Nig. J. Biotech. Vol. 37(2): 134-143 (Dec. 2020)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v37i2.13

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Assessment of Physicochemical and Fatty Acids Composition of Crude Seed Oil Extract of *Azadirachta indica* Adr. Juss. for its potential in Biodiesel Production

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Abstract

This study investigates the physico-chemical and fatty acids composition of crude seed oil extracts of *Azadirachta indica* . The main objective was to evaluate some biodiesel characteristics of the crude seed oil extract of *Azadirachta indica.* The procedures of the Association of Official and Analytical Chemist (AOAC) were used for assessment of some physical, biochemical, and fatty acids constituents of the test seed oil extract. The physical properties assayed for indicate that the oil is liquid at room temperature, non-drying, with specific gravity, with flash and melting points of 0.910±0.08 g/cm^3, 80±2.10°C and 76±1.60°C respectively. The chemical properties included 66.77±2.55 g/100g (iodine value), 1.465±0.07 (refractive index@ 30°C), 212.96±1.16 mgKOH/g (saponification value), 0.39±0.16 meq/Kg (peroxide value), 4.24±0.12 mgKOH/g (acid value), 2.20±0.12 mm^2/s (viscosity value), 56.91±2.19 (cetane number), 39.21±1.11 MJ/kg (calorific value) and 2.13±0.05% w/w (free fatty acids). Fatty acids composition of the crude seed oil of *A. indica* obtained were linoleic, hexadecanoic, octadecanoic and alpha linolenic acids, with retention time and % composition of 18.2 min and 10.8±0.50%, 22.2 min and 30.01±1.79%, 18.2 min and 59.10±2.22%, and 20.2 min and 0.09±0.02% respectively. The crude seed oil extract clearly presents a potential as a biodiesel substrate for incorporation as a proximate blend in auto-engines. This therefore would necessitate intensive afforestation efforts of the plant species for sustainable utilization.

Keywords: *Azadirachta indica,* Biodiesel, physico-chemical, fatty acids, crude seed oil, extracts

Introduction

The continuous increase in energy demand remains a global phenomenon. This increase has been largely traced to rise in human population and industrialization, which have continually placed pressure on basic economic infrastructure (Khan et al., 2014; Demirbas et al., 2015a). Fossil fuels which currently dominate the global energy landscape are faced with numerous threats ranging from price stability, issue of sustainability, dependence and energy security (Banik et al., 2018), environmental impacts and C- emissions as well as ecosystem stability (Sylvester et al., 2013). Biodiesel is a methyl or ethyl ester obtained through esterification of edible and non edible oils and fats of organic origins (Wilson, 2010). The imperativeness for consideration of biodiesel options against the fossil diesel commonly in use stemmed from its number of reported advantages ranging from reduced exhaust emissions, improved biodegradability, reduced toxicity, improved lubricity, higher flash point, and lower vapour pressure (Knothe and Steidley, 2005; Adewuyi et al., 2014; Syndia et al., 2015). Moreso, the fossilized diesels have recorded high oil prices coupled with high greenhouse gas emissions making its continuous use unattractive and consequently turning attention to investment in biofuels (Adewuyi et al., 2014).

The use of cheap, non-edible seed oils, animal fats, and waste oils as raw feedstock for manufacturing of non-fossil diesel provides a cost- saving process. So, the search for non-edible underutilized seed oils as feedstock for producing biodiesel is important (Adewuyi et al., 2014). Most of the feedstock currently incorporated in biodiesel were plant – based (Demirbas, 2015b). They include mustard seed, peanut, sunflower, and cotton seed. Soybean oil is commonly used in the United States, and rapeseed oil (Europe), coconut oil and palm oils (in Malaysia and Indonesia) for biodiesel production (Demirbas et al., 2016).

There has been a dramatic increase in prices of these vegetable oils, due to their rising demands as feedstock, competing for foods (Atabani et al., 2012). This definitely affects the economic viability of these substrates for the biodiesel industry (Keneni and Marchetti, 2017). This definitely rendered them not feasible and unsustainable, thereby engendering the consideration less expensive and less competitive and inedible oil-rich plant biomass (Avhad and Marchetti, 2015).

