



Original Research Article

Phytochemical, antioxidant and antibacterial potentials of *Abelmoschus esculentus* L. oil and its suitability in formulation of herbal cream

Received 3 February, 2023

Revised 12 April, 2023

Accepted 23 June, 2023

Published 7 December, 2023

Emmanuel M. Halilu^{1,2*}
Abdulrahman Lawal²
and
Thlama Ludiya³

¹Faculty of Pharmacy
(Pharmacognosy), Cyprus
International University, Nicosia,
Mersin 10 Turkey.

²Department of Pharmacognosy
and Ethnopharmacy, Faculty of
Pharmaceutical Sciences,
Usmanu Danfodiyo University,
Sokoto, Nigeria.

³Department of Pharmacognosy,
Faculty of Pharmacy, University
of Maiduguri, Nigeria.

*Corresponding Author Email:
emshelia2002@yahoo.com

Abelmoschus esculentus (okra) is used for its nutritional and health benefits. The research was designed to investigate the phytochemical, biological activities and suitability of the seed oil in the formulation of herbal cream. The seeds were ground to powder and was subjected to organoleptic and physicochemical evaluations. The oil was extracted using the Soxhlet apparatus and was subjected to organoleptic, physicochemical, phytochemical, GC-MS, oral acute toxicity, antioxidant and antibacterial studies. The organoleptic evaluation of the powder seeds revealed brownish colour with characteristic odour, taste and smooth texture. The physicochemical evaluation revealed moisture content (8 %,) total ash (3.67 %,) acid insoluble ash (3 %), alcohol extractive value (1.40 %) and water extractive value (8.8%). The percentage yield of the oil was 8.96 %. The organoleptic evaluation of the oil revealed yellowish colour with characteristic odour, taste and smooth texture. The oil was found to be soluble in organic in some organic solvents. The phytochemical screening of the oil revealed the presence of steroids. The GC-MS revealed the presence of fatty acids and their esters. The physicochemical analysis of the oil showed specific gravity of 0.876, Saponification value of 0.84 (mgKOH/g of oil), acid value of 169.7 (mgKOH/g of oil), ester value of 168.86 (mgKOH/g of oil) and iodine value of 79.31 (gI/100g of oil). The oral acute toxicity (LD₅₀) was greater than 5000 mg/kg in mice. The oil showed IC₅₀ of 0.46 mg/mL against DPPH radical while the ascorbic acid (standard) had IC₅₀ of 11.3 mg/mL. The antibacterial activity against *S. aureus* and *P. aeruginosa* showed zones of inhibitions between 9-13 mm. The herbal cream formulated showed the best texture when 50 % of the oil and beeswax were used. *A. esculentus* oil possess some therapeutic properties and it may be suitable for formulation of herbal creams.

Keywords: Antibacterial, Antioxidant, acute-toxicity, DPPH, Herbal, GC-MS and physicochemical

INTRODUCTION

Fixed oils have been used by humans since time immemorial for their nutritional and medicinal properties. In the recent times, there is an increase demand for the use of vegetable oils obtained from plants as biodiesel due to

their ecofriendliness (Shereena and Thangaraj, 2009). This demand has created competition between the human needs and the industrial demands of fixed oils to power machines and motor vehicles (Ayhan and Huseyin, 2006; Vrabie et al.,

2016). This new demands on fixed oils have created competition on oils which are mainly used for food, medicine and other industrial purposes. Therefore, due to the current demand of oil for usage as biodiesel, there is need to search and investigate oils from newer sources that can be exploited for their nutritional, medicinal and pharmaceutical applications.

Fixed oils and fats are of great importance in pharmaceutical preparations, industrially, as well as nutritionally (Antonio and Maria, 2000). Pharmaceutically, the fixed oils are used as vehicles to convey drugs to their sites of action (Antonio and Maria, 2000). An example is arachis oil which is used in formulating emulsions. Furthermore, fixed oils serve as solvents in the preparation of certain intramuscular injections e.g., sesame oil. Fixed oils are also used as stimulants, cathartic, purgative e.g., castor oil. Fixed oils are not only limited to internal use but are also used externally as emollient for ointments, liniment creams and other preparations e.g., almond oil and coconut oil. Industrially, fixed oils are used in the manufacture of detergents, soaps, paints, and varnishes as lubricants (Aruna et al., 2016). Nutritionally, fixed oil such as corn oil have been used in diet due to its high contents of unsaturated fatty acid.

