

Available online at [GSC Online Press Directory](https://www.gsonlinepress.com/)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsonlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



Phytochemical composition of desert date kernel (*Balanites aegyptiaca*) and the physical and chemical characteristics of its oil

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Publication history: Received on 08 June 2020; revised on 26 June 2020; accepted on 27 June 2020

Article DOI: <https://doi.org/10.30574/gscbps.2020.11.3.0166>

Abstract

The desert date (*Balanites aegyptiaca*) is an important tree found in some African countries. In this study the phytochemical composition of the desert date kernel and some physical and chemical properties of its oil were analyzed using standard procedures. The results of the phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, tannins, steroids and glycosides. While the results for the physical analysis of the kernel oil revealed the following: Color, pale yellow; density, 0.910 g/cm³; specific gravity, 0.907; refractive index, 1.458; and viscosity, 19.68. And on the other hand, the results for the chemical analysis of the kernel oil revealed the following: Acid value, 3.06 mgKOH/g; free fatty acid, 1.27; peroxide value, 3.71 mEq/Kg; saponification value, 198 mg/KOH/g; and iodine value, 98.73 100/g. All the results were compared and found to be within the FAO/WHO standards.

Keywords: Desert date; Kernel; Phytochemical composition; Chemical characteristics

1. Introduction

Desert date (*Balanite aegyptiaca* Linn.) is an important multipurpose tree found in most African countries (Clement *et al.*, 2011). *Balanites Aegyptica* has multiple applications with almost every part of the plant being useful. The tree is actually valued more for its fruits and seeds: The fruits and kernel are widely used in many ways in different countries including Nigeria especially during the dry season and drought periods (Lockett *et al.*, 2000). The kernel of this plant is traditionally used in the treatment of various ailments such as jaundice, intestinal worm infection, malaria, syphilis, epilepsy, dysentery, constipation, haemorrhoid among others (Daya and Vaghasiya, 2011). The stem of the plant is used as timber, poles, charcoal, firewood, fodder, food (Elseed *et al.*, 2002), gum, shade, gum and windbreak (Guinand and Lemessa 2001). The plant may be grown for its fiber, oil and/or for medicinal values. It is also used in treatment of several diseases and disorders since ages.

Balanites Aegyptica fruit is readily available in northern Nigeria and the seed which is obtained after it is being sucked is seen as a nuisance along markets and settlements in communities (Jock 2017). This is because no suitable application for the seed kernel has been found in most developing countries. However, the seed kernel oil of *Balanites aegyptiaca* is a good source of raw material for food, cosmetic and pharmaceutical industries (Jock 2017).

This multipurpose tree is used for food and fodder in almost all parts of Africa and South Asia (Billore 1988; Elseed *et al.*, 2002). Among the various useful parts of the plant, the fleshy pulp of the fruit is eaten fresh or dried; it also used as a food, beverage and medicines. The protein content of the fruit of this plant is believed to be superior to that in guava,

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mango, banana and papaya. The fleshy fruit contains high carbohydrates and steroidal saponins (Al-Thobaiti and Abu-Zeid 2018), vitamin A, vitamin C and other essential minerals for human. Seed kernel also widely used for oil. The kernel produces high quality edible oil (Obidah *et al.*, 2009) with large number of medicinal properties (Hanan *et al.*, 2009), and is also rich in protein and minerals contents (Elfeel and Warrag, 2011). In quality aspect, it is similar to sesame and groundnuts oils (Abu Al-Futuh, 1983; Obidah *et al.*, 2009), with the oil used as a biodiesel (Chapagain *et al.*, 2009; Gutti *et al.*, 2012; Kumawat *et al.*, 2012).

The aim of this study is to determine the phytochemical constituents of desert date kernel *Balanites aegyptiaca*, and also to investigate the physical and chemical characteristics of the desert date kernel oil.

2. Material and Methods

2.1. Sample Collection and Preparation

The fruits of *Balanites aegyptiaca* (desert date) were purchased from Rimi market in Kano State, Nigeria and brought to the department of Biological Sciences, Yusuf Maitama Sule University, Kano, for identification by a plant taxonomist. The plant was identified and authenticated and issued a voucher specimen number YUHAN 0058. The fruits were crushed using a steel hammer to obtain the kernels, which were then air-dried and then ground using mortar and pestle (Bayero *et al.*, 2019). The ground kernel was then packed in an air tight container and then stored in a desiccator (containing silica gel) ready for further analysis.

