



# Chemical Composition, Antimicrobial and Physicochemical Properties of *Treculia Africana* (Breadfruit) Seed Oil

Daniel I. E., <sup>1\*</sup> Ejiofor V. K. <sup>1\*\*</sup>

<sup>1</sup>Department of Chemistry University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.

\*Corresponding author, Email address: [imauoekwere@gmail.com](mailto:imauoekwere@gmail.com)

\*\*Corresponding author, Email address: [ejioforvitalis@gmail.com](mailto:ejioforvitalis@gmail.com)

Received 17 Jan 2024,

Revised 26 Feb 2024,

Accepted 27 Feb 2024

## Keywords:

- ✓ Saponification Value,
- ✓ Octadecanoic acid,
- ✓ *Candida albicans*,
- ✓ *Treculia africana*

**Citation:** Daniel I. E.,  
Ejiofor V. K. (2024)  
Chemical Composition,  
Antimicrobial and  
Physicochemical Properties  
of *Treculia Africana*  
(Breadfruit) Seed Oil, *J.  
Mater. Environ. Sci.*, 15(2),  
285-300

**Abstract:** The physicochemical properties such as moisture content, specific gravity, percentage yield, acid value, iodine value, saponification value, peroxide value and colour of oil obtained from the seeds of *Treculia africana* (breadfruit) was determined using Association of Official Analytical Chemists (AOAC) standard procedures. The chemical composition of the oil obtained from breadfruit seeds was determined using Gas chromatography Mass Spectroscopy (GC-MS). Antimicrobial activity of the oil against various pathogenic microorganisms was screened using disc diffusion method. The results obtained showed that all the physicochemical properties were within the permissible limits except moisture content, which was slightly higher than the permissible limit. The result of GC-MS analysis showed that about 56 compounds were detected in the seed oil of breadfruit. The most abundant compounds identified with respect to their % peak areas were n-hexadecanoic acid, 9, 12-Octadecadienoic acid (Z, Z)-, trans-13-Octadecenoic acid, Undecane, Decane, Octadecanoic acid, 1, 2, 3-trimethyl Benzene, and D-Limonene while about 29 compounds had % peak < 1. The antimicrobial activity of the seed oil of breadfruit showed appreciable broad spectrum activity against the pathogenic microorganisms tested at various concentrations. However, no inhibition activity was recorded against *Proteus sp.*, *E. coli*, *Micrococcus sp.*, *Staph aureus* and *Enterobacter aerogene*. Equally, test fungi (*Candida albicans* and *Aspergillus niger*) showed no susceptibility to the oil. The presence of these bioactive compounds in the seed oil of breadfruit corroborates its phytopharmaceutical as well as its industrial usage.

## 1. Introduction

Vegetable fats and oils are lipid materials obtained from plants, which are solids and liquids at room temperature, respectively (Adebayo *et al.*, 2012). These plants have been developed to maximize their oil production capacity, as they are traditional and economic products of most tropical and sub-tropical countries (Ononogbu, 2002). Nowadays, oils from plant origin are largely preferred to animal fat because of its low or complete absence of cholesterol, presence of unsaturated free fatty acids, complex carbohydrates and fat-soluble vitamins like as A, D, E, and K (Kostiket *et al.*, 2013; Wilcox, 2006). They also play other distinctive role in the natural flavour and palatability of a wide of food commodities, while some of its components (Linoleic, Linolenic and arachidonic acid) are essential in all diets for body growth and normal skin condition (Kadda *et al.*, 2022; Ebuehi *et al.*, 2006; Ihekoronye and Ngoddy, 1985). Apart from the nutritive aspects of plant oils (Jafari *et al.*, 2022; Belarbi-Benmahdi *et al.*, 2009), a wide range of oilseeds and other oil producing plants are high quality feedstock for biodiesel. Outside the realm of food manufacture, vegetable oils feature in a variety of

industrial uses ranging from the manufacture of soap to the production of paints, varnishes, lubricants and plastics. For instance, they have been used for illumination and lubricating purpose, production of detergents and cosmetics and for coatings and paint for many centuries before an abundant and cheap supply of mineral oil became available (Ibemesi, 1992). In spite of their uses, the characterization of oils is important because it helps in determining properties inherent in the oil, thereby ascertaining its suitability or otherwise for consumption and for other purposes. GC–MS is one of the best techniques to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compounds (Muthulakshmi *et al.*, 2012). Several studies indicate that medicinal plants which are rich reservoir of bioactive compounds contain compounds that are significant in therapeutic application against human and animal pathogen, including bacteria, fungi and viruses (Khan *et al.*, 2003; Pavrez *et al.*, 2005).

In an effort to discover new lead compounds many research groups have screened plant extracts to detect secondary metabolites with relevant biological activities (Diass *et al.*, 2023; Harish *et al.*, 2007). The search for new antimicrobial compounds is particularly important, since bacteremia remains a significant cause of morbidity and mortality in many nosocomial infections B worldwide (Davies 2007). This is because herbal medicines have been reported to be safe, affordable, acceptable, available and without any adverse side effect especially when compared with synthetic drugs (Ouahabi *et al.*, 2023; Britton *et al.*, 2002; Hussain *et al.*, 2010).

African breadfruit (*Treculia africana*) is a monoecious evergreen fruit belonging to the family of moraceae and is a native of the East Indies, tropical Africa; Sierra Leone, Nigeria etc. (Lim, 2012), (Ajiwe *et al.*, 1995). It is a common forest tree recognized by various tribal names such as “*afon*” (Yoruba), “*barafuta*” (Hausa), “*Ize*” (Bini) “*eyo*” (Igalala) “*ediang*” (Efik) and “*Ukwa*” (Igbo) (Anuonye *et al.*, 2012). The seeds from the fruit are edible and are rich source of protein carbohydrates, vitamins, mineral and contain high aromatic amino acids (Keay 1989; Giami *et al.*, 2004; Oyetayo and Omenwa, 2006). African breadfruit (*Treculia africana*) is commonly roasted, cooked, mashed and consumed either directly as snack food or as flour for use in soup thickening and cake (Fasasi *et al.*, 2004). The seeds are light brown in colour and roughly oval and spherical in shape. The major, intermediate and minor axial dimensions of the seed were found to be 11.9, 5.7 and 4.6 mm, respectively, while the density and bulk density of African breadfruit seeds are 979 kg m<sup>-3</sup> and 614 kg m<sup>-3</sup>, respectively (Omobuwajo *et al.*, 1999).

The oil yield of the seed compares well with that of cotton seeds, palm kernel and sunflower seeds. The fat and oil content of the seed makes it probable industrial raw materials in producing pharmaceutical drugs, vegetable oils, soaps, paints and perfumes (Nwabueze *et al.*, 2008) The seeds are found to have an excellent polyvalent dietetic value with biological value of its proteins exceeding that of vegetable cowpea and soybean (Enibe, 2001).