Several non-edible plant seed oils are being investigated to assess their suitability based on physico-chemical, fatty acids and other biodiesel properties. These include oils from *Jatropha* (Umaru and Aberuagba, 2012), *Sclerocarya birrea* (Ejilah *et al*., 2012), *Hevea brasiliensis* (Krishnakumar et al., 2013), *Balanites aegyptiaca* (Ogala et al., 2018), *Hura crepitans* (Sidohounde et al., 2019). The Neem tree (*Azadirachta indica A. Juss*), an evergreen member of the Meliaceae (Mahogany) family and native of India, grows in the tropical and sub tropical Africa (Abubakar, 2016). The species has been reported to have significant medicinal uses, as anti-dermatophytes, malaria, asthma, and intestinal worms (Nde et al., 2015), as well as other useful industrial utilizations (Syndia et al., 2015). Some efforts have been made to incorporate the seed oil extracts of this plant in biodiesels (Banu et al., 2018; Madai et al., 2020). The present study, therefore assesses the physico-chemical and fatty acid compositions of crude seed oil extracts of *Azadirachta indica* as potential biodiesel and industrial substrate.

Materials and Methods

Collection and Preparation of Kernels of Azadirachta indica

The seeds of neem (*Azadirachta indica*) called dogonyaro in Hausa (Adewoye and Ogunleye, 2012), sourced from Toro - Bauchi State of Nigeria, packed and transported in sterilized polythene bags and thereafter identified at the herbarium of the Federal College of Forestry, Jos (FCFJ), Plateau State, (with a voucher number of FHJ31720). They were cleaned and then depulped by soaking in water for 24 hrs. The depulped seeds were dried at room temperature (26.1°C) for 10 days and peeled to separate the shell from the kernel.

Extraction of Crude Seed Oil of Azadirachta indica

The extraction of the crude seed kernel oil of *A. indica* was carried out, using modified methods of Syndia et al*.,* (2015) and Hamadou et al*.,* (2020). The kernels (1.0kg) were mechanically decorticated (dehulling) to obtain the almonds, which were spread in a tray and air - dried for 10 days in the chemistry lab, FCFJ. The dried almonds were weighed, pulverized with mortar and pestle, at temperatures between 45-47°C to dryness and liquefied to ease extraction. (Tesfaye et al., 2018; Banik et al*.,* 2018). The oil was extracted by traditional methods by pouring the toasted kernels into hot water in a mortar and allowed to float, followed by stirring, using a pestle then decantation and drying (Abu-Al-Futuh,1989; Hamadou et al., 2020) (Figure 1). The extracted seed oil was assayed for some physical, biochemical, diesel and fatty acids constituents

