ChemSearch Journal 12(2): 70 – 80, December, 2021
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 22/11/2021 Accepted: 10/12/2021
http://www.ajol.info/index.php/csj

Physico-Chemical Properties, Chemical Composition, Biodiesel Production and Antibacterial Potential of *Terminalia catapa* Seed Oil

***Ogbeide, O. K., Eze, O. F., Akaeze, D. A., Akhigbe, I. U., Omoruyi, U., Iyekowa, O. and Owolabi, B. J.**
Department of Chemistry, University of Benin, Benin, Nigeria
***Correspondence Email:** kennedy.ogbeide@uniben.edu

ABSTRACT

Terminalia catapa (Almonds) are highly nutritional powerhouses full of healthy fats, fibres, phytochemicals, vitamins and minerals. Its usefulness includes food and for the production of some other desirable necessities to man. The aim of this study was to extract, characterize, determine the chemical constituents and investigate the antimicrobial potential of oil from almond seed, as well as biodiesel production. All analyses were done using established methods. The result obtained showed the proximate composition with moisture content (20.00%), crude fat (20.60%,) protein (25.60%,),carbohydrate (25.60%,), ash (4.80%) and crude fibre (3.40%). The physicochemical properties of the *T. catapppa* seed oil were moisture (0.20%), specific gravity (0.90g/cm^3), viscosity at 40^{o}C (27.50 mPa.s), cloud point (15.00^{o}C), pour point (11.00^{o}C), smoke point (170.00^{o}C), flash point (203.00^{o}C), fire point (265.00^{o}C), acid value (2.94 mgKOH/g), free fatty acid (FFA) (1.74 mgKOH/g), saponification value (326.08 mgKOH/g), Iodine value (131.37 gI2/100g) and Peroxide value (3.81meq/kg). The biodiesel produced had a percentage yield of 36.9% with the physicochemical properties including specific gravity (0.89g/cm^3), viscosity at 40^{o}C (5.8 mPa.s), cloud point (7.0^{o}C), pour point (6.0^{o}C), smoke point (161.0^{o}C), flash point (215.0^{o}C), fire point (186.0^{o}C), acid value (0.76 mgKOH/g), FFA (0.38 mgKOH/g), saponification value (313.45 mgKOH/g), Iodine value (54.56 gI2/100g) and Peroxide value (4meq/kg). A total of 21 compounds were identified from the *T. catapa* seed oil by the GC-MS, with the major constituents being fatty acids; n-Hexadecanoic acid (17.96%), Oleic acid (22.42%) and 2,9-octadecadienoic acid (22.82%)by composition. The oil also demonstrated a good antibacterial potential at all concentrations (50, 75, 100 and 125 mg/mL) against all tested isolates (Staph*ylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus sp*). The results obtained revealed that the oil from almond seed may possibly find its useful application in the cosmetic industry, and could also be a promising source of renewable energy.

Keywords: Antibacterial activity, Biodiesel, Chemical Composition, Physico-Chemical, *Terminalia catapa*

INTRODUCTION

Terminalia catappa (Almonds) belongs to the family of Combretaceae. It is widely found in the sub-tropic and tropic regions and particularly native to the Southeast Asians. It is majorly grown for its ornamental purposes and could attain a height of over 30 m with large leaves of 10-14 cm broad and 15-25 cm long. It has a drupe fruit (green to red on maturity) that encloses a seed. Itis rich in important dietary nutrients such as carbohydrates, minerals, lipid, vitamins and protein (Sarkar *et al*., 2020). The oil from the seed is edible and is used in many countries for cooking, and often applied on skin as an antifungal, anti-inflammatory and antiseptic agent (Ruttarattanamongkol and Petrasch, 2015). The nuts are often consumed fresh after separation from the shell and could be preserved for over a year when smoked or dried. Almond seeds are usually small in size and tough to extract, hence its use is very limited (Orhebva *et al*., 2016), however, it is often processed to get the oil which is useful in food preparation and industrial application. The natives of Ayurveda use extract from the leaves to prepare medicinal ointments for treating scabies and leprosy, and it is taken for head and stomach aches (Vijaya *et al*., 2015).

The plant has been reported to possess bioactive compounds like tannins, flavonoids, terpenoids, steroids and phenols (Terças *et al*., 2017). Compounds isolated from the plants include asiatic acid, gallic acid, ursolic acid, chebulagic acid, squalene, isovitexin, rutin, vitexin, geranin, punicalin, tercatain, tergallagin, corilagin and terflavins (Chung *et al*., 1998; Lin *et al*., 2000; Fan *et al*., 2004; Mininel *et al*., 2014). Studies have revealed the leaves to exhibit antibacterial (Taganna *et al*., 2011), antioxidant (Lin *et al*., 2001), anti-inflammatory (Fan *et al*. 2004), hepato-protective (Tang *et al*., 2006) analgesic (Ratnasooriya *et al*. 2002) and anticancer activities. The fruit and seed showed adiabatic and anticancer potentials (Nagappa *et al*., 2003; Ko *et al*., 2002). Other parts of the plant, like the root and bark

demonstrated antimicrobial (Pawar and Pal, 2002) and wound healing properties (Khan *et al*. 2013).

Pathogens are the agents for countless diseases and deaths around the world. The numerous medicines available are often either resistant to the pathogens or intolerable as followed with their side effects.

In addition to this concern, global warming and other sources of emissions are just a couple of the effects of our overreliance on fossil fuels. Researchers are regularly working with different green energy sources in order to create environmentally friendly alternatives. Because of the increased global oil demand and the need for renewable fuels and energy, biofuels have gained a lot of interest (Bateni and Karimi, 2016; Samadi *et al*., 2017). The use of biofuels instead of fossil fuels would slow the rate of global warming by lowering sulfur, carbon oxide, and hydrocarbon emissions (Krishnaprabu, 2019). Biodiesel is one of the attractive alternatives due to its lower carbon and sulphur emissions as compared to traditional petroleum-based fuels.

