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Research Article

Antimicrobial Activity of Different Concentrations of Hexane Extract of *Balanites aegyptiaca* (l.) Delile (Desert Date) Kernel Oil

Musa, H[*1]., Dauda, W[2]., Bissa, Anna[3] and Usman, S[4]
[1]Department of Botany, Ahmadu Bello University, Zaria, Nigeria.
[2]Department Agronomy, Federal University Gashua, Yobe State, Nigeria
[3]Department of Plant Science and Biotechnology, University of Jos, Nigeria.
[4]Department of Crop Protection, Ahmadu Bello University, Zaria, Nigeria
*** Corresponding Author:** *hannatumusa23@gmail.com*

ABSTRACT

Balanites aegyptiaca has been reported to be an anti-helminthic, a purgative, febrifuge, emetic and can also cure other types of ailments like skin boils, malaria, wounds, colds, syphilis, liver and spleen disorders. Various parts of this plant have their own traditional medicinal properties. In this study the phytochemical screening and antimicrobial activity of different concentrations (400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml) of hexane extracted oil from *Balanites aegyptiaca* kernel were investigated. The fixed oil was extracted by solvent semi-continuous extraction method (Soxhlet) with hexane. The phytochemical properties were determined according to standard methods. The percentage yield of the kernel oil was found to be 35.10%. The antimicrobial activity was investigated by agar well diffusion assay against two bacteria; *Staphylococcus aureus* and *Escherichia coli* and two fungi; *Candida albicans* and *Trichophyton tonsurans*. Gentamicin and Fluconazole served as control antibiotics for bacteria and fungi respectively. The phytochemical screening showed the presence of alkaloid, steroid, cardiac glycoside and carbohydrate. The antimicrobial activities of the oil extracted from *Balanites aegyptiaca* kernel showed that the oil extract exhibited inhibitory effects against the tested bacteria with zones of inhibition ranging from 13mm to 8mm and 8mm to 6mm for *Staphylococcus aureus* and *Escherichia coli* respectively. However, the oil did not show inhibitory effect against the tested fungi (*Trichophyton tonsurans* and *Candida albicans*).

Keywords: *Balanites aegyptiaca*, kernel oil, phytochemical, steroid, cardiac glycoside, *S. aureus*, *C. albicans*

Introduction

Natural products have been used since ancient times and in folklore for the treatment of many diseases and illnesses (Dias *et al*., 2012). They have been the source of most of the active ingredients of medicines. This is widely accepted to be true when applied to drug discovery in 'olden times' before the advent of high-throughput screening and the postgenomic era (Sneader, 1996). Many natural products and synthetically modified natural product derivatives have been successfully developed for clinical use to treat human diseases in almost all therapeutic areas (Newman and Cragg, 2007). The 19th century marked the isolation of numerous alkaloids from plants used as drugs, namely, atropine (*Atropa blladonna*), caffeine (*Cofifea arabica*), cocaine (*Erythroxylumcoca*), ephedrine (*Ephedra spp*), morphine and codein (*Papaver somniferum*), pilocarpine (*Pilocarpus jaborandi*), physostigmine (*Physostigma venenosum*), quinine (*Cinchona cordifolia*), salicin (*Salix spp*), theobromine (*Theobroma cacao*), theophylline (*Camellia sinensis*), and (+)-tubocurarine (*Chondodendron tomentosum*). Following these discoveries, bioactive secondary metabolites from plants were later utilized more widely as medicines, both in their original and modified forms (Salim *et al*., 2008).

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Medicinal plants are rich in secondary plant products, and it is because of these compounds that these are termed 'medicinal' or 'officinal' plants. Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. Ethnobotanical and traditional usage of medicinal plants serves as a source of information for the isolation of active compounds, e.g. as direct therapeutic agents (D-tubocurarine from *Chondrodendron tomentosum*), as the starting drug for semisynthesis (diosgenin from *Dioscorea floribunda*), the model drug for new synthetic drugs (cocaine from *Erythroxylumcoca*), for the synthesis of local anaesthetics and, lastly, as taxonomic markers for identification (Balunas and Kinghorn, 2005; Gurib-Fakim, 2006). Thus the ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics.

Balanites aegyptiaca, commonly referred to as desert date, is an important food and medicinal tree found in most African countries, stretching from arid and semi-arid regions to sub-humid savanna. The feasibility of commercial exploitation of *B. aegyptiaca* for pharmaceutical and food industry in the Blue Nile Province of Sudan was shown in the early 1980s by Abu-Al-Futuh (1983).