A. esculentus (Okra) also known as *Hibiscus esculentus* (Plate 1 and 2) is an important vegetable crop belonging to the Malvaceae family which grow in tropical and sub-tropical parts of the world (Gemede et al., 2015). Okra is a popular health food due to its high fiber, vitamin C, and folate content. It is also a good source of calcium and potassium (Kumar et al., 2013). It has a high content of mucilage and can be used as a plasma replacement. An infusion of the roots has been used traditionally for treatment of syphilis and the leaves is an emollient poultice. The juice of the roots is used externally in Nepal to treat cuts, wounds and boils. A decoction of the immature capsules is demulcent and diuretic (Kumar et al., 2013). It is used in the treatment of catarrhal infections, dysuria and gonorrhoea. The seeds are antispasmodic, cordial and stimulant. An infusion of the roasted seeds has sudorific properties (Kumar et al., 2013). Okra seeds are a potential source of oil, with concentrations varying from 20% to 40%, which consists of linoleic acid up to 47.4% (Tomar, 2017). Okra is also known for its antioxidant activity and has several potential health beneficial effects on some of the important human diseases like cardiovascular disease, type 2 diabetes, digestive diseases and some cancers (Gemede, 2015). Phytochemical constituents of *A. esculentus* have been reported by Jia et al. (2011) where steroids, triterpene and hexadecanoic acid have been identified. The most common bacteria species that have been implicated in skin infections are *Staphylococcus aureus* and *Streptococcus pyogenes* (Aly, 1996). Bacterial resistance to various antibiotics have been widely reported (Zhang and Cheng, 2022). The current research was aimed to evaluate the physicochemical, antioxidant and antibacterial activities of the oil extracted from *A. esculentus* seeds and investigate its suitability in the formulation of

herbal cream.

MATERIALS AND METHODS

Collection and identification of seeds

The seeds of *A. esculentus* were purchased in March, 2019 at Karah market in Sokoto metropolis, Sokoto state, Nigeria. The seed was identified by a Pharmacognosist at the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto.

Preparation of the Seeds

The seeds of *A. esculentus* were grounded into a moderately fine powder using milling machine and was stored in a plastic container for subsequent use.

Organoleptic Evaluation of Powdered Sample of *A. esculentus*

The colour, odour, texture and taste were examined using the appropriate sense organs (WHO,1998).

Physicochemical Studies

The moisture content, total ash, acid insoluble ash, alcohol-soluble extractive and water-soluble extractive values were determined as described in (AOAC, 2000).

Extraction of Oil

The powder sample (841.99g) was extracted with 800 mL of n-hexane with the aid of the Soxhlet extractor for duration of 6 hours. The extract was concentrated at room temperature and the percentage yield was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{Mass of oil}}{\text{Initial mass}} \times 100$$

Organoleptic Evaluation of the Fixed Oil

The colour, odour, texture and taste of the oil were determined as described in WHO (1998).

Solubility

The oil (1 mL) was added to chloroform, methanol/chloroform (50:50), dimethyl sulfoxide, diethyl ether, methanol, ethyl acetate, petroleum ether, benzene, n-hexane, and ethanol in separate test tubes and then observed for solubility/miscibility at room temperature (25 °C).

Specific Gravity

The specific gravity was determined as described in AOAC (2000).



Plate 1: *A. esculentus* in its habitat



Plate 2: *A. esculentus* seeds

Phytochemical Screening of the oil

Paper Test

The *A. esculentus* oil (1 drop) was placed on a filter paper. The formation of a greasy translucent spot which stains permanently, indicates the presence of fixed oil (Halilu et al., 2021).

Test for steroids

Lieberman Burchard's Test

The oil (1 mL) was dissolved in 3 mL of chloroform and an equal volume of acetic anhydride was added. This was followed by the addition of 3 drops of concentrated sulphuric acid by the walls of the test tube. The formation of reddish ring at the interphase and a greenish upper layer indicates the presence of steroidal nucleus (Shaikh and Patil, 2020).

Salkowski's Test

The oil (1 mL) was dissolved in 3 mL chloroform and 3 drops of concentrated sulphuric acid by the walls of the test tube. The formation of reddish ring at the interphase indicates the presence of steroidal nucleus (Shaikh and Patil, 2020).

Physicochemical analyses of the oil

The saponification value, acid value, iodine value and ester value were determined as described in AOAC (1998).

GC-MS Analysis

The method described by Debendranath and Sunita (2020) was followed. The analysis was carried out at Central Science Laboratory, Usmanu Danfodiyo University, Sokoto.

The GC-MS analysis was carried out on Agilent Technologies Intuvo 9000 GC System and Agilent Technologies 5977B Mass Selective Detector (MSD) coupled with 4513A Automatic Liquid Sampler (ALS).

Experimental Animals Ethical Clearance

Five (5) healthy female mice weighing about 20-40 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The animals were kept under standard environmental condition with free access to feed and water for 14 days before the commencement of the study. The ethical approval for the animal studies was granted by the animal research committee of the Department of Pharmacology and Toxicology. Ethical clearance certificate with number PTAC/Tc/(He)OT/47-22 was issued.

Acute Toxicity Study

The acute toxicity was carried out according to the Organization for Economic Co-operation and Development (OECD) guideline number 425. Prior to administration of a dose of 5000 mg/kg, the mice were fasted and the oil was orally administered to each rat over a period of 5 days (i.e., one mouse per day). The mice were observed for 30 minutes for signs of toxicity and the mortality. The mice were observed for 14 days and the LD₅₀ was recorded.