## 2. Methodology

### 2.1 Sourcing and preparation of African breadfruit seeds oil

African breadfruit (*Treculia africana*) seeds were purchased from Owerri main market, Imo State. The seeds were washed and sorted manually to remove bad ones and extraneous materials. The seeds were further dehulled manually without any heat treatment. The sample was sun dried for about 17 hours and then milled in a blender to fine flour (2 mm particle size). The flour was preserved in a tight polyethylene bag at room temperature from which samples were collected for different analyses (Fasasi, *et al.*, 2004; Ifeoma *et al.*; 2010).



**Photo 1;** African breadfruit (*Treculia africana*) seeds

## **2.2 Experiments**

The breadfruit seed oil was obtained from the resulting powder by continuous extraction in a Soxhlet apparatus for 6 hours using hexane as solvent according to method described by AOAC (2005). Solvent was recovered by rotary evaporator under reduced pressure and residual oil was oven-dried at 75°C for one hour. The oil was then transferred to a desiccator and allowed to cool before being weighed. The drying, cooling and weighing was repeated until a constant dry weight was obtained. The extracted oil sample was sealed in dark brown coloured glass bottle and kept for analytical tests.

## **2.3 African breadfruit seed oil characterization**

The breadfruit oil obtained from the extraction of the seed was characterized using gas chromatography- mass spectrometry (GC-MS). GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument. The derivatised sugars were separated on a Varian CP3800 GC equipped with a CP8400 auto sampler. A Varian Factor Four VF-5ms (cross linked 5% phenyl-methyl siloxane) column (30 m x 0.25 mm ID with DF=0.25 film thickness) was used. Column flow was set to 1.0 mL/min using helium as the carrier gas. The temperature program started with a temperature of 140°C held for 1 minute, a ramp of 2°C per minute to 218°C, followed by a ramp of 10°C per minute to 280°C.

The final hold was two minutes. Injection temperature was 280°C with a split less injection of 1µL. The transfer line was held at 280°C and the mass spectrometer had a delay of five minutes. Data were collected over a range of m/z 40–650.

The chemical components of the oil of breadfruit were identified by the GC-MS system by comparison of the mass spectra fragmentations, GC retention times, retention indices and percentage area composition of the compounds with the robust Library references (National Institute of Standards and Technology (NIST) Library 2014) by autonomously linear interpolating the component to the retention of the series of n-alkane in the data base.

## **2.4 Determination of physicochemical properties of Breadfruit oil**

### **2.4.1 Determination of melting point**

*Treculia africana* seed oil extracted was weighed into a test tube and kept in a refrigerator and a thermometer was inserted into the frozen oil sample. The temperature at which the oil turned into liquid was taken as the melting point (AOAC, 1984).

### 2.4.2 Moisture content

The sample of *Treculia africana* oil (1.0g) was weighed into an empty beaker with known weight and was kept in an oven for six hours at temperature of 105°C, after six hours the beaker was cooled, reweighed after cooling to obtain constant weight (AOAC, 1984)

Formula

$$\text{Moisture Content} = \frac{b-c \times 100}{b-a} \text{Eqn 1}$$

where

a = weight of empty beaker

b = weight of beaker + oil before drying

c = weight of beaker + oil after drying

### 2.5 Percentage Yield

The weight of then sample was obtained before the extraction. After isolation process, the oil recovered was weighed and yield of oil was calculated using formula:

$$\text{Yield of oil} = \frac{\text{weight of oil extracted} \times 100}{\text{weight of dried fruit}} \text{Eqn 2}$$

### 2.6 Determination of acid value

1.0g of oil sample of *Treculia africana* was added to carbon tetrachloride (10ml) in a conical flask. Phenolphthalein solution (3 drops) was added as an indicator to the solution and titrated with KOH (0.1M), until a colour change was observed. A blank determination was run (AOAC, 1984). The formula for calculating the acid value is given below.

$$\text{Acid value} = \frac{(\text{sample Titre (ml)} - \text{blank titre}) \times 5.61}{\text{weight of sample (g)}} \text{Eqn 3}$$

### 2.7 Iodine value

The iodine value was determined using Hanus method. The oil sample taken into a glass stopper iodine flask is dissolved in chloroform. The measured volume of Hanus reagent is accurately added and after thorough mixing, it is placed in the dark for exactly one hour. A corresponding blank reagent is simultaneously prepared. At the end of the specified time, the reaction is stopped by adding potassium iodide and diluted with water to prevent loss of the free iodine. The amount of free iodine is determined by titration with sodium thiosulfate using starch as indicator.

Formula:

$$\text{Iodine value} = \frac{1.269 (a-b)}{w} \text{Eqn 4}$$

Where

a = 0.1M Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for blank

b = 1M of 0.1 Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> for sample

w = weight of the sample

## 2.8 Determination of Specific Gravity

A clean 50 ml specific gravity bottle was weighted (M1). Then the bottle was filled to the brim with water and stopper was inserted. Extra water spilled out. The water on the stopper and bottle were carefully wiped off and reweighed (M2). Same process was repeated, but using oil samples instead of water and weighted again (M3).

$$\text{Specific Gravity} = \frac{M3-M1}{M2-M1} \text{Eqn 5}$$

## 2.9 Saponification value determination

2g of breadfruit seed oil was weighed in to a conical flask after that 25ml of 0.1N ethanolic KOH was added. The content mixture was constantly stirred and allowed to boil gently for 1hr. Reflex condenser was placed on the flask containing the mixture. A few drops of phenolphthalein indicator were added to the warm solution and then titrated with 0.43N HCl to the end point until the pink colour of the indicator just disappeared. The same procedure was used for blank.

$$\text{Saponification Value (SV)} = \frac{(b-s) 28.05}{w} \text{Eqn 6}$$

Where

b	=	Average blank titre
s	=	Average sample titre
w	=	Weight of the sample

## 2.10 Determination of peroxide value

1.2g of the oil sample was weighed into conical flask. Chloroform (6ml) and acetic acid (9ml) was added in a ratio of 2:3 respectively to saturate KI (5ml). This was allowed to stand for 1min and thereafter 30ml of distilled water was added with starch (2ml) as an indicator. It was titrated with 0.1N<sub>2</sub>S<sub>2</sub>O<sub>2</sub> until a yellowish colouration almost disappears to a colourless endpoint (AOAC, 1984). The formula for calculating the peroxide value (PV) is:

$$\text{Peroxide Value} = \frac{1000 (V1-V2)T}{M} \text{Eqn 7}$$

Where

M	=	Mass of the oil taken
V1	=	Volume of 0.1N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>
V2	=	Volume of Blank
T	=	Normality of N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> for blank

## 2.11 Free fatty acid

25ml of ethanol was added to 1.0g of the sample contained in conical flask. The sample mixture was boiled and allowed to cool. The essence of boiling was to enable some of the undissolved oil to dissolve. 1cm<sup>3</sup>(10 drops) of phenolphthalein indicator was added to the solution and titrated with NaOH (0.1M) until a purple colouration was observed (AOAC, 1984). The Formula:

$$\% \text{FFA} = \frac{V \times 0.0282 \times 100}{\text{weight of the sample}} \text{Eqn 8}$$

## 2.12 Colour determination:

Color of the respective oils was determined by physical observation in day light and under ultraviolet radiation of 254 and 366 nm using ultraviolet chamber (Bamgboye and Adejumo 2010).