A single dose of 200 mg/kg body weight of *B. aegyptiaca* fruit mesocarp also showed activity against *Schistosoma mansoni* in infected mice when compared with praziquantel (Koko *et al*., 2005). The methanolic extract of *B. aegyptiaca* fruits is reported to have anthelmintic action against different stages of *Trichinella spiralis* in rats compared with anthelmintic drug albendazole (Shalaby *et al*., 2010). The aqueous extract of *B. aegyptiaca* also has molluscicidal agent to juvenile and adult *Bulinus globosus* and *Bulinus truncatus* (Anto *et al.*, 2005). The acetone and methanolic extracts of stem bark of plant has reported an antivenin activity against saw-scaled (*Echis carinatus*) viper venom concentration at lethal dose (0.194 mg/ml) when administered intramuscularly to Wistar albino rats. Both extracts were found to be effective at 75 and 100 mg/ml concentrations (Wufen *et al*., 2007). It also indicated that the ethanolic extract of *B. aegyptiaca* exhibited more significant activity than petroleum ether in the treatment of pain and inflammation (Gaur *et al*., 2008). The aqueous extract of the mesocarp of fruits of *B. aegyptiaca* was reported to have antidiabetic effect in streptozotocin-induced diabetic mice (Mansour and Newairy, 2000). It is reported that whole and extracted pulp of *B. aegyptiaca* fruits has a hypocholesterolemic effect when tested on adult albino rats (Abdel-Rahim *et al*., 1986). The ethanol and methanolic extract of leaves of *B. aegyptiaca* reported diuretic effect when tested on Wistar albino rats with (150 and 300 mg/kg) oral doses. Frusemide was used as standard. The results indicate that ethanol and methanol extracts shows a significant ($p<0.05$) increase in the urine volume and electrolyte excretion ($p<0.001$) when compared with control (Wani *et al*., 2012).

Infectious diseases account for high proportion of health problems and are the leading cause of death worldwide (Parekh and Chanda, 2007). Even though pharmaceutical industries have produced a number of new antimicrobial drugs in the last years, resistance to these drugs by microorganisms has increased. This is due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases (Davies, 1994). Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns.

The seed kernel oil of *Balanites aegyptiaca* has been reported to be used in folk medicine for the treatment of wounds and tumours (Creach, 1940). *Balanites aegyptiaca* (Desert date) has a wide range of uses which include its medicinal properties. All parts of the plant- seed kernel, stem, root, bark, fruit, seed oil, flower and leaves are widely used in folk medicine.

The aim of this study is to investigate the phytochemical component and antimicrobial activity of the oil extracted from *Balanites aegyptiaca* kernel with the major interest of the utilization of the seed kernel oil for the treatment of various infectious diseases, which can be of great significance for this generation.

Materials and Method

Collection and Preparation of the Seeds

The seeds of *Balanites aegyptiaca* were purchased at Yan doya market Jos, plateau state. The fruits were washed and sun-dried. The seeds were manually crushed to remove the seed kernel from the husk shell. The seed kernel was pulverized using the laboratory pestle and mortar.

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Extraction of Oil

Two hundred grams (200g) of the pulverized seed kernel was weighed into 500 ml conical flask and was soaked in hexane. This was then left to stand overnight and shaked for 3 hours on a rotary mechanical shaker after which the content was filtered using a non-absorbent cotton wool on a Buchner funnel flask using a vacuum pump. The residue was subjected to several parts of rinsing and filtration with fresh solvent to attain some level of exclusive maceration (extraction). The collective filtrate was evaporated to dryness using a rotary evaporator and a water bath or drying cabinet at a controlled temperature of 60°C.

Preparation of Culture Media

Nutrient Agar

Two hundred and fifty milliliters (250 ml) of nutrient agar was prepared by weighing 7g. Of powdered nutrient agar dissolved in sterile distilled water. The dissolved agar was brought to the boil with the aid of hot plate for even mixture. The prepared nutrient agar was sterilized in the autoclave at 121°C for fifteen minutes and was brought out of the autoclave and allowed to cool on the work bench at about 45°C. The nutrient agar was dispensed into sterile Petri dishes and allowed to solidify on a sterilized surface.