Antioxidant Study

Qualitative and quantitative antioxidant test using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH solution 0.8 mM was prepared by dissolving in ethanol (Vadivukkarasi and Pavithra, 2014). The TLC plate was cut into a suitable size and the oil was spotted using a capillary tube. It was then sprayed with the DPPH solution

Table 1. The working formula

1 st Cream		2 nd Cream		3 rd Cream	
Ingredients	Quantity	Ingredients	Quantity	Ingredients	Quantity
Okra oil	10g	Okra oil	12g	Okra oil	8g
Bees wax	10g	Bees wax	8g	Bees wax	12g
Fragrance	2 mL	Fragrance	2mL	Fragrance	2mL

and was observed for immediate development of yellow or white spot against purple background. The quantitative estimation of the free radical scavenging activity of the oil on DPPH radical was carried out using UV spectroscopy as described by (Chatatikun and Chiabchalard, 2013).

Antibacterial activity of *Abelmoschus esculentus* Oil

Collected of test organisms

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were obtained at the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Susceptibility test

The cup plate method was used. The nutrient agar was prepared according to the manufacturer's specification and then sterilized at 121°C. It was allowed to cool and then transferred to sterilized Petri-dishes. The Petri-dishes were flooded with various dilutions of the test bacteria and drained using the Pasteur pipette. Wells measuring 4 mm in diameter were bored into the inoculated plates using a sterilized cork borer. The wells were filled with 0.1 mL each of 30 mg/mL, 40 mg/mL, 50 mg/mL and 60 mg/mL of the oil using dimethyl sulfoxide as diluent. The plates were allowed to stand for pre-diffusion time for 2 hours and then incubated for 24 hours at 37°C. The zones of inhibition were measured to the nearest milli meter using a metric rule. The readings were taken in triplicate and the mean and standard error of mean were recorded. Similarly, the plate of ofloxacin (5 µg) and DMSO were used as positive control and negative control respectively. The zone of inhibition was also recorded.

Formulation of herbal cream

Three (3) creams of weight of 20 g each were formulated. The ingredients used include *A. esculentus* oil, bees wax and fragrance. Variations were made in the concentrations of the *A. esculentus* oil as well as the beeswax. The first cream contained 50% of the oil and 50% of bees wax, the second cream contained 60% of the oil and 40% of bees wax while the third cream contained 40% of the oil and 60 % beeswax. The formula for the preparation of the three creams are presented in Table 1.

The creams were formulated as follows: The amount of

the oil and bees wax to be used was accurately weighed and transferred into an evaporating dish. The mixture was heated gently over water bath maintained at a temperature of 70° C. A glass rod was used to stir the mixture gently until the bees wax was completely melted. The mixture was continuously stirred in one direction and then cooled at room temperature until the mixture became cloudy and thickened. After formation of a thick consistency, the resulting cream was transferred into clean covered plastic containers and then stored at room temperature away from sunlight.

Preliminary physical evaluation of cream

The formulated creams were evaluated for physical appearance by human volunteers using their sense organs (the eyes and the skin).

Statistical analysis

The data were analysed using Microsoft Excel and the means of triplicate reading were expressed as mean ± standard error means. The IC₅₀ was obtained by plotting the graph of percentage inhibition against concentration.

RESULTS AND DISCUSSION

Organoleptic evaluation of powdered sample of *A. esculentus* seeds

The organoleptic evaluation of crude drugs is a basic requirement in crude drug research (WHO, 2011). The results of the various organoleptic tests carried out on the powdered sample are presented in Table 2. These observations agrees with the reports of Emmanuel and Baraka, (2022).

Physicochemical evaluation

The results of the physicochemical analysis on the powdered seeds are presented in Table 3. The moisture content of 8.0 % was obtained and this result deviated from earlier studies carried by Zerihun et al., 2020 which indicated moisture content of 4.72 %. This difference may be due to the moisture absorbed by the powdered seeds during storage. The ash content obtained in this study was lower than that reported by Zerihun et al., 2020. The total ash and acid insoluble ash value were found to be 3.67%

Table 2. Organoleptic evaluation of powdered sample

Parameters	Observation
Colour	Brownish
Odour	Characteristic
Taste	Characteristic
Texture	Fine

Table 3. Physicochemical evaluation of *A. esculentus*

Parameter	Result (%)
Moisture content	8.0
Total ash	3.7
Acid insoluble ash	3.0
Water extractive value	8.8
Alcohol extractive value	1.4

Table 4. Organoleptic evaluation of the fixed oil

Parameters	Observation
Colour	Yellowish
Odour	Characteristic
Taste	Characteristic
Texture	Smooth and greasy

Table 5. Solubility *A. esculentus* oil in organic solvents

Solvent	Inference
Chloroform	+
100% Petroleum ether	+
100% Benzene	+
95% Alcohol	+
100% Methanol	+
100% Ethylacetate	+
100% n-hexane	+
100% n-butanol	+
Diethyl ether	-
Dimethyl sulfoxide	+

Key: + = soluble; - = Insoluble

Table 6. Oil analysis

Parameter	Results
Specific gravity	0.876
Saponification value	0.84 mgKOH/g
Acid value	169.7 mgKOH/g
Ester value	168.86 mgKOH/g
Iodine	79.31 gI ₂ /100g of oil

and 3% respectively. Ash values reflect the level of adulteration or handling of the drug. Direct adulteration by sand or earth is immediately detected as the total ash which is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. Therefore, the low values of total ash and acid insoluble ash obtained in this study indicate that there were low levels of contamination

(Emeje et al., 2011). The water soluble extractive value of 8.8 % and alcohol extractive value of 1.4 % were obtained from this study. The water soluble extractive value indicates the presence of acids and inorganic compounds (Kumar et al., 2013) whereas alcohol extractive values represent the presence of polar constituents like phenols, alkaloids, steroids, glycosides and flavonoids. The physicochemical parameters play a major role in ensuring quality of crude drugs.