Overcoming these problems will require continuous search for better alternatives particularly from natural sources.

Many studies have been carried out on different parts (leave, nut and seed) of *T. catappa*, however, there are limited information about the chemical compounds present and the antibacterial potential of the seed oil. To this end, this study aims at investigating the physicochemical properties, chemical composition and antibacterial potential of *T. catappa* seed oil, as well as to explore its use in the production of biodiesel

MATERIALS AND METHODS

Sample collection and preparation

Almond-seed (*T. catappa*) fruits was collected from the garden in Faculty of Arts, University of Benin, Nigeria (September, 2020). The fruit pericarps were removed mechanically with a knife and shade-dried (35.5^{o}C) for 14 days after which their endocarps (dried shells) were removed to expose the seeds. The seeds were ground to powder with a mechanical grinder, and stored into an air tight container for further laboratory analyses when needed.

Extraction of oil for almond seed

The almond seed powder (650 g) was extracted with n-hexane with a Soxhlet apparatus. After several cycles, the extracted seed oil was then taken out and concentrated using a rotary evaporator, and the percentage yield calculated.

Physicochemical analysis of almond seed oil

Physical and chemical analyses on the oil were carried out using methods described by AOAC (2005).

Oil pre-treatment and biodiesel production

For the dosage of FFA pre-treatment, most industrial biodiesel manufacturers adopt the dosing regimen recommended by the National Renewable Energy laboratory (NREL) report, which is 2.59 of methanol and 0.05g of sulphuric acid for every gram of FFA in the oil (equivalent to 19.8:1 of methanol-to-FFA molar ratio and 5% of acid-to-FFA weight percentage).

During the production of biodiesel, two phases were undergone, they are: Trans-esterification phase and Separation and washing phase

Phase 1: Trans-Esterification Phase

The reactions in the trans-esterification phase involve glyceride (oil) and alcohol in the presence of catalyst. 100cm^3 of the oil was measured with a measuring cylinder, using oil to alcohol ratio of 4:1, 25cm^3 of methanol (alcohol) was measured into a beaker and 0.7g of KOH (catalyst) was dissolved inside the methanol. 0.7g of KOH was used because for standard 7g of KOH is required for 1 litre of oil. The oil and the methanol and KOH were mixed inside a round bottom flask attached to a magnetic stirrer. The magnetic stirrer temperature was set to 50°C and stirring was done for 30 minutes.

Phase 2: Biodiesel Separation and Washing Phase

After stirring the mixture for 30 minutes, it was then poured into a separating funnel. A 2-phase solution was obtained in which the biodiesel was below while the residual catalyst (glycerol) as the by-product was above, the biodiesel was removed and washed to purify it, while the glycerol i.e. the by-product was not utilized. Finally, the biodiesel was then dried in an oven for 30 minutes.

Microorganisms

The microorganisms employed in this study were clinical isolate (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus spp.*) procured from the University of Benin Teaching Hospital, Benin City. Isolates were collected using sterile containers. All isolates were characterized using cultural morphological characteristics.

Anti-microbial agents

Augmentin(10µm), Ciprofloxacin (30µm), Amoxicillin (30µm), Pefloxacillin (10µm), Streptomycin (10µm).

Agar well diffusion

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldis *et al.,* 2004; Valgas *et al*., 2007). Similarly to the procedure used in disk-diffusion method, the agar plate surface was inoculated by spreading a volume of the microbial

inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibited the growth of the microbial strain tested.

Minimum inhibition concentration (MIC)

Antimicrobial sensitivity test was carried out using the method described by Vinothkumar *et al*. (2010) with slight modification. Varied concentrations (50, 75, 100, and 125mg/mL) were made from each extract. Molten Mullen Hinton Agar was poured on sterile petri dish and allowed to solidify. Upon solicitation, test isolates of microbes were streaked on it, after which a corkborer (sterile) was used to bore hole on the agar and labelled appropriately.

After this, 1 mL from the concentrations made was transferred to the hole created and allowed to diffuse (making sure the hole does not get to the bottom of the Agar). All petri dishes were incubated at a temperature of 37°C for 24hrs. Upon incubation, all emergent clear zone of inhibition was measured using a measuring ruler and recorded in millimetre. The smallest value represents minimum inhibition concentrations.

Antibiotic susceptibility test

Mullen- Hinton Agar was prepared and poured on sterile petri dishes and allowed to solidify. Each isolate was streaked on plates using sterile wire loop. Each antibiotic disc of +ve gram was placed using a sterile forceps on each plate. All micro-organisms plates were incubated at a temperature of 37°C for 24hrs. After incubation, zones of inhibition were measured for each organism using a ruler and later recorded in millimetre (mm).