## **2.13 Antimicrobial activity**

### **2.13.1 Collection of test organisms**

Microorganisms used were obtained from the microbial stock collection unit of department of microbiology, university of Uyo, the university health centre and University of Uyo teaching Hospital, Akwa Ibom State. The test organisms used were three Gram –positive, five Gram –negative bacteria, one yeast and one mold. (*Staphylococcus aureus*, *Bacillus subtilis*, *Shigella sp*, *Salmonella sp*, *Echerichia coli*, *Candida albicans*, *Aspergillus niger*, *serratia sp*, *Micrococcus sp* and *Proteus sp*.) These organisms were sub cultured to obtain pure and fresh isolates. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Isolates were identified using standard microbiological procedures by carrying out gram's reaction and biochemical tests to confirm the species.

### **2.13.2 Preparation of test organisms before inoculation**

McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions. The bacterial suspensions were standardized following the CLSI guidelines for aerobic bacteria. All of the test microorganisms were grown in Mueller Hinton broth for 18–24 h, followed by the matching of bacterial suspension to the turbidity equivalent to 0.5 McFarland solutions ( $1-2 \times 10^8$  cfu/ml). Different concentrations (40, 80, 120, 160 and 200mg/ml) of the extracts were prepared and kept in corked test tubes. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Isolates were identified using standard microbiological procedures by carrying out gram's reaction and biochemical tests to confirm the species.

### **2.13.3 Seeding of Muller – Hinton agar plates**

0.1 ml of each diluted isolates was aseptically transferred into Muller – Hinton agar (Oxoid, UK) plates and aseptically spread evenly using sterile Hockey stick. The seeded plates were left for 30 minutes for the isolates to diffuse into the medium. Sterile cork borer of 5mm was used to bore holes on the agar plates. 0.1 mL of each of the extracts was then dropped in the holes and labeled accordingly. Control experiments were set up alongside with the extracts using commercial antibiotics and antifungal drugs (Gentamycin and Nystatin) 20mg and 50mg for bacterial and fungal respectively. After incubation, antibacterial activity of the extract was determined by measuring the different diameters of inhibition zone ([Rahmoun et al., 2014](#); [Karthikeyan et al., 2009](#)).

## **3. Results and Discussion**

The results of the various physicochemical analysis carried out on the seed oil of breadfruit are presented in Table 1. The result of the oil yield of breadfruit seeds (15.69%) shows that breadfruit seeds may be moderate oil yielding plants when compared to other oil yielding plants such as groundnuts with percentage yield of 43% ([Apta and Ologhobo, 1994](#)).

### **3.1 Colour**

From the results obtained, the color of the oil extracted was golden yellow and was liquid at room temperature ( $28 \pm 2^\circ\text{C}$ ).

### **3.2. Specific gravity**

Specific gravity is the ratio of the density of a respective substance to the density of water at  $4^\circ\text{C}$  ([Bangboye and Adejumo, 2010](#)). The density of vegetable oils is dependent on their fatty acid composition, minor components and temperature ([Fakhri et al 2011](#)). The result obtained in this

research indicated that breadfruit seed oil had a specific gravity of 0.635, showing that the oil of breadfruit was less dense than water.

**Table 1.** Results of Physico-chemical screening of *Treculia africana*

Physicochemical properties	values
Percentage yield (%)	15.69
Acid value (mg KOH g <sup>-1</sup> oil)	7.25
Iodine value (g iodine 100 g <sup>-1</sup> of oil)	52.60
Peroxide value (Meq KOH/g)	1.75
Free Fatty acid (meq/g)	2.75
Saponification value (mg KOH/goil)	245.99
Specific gravity at 25 °C (g mL <sup>-1</sup> )	0.635
Colour	Golden yellow
Unsaponifiable matter	4.34
Moisture	2.95

### 3.3. Moisture content

The determination of water content in oils is quite valuable because the conversion of the triglycerides in oils to free fatty acids is just one of the side-reactions that lead to oil decomposition. From the result obtained, the moisture content in breadfruit oil (2.95) was above 0.2% permissible limit for oil.

### 3.4. Acid value

Acid value is used as an indicator for edibility of an oil and suitability for use in the paint and soap industries (Aremu *et al.*, 2006). The acid value which is an indicator of the presence of free fatty acid content due to enzymatic activity in the samples was found to be moderate (7.25mg KOH/g oil). However, the value was higher compared to 2.63mg/KOH/g oil reported by Okorie and Nwachukwu (2014) for breadfruit seed oil. This difference may be attributed to different extraction procedures among other things. Low acid value in oil indicates that the oil will be stable over a long period of time and may resist rancidity and peroxidation. This may be attributed to presence of natural antioxidants in the seeds such as vitamins C and A as well as other possible phytochemical like flavonoids.

### 3.5. Iodine value

The iodine value is a measure of the degree of unsaturation and it is an identity characteristic of seed oils making it an excellent raw material for soaps cosmetics industries (Hamilton, 1999; Akbar *et al.*, 2009). Oils with iodine value less than 100 gI<sub>2</sub>/100g of oil are non-drying oils. The iodine value in this study was 52.60 g iodine 100 g/1 of oil, indicating that the oil is non-drying oil. The low iodine value indicates that the oil has low content of unsaturated fatty acids. Aremu *et al.* (2006) reported that

the lower the iodine value the lesser the number of unsaturated bonds; thus, the lower the susceptibility of such oil to oxidative rancidity.

### 3.6. Peroxide value

Peroxides are the primary reaction products formed in the initial stages of oxidation of oil and therefore give an indication of the process of lipid peroxidation (Shaker, *et al.*, 2009). Peroxide value is an index of rancidity, thus the high peroxide value of oil indicates a poor resistance of the oil to peroxidation during storage (Mohammed & Hamza, 2008). The peroxide value of breadfruit seed oil obtained in this study was 1.75 meq KOH/g, which was below the maximum acceptable value of 10 meq/KOH/g set by the Codex Alimentarius Commission for such oils as groundnut seed oils. The low acid and peroxide values in this study shows that breadfruit oil has the ability to resist lyplitic hydrolysis and oxidative deterioration (Akanni *et al.*, 2005).