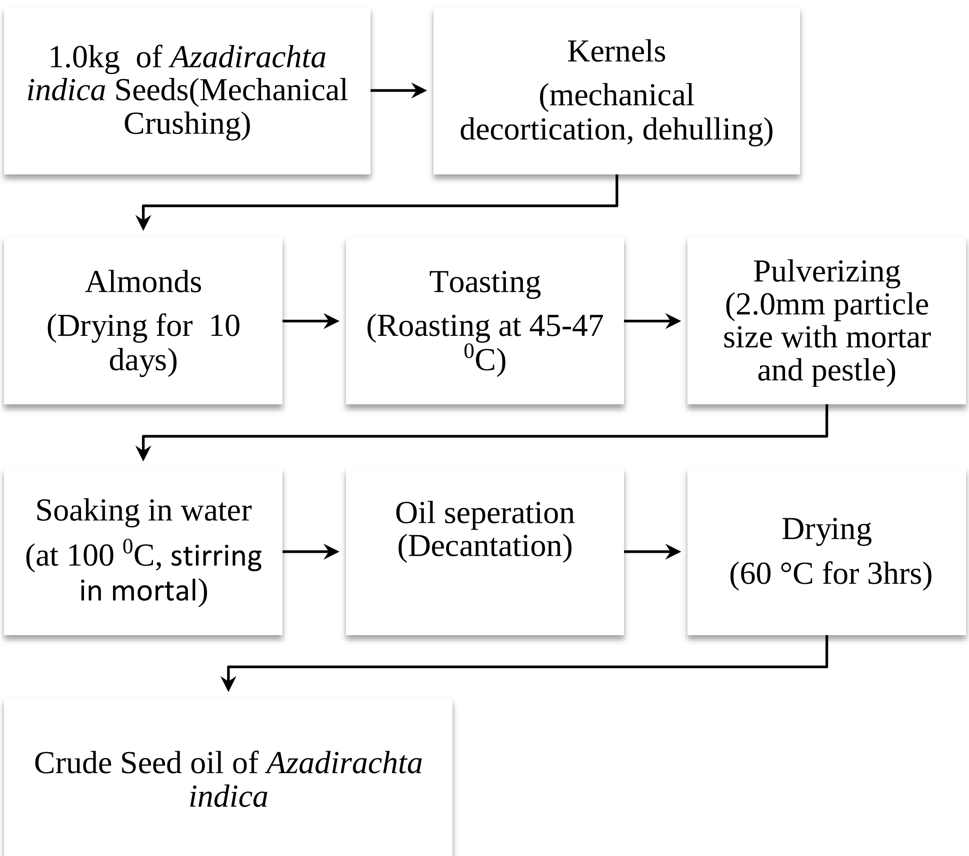

Figure 1: Flow chart of Extraction Process of Crude Seed oil of *Azadirachta indica*

Determination of Physical and biochemical Composition of Crude Seed oil extract of Azadirachta indica

The crude seed oil extract of *A. indica* was subjected to standard methods of the American Society of Testing Materials (ASTM 2003 ASTM D6751-08) as described by Umaru and Aberuagba, (2012), to determine the physical and biochemical properties. The acid value was assessed using titration method , involving dissolution of 2.0 g of the oil samples in 50 cm^3 of mixed solvent (25 cm^3 dimethyl ether with 25 cm^3 of ethanol precisely made up to pH 7.0, by addition of 0.1M NaOH, and 1% phenolphthalein solution as an indicator). Relative density was determined as described by Jibril et al. (2012), viscosity, using Clandon viscometer, model: VT-03 viscometer (Umaru and Aberuagba, 2012). The saponification value, peroxide, iodine value, specific gravity were determined according to the methods of Umaru and Aberuagba (2012).

Determination of Fatty Acids Composition of Azadirachta indica

The fatty acids composition of the crude seed oil of *Azadirachta indica* was determined by modified methods of Rizvi (2009), using Gas chromatograp hy and Mass Spectrometry (GC-MS) of models: QP2010 and HP5973 respectively at the National Research Institute for Chemical Technology (NARICT), Zaria - Kaduna State.

Determination of Some Other Measurable Quantities of Azadirachta indica crude oil extract

Some important biodiesel properties such as refractive index (R1), cetane number (CN), calorific value (CV) and were determined using reference formulae as follow:-

The refractive index (R1) was determined using the Perkins formula (1) reported by Babatunde and Bello (2016).

$$RI = 1.45765 + 0.0001164\, IV \quad \ldots\ldots..(1)$$

Where RI = Refractive Index; IV = iodine value

The cetane number (CN) of the oil extract was evaluated using the formula of Krisnangkura (1986) reported by Adewuyi et al. (2014) in equation (2) below:-

$$CN = 46.3 + \frac{5458}{SV} - (0.225 \times IV) \quad \ldots\ldots\ldots\ldots\ldots(2)$$

Where SV = Saponification value; IV = iodine value

The calorific value (CV) was computed using the equation of Batel et al. (1980), reported by Adewuyi et al. (2014) in the relationship (3) below:-

$$CV = - 4.187\, IV - 38.31 SV \left(in \frac{Kj}{kg}\right) \quad \ldots\ldots(3)$$

Where IV = iodine value; SV = Saponification value;

Results

The results of some of the physical properties of crude seed oil of *Azadirachta indica* showed that the oil is a brown liquid at ambient temperature. The melting and flash points of the crude seed oil extract of A. indica are 76±1.60°C and 80±2.10°C, respectively. The oil is of a non-drying class, with a specific gravity of 0.910±0.08 g/cm^3 at 25°C. The details of the results are shown on Table 1.