RESULTS AND DISCUSSION

Proximate analysis of almond seed

The result for proximate analysis is presented in Table 1. The moisture content of almond seed was observed to be high (20%) compared to cashew nut; 7.3% (Akinhanmi *et al*., 2008) and African oil bean 5.7% (Igwenyi *et al*., 2015) and is in agreement with(25%) Akpankpan and Akpabio (2012). The moisture content of food source typically serves as basis of stability and susceptibility to microbial action. Hence due to the high moisture content, almond seed may be susceptible to degradation and microbial spoilage when preserved over a long period of time (Barku *et al*., 2012). An appreciable amount of inorganic minerals was attested by a high ash content of 4.8%, which is in agreement with values (4.27%, (4.55% and 4%) reported by Matos *et al*. (2009), Omeje *et al*. (2008) and Guillermo *et al*. (2008) respectively. There was a high concentration of fat and protein with values of 20.6% and 25.6% respectively. Although the fat content reported by Justina *et al*. (2015) was higher at 44.64%, however, this along with other reports on almond seed, confirms it to be a rich source of fats and protein (Akpabio, 2012; Akpankpan and Akpabio 2012). Fat is important in diets for the absorption of fat soluble vitamins and the high fat content could make almond seed a good source of oil for industrial applications. The protein content is a good source of amino acids, and it can supplement the required daily amount of 23.6g for adults (National Research Council, 1929; Akinhanmi *et al*., 2008).

The fibre content of the almond seed (3.4%) obtained in this study is similar to the findings obtained from previous reports (Akpabio, 2012; Akpankpan and Akpabio 2012; Udotong and Bassey2015). Fibre mostly consists of cellulose in addition with lignins (Saratale and Oh 2012). They have beneficial effects on muscles of the intestine during peristalsis. They are not digestible by man, but however act as roughages which aid digestion (Udotong and Bassey, 2015).

The carbohydrate content of almond seed was 25.6%. Carbohydrates are important source of energy for cellular metabolism. It also performs functional and structural roles in the animal body (Udotong and Bassey, 2015).

The percentage yield of oil obtained (Table 2) from tropical almond seed was 36.90%. The yield was lower than 51.80% (Matos *et al.*, 2009), 52.11% (Barku *et al.*, 2012) and 54.00% (Adu*et al*., 2013) from other reports. The decreased output may be due to the length of oil extraction, variations in plant species, growing environment, ripening stage, seed harvesting period, and extraction process employed (Dutta *et al*., 2015). The extracted oil was liquid at room temperature, which makes it suitable for biodiesel manufacturing. The average percentage yield of biodiesel was 60.60% (Table 2).

Physical properties of almond seed oil and its biodiesel

The physical analysis of the almond seed oil and biodiesel are outlined in Table 2. The recommended moisture content of an oil is 0.05% (ASTM, 2003). A high deviation could result in microbial growth during storage or within transporting equipment (Galadima *et al*., 2008; Onyezeka *et al*., 2020). The value of moisture content obtained is much lower than the values reported by Orhevba *et al*. 2016 (2.04 %) and Matos *et al*. 2009(4.13 %), however it is still very much higher (0.21%) than the recommended America Society for Testing and Materials (ASTM) limit (0.05%). This deviation may have been caused by absorption of moisture present in

the atmosphere upon storage or improper treatment after processing. (Gerpen, 2005).

The specific gravity of the oil (0.9 g) and the biodiesel (0.89 g) are very close, as similar result was obtained by Orhevba *et al.* (2016). The value for the biodiesel falls within the range recommended by the ASTM, (2003) standard. From literature, the specific gravity of biodiesels is attributed to the fatty acids present, and hence their density will vary upon the difference in fatty acid composition (Tat and Gerpen, 2000). The specific gravity is important, as it determines ease of air to fuel ratios for complete combustion, hence a value less or higher may cause incomplete combustions and lead to pollutant emission (Galadima *et al*., 2008; Onyezeka *et al*., 2020)

Viscosity, the resistance to fluid flow under gravity is one of the basic conditions for fuel injectors used in diesel engines and when viscosity is high, injectors perform poorly (Yunus *et al*., 2013). The viscosity of the oil at 40 °C was 27.5mPas, this was higher than 14.1 mPas reported by Orhevba *et al*.(2016), however, the viscosity measured (5.8 mPas at 40^{o}C) for biodiesel from almond seed oil is in conformity with the ASTM,(2003) standard (1.9-6.0). It is worthy to note that the viscosity of a liquid decreases with increase in temperature. The viscosity at 40 °C and 100 °C may be used to calculate the biodiesel's viscosity index. The viscosity index (VI) is an arbitrary, unit-less measure of a fluid's viscosity variation as temperature changes. It is mostly used to describe the viscosity-temperature behaviour of lubricating oils.

The smoke point, also referred to as the burning point, is the temperature at which an oil or fat begins to produce a continuous bluish smoke that becomes clearly visible under certain conditions. The more free fatty acid (FFA) an oil contains, the quicker it will break down and start smoking (Thomas, 2002). The flash point of a volatile material is the lowest temperature at which its vapours ignite if given an ignition source and consequently an essential safety requirement in transportation and storage. The flash point of pure biodiesels is normally greater than the ASTM (2003) limitations, but it drops fast as the proportion of methanol increases (Bello and Agege, 2012) The fire point of a fuel is the lowest temperature at which the vapour of that fuel will continue to burn for at least five seconds after ignition by an open flame (Steven *et al*., 2015).

The oil's smoke point, flash point, and fire point were 170.0°C, 203°C, and 265.0°C respectively, while the biodiesel's were 161°C, 186°C, and 215°C.

Cloud point and pour point are indicators of the lowest temperature of utility for petroleum products. The highest temperature at which haziness is observed (cloud point), or the lowest temperature at which movement of the oil is observed (pour point), is reported as the test result. The cloud point and pour point of the oil were 15.0°C and 11.0°C, respectively. This result indicates that when the oil cools to 15.0°C, a wax or cloud forms, and that if the temperature continues to drop to about 11.0°C, the oil becomes semi-solid. The biodiesel's cloud point and pour point were 7.0°C and 6.0°C, respectively. The results demonstrated that a wax was generated at 7.0°C and the biodiesel will seize to flow at 6.0 °C. The cloud point and pour point values obtained were within the range prescribed by the ASTM (2003) standard, indicating that biodiesel from almond has good cloud and pour point properties. The cloud point and pour point are the two most critical properties of biodiesel, as frozen fuel can clog fuel lines and filters, depriving the engine of fuel. These findings also indicate that biodiesel from almond seed oil should not be utilized in areas where the average temperature is less than 7.0 °C, as this will cause the fuel to freeze during use. The results obtained from this study are similar with those reported by Orhevba *et al.*, 2016 (16.0°C and 11.5°C) for oil and (7.0°C and 6.0°C) for biodiesel.