Potato Dextrose Agar (PDA)

Commercialized potato dextrose agar (PDA) (9.75g) was added to 250 ml of distilled water. The dissolved PDA was boiled on a hot plate while mixing to dissolve completely. It was then sterilized by autoclaving at 121°C for fifteen minutes and allowed to cool at about 45°C before dispensing. The PDA was aseptically dispensed into sterile Petri dishes.

The Test Microorganisms

The test microorganisms were collected at the Department of microbiology, University of Jos. These microorganisms include: Two bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*) and two fungal pathogens (*Trichophyton tonsurans* and *Candida albicans*).

Preparation of Different Concentrations of Oil Extract

Four grams (4.00g) of oil extract was weighed and dissolved in 10.00 ml of sterile distilled water and was shaken vigorously to obtain a concentration of 400 mg/ml in a sterile sample container. Similarly another 4.00g of oil extract and 1.00ml of Tween 80 (emulsifier) was dissolved in 10.00ml of sterile distilled water to form a stock solution of concentration 400 mg/ml. Different concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml were obtained from the stock solution through serial dilution. That is, backward solution factor of the highest concentration was 400 mg/ml. The concentrations were used immediately and the remaining oil extract was returned to the refrigerator and stored until required again.

Antimicrobial Assay

The antimicrobial activity of different concentrations of the oil was determined by modified agar-well diffusion method described by Perez *et al*. (1990) and Adeniyi *et al*. (1996). In this method, nutrient agar plates were seeded with 0.2ml of 24h broth cultures of each isolate (Potato Dextrose Agar was used for *Trichophyton tonsurans* and *Candida albicans* strains). The plates were allowed to dry for 1h.

A sterile 9mm cork-borer was used to cut five wells of equidistance in each of the plates; 0.5 ml of different concentrations (400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml) of the desert date seed oil was introduced into four of the five wells while the same amount of gentamicin 40 mg/ml for the plates of bacteria (*Staphylococcus aureus* and *Escherichia coli*) and fluconazole 50 mg/ml for the plates of fungi (*Trichophyton tonsurans* and *Candida albicans*) was introduced into the fifth well at the center of the plates as control. The plates were incubated at 37°C for 24h (48h for yeast species). The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (in mm). All the plates were made in triplicates.

Preparation of the Test Organisms

Bacterial Suspension

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated at 37ºC for 24 hours.

The bacterial broth was harvested and washed off with 100ml sterile normal saline to opacity of matched barium chloride turbidity standard the suspension was stored in the refrigerator at 4ºC till used.

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Fungal Suspension

The fungal cultures were maintained on Sabouraud Dextrose Agar, incubated at 25ºC for 4 days.
The fungal growth was harvested and washed off with 100 ml sterile normal saline and the suspension was stored in the refrigerator at 4ºC till used.

Antimicrobial Activity Assay

The antimicrobial activity test was conducted in order to know the type of activity demonstrated by the kernel oil extract of *Balanites aegyptiaca*; whether bactericidal/fungicidal or bacteriostatic/fungistatic. In doing this, fresh growth media were prepared for each microorganism. The test tube showing minimum inhibitory concentration (MIC) for each test microorganism was noted. Broth from each test tube was incubated at 37°C for 24 hours for bacteria (*Staphylococcus aureus* and *Escherichia coli*) and 28°C for 72hours for fungi (*Trichophyton tonsurans* and *Candida albicans*). Growth indicated bacteriostatic/fungistatic activity while the absence of growth indicated bactericidal/fungicidal activity.

Determination of Minimum Inhibitory Concentration (MIC)

Serial dilution of the extract oil was carried out using nutrient broth so that different concentrations were obtained. Another serial dilution of the extract oil and emulsifying agent (tween 80) was carried out using nutrient broth for bacteria and potato dextrose broth for fungi so that different concentrations were obtained in various test tubes.

The concentrations include 400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml which were inoculated with the test microorganisms. A fifth test tube containing broth without extract was also inoculated with the test microorganisms as control. The test tubes were properly labelled and incubated at 37°C for 24 hours for bacteria (*Staphylococcus aureus* and *Escherichia coli*) and 28°C for 72 hours for fungi (*Trichophyton tonsurans* and *Candida albicans*). They were later observed for turbidity.