All the chemicals and reagent used were of analytical grade.

2.2. Extraction of the Sample

The powdered sample (50 g) was soaked in 300 ml of absolute ethanol for 72 hrs, and stored away from direct light. The supernatant was decanted and filtered using filter paper and the filtrate was evaporated to dryness, and then stored in sample bottles at room temperature to avoid any biological degradation (Bayero *et al.*, 2019).

2.3. Phytochemical Analysis

The crude ethanol extract was phytochemically screened for the presence of secondary metabolites such as alkaloids, saponins, phenols, flavonoids, tannins, steroids, reducing sugars, terpenoids, glycosides, volatile oils and anthraquinones in accordance with standard methods by Harborne (1998) with slight modifications by Tripathi and Mishra (2015) and Alhassan *et al.*, 2018.

2.3.1. Test for Alkaloids

The ethanol extract (2 g) was dissolved in dilute 1% hydrochloric acid and filtered.

Mayer's Test: A portion of the filtrate (1 ml) was treated with 4 drops of Mayer's reagent (potassium iodide). Formation of white or pale yellow precipitate indicates the presence of alkaloids.

2.3.2. Test of Saponin

Froth Test: A portion of the ethanol extract (2 ml) was diluted with distilled water to 5 ml and then shaken in a graduated cylinder for 5 minutes. Formation of honeycomb froth confirms the presence of saponin.

2.3.3. Test for Phenols

A portion of ethanol extract (2 ml) was treated with 4 drops of ferric chloride (FeCl₃) solution. Formation of a blue-green colour confirms the presence of phenols.

2.3.4. Test for Flavonoids

Alkaline Reagent Test: A fraction of the extract (2 ml) was treated with 4 drops of 1% sodium hydroxide. Formation of yellow colour indicates the presence of flavonoids.

2.3.5. Test for Tannins

Lead Acetate Test: A portion of the filtrate (1 ml) was treated with 3 drops of 1% lead acetate in a test tube. Formation of a blue-black colour indicates the presence of tannins.

2.3.6. Test for Steroids

Salkowski Tests: Chloroform (5 ml) was added to 0.5 ml of the filtrate in a test tube, and then an equal volume of concentrated sulphuric acid (5 ml) was added by the sides of the test tube. Formation of a red colour on standing indicates the presence of steroids.

2.3.7. Test for Reducing Sugars

Benedict's Test: Benedict's solution (2.5 ml) was added to the extract (1 ml) in a test tube, and then warmed over water bath for about 5 minutes. Formation of a green, red or yellow coloration indicates the presence of reducing sugar.

2.3.8. Test for Terpenoids

Chloroform (2 ml) was added to 5 ml of the ethanol extract (5 ml), and carefully followed by 3 ml of Conc H₂SO₄ to form a layer. Appearance of a reddish brown coloration indicates the presence of terpenoids.

2.3.9. Test for Glycosides

The sample extract (5 ml) was mixed with 2 ml of glacial acetic acid containing 1 drop of FeCl₃ solution, and then 1 ml of Conc. H₂SO₄ was further added. Formation of a brown ring of interface indicates the presence of glycosides.

2.3.10. Test for Volatile Oils

Five (5) grams of the ethanol extract was dissolved in 90% alcohol and 4 drops of ferric chloride were added. Formation of green coloration indicates the presence of volatile oils.

2.3.11. Test for Anthraquinones

Sulphuric acid (10 ml) was added to 0.5 g of the extract and then boiled and filtered while hot. The filtrate was further shaken with 5 ml of chloroform, and the chloroform layer transferred to another test tube and 1 ml of dilute ammonia solution was added. Formation of a pink, red or violet colour indicates the presence of anthraquinones.

2.4. Extraction and Purification of the Oil

The oil was extracted from the kernels of the desert date using the solvent extraction process, using petroleum ether as a solvent by soxhlet apparatus (Karamat *et al.*, 2003; Adejumo *et al.*, 2013; Ratna *et al.*, 2014). For purification, the oil was taken in a separating funnel along with water (100 ml), ether (200 ml) and saturated sodium chloride the content was well shaken and then allowed to stand. The aqueous layer was then discarded and the process was repeated three times with organic layer. Finally the ethereal extract was taken in a conical flask and then dried over 20 g anhydrous sodium sulfate and was evaporated at a temperature of 40°C to get the purified oil (Karamat *et al.*, 2003; Ratna *et al.*, 2014).