Chemical properties of almond seed oil and its biodiesel

The chemical properties of the almond seed oil and biodiesel are presented in Table 3.

The acid value is the amount of potassium hydroxide (KOH) in mg required to completely neutralize 1g of a fat. The acid value of oil indicates the level of spoilage that has occurred in an oil sample, and is usually indicated in the formation of free fatty acids caused by enzymatic hydrolysis.

The oil has an acid value of 2.94 mgKOH/g and a free fatty acid (FFA) content of 1.47 mgKOH/g. This is lower than 3.37 mgKOH/g and 1.68 mgKOH/g reported by Orhevba *et al.*, (2016). It is however greater than 0.78 mgKOH/g acid and 0.38 mgKOH/g (Barku *et al.*, 2012). The acid value of the biodiesel is 0.76 mgKOH/g, which is within the ASTM specification of 0.80 mgKOH/g. The biodiesel has 0.38 mgKOH/g. According to Jaichandar and Annamalai, (2011), the type of fatty acids can alter the properties of biodiesel. In the case of very acidic raw materials, Romano *et al*., (2013) showed that basic transesterification is feasible if the value of free fatty acids is less than 2%.

The oil's saponification value obtained (326.08mgKOH/g) was higher than 168.27 mgKOH/g (Barku *et al.*, 2012), and 199.19 mgKOH/g (Orhevba *et al.*, 2016).This might be because of the method used to extract the oil and the method used to calculate the saponification value. This demonstrates that more alkali is necessary to neutralize the available free fatty acid generated by the oil. The higher the saponification value, the easier the oil may be utilized to make soap, shampoos, and shaving creams (Schumann and Siekmann 2000; Auwal *et al*. 2010). The

saponification value of the biodiesel was 313.45mgKOH/g, which was lower than that of the oil. However, there is no set standard number for biodiesel saponification value.

The iodine value is a measure of the amount of unsaturation in fats, oils and waxes (Thomas, 2002). It is used to quantify the amount of double bond in oil which reflects the susceptibility of the oil to oxidation. The oil has an iodine value of 131.37 gI2/100g, and the biodiesel was recorded at 54.56 gI2/100g, Oils with iodine value less than 100 mg I_2/100g are non-drying oils, and consequently, the lesser the number of unsaturation the lower the susceptibility of such oil to oxidative rancidity (Aremu *et al*., 2006). The oil obtained may not be suitable as alkyl resins for paint production or used as varnishers. Moreover, high percentage of unsaturation is typically useful as raw materials in the manufacture of vegetable oil-based ice cream (Oderinde *et al*., 2009).

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. It is a measure of the extent to which an oil sample has undergone primary oxidation, extent of secondary oxidation may be determined from p-anisidine test (Chakrabarty, 2016).

The peroxide value obtained for the oil (3.80 meq/kg) is lower than 5.0 meq/kg (Orhevba *et al*.,2016) and 4.073meq/kg (Barku *et al.*, 2012). High peroxide values indicates high levels of oxidative rancidity of the oil which suggest low levels or absence of antioxidant (Kyari, 2008). The World Health Organisation (WHO) in 1994, specified a permitted maximum peroxide level of not higher than 10 meq/kg for oils (Aremu *et al*., 2015), hence, this value (3.80 meq/kg) is within the WHO tolerable range.

Table 1: Proximate Composition of *T. catappa* seed

Composition	Values(%)
Moisture content	20.00
Ash content	4.80
Carbohydrate	25.60
Crude fat	20.60
Protein	25.60
Crude fibre	3.40

Table 2: Physical properties of *T. catappa* seed oil and its Biodiesel

S/N	Properties	*T. catappa* seed oil	Biodiesel
1	Moisture content (%)	0.20	-
2	Specific gravity	0.90	0.89
3	Density (g/cm^3)	0.90	0.88
4	Percentage yield (%)	36.90	60.60
5	Colour	Yellow	Pale yellow
6	Odour	-	-
7	Viscosity (mPas)at 40^{o}C	27.50	5.80
8	Cloud point (^{o}C)	15.00	7.00
9	Pour point (^{o}C)	11.00	6.00
10	Smoke point (^{o}C)	170.00	161.00
11	Flash point (^{o}C)	203.00	186.00
12	Fire point (^{o}C)	265.00	215.00

Table 3: Chemical properties of *T. catappa* seed oil and its Biodiesel

S/N	Properties	Almond seed oil	Biodiesel
1	Acid value (mgKOH/g)	2.94	0.76
2	Free Fatty Acids (mgKOH/g)	1.47	0.38
3	Saponification value (mgKOH/g)	326.08	313.45
4	Iodine value (gI/100g)	131.37	54.56
5	Peroxide value (meq/Kg)	3.81	4.0

GC-MS analysis of almond seed oil

The result from the GC-MS (Table 4) revealed the 21 chemical constituents in the Almond oil consisting of fatty acids, hydrocarbons, ester, aldehyde, alcohols and aromatic compounds. The fatty acid was the highest in proportion with over 60% in the Almond oil. 2,9-octadecadienoic acid had the highest in percent composition (22.82), followed by Oleic acid (22.42%) and n-Hexadecanoic acid (17.96%).