Reading of the result was done by recording the averages of the zone of inhibition for each well containing oil extract at different concentrations in millimeter (mm) using a transparent meter rule. The concentration that was observed as having the MIC value for each isolate was recorded as well.

Phytochemical Screening

Molisch's Test for Carbohydrates

Few drops of Molisch's reagent was added to 1 ml of oil sample dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was a positive test (Sofowora, 1993).

Test for Tannins

About 1 ml of oil sample was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002).

Borntrager's Test for Anthraquinones

About 1 ml of oil sample to be tested was shaken with 10 ml of benzene and then filtered. Five millilitres of the 10% ammonia solution was then added to the filtrate and thereafter the shaken. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase was taken as the presence of free anthraquinones (Sofowora, 1993).

Test for Terpenoids

A little of oil sample was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids (Sofowora, 1993).

Test for Saponins

An aliquot (1ml) of oil sample was boiled with 5ml of distilled water, and filtered. To the filtrate, about 3ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as evidence for the presence of saponins (Sofowora, 1993).

Sodium Hydroxide Test for Flavonoids

Few drops of the oil sample were dissolved in water; to this 2ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

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Test for Alkaloids

Few drops of oil sample was stirred with 5 ml of 1% aqueous HCl on water bath. An aliquot (1ml) of the mixture was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids (Sofowora, 1993).

Test for Steroids (Salkowski Test)

An aliquot (1.0ml) of oil sample was dissolved in 2.0ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interphase is indicative of the presence of steroidal ring (Soforowa, 1993).

Keller Kilhani Test for Cardiac Glycoside

An aliquot (1.0ml) of oil sample was dissolved in 1.0ml of glacial acetic acid containing one drop of Ferric chloride solution. This was then under-layed with 1.0ml of conc. Sulphuric acid. A brown ring obtained at the interphase indicated the presence of a deoxy sugar characteristic of cardenolides (Soforowa, 2008).

Determination of percentage yield

Determination of percentage yield (Warra *et al*., 2011). The oil obtained from the extraction was transferred into a measuring cylinder which was placed over a water bath for 30 minutes at70°C so as to ensure complete evaporation of the solvent and then the volume of the oil was recorded.

% =1/2× 100

Where: W1= weight of oil extracted; W2= weight of sample used.

Results

The result of the Phytochemical Screening of the hexane extracted seed kernel oil of *Balanites aegyptiaca* showed that, the seed kernel oil of *Balanites aegyptiaca* are composed of phytochemical components which included alkaloids, carbohydrates, steroids and cardiac glycosides. While anthraquinones, saponins, tannins, terpenoids, and flavonoids were absent in the oil.

The result of the antimicrobial activity of the hexane extract of the seed kernel oil of *Balanitesa egyptiaca* (desert date) against two bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*) and two fungal pathogens (*Trichophyton tonsurans* and *Candida albicans*) is shown in Table 1.

The result of the Zones of inhibition of against microorganisms at different concentrations of *B. egyptiaca* oil extract emulsified with tween 80 is shown in Figure 1.

Table 1: Sensitivity of different concentrations (mg/ml) of *Balanitesa egyptiaca* (desert date) kernel oil emulsified with Tween 80 on tested micro-organisms

Test Microorganism	Concentration of *Balanitesa egyptiaca* oil (mg/ml)				Gentamicin (Control)	Fluconazole (Control)
	400	200	100	50		
Staphylococcus aureus	+	+	+	-	+	
Escherichia coli	+	+	-	-	+	
Trichophyton tonsurans	-	-	-	-		+
Candida albicans	-	-	-			+

Key: '+' implies 'Susceptible'; -'implies 'Not Susceptible'