2.5. Physical Characteristics of the Oil

2.5.1. Color of Oil

The color of the extracted oil from the desert date kernel was observed visually as reported by Ogala *et al.*, (2018).

2.5.2. Determination of Density and Specific Gravity of the Oil

The density of the desert date oil was determined using pre-washed, dried and labeled density bottles. The density bottled was then filled up to the volume mark with the oil sample, and the weight of the density bottle with the oil was determined. The density bottled was also filled up to the volume mark with the water, and the weight of the density bottle with the water was determined (Myles 2001; Brosk 2014). The density and the specific gravity of the oil were calculated using the formulae:

$$\text{Density} = \frac{W_2 - W_1}{V}$$

$$\text{Specific Gravity} = \frac{W_2 - W_1}{W_3 - W_1}$$

Where;

W1 = Weight of Empty Density Bottle
 W2 = Weight of Density Bottle + Oil
 W3 = Weight of Density Bottle + Water
 V = Volume of Oil.

2.5.3. Refractive Index of the Oil

The refractive index of the oil was determined using Abbe-60 refractometer (NYRL 3-Leica Mark, Leica Inc., Buffalo, New York) as described by AOAC (2008).

2.5.4. Viscosity of the Oil

The viscosity of the oil sample was investigated using the Ostwald-U-tube viscometer according to AOAC (2008). The viscometer was first suspended in a constant temperature water bath so that the capillary was vertical. The instrument was filled with the oil to the mark at the top of the lower reservoir with the aid of a pipette inserted in the side arm in such a way that the tube wall above the mark was not wetted. The instrument was then left to stand for 3 minutes before reading in order to equilibrate the sample temperature with that of the instrument (35°C). By means of pressure on the respective arm of the tube, the oil moved into the other arm so that the meniscus was above the mark at the top of upper reservoir. Finally, the liquid was allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at the bottom of the upper reservoir was recorded.

2.6. Chemical Characteristics of *B. aegyptiaca* Kernel Oil

2.6.1. Acid Value of the Oil

Here 2 g of the test sample was transferred into a conical flask, and then 50 cm³ petroleum ether added and gently mixed. Ethanol (50 cm³) was then added into the mixture and titrated with 0.1 M KOH to pink colour (AOAC, 2008). The Acid value was calculated using the formula:

$$\text{Acid Value (mg KOH/g)} = \frac{\text{Titre Value} \times \text{Normality} \times 56.1}{\text{Weight of Sample}}$$

$$\% \text{ Free Fatty Acid} = \frac{\text{Titre Value} \times 28.2 \times \text{Normality}}{\text{Weight of Sample}}$$

$$1 \text{ cm}^3 \text{ of } 1 \text{ M KOH} = 56.1 \text{ mg of KOH}$$

2.6.2. Peroxide Value of the Oil

The peroxide value (PV) of desert date kernel oil was determined according to the procedure reported by Wail *et al.*, (1995) and adopted by Mohamed and Mohammed (2018), where 1 g of the test sample was transferred into 250 ml conical flask, then 30 ml of a glacial acetic acid/chloroform solution (ratio 3:2) was added, and the flask was gently shaken until the sample was dissolved, and then 0.5 ml of saturated potassium iodide was added. The solution was one again gently shaken for 1 minute, and 30 ml of distilled water was added followed by 0.5 ml of 1% starch solution. The content of the flask was later titrated with 0.1 N sodium thiosulphate with constant and vigorous shaking until the blue colour just disappeared. A blank test was also carried out in similar manner. The volume of the 0.1 N sodium thiosulphate required was recorded.

$$PV = \frac{(Va - Vb) N \times 100}{W}$$

Where;

Va = Volume of sodium thiosulphate solution used in the titration
 Vb = Volume of sodium thiosulphate solution used in the blank test
 W = Weight of the sample in grams
 N = Normality of sodium thiosulphate.