The presence of 2,9-octadecadienoic acid and oleic acid suggests that the almond oil could be useful in pharmaceutics and in cosmetics. Reports have shown 2,9-Octadecadienoic acid, plays a very important role in medicine for treatment of atherosclerosis and hyperlipoidemia (Lan *et al*., 2017). Oleic acids reduce cholesterol levels, improve heart conditions and exhibit anti-inflammatory potency when applied on skin to treat injuries. Industrially, it is used in producing detergents, coatings and lubricants. (Roestorf, 2017; Wentworth, 2018). The n-Hexadecanoic acid also has an anti-inflammatory capacity as well as an antioxidant and anti-bacterial properties (Vasudevan *et al*., 2012). Industrially, n-Hexadecanoic acid is used to produce cosmetics molding agents and soaps.

The organo-silicon compounds had the second highest percentage composition in the almond oil. Reports have shown these compounds (cyclotetrasiloxane, octamethyl, cyclopentasiloxane, decamethylcyclohexasiloxane, dodecamethylcyclooctasiloxane, hexadecamethyl and cyclohexasiloxanedodecamethyl) to find use majorly in the cosmetic industries; in the manufacturing of deodorants, shampoos, antiperspirants and skin creams (Klykken *et al*., 1999; Johnson *et al*., 2011).

The aromatic compound (7-Hydroxy-3-(1,1-dimethyl prop-2-enyl coumarin) is among the class of umbelliferone derivatives, known to be present in edible fruits and vegetables. Owing to their beneficial medicinal effects to man, they act as cancer preventive agents, antimicrobial, and anti-inflammatory and antioxidants (Preziuso *et al*., 2020).

Phytol is a diterpene alcohol and one of the principal product of chlorophyll metabolism in plants, and it is widely present in nature. It possesses an antibacterial activity as reported (Inoue *et al*., 2005) to inhibit the growth of *Staphylococci aureus*. It was shown also to exhibit *in vitro* and *in vivo* Antinociceptive and Antioxidant Activities (Santos *et al*. 2013).

Antibacterial zone of inhibition test of almond seed oil

Almond seed oil exhibited bacterial growth inhibitions against the test isolates (*Staphylococcus aureus, Bacillus sp, Pseudomonas aeruginosa* and *Escherichia coli*) at different concentrations, with the highest observed at 125 mg/mL (Table 5). Based on Bauer *et al*., the microbicidal activity is classified as resistant if the zone of inhibition is less than 7 mm, intermediate (7-9 mm), and sensitive (10 or more). The antibacterial activity of the almond seed oil could be attributed to its fatty acid identified by the GC-MS. In addition, when comparing the Antibiotic sensitivity profiles of bacteria strain (Table 6), with the zone of inhibition of almond seed oil (Table 5), it could be said that the almond seed oil has very good antibacterial activity.

Table 4: GC-MS analysis of *T. catappa* seed oil

Compound name	Molecular formula	Molecular weight	Retention index	%Composition
Cyclotetrasiloxane, octamethyl	$C_8H_{24}O_4Si_4$	296	827	3.06
Cyclopentasiloxane, decamethyl	$C_{10}H_{30}O_5Si_5$	370	1034	0.99
Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	1240	1.28
Cyclopentanetridecanoic acid	$C_{19}H_{36}O_2$	296	2120	0.41
Oleic acid	$C_{18}H_{34}O_2$	282	2175	22.42
Dodecanal	$C_{12}H_{24}O$	184	1402	0.18
3-hydroxy,dodecanoic acid	$C_{12}H_{24}O_3$	216	1733	0.11
Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	1654	2.56
2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$	306	2001	0.23
2-bromo, octadecanal	$C_{18}H_{35}BrO$	346	2231	0.39
Dodecanal	$C_{12}H_{24}O$	184	1402	0.18
2,9-octadecadienoic acid	$C_{18}H_{32}O_2$	280	2183	22.82
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1968	17.96
Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	1240	1.28
1,6,10,14,18,22-Tetracosahexane-3-ol,2,610,15,19,23-hexamethyl	$C_{30}H_{50}O$	426	3058	3.44
7-Hydroxy-3-(1,1-dimethyl prop-2-enyl coumarin	$C_{14}H_{14}O_3$	230	1987	1.48
10-Octadecanal	$C_{18}H_{34}O$	266	2007	2.97
9,12-Octadecadienoic acid	$C_{18}H_{34}O_2$	282	2183	1.81
Phytol	$C_{20}H_{40}O$	296	2045	3.15
9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	2085	2.29
9,12-Octadecadienioc acid	$C_{18}H_{32}O_2$	280	2183	1.81

Table 5: Zones of Inhibition of Almond seed oil

	Zone of Inhibition (mm)			
Bacteria Isolates	**50 mg/mL**	**75 mg/mL**	**100 mg/mL**	**125 mg/mL**
Staphylococcus aureus	13	19	21	32
Escherichia coli	10.5	11	22	28
Pseudomonas aeruginosa	17	25	27	43
Bacillus sp	29	34	34	36

Table 6: Standard Antibiotic Susceptibility Test

Bacteria Isolate	GEN	OFL	NIT	CTR	AUG	CRX	CAZ	CXM
Escherichia coli	S (22)	R (11)	S (19)	R (11)	R (9)	R (10)	R (11)	S (20)
Pseudomonas sp	S (20)	R (10)	S (20)	S (31)	S (31)	S (19)	S (22)	S (35)
Staphylococcus aureus	S (22)	S (31)	R (10)	R (11)	S (26)	R (11)	R (12)	R (12)
Bacillus sp	S (21)	S (21)	S (16)	R (12)	R (8)	R (12)	R (10)	S (28)

Key: GEN=Gentamycin, CXM=Ceftriaxone, NIT=Nitrofurantoin, OFL=Ofloxacin, AUG=Augmentin, CAZ=Ceftazidime, CRX=Cefuroxime, S=Susceptible, R=Resistant, 0-13=Resistant, 14 and above=Susceptible

CONCLUSION

The results showed that aside the dietary nutrients in *T. catappa* seed oil, it also contains chemical compounds with important characteristics employed in both pharmaceutical and cosmetic production as well as good antibacterial activity. In addition to these qualities, the oil obtained from almond could be a promising source of renewable energy and as a result, it should be given more attention to explore its use in the production of biodiesel.