Oil Extraction

The percentage yield of the fixed oil in n-hexane was 8.96%. *A. esculentus* seeds contain considerable amount of oil, ranging from 20%-40% (Zerihun et al., 2020; Halilu et al., 2021). The oil content may vary between species due to environmental factors and geographical location.

Organoleptic Evaluation of the Oil

The organoleptic evaluation of *A. esculentus* oil revealed a yellowish colour, characteristic odour, characteristic taste and smooth and greasy texture (Table 4). The yellowish colour may be as result of lipophilic soluble plant pigments (carotenoids) in the oil which impart the yellowish colour (Cristina et al., 2016; Lazzarini and Domenici 2017).

Solubility studies

The solubility of the oil determines its suitability and the scope of applications. The solubility of oils depends on the concentration and temperature (Ramalingam, 1957). Table 4 showed the solubility of the oil in most of the various organic solvents and this observation was in agreement with the Alam (2019). However, the oil was not soluble in diethyl ether. Furthermore, it was observed that the degree of solubility of the oil was not the same in all the solvents.

Phytochemical Screening

The qualitative phytochemical screening revealed that the oil contains steroids as it gave positive tests for Liebermann Burchard's and Salkowaski's test. This may suggest the presence of steroids such as cholesterol, cholestanol, ergosterol, campesterol, stigmasterol and sitosterol that have been reported in many vegetable oils (Ruinan et al., 2019).

Physicochemical Analysis of the oil

The physicochemical parameters of the oil are presented in Table 6. The specific gravity of the oil was found to be 0.876 and it indicates the molecular weight of the fatty acid composition of the oil. Specific gravity is employed with other parameters to determine the purity of oil and its identity (Ichu and Nwakanma, 2019). The oil analysis revealed saponification value of 0.84 (mgKOH/g of oil), acid value of 169.7 (mgKOH/g of oil), ester value of 168.86 (mgKOH/g of oil) and iodine value of 79.31 (gI₂/100g of oil).

Oil analysis conducted on *A. esculentus* revealed saponification value to be 0.84 (mgKOH/g of oil). The saponification value of (200 mgKOH/g) indicates a high proportion of fatty acid of low molecular weight. This shows the presence of low molecular weight fatty acids which is good for human health. The results indicated that the saponification value (0.84 mgKOH/g) was lower than those of cotton oil (181.03-199.32 mgKOH/g), groundnut oil (191.33-199.05 mgKOH/g), and sesame oil (184.47-199.60 mgKOH/g). The acid value is an index of free fatty acid content due to enzymatic activity. The acid value obtained was 169.70 g which indicates the presence of high proportion of fatty acids in the oil. The iodine value is a measure of the degree of unsaturation of fatty acids content of any fat or oil and as the value increases the unsaturation increases. The iodine value obtained from this study was 79.31 gI₂/100g of oil and that of the ester value was 168.86 mgKOH/g.

GC-MS Analysis of *A. esculentus* oil

The results of the GC-MS analysis (Table 7) revealed the presence of some fatty acids including: propionic acid, 2-butenedioic acid, n-hexadecanoic acid, phosphoric acid phenyl ester, and 9,12-Octadecadienoic acid. Earlier analysis has shown that okra seed oil contains palmitic acid, oleic acid stearic acid, linoleic acid and nonadecanoic acid (Georgia et al., 2012). This result agrees with the findings from the present study.

Acute toxicity studies

The result showed that the five (5) mice survived each administration of 5000 mg/kg dose of the oil and no mortality was recorded. Therefore, the LD₅₀ is greater than or equal to 5000mg/kg. Although the rats scratched their mouth, they had heavy breathing and were calm. Therefore, the LD₅₀ was greater than or equal to 5000 mg/kg. Hence, it may be suggested that the oil is relatively non-toxic and can be consumed orally (Emmanuel and Baraka, 2022).

Antioxidant studies

The qualitative screening of antioxidant activity showed yellow spot against purple (DPPH) background. This is shown in Plate 4. The qualitative screening of the antioxidant activity showed yellow spot against purple background on the TLC plates. This provided preliminary evidence that the oil possesses compounds that can exhibit antioxidant activity (Kojima et al., 1998). The activity demonstrated may be due to some terpenes and vitamin E (Sibel et al., 2005). The oxidative stability is an important quality and safety parameter of oils for their potential commercial applications and utilizations in food and other commercial products (Parker et al., 2003). 2,2-diphenyl-1-picrylhydrazyl is a stable free radical and has been commonly used to screen phenolic compounds containing reactivity of the sample being tested with a stable free

radical, is an information provided by the DPPH test. DPPH produces a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the colour changes from deep violet to light yellow. As the absorbance measurement reduces, the radical scavenging power of the extract increases (Mshelia et al., 2017).