2.6.3. Saponification Value of the Oil

Determination of the saponification value of the desert date kernel oil was carried out according to the AOAC (2008) method, where 1 g of the oil sample was transferred into 200 ml conical flask, and the 25 ml of 0.1N alcoholic KOH

solution was added. The flask and its content were boiled under reflux for one hour with frequent rotation, then 1 ml of phenolphthalein indicator was added while the solution was still hot, and the excess alkali was titrated with 0.5N HCl, with the volume of HCl required to complete the titration recorded. The same procedure was repeated for the blank and the volume of HCl required to complete the titration also recorded.

$$\text{Saponification Value} = \frac{(b - a) \times 0.02805 \times 1000}{S}$$

Where;

a = Volume of HCl used to titrate the sample

b = Volume of HCl used to titrate the blank

S = Weight of oil in gram.

2.6.4. Iodine Value of Oil

The iodine value (IV) of the oil sample was determined according to the BSI (1985) method as adopted by Mohamed and Mohammed (2018), where 0.26 g of the sample was weighed into a glass stoppered flask and then dissolved with 10 ml cyclohexane. Then 20 ml of Wijs solution (iodine monochloride dissolved in acetic acid) was added and the flask was stoppered and then allowed to stand in the dark for 30 minutes at a temperature of 25°C after which 20 ml of 10% potassium iodide solution was added. The mixture was then titrated against 0.1 M Na₂S₂O₃ using starch as the indicator. The analysis was then carried out using a blank, and the iodine value was calculated using the following formula (AOAC, 2008).

$$\text{Iodine Value} = \frac{12.69 \times C(V1 - V2)}{\text{Weight of Sample in gram}}$$

Where;

C = Concentration of Na₂S₂O₃ solution,

V1 = Volume of Na₂S₂O₃ used for the blank,

V2 = Volume of Na₂S₂O₃ used for the sample

2.6.5. Determination of Fat Content

The Gerber method for the determination of the fat content of the desert date kernel oil was employed in accordance with the procedure reported by Richardson, (1985) and adopted by Gemechu *et al.*, (2015). Here 5 ml of each of the oil sample was mixed with 10 ml of sulphuric acid (specific gravity 1.82) into butyrometer and 1 ml of amyl alcohol was then added. The butyrometer was then closed with rubber cork, and the content was vigorously shaken until all the oil was digested by the acid. The butyrometer was then placed in a water bath at 65°C for 5 minutes. The sample was centrifuged for 5 minutes, and then transferred back to the water bath at 65°C for 5 minutes, and the percentage fat was recorded from the butyrometer.

2.6.6. Free Fatty Acid (FFA) of the Oil Sample

To determine the free fatty acid content of the desert date kernel oil, 2 g of the oil was placed in a 250 ml conical flask and warmed, and 2.5 mL of methanol was added with constant stirring, followed by 3 drops of phenolphthalein indicator, and this was then titrated against 0.14 M potassium hydroxide solution with vigorous shaking until a permanent light pink color, which persisted for 1 min, was observed (Afolabi, 2008; Ogala *et al.*, 2018). The end point was recorded, and the free fatty acid value was calculated using equation.

$$\% \text{ Free Fatty Acid} = \frac{V \times M \times 28.2}{W}$$

Where;

V = Volume of potassium hydroxide used

M = Molarity of potassium hydroxide used

W = Weight of the Sample.

3. Results

The results for the phytochemical constituent of the desert date kernel are presented in Table 1, while the physical and chemical characteristics of the desert date kernel oil are presented in Tables 2 and 3 respectively.

Table 1 Phytochemical Constituent of the Kernel of the Desert Date

S/NO	Phytochemical	Observation
1	Alkaloids	+
2	Saponins	+
3	Phenols	-
4	Flavonoids	+
5	Tannins	+
6	Steroids	+
7	Reducing Sugars	-
8	Terpenoids	-
9	Glycosides	+
10	Volatile Oils	+
11	Anthraquinones	-

Key: + = Positive; - = Negative

Table 2 Physical Characteristics of the Oil

S/NO	Parameter	FAO/WHO STANDARD
1	Color	Pale Yellow
2	Density g/cm ³	0.910
3	Specific Gravity	0.907
4	Refractive Index	1.458
5	Viscosity	19.68