REFERENCES

Adu, O.B, Omojufehinsi, M., Esanboro, M.O., Abe, D.A., Shofolahan, A.O., Uzodinma, E., Badmus, K., Martins, O. (2013): "Effect of processing on the quality, composition and antioxidant properties of *Terminalia catappa* (Indian Almond) seed oil." *African Journal of Food, Agriculture, Nutrition and Development*. 13:7662-7678

Akinhanmi, T.F., Atasie, V.N., Akintokun, P.O. (2008): Chemical Composition and Physicochemical Properties of Cashew nut (*Anacardium occidentale*) Oil and Cashew nut Shell Liquid. *J Agric Food and Environ Sci*. 2(1):1 – 10.

Akpabio, U.D. (2012): Evaluation of proximate composition, mineral element and anti-nutrient in almond (*Terminalia catappa)* seeds. Advances in *Applied Science Research*. 3(4): 2247-2252

Akpakpan, A.E and Akpabio, U.D. (2012): Evaluation of Proximate Composition Mineral Element and Anti-Nutrientin Almond (*Terminalia catappa*) Seed. *Res J Appl Sci*. 7(12): 489-493.

America Society for testing and Materials (ASTM) Standard D6751-12, (2003) "Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels," ASTM International, West Conshohocken, PA. doi:10.1520/C0033-03, astm.org.

AOAC International. Official methods of analysis of AOAC international, 18th ed. USA: AOAC International. 2005.

Aremu, M.O., Olaofe, O., Akintayo, E.T. (2006): Chemical composition and physicochemical characteristics of two varieties of bambara groundnut (*Vignasub terrenea*) flours. *J. Applied Sciences*. 6(9): 1900–1903.

Aremu, M.O., Ibrahim, H., Bamidele T.O. (2015): Physicochemical Characteristics of the Oils Extracted from Some Nigerian Plant Foods – A Review. 32:36-52.

Auwal, A., Edward, K., Abdulhamid, H. (2010): Extraction and Characterization of Landolphia Seed Oil. *Advances in Applied Science Research*. 1(3): 265-268.

Barku, V.A., Nyarko, H., Dordunu, P. (2012): "Studies on the physicochemical characteristics, microbial load and storage stability of oil from Indian almond nut (*Terminalia catappa L.*)," *Studies*. 8: 9-17.

Bateni, H and Karimi, K. (2016): Biodiesel production from castor plant integrating ethanol production via a biorefinery approach. *Chem. Eng. Res. Des*. 107:4-12.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin Pathol.,* 44: 493-496.

Bello, E and Agge, M. (2012): "Biodiesel production from ground nut oil." *Journal of Emerging Trends in Engineering and Applied Science*. 3: 276-280.

Biego, G.H.M., Konan, A.G., Douati, T.E., Kouadio. L.P. (2012): Physicochemical Quality of kernels from *Terminalia catappa* L. and sensory evaluation of the

Concocted kernels. *Journal of Sustainable Agriculture Research*, 1(2): 1 – 6.

Chung, K.T., Wong, T.Y., Wei, C.L., (1998): Huang, Y.W., Lin, Y. "Tannins and human health: a review," *Critical Reviews in Food Science and Nutrition*. 38(6): 421-464.

Dutta, R., Sarkar, U., Mukherjee, A. (2015): Soxhlet extraction of *Crotalaria juncea* oil using cylindrical and annular packed beds. *International Journal of Chemical Engineering and Applications*, 6(2): 130-133.

Fan, Y.M., Xu, L.Z., Gao, J., Wang, Y., Tang, X.H., Zhao, X.N., Zang, Z. X. (2004): Phytochemical and anti-inflammatory studies on *Terminalia catappa*. Fitoterapia. 75: 253-60.

Galadima, A., Garba, N.Z., Ibrahim, B. M. (2008): Homogeneous and Heterogeneous Transesterification of Groundnut Oil for Synthesizing Methyl Biodiesel. *International Journal of Pure and Applied Sciences*.2: 138–144.

Gerpen, J.V. (2005): Biodiesel Processing and Production. *Fuel Processing Technology*.86: 1097–1107

Guillermo A.P., Buelvas H.D., Arrieta Y.D, (2008): Nutritional characteristics of the indian almond (*Terminiala catappa* L.) as supplement in animal feeding. Revista MVZ Cordoba, 13(1): 1205-1214.

Igwenyi, I.O., Isiguzo, O.E., Aja, P.M. (2015): Proximate Composition, Mineral Content and Phytochemical Analysis of the African Oil Bean (*Pentaclethramacrophylla*) Seed. *American-Eurasian J. Agric. & Environ. Sci*. 15(9): 1873-1875.

Inoue, Y., Hada, T., Shiraishi, A., Hirose, K., Hamashima, H. (2005): Kobayashi S. Biphasic effects of geranylgeraniol, teprenone, and phytol on the growth of *Staphylococcus aureus*," *Antimicrobial Agents and Chemotherapy*. 49(5): 1770–1774.