### 3.7. Free fatty acid

Free fatty acid is the percentage by weight of a specified fatty acid such as percent oleic acid in oil. The free acid value for breadfruit seed oil in this study (2.75 meq/g) was within the permissible limit recommended by both FAO/WHO and Ethiopian Standards (ES) (1.0-3.0%). This low value may be as a result of lower hydrolysis of triglycerides, making it less susceptible to rancidity (Li *et al.*, 2007). It also signified that the oil may have a long shelf life, thus making it edible.

### 3.8. Saponification value

Saponification number is an indication of the molecular weight of triglycerides in oil. Saponification value obtained in this study was 245.99 mg KOH/g oil. The value obtained for the saponification index indicates that breadfruit oil is composed mainly by short chain triglycerides, which makes it suitable for sources of essential fatty acids required in the body, preparation of liquid soaps, shampoos and cosmetic products. (Nehdi, 2011; Akanni *et al.*, 2005; Eromosele *et al.*, 1994). According to Muhammad *et al.*, (2011), oil with higher saponification values contains high proportion of lower fatty acid. Ezeagu *et al.*, (1998) equally stated that a saponification value of 200 mg KOH/g indicates high proportion of fatty acids of low molecular weight.

### 3.9. Unsaponifiable matter

Unsaponifiable matters which include hydrocarbons, sterols, vitamins and pigments compounds usually play crucial roles in the oil stability. This is so because they are known to impact undesirable taste and flavor in products during processing into creams and vegetable oil (Umezuruike *et al.*, 2016). The low unsaponifiable matter reported for breadfruit seed oil in this study indicates that the oil is safe for consumption and industrial uses.

**Table 2.** Results of GC-MS analyses of the seed oil of *Treculia africana*

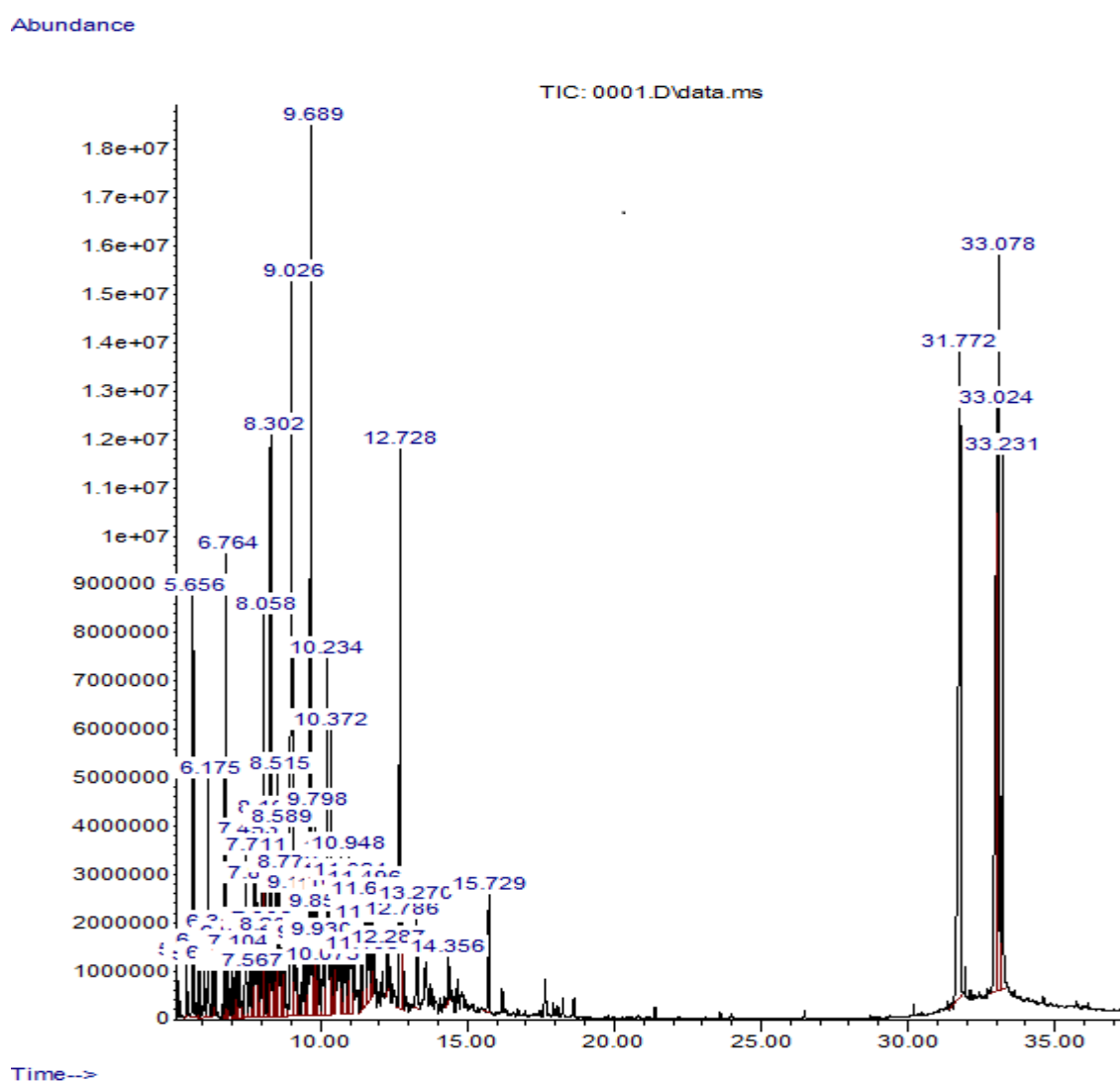
S/N	PK	RT	Area Pct	Library/ID
1	1	5.4369	0.2729	Ethylbenzene
2	2	5.6561	2.8683	p-Xylene
3	3	5.8654	0.3638	Octane, 2-methyl-
4	4	6.0299	0.4232	2-Decene, 5-methyl-, (Z)-
5	5	6.1751	1.3335	Benzene, 1,3-dimethyl-