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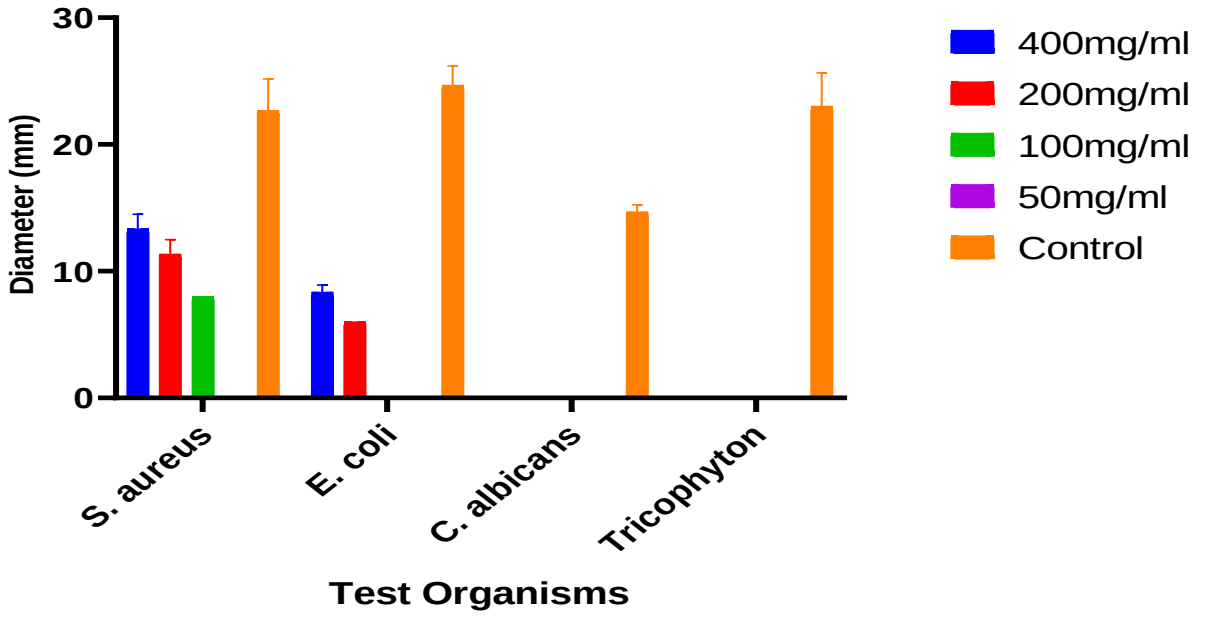

Fig. 1: Zones of inhibition of different concentrations of *B. egyptiaca* oil extract against microorganisms

Figure 1 showed that *S aureus* was most inhibited by the oil emulsified with tween 80 at all concentrations except 50mg/ml which is the lowest concentration. It showed no of zone of inhibition on *Cadida albicans* and *T. tonsurans.*

The results of the Sensitivity of different concentration of desert date seed oil (mg/ml) with no emulsifying agent (Tween 80) on tested micro-organisms is shown in Table 2 while the result of the measured zones of inhibition (mm) against the test micro-organisms at different concentrations (mg/ml) of oil extract with no emulsifying agent (Tween 80) is shown in Figure 2. These results showed that *S. aureus* was susceptible to the *Balanitesa egyptiaca* (desert date) kernel oil extract at 400mg/ml, 200mg/ml and at 100mg/ml while *E. coli* was susceptible only at 400mg/ml concentration. None of the organisms were susceptible to the plant extract at the lowest concentration 50mg/ml. *Candida albicans* and *T. tonsurans* were not susceptible to the oil extract at all concentrations. All the sampled organisms were susceptible to the standard drugs (control) gentamicin (for bacteria) and fluconazole (for fungi).

Table 2: Sensitivity of different concentration of *Balanitesa egyptiaca* (desert date) seed oil (mg/ml) with no emulsifying agent (Tween 80) on tested microorganisms

Test Microorganism	Concentration of *Balanitesa egyptiaca* oil (mg/ml)				Gentamicin (Control)	Fluconazole (Control)
	400	200	100	50		
Staphylococcus aureus	+	+	+	-	+	
Escherichia coli	+	-	-	-	+	
Trichophyton tonsurans	-	-	-	-		+
Candida albicans	-	-	-			+

Key: '+' implies 'Susceptible'; '-' implies 'Not Susceptible'