Jaichandar, S and Annamalai, K. (2011): The Status of Biodiesel as an Alternative Fuel for Diesel Engine-An Overview. *Journal of Sustainable Energy and Environment*. 2: 71-75.

Johnson, W., Bergfeld, W.F., Belsito, D.V., Ronald, A., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.M., Shank, R.C., Slaga, T.J., Snyder, P.W., Andersen, F.A. (2011): Safety Assessment of Cyclomethicone, Cyclotetrasiloxane, Cyclopentasiloxane, Cyclohexasiloxane, and Cycloheptasiloxane. *International Journal of Toxicology* 30 (Supplement 3). 149S-227S.

Khan, A.A., Kumar, V., Singh, B.K., Singh, R. (2014): Evaluation of wound healing property of *Terminalia catappa* on excision wound models in Wistar rats. Drug Res (Stuttg). 64: 225-8.

Klykken, P.C., Galbraith, T.W., Kolesar, G.B., Jean, P.A., Woolhiser, M.R., Elwell, M.R., Burns-Nass, L.A., Mast, R.W., Maccay, J.A., White, J.K.A., Munson, A.E. (1999): Toxicology and humoral immunity assessment of octamethylcyclotetrasiloxane (D4) following a 28-day whole body vapor inhalation exposure in Fischer 344 rats. *Drug ChemToxicol*. 22(4): 655 – 677.

Ko, T.F., Weng, Y.M., Chiou, R.Y. (2002): Squalene content and antioxidant activity of *Terminalia catappa* leaves and seeds. *J Agric Food Chem*. 50: 5343 – 8.

Krishnaprabu S. (2019): A Review on Biodiesel Production as Alternative Fuel. *Int. J. Pure App. Biosci.* 2019; 7(2):258 – 266.

Kyari, M.Z. (2008): Extraction and characterization of seed oils. *International Agrophysics*.22: 139–142.

Lan. W., Lin, S., Li, X., Zhang, Q., Qin, W. (2017): Chemical composition of the leaf and stem essential oil of Adenophorae Radix. *Journal of Physical and Chemical Reference Data*. 40:043101.

Lin, C.C., Hsu, Y.F., Lin, T.C. (2001): Antioxidant and free radical scavenging effects of the tannins of *Terminalia catappa* L. *Anticancer Res*. 21: 237-43.

Lin, Y.L., Y.H., Shiao, M.S., Chen, C.C., Ou, J.C. (2000): "Flavonoid glycosides from *Terminalia catappa* L," *Journal of the Chinese Chemical Society*. 47: 253–256.

Magaldi, S., Mata-Essayag. S., de Capriles, H.C., Perez, C., Colella, M.T., Olaizola, C., Ontiveros, Y. (2004): Well diffusion for antifungal susceptibility testing. *Int J Infect Dis*. 8(1):39-45.

Matos, L., Nzikou, J.M., Kimbonguila. A., Ndangui, C.B., Pambou-Tobi, N.P.G., Abena, A.A., Silou, T.H., Scher, J., Desobry, S. (2009): Composition and Nutritional properties of seeds and oil from *Terminalia catappa* L. *Advance Journal of Food Science and Technology*. 1(1): 72-77.

Mininel, F.J., Leonardo Jr. C.S., Espanha, L.G., Resende, F.A., Varanda, E.A., Leite, C.Q.F., Vilegas, W., dos Santos LC. (2014): Characterization and Quantification of Compounds in the Hydroalcoholic Extract of the Leaves from *Terminalia catappa* Linn. (Combretaceae) and Their Mutagenic Activity. Evidence-Based *Complementary and Alternative Medicine*. Volume 2014: 1 – 12.

Nagappa, A.N., Thakurdesai, P.A., Venkat, R.N., Singh, J. (2003): Anti-diabetic activity of *Terminalia catappa* Linn fruits. *J Ethnopharmacol* 88: 45-50.

Oderinde, K.A., Ajayi, I.A., Adewuyi, A. (2009): Characterization of Seed and Seeds oil of *Huracrepitans* and the Kinetics of Degradation of the Oil during Heating, *Electronic Journal of Environment, Agriculture & Food Chemistry*. 8(3): 201–208.

Omeje E.O., Okide, G.B., Esimone C.O., Ajali, U.(2008): Kinetics of autoxidation of an oil extraction from *Terminalia catappa*. *India J. Pharm.*, 70(2):260-262.

Onyezeka, E.G., Nnaji, J.C., Omotugba, S.K., Garba, M.H. (2020): Physicochemical Properties of Biodiesel Produced by Transeterification of Various Esterified Waste Frying Oils. *Nigerian Journal of Chemical Research*. 25(1): 44-58.

Orhevba, B.A, Adebayo, S.E, Salihu, A.O. (2016): Synthesis of Biodiesel from Tropical Almond (*Terminalia catappa*) Seed Oil.*Current Research in Agricultural Sciences*. 3(4): 57-63.

Pawar, S.P and Pal, S.C. (2002): Antimicrobial activity of extracts of *Terminalia catappa* root. *Indian J Med Sci*. 56: 276-8.

Preziuso. F., Genovese, S, Marchetti, L., Sharifi-Rad. M., Palumbo, L., Epifano, F., Fiorito, S. (2020): 7-Isopentenyloxycoumarin: What Is New across the Last Decade. *Molecules*. 5: 5923 doi:10.3390/molecules25245923.

Ratnasooriya W.D., Dharmasiri M.G., Rajapakse, R.A., De Silva, M.S., Jayawardena, S.P., Fernando, P.U.D., De Silva W.N., Nawela, A.J.M.D.N.B., Warusawithana, R.P.Y.T., Jayakodi, J.R.C., Digana, .M.C.B. (2002): Tender Leaf Extract of *Terminalia catappa* antinociceptive activity in rats. *Pharm Biol*. 40: 60-6.