6	6	6.3478	0.5274	1-Ethyl-4-methylcyclohexane
7	7	6.4054	0.2534	Cyclohexane, 1-ethyl-4-methyl-, cis-
8	8	6.7644	2.7452	Nonane
9	9	6.8432	0.459	3,5-Dimethyl-3-heptene
10	10	7.0079	0.2554	Benzene, (1-methylethyl)-
11	11	7.1037	0.383	Cyclopropane, (2-methylenebutyl)-
12	12	7.158	0.3226	Cyclohexane, (1-methylethyl)-
13	13	7.2597	0.3652	3,4-Octadiene, 7,7-dimethyl-
14	14	7.4527	1.3049	Cyclohexane, propyl-
15	15	7.5665	0.4332	n-Heptadecanol-1
16	16	7.7111	1.1895	Nonane, 3-methyl-
17	17	7.8168	1.1547	Benzene, propyl-
18	18	7.9062	0.6209	Heptane, 3-ethyl-2-methyl-
19	19	8.0581	3.0072	Benzene, 1-ethyl-3-methyl-
20	20	8.1048	1.0005	Benzene, 1-ethyl-4-methyl-
21	21	8.2057	0.4402	Cyclohexane, 1,1,2,3-tetramethyl-
22	22	8.3022	4.4795	Benzene, 1,2,4-trimethyl-
23	23	8.515	1.685	Nonane, 4-methyl-
24	24	8.5894	1.2024	Nonane, 2-methyl-
25	25	8.772	1.2197	Nonane, 3-methyl-
26	26	9.0263	<b>7.6755</b>	Benzene, 1,2,3-trimethyl-
27	27	9.1124	0.9543	m-Menthane, (1S,3R)-(+)-
28	28	9.4081	0.7137	Benzene, (2-methylpropyl)-
29	29	9.5011	0.6977	Benzene, (1-methylpropyl)-
30	30	9.689	<b>8.5505</b>	Decane
31	31	9.7979	2.0047	Benzene, 1,2,4-trimethyl-
32	32	9.855	0.687	p-Cymene
33	33	9.9297	0.6164	p-Cymene
34	34	10.0727	0.568	Indane
35	35	10.2337	3.15	D-Limonene
36	36	10.3721	1.8315	Decane, 4-methyl-
37	37	10.4603	0.9446	Cyclohexane, butyl-
38	38	10.7216	1.2166	Benzene, 1-methyl-3-propyl-
39	39	10.8363	1.1837	2-Tolyloxirane
40	40	10.9484	1.1162	Benzene, 4-ethyl-1,2-dimethyl-
41	41	11.0335	1.4014	Naphthalene, decahydro-, trans-
42	42	11.4053	0.4299	Decane, 5-methyl-
43	43	11.4963	1.3208	Benzene, 2-ethyl-1,3-dimethyl-
44	44	11.6152	0.8713	Decane, 2-methyl-
45	45	11.7297	0.5498	Benzene, 4-ethyl-1,2-dimethyl-
46	46	11.7982	0.4871	Decane, 3-methyl-
47	47	12.2866	0.2786	Benzene, 1,2-diethyl-
48	48	12.7282	5.0716	Undecane
49	49	12.7861	0.719	Benzene, 1-ethyl-2,4-dimethyl-

50	50	13.2699	0.9463	1-Methyldecahydronaphthalene
51	51	14.356	0.3744	Azulene
52	52	15.7292	0.8986	Dodecane
53	53	31.7719	<b>8.7234</b>	n-Hexadecanoic acid
54	54	33.0241	<b>7.8435</b>	9,12-Octadecadienoic acid (Z,Z)-
55	55	33.0782	5.8044	trans-13-Octadecenoic acid
56	56	33.2312	4.0589	Octadecanoic acid

The total ion chromatogram (TIC) of the fixed oil of breadfruit seeds showing the GC-MS profile of the compounds identified is given in Figure 2 and summarized in Table 2. GC/MS was used in identifying bioactive compounds present in the oil obtained from breadfruit seeds. The GC-MS analyses indicated that the African breadfruit oil contained a wide range of bioactive compounds as presented in Table 2 and Figure 1.



**Figure 2:** Chromatogram of chemical components in breadfruit oil

From the results obtained, fifty-six (56) compounds comprising of alkanes, alkenes, alcohols, terpenoids, saturated and unsaturated fatty acids, fatty acid esters and aromatic hydrocarbons were identified. The most abundant compounds identified with respect to their % peak areas were n-hexadecanoic acid, 9, 12-Octadecadienoic acid (Z, Z)-, trans-13-Octadecenoic acid, Undecane,

Decane, Octadecanoic acid, 1, 2, 3-trimethyl Benzene, and D-Limonene. Studies have shown that most of these bioactive compounds such as free fatty acids including long chain unsaturated fatty acids display significant biological activity against certain diseases, prevention of many diseases and health promoting properties (Olowokudejo *et al.*, 2008), (Aboul-Eneinet *et al.*, 2014).

N-Hexadecanoic acid commonly known as Palmitic acid has anti-alopecic, nematocidal, pesticidal, antioxidant and anti-androgenic properties. They also act as hemolytic 5-alpha reductase inhibitor and lubricant, and also possess hypo-cholesterolemic properties, (Komansilan *et al.*, 2012), (Isaiah *et al.*, 2016). 9, 12-Octadecadienoic acid, an ethyl ester, (also known as linoleic acid) belongs to omega 6-fatty acids used in the biosynthesis of arachidonic acid and thus some prostaglandins, thromboxane and leukotrienes collectively known as eicosanoids. It is a polyunsaturated fatty acid that plays a key role in support of heart vitality by lowering LDL cholesterol and reduces risk of developing heart disease (Farvid *et al.*, 2014). Linoleic acid also has anti-inflammatory, hypo-cholesterolemic, antiarthritic, 5-alpha reductase inhibitor, antihistaminic, insectifuge, anti-eczemic, anticancer, and anti-acne properties (Venkata-Raman *et al.*, 2012; Aneesh *et al.*, 2013; Rajeswari *et al.*, 2013; Rani *et al.*, 2009; Ponnamma and Manjunath 2012; Uma *et al.*, 2009).

It has been suggested that a combination of palmitic and linoleic acids displays antioxidant properties and can help prevent atherosclerosis (underlying pathogenesis of myocardial infarction) in rats (Cho *et al.*, 2010).

Octadecanoic acid (stearic acid), a saturated fatty acid has a hypocholesterolemic properties associated with low density lipoprotein (LDL) cholesterol levels (Mensink, 2005). They are equally known to possess Anti-inflammatory, anti-androgenic, dermatitogenic, anaemiagenic, insecticidal properties (Duke 2018).

Trans-13-Octadecenoic acid, a fatty acid methyl ester is known to inhibit the production of uric acid (Adegoke *et al.*, 2019). Studies have also shown that most octadecenoic acids have anti-inflammatory, pesticidal, antileukotriene, antiandrogenic, anticancer, dermatitogenic, -alpha reductase inhibitor, anemiagenic and hypocholesterolemic properties (Abubakar and Majinda 2016).

Limonene is reported in many studies to possess antioxidant, anti-inflammatory, antinociceptive, anticancer and insecticidal properties (Keinan *et al.*, 2005; Golshani *et al.*; 2004).