Table 3 Chemical Characteristics of *B. aegyptiaca* Kernel Oil

S/NO	Parameter	Desert Date Kernel Oil	FAO/WHO Standard
1	Acid Value (mgKOH/g)	3.06	4
2	Free Fatty Acid	1.27	5.78 – 7.28
3	Peroxide Value (mEq/Kg)	3.71	< 10
4	Saponification Value (mg/KOH/g)	198 ± 0.16	195-205
5	Iodine Value 100/g	98.73	80 – 106

4. Discussion

The results of phytochemical screening test of the desert date kernel oil revealed the presence of alkaloids, saponins, flavonoids, tannins, steroids, glycosides and volatile oils, while phenols, reducing sugars, terpenoids and anthraquinones were absent. These findings agree with many similar findings as reported by some researchers (Hanan *et al.*, 2009; Yadav and Panghal 2010; Chothani and Vaghasiya 2011; Gupta *et al.*, 2012; Kumawat *et al.*, 2012; Jock 2017; Al-Thobaiti and Abu-Zeid 2018; and Selouka *et al.*, 2020). These phytochemicals may be responsible for the application of the powdered kernel in treating certain skin infections (Daya and Vaghasiya 2011). Alkaloids may be toxic at a very high amount, but are often non-toxic and may have dramatic physiological activities hence they are widely used in medicine (Olaofe and Sanni, 1988). Lower doses of alkaloids have important pharmacological activities, such as reducing blood pressure, killing tumour cells, analgesic activity, stimulating circulation and respiration (Egbuna and Ifemeje 2015). Saponins are nonpoisonous to warm blooded animals, but at a high amount they are believed to cause gastroenteritis (Awe and Sodipo, 2001). Saponins have many health benefits which might have accounted for some of the reported therapeutic ability of *Balanites aegyptiaca* kernel (Gloria *et al.*, 2011), including anti-inflammatory, anti-diabetic, anti-HIV, anti-atherosclerotic, hepatoprotective and hypolipidemic (Egbuna and Ifemeje, 2015). Flavonoids sometimes serve as flavoring ingredients of spices and vegetable (Olaofe and Sanni, 1988), but may also have protective effects including anti-oxidant, anti-inflammatory, anti-microbial, anti-viral, anti-allergic, anti-cancer and anti-diarrheal activities (Egbuna and Ifemeje 2015). Tannins, the water-soluble polyphenols are present in many plant foods, have been reported to be responsible for decreases in feed intake, feed efficiency, growth rate and protein digestibility. However, reports have indicated some carcinogenic activities of tannins (Chung *et al.*, 1998). Steroids are known to possess many interesting medicinal, pharmaceutical and agrochemical activities, ranging from antibacterial, hepatoprotective, anti-tumor, antihelminthic, immunosuppressive, plant-growth hormone regulator, sex hormone, cytotoxic and cardiogenic activity (Patel and Savjani 2015). Glycosides family has been in clinical use for many years for the treatment of heart failure and atrial arrhythmia, and they are known to have a strong direct action on the heart and support and strengthen the rate of contraction (Vaidehi and Rajesh 2016). From ancient times, humans have used glycoside-containing plants and their crude extracts as arrow coatings, homicidal or suicidal aids, rat poisons and emetics, while in the modern times they have been adapted for the treatment of congestive heart failure and cardiac arrhythmia (Yubin *et al.*, 2014). Volatile oils are also called essential oils or ethereal oils, and they are natural metabolic secretions of plants that are considered to be true plant hormones, as well as the fluid manifestations of the immune system of the plants, since they contribute to the removal of pests, attracting, instead, pollinating agents, which are some insects and birds (Monica and Ioan 2018). The most important feature of the volatile oils which gives them special economic value is their specific smell, and this form the basis for their use in perfumery, cosmetics and the food industry. Many essential oils have special therapeutic qualities, some of which have been known and used since ancient times (Monica and Ioan 2018).

The quality assessment of *Balanites aegyptiaca* kernel oil was carried out by analyzing some physical such as the oil color, its density, specific gravity, refractive index and viscosity. The observed colour of the oil was pale yellow, and this has been the findings of Ogala *et al.*, (2018). The density of the kernel oil studied was found to be 0.910 which agrees with that reported by Babeker (2013) and just above that reported by Ogala *et al.*, (2018) who reported 0.87, and just below than that reported by Manji *et al.*, (2013) who reported a value of 1.001. However, the finding of this study is within the FAO/WHO standards of 0.909. The specific gravity of the desert date kernel oil in this study was found to be 0.907, and this result is in agreement with that reported by Jock (2011), Manji *et al.*, (2013), Haftu, (2015), Sara and Mahdi (2016), Elbadawi *et al.*, (2017), and within the 0.9–1.16 range set by the FAO/WHO standards. The refractive index of 1.458 is just a little below the FAO/WHO standard of 1.4677–1.4705, but still near similar to that reported by Babeker (2013) and Manji *et al.*, (2013). The viscosity of 19.68 recorded in this however found to be in agreement with similar reports by Babeker (2013).