The quantitative antioxidant study is presented in Tables 8, 9 and Figure 1. The free radical scavenging activity of *A. esculentus* oil showed considerable level of activity. Statistical studies carried out to compare the free radical scavenging activity of the oil and ascorbic acid to that of the control (blank) showed that both the oil and ascorbic acid has significantly higher antioxidant activity than the control. Also comparing the oil and ascorbic acid at respectively similar concentrations gave significantly higher activity for ascorbic acid except at 20 mg/mL which showed significantly higher antioxidant activity for the oil. The IC₅₀ values of the oil and ascorbic acid were 0.46 mg/mL and 11.3 mg/mL respectively. The lower the IC₅₀ value the more potent is the extract (Mshelia et al., 2017). This result indicated that the oil has more potent antioxidant activity than ascorbic acid. The antioxidant activity may be attributed to the vitamin E content and the phenolic contents (Al-kanani et al., 2019).

Antibacterial study

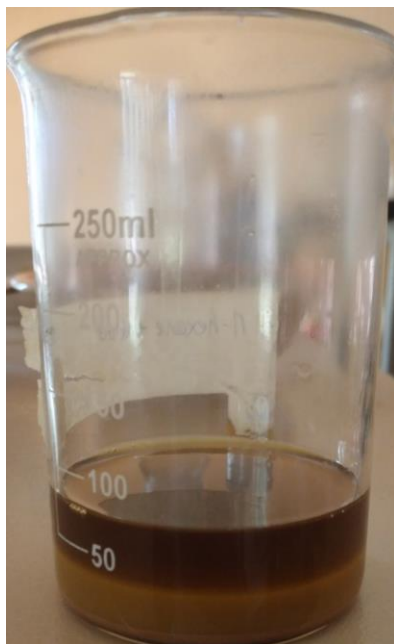
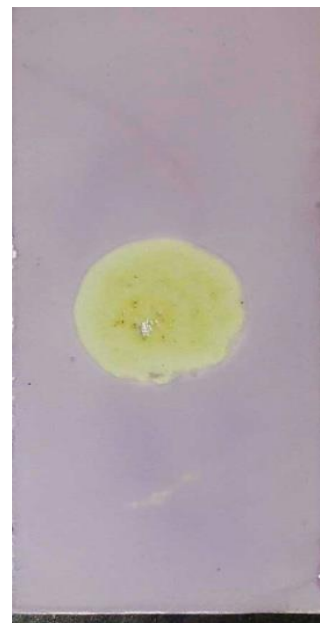
The result of antibacterial activity of the oil is presented in Table 10. Antibacterial studies on the oil showed some activity against *S. aureus* (at 40, 50, and 60 mg/mL) and at 60 mg/mL, it showed activity against *P. aeruginosa* with no activity against *B. subtilis* and *E. coli*. The zones of inhibition of growth of the microorganisms range between 9mm to 13mm. Ofloxacin 5µg used as positive control displayed zones of inhibition ranging between 24mm to 27mm. DMSO (negative control) which served as the diluent was also tested for antibacterial activity and it showed no activity on the microorganisms used. It is also observed that, the zone of inhibition produced by the oil on bacterial growth was concentration dependent. It is evident from the result that the zone of inhibition of the oil on *S. aureus* increases from 9mm to 12mm as the concentration increases. Also, only the highest concentration (60 mg/mL) showed zone of inhibition against *P. aeruginosa*. The antibacterial activity demonstrated by the oil may be due to secondary metabolites present in the oil (Mshelia et al., 2017).

Formulation of Herbal Cream and Preliminary Evaluation

The result of formulated antioxidant/antibacterial cream is presented in Plate 5. Three creams were formulated. The formulation containing 50% oil was the smoothest, then followed by 60% oil and the least smooth was the formulation containing 40% oil. It was observed, from this research that as the percentage of beeswax increases, the

Table 7. GC-MS Analysis of *A. esculentus* oil

Retention time (Sec)	Constituents	Quality (%)
20.044	2-butenedioic acid	53
28.015	Propionic acid	64
35.757	n-Hexadecanoic acid	99
38.172	Phosphoric acid, phenyl ester	50
38.893	9,12-Octadecadienoic acid	99

**Plate 3:** *A. esculentus* oil**Plate 4:** TLC plate of DPPH free radical scavenging activity of oil showing yellow spot**Table 8.** Percentage inhibition of ascorbic acid and okra oil, and their IC₅₀

Concentration (mg/mL)	% Inhibition ascorbic acid	% Inhibition of okra oil
1.25	65.48	52.86
2.5	64.88	63.21
5	59.41	38.45
10	58.81	37.02
20	39.88	69.05
IC ₅₀	11.3 mg/mL	0.46 mg/mL

Table 9. Percentage inhibition of ascorbic acid compared to the okra oil

Conc. (mg/ml)	Ascorbic acid Mean ± SEM	Okro oil Mean ± SEM	P value
1.25	* 65.50 ± 1.85	52.86 ± 0.90	0.004
2.5	* 64.88 ± 0.12	63.21 ± 0.21	0.002
5	* 59.41 ± 0.43	38.45 ± 1.06	<0.001
10	* 58.81 ± 1.02	37.02 ± 0.32	<0.001
20	39.88 ± 0.32	* 69.05 ± 0.60	<0.001