Hydrocarbons are assumed to be formed from fatty acid by lipoxygenase catalysis (Belitz *et al.*, 2009). This may explain their abundance in the African breadfruit seed oil, since the seeds are rich in oil.

The results of antimicrobial activity of the seed oil of breadfruit against different microorganisms are presented in Table 3. The antibacterial efficacy of breadfruit seed oil against these human pathogenic bacteria showed different selectivity for each microorganism. The oil of breadfruit showed a relatively good antimicrobial activity against test bacteria ranging from 9mm to 24mm. The results obtained in this study indicated that the antimicrobial activity of breadfruit oil was directly proportional to the concentration of the extracts, with the highest concentration recording the highest activity. Breadfruit oil showed maximum inhibition at 200 mg/ml against *Klebisella sp* (24mm), *Ps. Aeruginosa*, *Serratia sp* and *Shigella sp* at 20mm each, while the least inhibition at 200mg/ml was shown by *Bacillus sp* at 15mm. *Staphylococcus aureus*, *Proteus sp*, *E. coli*, *Enterobacter aerogenes* and *Micrococcus sp* were resistant to breadfruit oil as no activity was recorded at different concentrations. Equally, test fungi (*Candida albicans* and *Aspergillus niger*) showed no susceptibility to the oil.

**Table 3.** Results of Antimicrobial activity of *Treculia Africana* seed oil

Test organisms	Extract concentration(mg/ml)/zones of inhibition				
	200mg/ml	160mg/ml	120mg/ml	80mg/ml	40mg/ml

<i>Klebisellasp</i>	24mm	22mm	20mm	18mm	15mm
<i>Proteus sp</i>	-	-	-	-	-
<i>Bacillus sp</i>	15mm	10mm	9mm	-	-
<i>Ps. aeruginosa</i>	20mm	18mm	16mm	15mm	13mm
<i>E. coli</i>	-	-	-	-	-
<i>Enterobacter aerogenes</i>	-	-	-	-	-
<i>Serratia sp</i>	20mm	16mm	14mm	11mm	10mm
<i>Staph. aureus</i>	-	-	-	-	-
<i>Micrococcus sp</i>	-	-	-	-	-
<i>Shigella sp</i>	20mm	17mm	15mm	14mm	10mm
<i>Candida albicans</i>	-	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-

## Conclusion

The physicochemical properties of breadfruit seed oil were determined to ascertain its suitability or otherwise for consumption and industrial uses. GC-MS chromatography was used to determine the bioactive compounds present in the oil. The results revealed the breadfruit seed is a moderate oil yielding plant. It was further revealed that most of the physicochemical properties of seed oil of breadfruit were within permissible limits except moisture content, which was slightly higher than the permissible limit. The result of GC-MS analysis showed that about 56 compounds were determined. The most abundant compounds identified with respect to their % peak areas were n-hexadecanoic acid, 9, 12-Octadecadienoic acid (Z, Z)-, trans-13-Octadecenoic acid, Undecane, Decane, Octadecanoic acid, 1, 2, 3-trimethyl Benzene, and D-Limonene. The antibacterial activity of breadfruit seed oil against tested bacteria and fungi strains shows that it has the potential to be used as broad spectrum antibiotics for the treatment of various ailments. The results revealed that the breadfruit seed oil has many bioactive compounds of biological importance; hence it's fit for consumption and industrial uses.

**Acknowledgement:** The technical inputs of Mr Aniekan of the department of biochemistry are acknowledged

**Disclosure statement:** *Conflict of Interest:* The authors declare that there are no conflicts of interest.

*Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects.

## References

- Aboul-Enein AE., Abu, F, Shalaby E. and H El-Shemy, H(2014). Potent anticancer and antioxidant activities of active ingredients separated from *Solanum nigrum* and *Cassia italica* extracts. *Journal of Arid Land studies*, 24(1), 145 -152
- Abubakar M., Majinda R. (2016). GC-MS analysis and preliminary antimicrobial activity of *A. adianthifolia* (Schumach) and *P. angolensis* (DC). *Med* 3(1), 3, [doi:10.3390/medicines3010003](https://doi.org/10.3390/medicines3010003)
- Adebayo S.E, Orhevba B., Adeoye P. Musa J., Fase O. (2012). Solvent extraction and characteri-zation of oil from African star apple (*C. albidum*) seed. *Acad Res Inter.*, 3(2), 178– 183.