Roestorf, J. (2017): Facial Oils: Are You Using the Correct Percentage of Oleic and Linoleic Acids? https://luxebotanics.com/blogs/luxe-botanics/facial-oils-are-you-using-the-correct-percentage-of-oleic-and-linoleic-acidsAccessed12 November 2021.

Romano. S., Sorichetti, P.A, Lee, W.H., Cho, V.G. (2011): Estimation of methanol content in biodiesel by measurements of electrical properties and flash point determination. Handbook of sustainable energy. New York*: NOVA Science Publishers Inc*. 679 – 691.

Ruttarattanamongkol, K and Petrasch, A. (2015): Antimicrobial activities of *Moringa oleifera* seed and seed oil residue andoxidative stability of its cold pressed oil compared with extra virgin olive oil. *Songklanakarin J. Sci. Technol*. 37(5):587-594.

Samadi, S., Karimi, K., Behnam, S. (2017): Simultaneous biosorption and bioethanol production from lead-contaminated media by *Mucorindicus*. *Biofuel Res. J.* 4(1): 545-550.

Santos, C.C.M.P., Salvadori, M.S., Mota, V.G., Costa LM, Antonia ACA, Oliveira GAO, Costa JP, Damião PS, Rivelilson, M.F, Reinaldo NA. Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. *Neuroscience Journal* Volume 2013: 1 – 9.

Saratale, G and Oh, S.(2012): Lignocellulosics to ethanol: The future of the chemical and energy industry. *Afr J Biotch*. 11(5):1002-1013.

Sarkar, S.K., Miyaji, T., Sasaki, J., Biswas, S.N., Ali, S., Salam, A. (2020): Fatty Acid Composition, Physico-Chemical and Anti-Oxidant Properties of Almond Seed (*Terminalia catappia* L) Oil and its Therapeutic Uses. *Journal of Global Biosciences*. 5: 7419-7433.

Schumann, K and Siekmann, K. (2000): Soaps. in Ullmanns Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim. 2000.doi.org/10.1002/14356007.a24_247

Steven, A.T., Peter, R.P., David, S.J.J. (2015): Handbook of Petroleum Processing (2 ed.). Springer. p1736.

Tang, X., Gao, J., Wang, Y., Fan, Y.M., Xu, L.Z., Zhao, X.N., Xu, Q., Qian, Z.M. (2006): Effective protection of *Terminalia catappa* L. leaves from damage induced by carbon tetrachloride in liver mitochondria. *J Nutr Biochem*. 17: 177-82.

Taganna, J.C., Quanico, J.P., Perono, R.M., Amor, E.C., Rivera, W.L. (2011): T*erminalia catappa*Tannin-rich fraction from *Terminalia catappa* inhibits quorumsensing (QS) in *Chromobacterium violaceum* and the QS-controlled biofilm maturation and Las Astaphylolytic activity in *Pseudomonas aeruginosa*. *J Ethnopharmacol*. 134: 865-71. doi: 10.1016/j.jep.2011.01.028

Terças, A.G., Monteiro, A.S., Moffa E.B., dos Santos, J.R.A., de Sousa, E.M., Pinto, A.R.B., Costa, P.C.S., Borges, A.C.R., Luce M. B. Torres, L.M.B., Filho, A.K.D.B., Fernandes, E.S., Cristina de Andrade Monteiro, C.A. (2017): Phytochemical Characterization of *Terminalia catappa* Linn. Extracts and Their antifungal Activities against *Candida* spp. *Front. Microbiol*. 8:595. doi: 10.3389/fmicb.2017.00595

Tat, M.E. and Gerpen, J.H.V. (2000): The Specific Gravity of Biodiesel and Its Blends with

Diesel Fuel. *Journal of the American Oil Chemists Society*. 77(2):115–119.

Thomas, A. (2000): Fats and Fatty Oils. *Ullmann's Encyclopedia of Industrial Chemistry*. Wenheim: Wiley-VCH. 22

Udotong, J.I.R and Bassey, M.I. (2015): Evaluation of the Chemical Composition, Nutritive Value and Anti-nutrients of *Terminalia catappa* L. Fruit (Tropical Almond). *International Journal of Engineering and Technical Research*.3(9):96-99.

Valgas, C., de Souza, S.M., Smânia, E.F.A., Smânia Jr, A. (2007): Screening Methods to Determine Antibacterial Activity of Natural Products. *Brazilian Journal of Microbiology*. 38: 369-380.

Vinothkumar, P., Sivaraj. A., Ahmed, K.S.Z., Sivamani, P., Devi, K., Senthilkumar, B. (2010): Evaluation of antibacterial activities of *Andrographis paniculata* leaf extract against gram negative and gram positive species by in vitro methods. *Journal of Pharmacological Research*. 3:1513-1515.

Vasudevan, A., Vijayan, D., Mandal, P., Haridas, M., Karthe, P., Sadasivan, C. (2016): Anti-Inflammatory Property of n-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. *Chemical Biology & Drug Design*. 80(3): 434-9.

Wentworth, D. (2018): What is Oleic Acid 2018**.** https://www.twinriverstechnologies.com/blog/oleic-acidAccessed12 November 2021.

Yunus MM, Zuru AA, Faruq UZ, Aliero AA. (2013): Assessment of Physicochemical Properties of Biodiesel from African Grapes (*Lanneamicro carpa* Engl & K.Krause). *Nigerian Journal of Basic and Applied Science*. 21(2):127-130.