The acid value of 3.06 mgKOH/g and observed in this study is just below the FAO/WHO standard of 4 mgKOH/g, but still shows that the oil is stable (Haftu 2015). While the free fatty acid content of 1.27% is far below the FAO/WHO standard limits of 5.78–7.28%. Oils with high acid value (above 4 mgKOH/g), also implying a high percentage free fatty acid content, will undergo rancidity due to the hydrolysis of the free fatty acids on storage. The acid value and the percentage free fatty acid of *Balanites aegyptiaca* seed kernel oil are all lower than FAO/WHO standard for edible oils. The lower the percentage free fatty acid content the less the tendency of the oil to undergo hydrolytic activities (Haftu 2015). However, the results for the acid value and the free fatty acids obtained in this study are in agreement with similar findings by Manji *et al.*, 2013 and Jock 2017, but lower than that reported by Ogala *et al.*, 2018.

The peroxide value, used as a measure of the extent to which rancidity reactions have occurred during storage, is used as an indication of the quality and stability of fats and oils. The peroxide value determined for the kernel oil of *Balanites*

aegyptica was found to be 3.71 mEq/g and is within the FAO/WHO standard of less than 10 mEq/g, and the lower the peroxide value the more suitable is the oil for a long storage due to low level of oxidative and lipolytic activities (Adegbe *et al.*, 2016). The results of this study agree with similar reports by Mohammed *et al.*, (2017) and Mohamed and Mohammed (2018), but far lower than reported by Babeker (2013) and Manji *et al.*, (2013) who respectively reported 8.0 and 6.0 mEq/g).

The saponification value, which is an index of average molecular mass of fatty acid in the oil sample, was found to be 198 mgKOH/g for *Balanites aegyptica* kernel oil analyzed, and this value is within the FAO/WHO range of 195–205 mg KOH/g for edible oils. The result agrees with similar reports by Babeker (2013), Manji *et al.*, (2013), Mohammed *et al.*, (2017) and Mohamed and Mohammed (2018). The saponification value of oil is as an important parameter in determining the suitability of the oil for soap making.

The iodine value measures the degree of unsaturation in a fat or oil, and it determines the stability of oils to oxidation and allows the overall unsaturation of the fat to be determined qualitatively. The iodine value of the desert date kernel oil analyzed in this study was found to be 98.73 100/g and within the range of the FAO/WHO standard of 80-106 100/g. The result was in agreement to similar reports by Babeker (2013), Manji *et al.*, (2013), Mohammed *et al.*, (2017) and Mohamed and Mohammed (2018). Oils with iodine values below 100 are non-drying, while those having values between 100 -130 are semi-drying and those with values above 130 are termed drying oils (Jock 2018).

5. Conclusion

This research analyzed the phytochemical properties of the seed kernel of *Balanites aegyptiaca* and the physical and chemical properties of the oil extracted from the seed. The results suggest that the seed kernel has high potentials for medicinal applications, while the kernel oil can serve as raw material for many oil related purposes. The oil can be used for human consumption, as well as in the production of industrial products like biodiesel, lubricants, soaps, shampoos and many other cosmetic products.

Compliance with ethical standards

Acknowledgments

Our acknowledgment goes to the management of Yusuf Maitama Sule University, Kano who gave full financial, moral and technical support to this research.

Disclosure of conflict of interest

Some of the materials required for the research had to be obtained from a sister University, and this has not been as easy as it sounds, because it contradicts the policy of our University.

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How to cite this article

Datti Y, Tijjani YA, Koki IB, Ali UL, Labaran M, Ahmad UU and Tasi'u N. (2020). Phytochemical composition of desert date kernel (*Balanites aegyptiaca*) and the physical and chemical characteristics of its oil. GSC Biological and Pharmaceutical Sciences, 11(3), 197-207.