- Adegoke A.S., Oke V., Ademola G. (2019). GC-MS Analysis of Phytochemical Constituents in Methanol Extract of Wood Bark from *Durio Zibethinus Murr* *International Journal of Medicinal Plants and Natural Products*, 5(3), 1- 11, <http://dx.doi.org/10.20431/2454-7999.05030019>
- Ajiwe V., Okeke C. Agbo H. (1995). Extraction and utilization of breadfruit seed oil (*Treculia africana*). *Bioresource Technology*. 53,183–184
- Akanni M., Adekunle, A., Oluyemi E. (2005). Physico-chemical Properties of Some Nonconventional Oilseeds. *Journal of Food Technology*, 3(2), 177 – 181.
- Akbar E.Yaakob Z.Kamarudin S., Ismail M, Salimon M.(2009). Characteristic and Composition of *Jatropha Curcas* Oil Seed from Malaysia and its potential as biodiesel feedstock. *European Journal of Scientific Research*, 29(3), 396-403
- Aneesh T., Thomas E., Thomas D., Anandan R. (2013). GC-MS Analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* GAUD. *Asian J Pharm Clin Res*. 6(3), 68-74
- Anuonye J., Jigam A., Ndaceko, G. (2012). Effect of extrusion-cooking on the nutrient and anti-nutrient composition of pigeon pea and unripe plantain blends. *J. App. Pharm. Sci*, 2(5), 158-162
- A.O.A.C 2005 Association of Official Analytical Chemists, 18<sup>th</sup> edition, Washington, D.C.
- A.O.A.C, (1984). Association of Official Analytical Chemists, 14<sup>th</sup> edition, Washington, D.C. USA, pp 505-532.
- Apata D., Ologhobo A. (1994) Biochemical evaluation of some Nigerian legumes seeds. *Food Chem.*, 49: 333-338.
- Aremu M., Olonisakin A., Bako D., Madu P. (2006). Compositional studies and physicochemical characteristics of cashew nut (*Anacardium occidentale*) flour. *Pakistan Journal of Nutrition*, 5(4), 328-333, [doi:10.3923/pjn.2006.328.333](https://doi.org/10.3923/pjn.2006.328.333)
- Bamgboye A., Adejumo, O. (2010). Physicochemical properties of Roselle seed oil. *Nutrit and Food Science*, 40(2), 186-192. [doi.10.1108/00346651011029219](https://doi.org/10.1108/00346651011029219)
- Belarbi-Benmahdi M., Khaldi D., Beghdad C., Gouzi H., Bendimerad N., Hammouti B. (2009), Physicochemical and nutritional study of argan oil (*Argania spinosa* L.) in south-western Algeria, *Pigment and Resin Technology*, 38N<sup>o</sup>2, 96-99.
- Belitz H.D., Grosch W. Schieberle P. (2009) *Aroma Compounds in Food Chemistry*. 4th Edition, Springer, Berlin, 340-400.
- Britton R. S., Leicester K., L., Bacon B. R. (2002).. Iron toxicity and chelation therapy. *International journal of hematology*. 76(3),219-28. [doi:10.1007/BF02982791](https://doi.org/10.1007/BF02982791).
- Cho K. H., Hong J. H., Lee, K.T. (2010). Monoacylglycerol (MAG)-oleic acid has stronger antioxidant, anti-atherosclerotic, and protein glycation inhibitory activities than MAG-palmitic acid. *Journal of Medicinal Food*, 13(1), 99-107. [doi:10.1089/jmf2009.1024](https://doi.org/10.1089/jmf2009.1024)
- Davies J. (2007). Microbes have the last word. A drastic reevaluation of antimicrobial treatment is needed to overcome the threat of antibiotic –resistant bacteria. *EMBO Report*, 8 (7), 616-621.
- Diass K., Merzouki M., El Fazazi K., *et al.* (2023). Essential oil of *Lavandula officinalis*: Chemical composition and antibacterial activities, *Plants*, 12, 1571. [doi.org/10.3390/plants12071571](https://doi.org/10.3390/plants12071571)
- Dr. Duke's Phytochemical and Ethnobotanical Databases. Homepage, <https://phytochem.nal.usda.gov/> accessed on 10/12/2018
- Ebuehi O., Umeh R. Oletu F. (2006). Physico-chemical and fatty acid composition of two common edible vegetable oils in Nigeria. *Niger. Food J.*, 24(1), 17-2
- Enibe S. O. (2001). Design, construction and testing of African breadfruit depulping machine.
- Eromosele I. C., Eromosele C. O. Akintoye A, Komolafe T. (1994). Characterization of oils and chemical analysis of the seed of wild plants. *Plant Food for Human Nutrition*,46,361 – 365
- Ezeagu I., Petze K., Lange E., Metges C. (1998) Fat content and fatty acid composition of oils extracted from selected wild – gathered tropical plant seeds from Nigeria. *Journal of the American Oil Content*. 75(8), 1031 – 1035

- Fakhri. N., QadirH. (2011). Studies on various physico-chemical characteristics of some vegetable oils. *Journal of Environmental Science and Engineering*, 5: 844- 849.
- Farvid M., Ding M., Pan A, Sun Q., *et al.* (2014). Dietary linoleic acid and risk of coronary heart disease: A systematic review and meta-analysis of prospective *cohort studies*. *Circulation*, 130(18), 1568-1578, doi: [10.1161/CIRCULATIONAHA.114.01023](https://doi.org/10.1161/CIRCULATIONAHA.114.01023)
- Fasisi, O.S., Eleyinmi, A.F., Fasisi, A.R. and Karim, O.R. (2003). Chemical Properties of Raw and Processed Breadfruit (*Treculia africana*) Seed Flour. *African Crop Science Conference Proceedings*, 6, 547-551.
- Fasisi O., Eleyinmi A., Fasasi A. Karim O. (2004). Chemical properties of raw and processed breadfruit *Treculia Africana* seed flours. *Food, Agri. Environ.* 2, 65-68
- Giami S., Amasisi, T., Ekiyor G. (2004). Comparison of bread making properties of composite flour from Kernel of roasted and boiled African breadfruit (*Treculia africana*) Seeds, *Material Review*,1(1), 16-25.
- Golshani S., Karamkhani F., Monsef-Esfehani H., Abdollahi M. (2004). Antinociceptive effect of the essential oil of *Dracocephalum kotschy* in the mouse writhing test. *Journal of Pharmacology and Pharmaceutical Sciences*,7, 76-79.
- Hussain J., Ullah R., RehmanN, Khan A., Muhammad Z. Hussain F., Anwar S. (2010) Endogenous transitional metal and proximate analysis of selected medicinal plants from *Pakistan*. *Journal of Medicinal Plants Research*,4(3),267-70.
- Ibemesi J. (1992). *Vegetable oils as industrial raw materials; The Nigerian Perspective* (A Monograph). Enugu: SNAAP Press
- Ifeoma I., Chukwunonso E., Obinna E., Nkwonta M., Bryan C. (2010) Effect of Traditional Processing Techniques on the Nutritional and Phytochemical Composition of African Breadfruit (*Treculia africana*) Seed. *Journal of Applied Sciences and Environmental Management*,14, 169-173
- Ihekoronye A., Ngoddy P.O. (1985). *P Integrated Food Science and Technology for the Tropics*. Macmillan Publishers. London. pp 58-85
- Isaiah S., Arun Kumar, C., Senthamizh Selva N. (2016). Phytochemical Screening, Antimicrobial Activity and GC-MS Analysis of *Corchorus tridens* L. *International Journal of Pharmacology Research*,6 (12), 353-357.
- Jafari, S., Ebrahimi, M., Assatarakul, K., Jafari, S.M. (2022). Plant Oils Rich in Essential Fatty Acids. In: Jafari, S.M., Rashidinejad, A., Simal-Gandara, J. (eds) *Handbook of Food Bioactive Ingredients*. Springer, Cham. [https://doi.org/10.1007/978-3-030-81404-5\\_21-1](https://doi.org/10.1007/978-3-030-81404-5_21-1)
- Karthikeyan A., ShanthiV.,NagasathayaA.(2009). Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica*. L. *International J. Green Pharmacy*, 3:78-79. <https://doi.org/10.22377/ijgp.v3i1.62>
- Kadda, S., Belabed, A., Loukili, E.H., Hammouti B., Fadlaoui S. (2022), Temperature and extraction methods effects on yields, fatty acids, and tocopherols of prickly pear (*Opuntia ficus-indica* L.) seed oil of eastern region of Morocco. *Environ Sci Pollut Res* 29, 158-166. <https://doi.org/10.1007/s11356-021-16752-8>
- Keay R. W. J. (1989) *Trees of Nigeria*. A revised version of Nigerian trees (Vols. 1 and 2); keay RWJ, Onochie CFA, Stanfield DP (eds); Clarendon Press, Oxford.; 476.
- Keinan E., Att A., Amir G., Bentur L., Bibi H., Shoseyov D. (2005) Natural ozone scavenger prevents asthma in sensitized rats. *Bioorganic and Medicinal Chemistry*, 13(2), 557-562, doi: [10.1016/j.bmc.2004.09.057](https://doi.org/10.1016/j.bmc.2004.09.057)
- Khan M., Kihara M., Omoloso A. (2003). Antimicrobial activity of the alkaloidal constituents of the root bark of *Eupomatia aurina*. *Pharmaceutical Biology*, 41(4), 277-80. doi: [10.1076/phbi.41.4.277.15671](https://doi.org/10.1076/phbi.41.4.277.15671)