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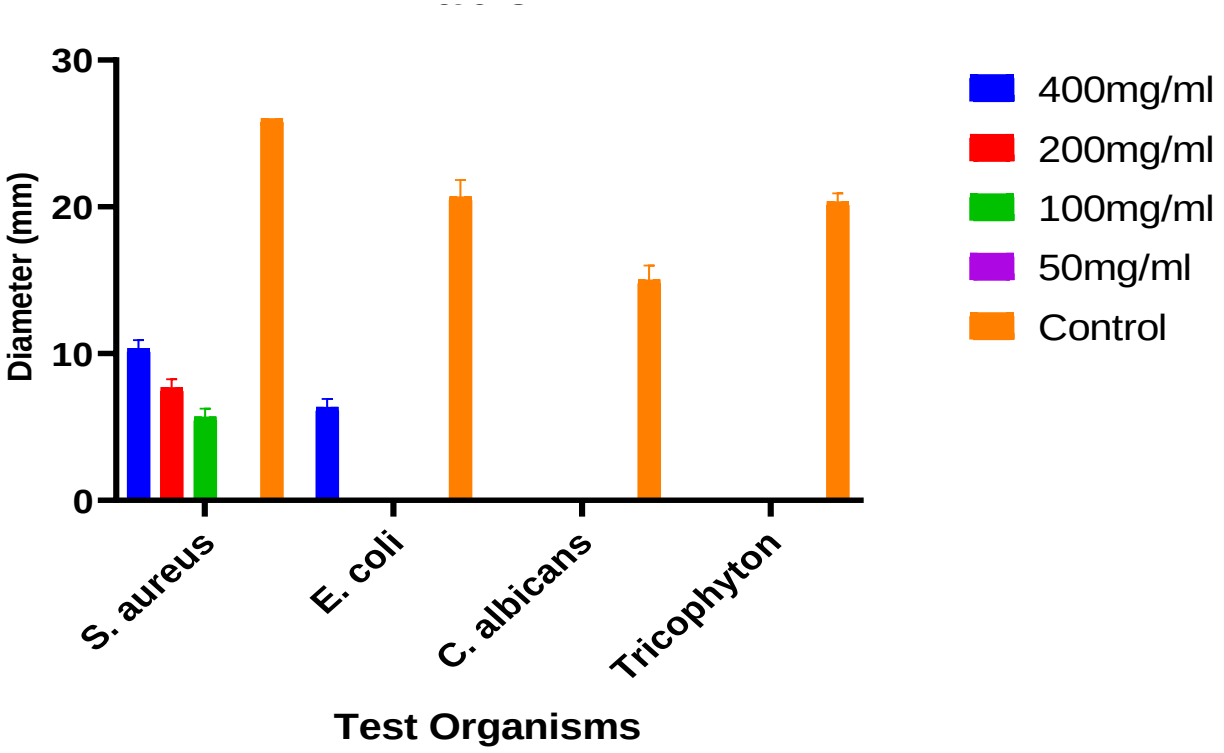

Fig. 2: Zones of inhibition against the test micro-organisms at different concentrations (mg/ml) of oil extract with no emulsifying agent (Tween 80)

Figure 2 indicated that *S. aureus* was most inhibited by oil extract with no emulsifying agent at concentrations 400mg/ml, 200mg/ml and 100mg/ml, while *E. coli* was inhibited at concentration 400mg/ml only. *Candida albanicans* and *E. coli* showed no inhibition all concentrations.

The result of the determination of the minimum inhibitory concentration (MIC) of the emulsified with Tween 80 against the test microorganisms is shown in Table 3. The results showed that only *S. aureus* and *E. coli* were inhibited, at concentrations 400mg/ml and 200mg/ml. *Candida albicans* and *T. tonsurans* were not inhibited at all concentrations. *S. aureus* was inhibited at 100mg/ml which showed no turbidity. This formed the MIC. The other test microorganisms showed no turbidity at concentration 100mg/ml.

Table 3: Determination of the minimum inhibitory concentration (MIC) of the emulsified with tween 80 against the test microorganisms

Test Microorganisms	Concentration of *Balanitesa egyptiaca* oil (mg/ml)				Gentamicin (Control)	Fluconazole (Control)
	400	200	100	50		
Staphylococcus aureus	+	+	+	-	+	
Escherichia coli	+	+	-	-	+	
Trichophyton tonsurans	-	-	-	-		+
Candida albicans	-	-	-			+

Key: '+' indicated 'Inhibition' (Clear broth); '-'indicated 'No Inhibition' (Turbid broth)

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Discussion

The study has shown that the seed kernel oil of *Balanites aegyptiaca* are composed of phytochemical components which include alkaloids, carbohydrates, steroids and cardiac glycosides. While anthraquinones, saponins, tannins, terpenoids, and flavonoids were absent in the oil. These phytochemicals may be responsible for the application of the oil in treating certain skin infections (Daya *et al*., 2011).
It was observed that only bacterial pathogens of the tested micro-organism were susceptible to the oil extract emulsified with Tween 80 while the fungal pathogens were not susceptible to any concentration of it. Results obtained in this study showed that, *S. aureus* was susceptible to the *Balanitesa egyptiaca* (desert date) kernel oil extract at 400mg/ml, 200mg/ml and at 100mg/ml while *E. coli* was susceptible only at 400mg/ml concentration. None of the organisms were susceptible to the plant extract at the lowest concentration 50mg/ml. *Candida albicans* and *T. tonsurans* were not susceptible to the oil extract at all concentrations. The highest inhibitory zone at 13.00mm at 400mg/ml was observed on *S. aureus*. Also the test microorganisms were susceptible to the conventional standard drugs, Gentamicin (for bacteria) and fluconazole (for fungi).