Key; *= has significantly higher antioxidant activity using independent sample t-test at $p < 0.05$

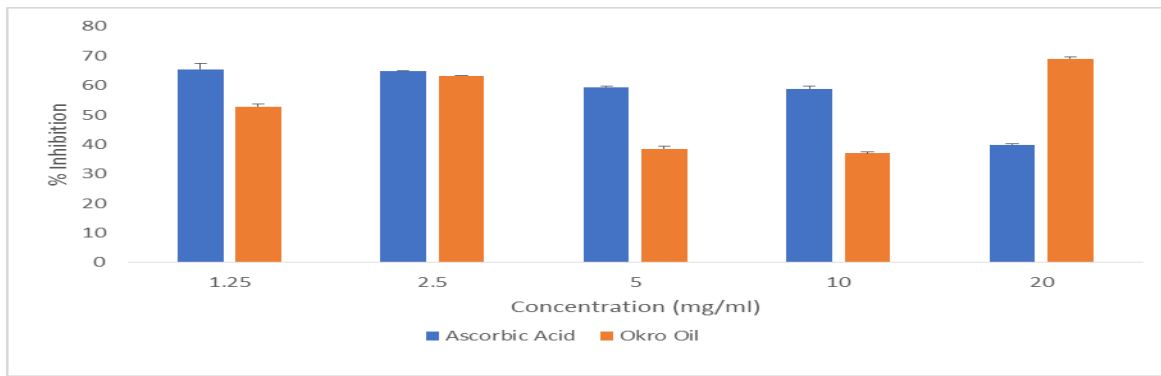
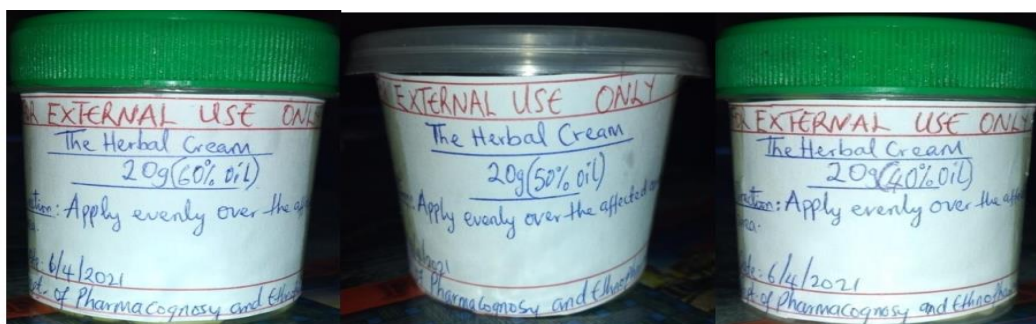


Figure 1: DPPH free radical scavenging activity of ascorbic acid compared to the oil

Table 10. Antibacterial activity of *A. esculentus* oil

Treatment/Concentrations	Bacteria strains	Mean ± SEM (Zone of inhibition in mm)
DMSO	SA	-
	BS	-
	PA	-
	EC	-
Ofloxacin 5µg (Control)	SA	25.67 ± 0.33
	BS	24.67 ± 0.33
	PA	26.33 ± 0.33
	EC	25.67 ± 0.33
A.E (30µg/ml)	SA	-
	BS	-
	PA	-
	EC	-
A.E (40µg/ml)	SA	9.67 ± 0.33
	BS	-
	PA	-
	EC	-
A.E (50µg/ml)	SA	11.00 ± 0.58
	BS	-
	PA	-
	EC	-
A.E (60µg/ml)	SA	12.00 ± 0.00
	BS	-
	PA	13.00 ± 0.00
	EC	-

Key: - = No zone of inhibition; SEM= Standard Error Mean; A.E= *A. esculentus* DMSO = Dimethyl Sulfoxide; SA= *Staphylococcus aureus*; BS= *Bacillus subtilis*; PA= *Pseudomonas Aeruginosa*; EC= *Escherichia coli*



60% oil/40% Beeswax

50% oil /50 Beeswax

40% oil/60% Beeswax

Plate 5. Cream fomulations with different concentrations of *A. esculentus* oil herbal cream

smoothness decreases. This finding agrees with the report of Girdre et al. (2016).

Conclusions

Macroscopically, *A. esculentus* seed powder was found to have a brownish colour, it has characteristic taste and odour. It also has a fine texture. The percentage yield of oil in *A. esculentus* seeds was 8.96 %. The results of saponification value suggest the presence of low molecular weight fatty acids which makes it good for human health. This study showed that *A. esculentus* oil is soluble in 9 solvents including petroleum ether, benzene, alcohol, methanol, ethanol, n-hexane, n-butanol, ethyl acetate, DMSO and insoluble in distilled water and diethyl ether. *A. esculentus* oil contains steroids and triterpenes while the aqueous extract of the seeds contains alkaloids. GC-MS analysis of *A. esculentus* oil revealed the presence of fatty acid and esters. The acute toxicity study of *A. esculentus* oil showed that the oil is non-toxic at a dose of 5000 mg/kg and hence can be used for cooking, pharmaceutical and cosmetic formulations. *A. esculentus* oil demonstrated antioxidant activity by scavenging DPPH free radical. Furthermore, the oil demonstrated antibacterial activity against *S. aureus* and *P. aeruginosa*. The oil was found to be suitable for formulation of creams as seen in the three (3) creams formulated by varying the oil concentration.