- Komansilan A., AbadiA., L., Yanuwidi B., Kaligis D. A. (2012). Isolation and Identification of Biolarvicide from Soursop (*Annona muricata* Linn) Seeds to Mosquito (*Aedes aegypti*) Larvae. *International Journal of Engineering & Technology*. 12(03), 28–32.
- Kostik V., Memeti S., Bauer B., (2013). Fatty acid composition of edible oils and fats. *Journal of Hygienic Engineering and Design*, 4, 112-116
- Li S.G., Zhang H., Xue W.T. (2007): A novel method for the determination of acid value of vegetable oils. *Eur. J. Lipid Sci. Tech.*, 109, 1088-1094. doi: 10.1002/ejlt.200700058
- Lim T. K. (2012). *Treculia africana*. Edible medicinal and non-medicinal plants. 3, 446–452, doi: 10.1007/978-94-007-2534-8
- Mensink R. P. (2005). Effects of stearic acid on plasma lipid and lipoproteins in humans. *Lipids*, 40: 1201-1205, doi: 10.1007/s11745-005-1486-x
- Muhammad N. O., Bamishaiye E.B., Usman L., A., Salawu M.O., Nafi M.O., Oloyede, O.B. (2011). Physicochemical properties and fatty acid composition of *C. esculentus* (Tiger nut) tuber oil. *Bioresearch Bulletin*, 5, 51-54.
- Mohammed M. I., Hamza, Z. U. (2008). Physicochemical Properties of Oil Extracts from *Sesamum Indicum* L. Seeds Grown in Jigawa State – Nigeria. *Journal of Applied Science and Environmental Management*, 12(2), 99 – 101. doi:10.4314/jasem.v12i2.55542
- Muthulakshmi A., Jothibai M.R., Mohan V.R. (2012). GC-MS Analysis of Bioactive Components of *Feronia elephantum* Correa (Rutaceae). *Journal of Applied Pharmaceutical Science*; 2(2), 69-71
- Nehdi I. (2011). Characteristics, chemical composition and utilization of *Albizia julibrissin* seed oil. *Industrial Crops and Products* 33, 30–34. doi: 10.1016/j.indcrop.2010.08.004
- Nwabueze I. T. U., Iwe, M. O., Akobundu, E. N. T. (2008) Physical characteristics and acceptability of extruded African breadfruit-based snacks. *Journal of Food Quality*, 31: 142-155. doi:10.1111/j.1745-4557.2008.00194.x
- Okorie S.U., Nwachukwu, C.N. (2014). Comparative Evaluation of Quality characteristics of oils Extracted from some selected Legumes and cereal. *Journal of Environmental Science, Toxicology and Food Technology*. 8(7), 66-69, doi:10.9790/2402-08726669
- Olowokudejo J. D., Kadiri A. B., Travih V. A. (2008). An ethnobotanical survey of herbal markets and medicinal plants in lagos state of Nigeria. *Ethnobotanical Leaflets*, 12: 851-865
- Omobuwajo T.O., Akande E.A., Sanni L.A. (1999) Selected physical, mechanical and aerodynamic properties of African breadfruit (*Treculia africana*) seeds. *Journal of Food Engineering* 40: 241-244.
- Ononogbu I. C. (2002). Lipids in human existence. Nsukka, Nigeria: AP Express Publishers, pp. 2–9.
- Ouahabi, S., Loukili, E.H., Elbouzidi, A., et al. (2023) Pharmacological Properties of Chemically Characterized Extracts from Mastic Tree: In vitro and in silico assays. *Life*, 12, 1393; <https://doi.org/10.3390/life13061393>
- Oyetayo V.O., Omenwa V.C. (2006). Microbial and Chemical Qualities of Raw and Trona Processed African Breadfruit (*Treculia Africana* Decne). *American Journal of Food Technology*, 1: 77-80
- Parvez M., Khan M., Islam M., Hasan S. (2005). Antimicrobial activities of the petroleum ether, methanol and acetone extracts of *Kaempferia galanga* L. rhizome. *J. Life Earth Sci.* 1, 25-29
- Ponnamma S. U., Manjunath K. (2012). GC-MS Analysis of phytochemicals in the methanolic extract of *Justicia wynaadensis* (nees) T. anders. *Int J Pharm Bio Sci* 3(3), 570–576
- Rahmoun N.M., Ziane H., Boucherit-Otmani Z. (2014). Antibacterial and antifungal screening of four medicinal plants. *J. Coast Life Med.* 2(12), 975-9.
- Rajeswari G., Murugan M., Mohan V.R. (2013). Bioactive components of *Hugoniamystax* bark. *Journal of pharmaceutical and biomedical sciences*, 29(29), 818-824
- Rani L. S., Mohan V. R., Regini G. S., Kalidass C. (2009) GC-MS analysis of ethanolic extract of *Pothos scandens* leaf. *J Herb Medi Toxicol* 3, 159–160

- Shahidi F., Shukla V.K.S. (1996) Nontriacylglycerol constituents of fats/oil. *Inform*, (7), 1227-1232.
- Shahidi F., Cadwallader K. R., Weenan H. (2002) Freshness and Shelf Life of Foods, *ACS symposium series 836*, American Chemical Society Washington D.C.; 201-211.
- Shaker M.A., Ahmed M.G., Amany M.B., Shereen L.N. (2009). Chufatubers (*C. esculentus* L.) as a new source of food. *World Applied Sciences Journal*, 7(2), 151-156
- Uma B., Prabhakar K, Rajendran S., Sarayu L.Y. (2009). Studies on GC/MS spectroscopic analysis of some bioactive antimicrobial compounds from *Cinnamomum zeylanicum*. *J. Med. Plants*, 8(31), 25–131
- Umezuruike C. A., Titus U. N., Enoch N. T. A (2016). Optimization and assessment of physiochemical properties and use of oil of African breadfruit (*Treculia africana*) seeds. *Sky Journal of Agricultural Research*, 5(8), 168 – 173
- Venkata-Raman B., Samuel L.A., Pardha S. M., Narashimha R. B., Naga V.K.A., Sudhakar M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian J. Pharm. Clin Res.*, 5(2), 99-106.
- Wilcox B. F (2006). *Fundamental of food chemistry*. Paraclete Pub. Yola Nigeria

---

(2024) ; <http://www.jmaterenvironsci.com>