foods Hum. Nutr.* **2002**, *57* (2), 179–189.
[13]. Datti, Y.; Yahya, A. T.; Koki, I. B.; Lado, U. A.; Musbahu, L.; Shuaibu, M. M.; Ahmad, U. U.; Adamu, G. A.; Umar, A.; Nura, T. *Fudma J. Sci.* **2020**, *4* (2), 250–259.
[14]. Cocks; Victor., L.; Rede; Van, C. *Laboratory Handbook for Oil and Fat Analysts*; Cocks, L. V., Van Rede, C., Eds.; Academic Press: San Diego, CA, 1966.
[15]. Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. *J. Agric. Food Chem.* **1992**, *40* (6), 945–948.
[16]. Lopez-Bote, C. J.; Gray, J. I.; Gomaa, E. A.; Flegal, C. J. *Br. Poult. Sci.* **1998**, *39* (2), 235–240.
[17]. Bouanga-Kalou, G.; Nitou, J. G.; Nzikou, J. M.; Ganongo-Po, F. B.; Hounounou, C. H.; Diabangouaya, B.; Mpika-Bouesso, J.; Silou, T.; Desobry, S. *Australian J. Basic Appl. Sci.* **2011**, *5* (3), 475–482.
[18]. Ogala, H.; Elinge, C. M.; Wawata, I. G.; Adegoke, A. I.; Muhammad, A. B.; Ige, A. R. *Inter. J. Adv. Acad. Res.* **2018**, *4* (4), 1–12.
[19]. Kirschenbauer, H. G. Fats and oils – an outline of their chemistry and technology. Reinhold Publishing Corporation, New York 1960. 240 S., *Fette, Seifen, Anstrichm.* **1962**, *64* (10), 956–956.
[20]. Yadav, J. P.; Panghal, M. *Int. J. Green Pharm.* **2010**, *4* (3), 140–146.
[21]. Fregon, S. M. MSc Thesis, Physicochemical Properties of Balanites aegyptiaca (Laloub) Seed Oil, Sudan University of Science and Technology, 2015.
[22]. Manji, A. J.; Sarah, E. E.; Modibbo, U. U. *Int. J. Phys. Sci.* **2013**, *8* (33), 1655–1660.
[23]. Diedhiou, D.; Faye, M.; Candy, L.; Vandenbossche, V.; Vilarem, G.; Sock, O.; Rigal, L. *Afr. J. Biotechnol.* **2021**, *20* (4), 150–158.
[24]. Hassan, D. M. *MOJ Bioorg. Org. Chem.* **2017**, *1* (6), 228–232.
[25]. Adebayo, S. E.; Orhevba, B. A.; Adeoye, P. A.; Musa, J. J.; Fase, O. J. *Academic Res. Inter*. **2012**, *3* (2), 178–183.