The present study also revealed that the oil extract of desert date seeds had antimicrobial effects on only the bacteria species of the test microorganisms. Clear zones of inhibition were observed on some of the agar plates. Agar plates containing the stock solution of desert date oil, Tween 80 (emulsifying agent) and sterile distilled water at different concentrations inhibited the growth of the bacterial pathogens with higher zones of inhibition while the agar plates containing the stock solution of desert date oil and sterile distilled water only inhibited the Bacterial pathogens with lower zones of inhibition. The minimum inhibitory concentration (MIC) conducted for this study revealed that the bacterial species had minimum inhibitory concentration of 100 mg/ml for *Staphylococcus aureus* while *Escherichia coli* was inhibited at higher concentrations. The fungal pathogens were not inhibited by all the concentrations.

A test on the type of activity demonstrated by the desert date oil extract on the test microorganisms revealed that the oil extract was bacteriostatic on *Escherichia coli*. Other inhibitions by the extract were bactericidal. The standard drugs used as control for this study are gentamicin (for bacteria) and fluconazole (for fungi) which inhibited the growth of all the sampled organisms.

The phytochemical analysis of the desert date seed oil extracted with n-hexane had bioactive constituents. These phytochemicals must have been responsible for the zones of inhibition around the test micro-organisms. The antimicrobial activity test carried out in this study has shown that the seed kernel oil of *Balanites aegyptiaca* has inhibitory effect on the tested bacterial pathogens (*S. aureus* and *E. col*i) the fungal pathogens (*T. tonsurans* and *C. albicans*) showed no inhibition.

Daya and Vaghasiya (2011) reported the use of the oil in the treatment of some bacterial and fungal infections, but lack of inhibition of the tested fungal pathogens shown in the analysis could be as a result of the differences in the method of oil extraction and variation of the properties of the seed kernel oil of *Balanites aegyptiaca* as reported by Elfeel (2010). Mohammed *et al*. (2002) also reported a morphological variation and chemical characteristics of fruits and seeds of *Balanites aegyptiaca* within different geographical regions in Sudan. Mohammed *et al*. (1999) also reported the antibacterial activity of *Balanites aegyptiaca* seed oil, which is similar to the results shown in this study even though a slight modification was made in the method of the experiment and the emulsifying agent used.

The results of this study also supported the point of view that different pathogenic bacterial species exhibited different sensitivities towards medicinal plants. Scientists from divergent fields are investigating plants a new with an eye to their antimicrobial usefulness. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effect on bacteria and fungi in vitro. It would be advantageous to standardize methods of extraction and in-vitro testing so that search could be more systematic and interpretation of results would be facilitated. This study confirms that seed extract have in vitro antibacterial activity. This obviously justifies the use of above seed oil extract of this plant in traditional medicine and further studies are needed to isolate and characterize antibacterial moieties in these for practical disease control *in vivo*.

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In conclusion, this present study has proved that *Balanites aegyptiaca* possesses several bioactive phytochemical compounds namely: Alkaloids, steroids, cardiac glycosides, and carbohydrates which are responsible for the antimicrobial activity. The antimicrobial investigation revealed that the oil extract have moderate inhibitory effects against the tested bacterial species (*Staphylococcus aureus* and *Escherichia coli*), while it revealed a very weak inhibitory effect against the tested fungal species (*Trichophyton tonsurans and Candida albicans*). The good use of old knowledge about desert date oil as well as modern approaches to develop and formulate drugs and skin care products such as soap, creams and lotions is very important. It is suggested that the oil should be tested on other microorganisms especially dermatophytes and other causal agents of skin infections, as it has shown only moderate inhibitory effect on some of the test microorganisms. Further investigations regarding the mode of action and other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended.

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