Declarations of interest

The authors declare that there is no conflict of interests regarding the publication of the paper.

Acknowledgments

The authors express their profound gratitude to Aliyu and Abdullahi of the faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto for their technical support.

Credit author statement

Emmanuel M. Halilu: conceptualization, writing and editing of the final manuscript; **Abdulrahman Lawal:** Extracted the oil, phytochemical analysis, toxicity studies; **Thlama Ludiya:** antioxidant & antibacterial studies & formulated the herbal cream.

REFERENCES

- Al-Ashaal HA, Farghaly AA, Abd El Aziz MM, Ali MA, (2010). Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). *Journal of Ethnopharmacology*, 127(2):495–501.
- Albuquerque MBD, Raul R, Ant´onio GS, Darlene CP, Carlos AAG, Maria JCC, Tatiane SG, (2012). Spectroscopic and Thermooxidative Analysis of Organic Okra Oil and Seeds from *Abelmoschus esculentus*. *The Scientific World Journal* Volume 2012, Article ID 847471, 6 pages doi:10.1100/2012/847471
- AL-Kanani EAS, (2019). The nutritional composition and vitamin E of three Iraqi okra (*Abelmoschus esculentus* L.) seeds oil, *IOP Conf. Ser.: Earth Environ. Sci.* 388 012058 (IOP Conference Series: Earth and Environmental Science, 388:012058
- Aly R, (1996). Microbial Infections of Skin and Nails. In: Baron S, editor. *Medical Microbiology*. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; Chapter 98. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK8301/>
- Antonio MRA, María LGR, (2000). Lipids in pharmaceutical and cosmetic preparations. *Grasas y Aceites* Vol. 51;(1-2); 74-96
- AOAC (1998). *Official Methods of Analysis of AOAC International*, 16th Edition, 4th Revision. Washington D.C. U.S.A; 70-90.
- Aruna KAS, Kailash CU, (2016). Vegetable Oil: Nutritional and Industrial Perspective. *Current Genomics*, 17, 230-240
- Ayhan D, Huseyin K, (2006) New Options for Conversion of Vegetable Oils to Alternative Fuels, *Energy Sources*, Part A, 28:7, 619-626, DOI:10.1080/009083190951357
- Chatatikun M, Chiabchalard A, (2013). Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts. *Journal of Chemical and Pharmaceutical Research*, 5(4):97–102.
- Cristina L, Mario C, Valentina D, (2016) Pigments in Extra-Virgin Olive Oil: Authenticity and Quality. Chapter 6 IntechOpen, <http://dx.doi.org/10.5772/64736>
- Debendranath M, Sunita P, (2020). GC-MS Analysed Phytochemicals and Antibacterial Activity of *Withania Somnifera* (L.) Dunal Extract in the Context of Treatment to Liver Cirrhosis, *Biomedical & Pharmacology Journal*, Vol. 13(1): 71-78
- Emeje M, Isimi C, Byrn S, Fortunak J, Kunle O, Ofoefule S, (2011). Extraction and physicochemical characterization of a new polysaccharide obtained from the fresh fruits of *Abelmoschus esculentus*. *Iranian Journal of Pharmaceutical Research*, 10(2), 237–246.
- Emmanuel MH, Baraka M (2021). Phytochemical and antioxidant studies of *Hibiscus cannabinus* seed oil, *Physical Sciences Reviews*, <https://doi.org/10.1515/psr-2021-0184>
- Gemedede HF, (2015). Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A Review. *Journal of Food Processing & Technology*, 06(06). <https://doi.org/10.4172/21577110.1000458>
- Georgia SFS, Vinicius MG, Anderson RA, Manoel BD, Raul R., Ant´onio GS, Darlene CP, Carlos AAG, Maria JCC, Tatiane, SG (2012). Spectroscopic and Thermooxidative Analysis of Organic Okra Oil and Seeds from *Abelmoschus esculentus*. *The Scientific World Journal* Volume 2012, Article ID 847471, 6 pages doi:10.1100/2012/847471

- Giedre K, Arunas S, Zenona K, Saule V, Loreta K, Jurga B, (2016). Evaluation of Beeswax Influence on Physical Properties of Lipstick Using Instrumental and Sensory Methods. Hindawi Publishing Corporation *Evidence-Based Complementary and Alternative Medicine*, Volume 2016, Article ID 3816460, 8 pages <http://dx.doi.org/10.1155/2016/3816460>
- Halilu ME, Abacha YZ, Samagoro C, Bello SS, Jibril SA, (2021). Evaluation of Physicochemical and Antioxidant Potential of Fixed Oil from *Curcuma Longa* Linn. *Trends in Natural Products Research* Vol. 2 (2): 66-74
- Ichu CD, Nwakanma HO, (2019). Comparative Study of the physicochemical characterization and quality of edible vegetable oils. *Inter. J. Res. in Informative Sci. Appl. Techniq.* 3 (2):19321-19329.
- Jia L, Guo M, Li D, Jing L, (2011). Chemical constituents from petroleum ether portion of *Abelmoschus esculentus* II. *Zhongguo Zhongyao Zazh*, 36(7):891-895. <https://doi.org/10.4268/cjcm20110715>
- Jia L, Guo M, Li D, Jing L, (2011). Chemical constituents from petroleum ether portion of *Abelmoschus esculentus* II. *Zhongguo Zhongyao Zazhi*, 36(7):891-895.
- Kaithwas G, Majumdar DK, (2010). Evaluation of antiulcer and antisecretory potential of *Linum usitatissimum* fixed oil and possible mechanism of action. *Inflammopharmacology*, 18(3), 137-145.
- Kojima H, Yanai T, Toyota A, (1998). Essential oil constituents from Japanese and Indian *Curcuma aromatica* rhizomes. *Planta Medica*, 64(4), 380-381.
- Kumar DS, Tony DE, Kumar AP, Kumar KA, Rao DBS, Nadendla R, (2013). *A Review On : Abelmoschus esculentus* (Okra), 3(4): 129-132.
- Kumar R, Patil MB, Patil SR, Paschapur MS, (2015). *Evaluation of Abelmoschus Esculentus Mucilage as Suspending Agent in Paracetamol Suspension. August 2002.*
- Lazzerini C, Domenici V, (2017). Pigments in Extra-Virgin Olive Oils Produced in Tuscany (Italy) in Different Years. *Foods* ; 6: 25; 1-11
- Lee JM, Chung H, Chang PS, Lee JH, (2007). Development of a method predicting the oxidative stability oils using 2,2-diphenyl-1-picrylhydrazyl (DPPH). *Food Chemistry*, 103(2), 662-669.
- Mshelia HE, Sani J, Abdullahi S, Umaru ML, Dauda J, (2017). Phytochemical screening , free radical scavenging and antibacterial activity of *Cassia sieberiana* root bark extracts. *Journal of Pharmacy and Bioresources*.14(1):75-82.
- Organization for Economic Community and Development. Principles of Good Laboratory Practice, In: hand book of Good Laboratory Practice (GLP) TDR, PRD/GLP/ 2008; 01:2.
- Parker TD, Adams DA, Zhou K, Harris M, Yu L, (2003). Fatty Acid Composition and Oxidative Stability of Cold-pressed Edible Seed Oils. *Journal of Food Science*, 68(4):1240-1243.
- Ramalingan K, Chari KS, (1959). Solubilities of vegetable oils in aqueous ethanol and ethanol-hexane mixtures. Presented at fall meeting, American Oil Chemists' Society, Cincinnati, 37(2): 77-80.
- Ruinan Y, Li X, Liangxiao Z, Xuefang W, Xin Q, Jun J, Li Y, Xiupin W, Wen Z, Qi Z, and Peiwu L, (2019). Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, (2019) Phytosterol Contents of Edible Oils and Their Contributions to Estimated Phytosterol Intake in the Chinese Diet, *Foods* 2019, 8, 334; doi:10.3390/foods8080334
- Shaikh, RJ, Patil MK (2020). Qualitative tests for preliminary phytochemical screening: An overview, *International Journal of Chemical Sciences*; 8(2): 603-608
- Shereena KM, Thangaraj T, (2009). Biodiesel: An Alternative Fuel Produced from Vegetable Oils by Transesterification. *Electronic Journal of Biology*, 5 (3): 67-74
- Sibel K, Hüsniye S, Bijen K. 2005. α -Tocopherol, Flavonoid, and Phenol Contents and Antioxidant Activity of *Ficus carica*. Leaves. *Pharm. Biol.* 43 (8), 683-686.
- Singh S, Malhotra M, Majumdar DK, (2005). Antibacterial activity of *Ocimum sanctum* L. fixed oil. 43, 835-837.
- Tomar A, (2017). Medicinal use of *Abelmoschus esculentus* (Linn .) Moench . (Bhindi) to cure fever. 6(4), 596-597.
- Vadivukkarasi S, Pavithra K, (2014). Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from *Kedrostis foetidissima* (Jacq.) Cogn, *Biochemistry & Analytical Biochemistry*, 3:2 pp-2-7 DOI: 10.4172/2161-1009.1000150
- Vrabie V, Scarpete D, Zbarcea O, (2016). Vegetable Oils as Alternative Fuel for New Generation of Diesel Engines: A Review. Scientific Proceedings Xxiv International Scientific-Technical Conference "trans & MOTAUTO '16". 1:105-109.
- WHO. (2011). *Quality control methods for medicinal plant materials*. World Health Organization, Printed in Malta, Geneva pp 5-43.
- Zerihun, M., Berhe, H., Mulu, M., Argahgn, Z., & Demelie, M. (2020). *Nutritive Composition and Physicochemical Properties and Oil Contents of Okra (Abelmoschus esculentus L.) Seed in Middle Awash , Ethiopia. November.* <https://doi.org/10.35248/2157-7110.20.11.848>
- Zhang F, Cheng W, (2022). The Mechanism of Bacterial Resistance and Potential Bacteriostatic Strategies, *Antibiotics*; 11(9): 1-23