The average chemical constituents of the crude seed oil extract of *A. indica* revealed that it has saponification , peroxide , and acid values of 212.96±1.16 mgKOH/g, 0.39±0.16 meq/Kg and 4.24±0.12 mgKOH/g, respectively. While 2.20±0.12 mm^2/s, 66.77±2.55 g/100g, and 2.13±0.05% (w/w) were the viscosity , iodine and free fatty acids values, respectively (Table 2).

Refractive Index, Cetane Number and Calorific Value

The refractive index, cetane number (CN), and the calorific value (CV) of the crude kernel oil extract of *A. indica* obtained were 1.465±0.07 (@ 30°C), 56.91±2.19 and 39.21±1.11 MJ/kg, respectively (Table 2).

Fatty Acids Composition of the Crude Seed Oil Extract of Azadirachta indica

The fatty acids composition of the crude seed oil extract of *Azadirachta indica* showed the presence of linoleic acid ($C_{18}H_{32}O_2$), hexadecanoic acid ($C_{16}H_{32}O_2$), octadecanoic acid ($C_{19}H_{32}O_2$) and alpha-linonoic acid ($C_{18}H_{30}O_2$). These have retention time and percentage composition of 18:2 min and 10.8±0.50%, 22:2 min and 30.01±1.79%, 18:2 min and 59.10±2.22% and 22:2 min and 0.09±0.02%, respectively (Table 3).

Table 1: Physical Properties of Crude Seed Oil Extract of *Azadirachta indica*

Physical property	***Azadirachta indica*** [1]	***Jatropha curcas*** [2]	***Hura crepitans*** [3]
Colour	Brown	Golden	Pale yellow
Oil class	Non-drying		
Melting point(°C)	76±1.60		220-330**
Flash point(°C)	80±2.10	108	152
Specific gravity(g/cm^3)	0.910±0.08	0.913	0.913

[1](Present study), [2](Umaru and Aberuagba, 2012), [3](Ottih *et al.*, 2015) melting point**
Mean values ± standard deviation for n = 3

Table 2: Comparative Average Chemical Composition of Crude Seed Oil Extract of *Azadirachta indica*

Parameter	***Azadirachta indica*** [1]	***Jatropha curcas*** [2]	***Hura crepitans*** [3]	**Reference** [4]
Refractive index(@ 30°C)	1.465±0.07	1.47	1.36	
Saponification value (mg KOH/g)	212.96±1.16	190	220.19	
Peroxide value (meq/Kg)	0.39±0.16	2.0	20.00	
Acid value (mgKOH/g)	4.24±0.12	36.2	7.09	0.60(EN rec)
Iodine value (g/100g)	66.77±2.55	105	149.64	120(g/100g) (EN rec)
Viscosity value (mm^2/s)	2.20±0.12	40	5.91	3.5-5.9
Cetane number	56.91±2.19		45.62*	51(EN rec)
Calorific value(MJ/kg)	39.21±1.11	42	39.10*	35.00(EN rec)
Free fatty acids(%w/w)	2.13±0.05	18.1	4.61	0.5(EN/ASTM) **

[1] (Present study); [2](Umaru and Aberuagba, 2012); [3](Ottih et al., 2015); * (Adewuyi et al., 2014); [4](Sidohounde et al., 2018); Mean values ± standard deviation for n = 3.**(Zahan and Kano, 2018)

Table 3: Fatty Acids Composition of Crude Seed Oil Extract of *Azadirachta indica*

s/n	**Retention Time (min)**	**Compound**	**Molecular formula**	**% composition**
1	18.2	Linoleic	$C_{18}H_{32}0_2$	10.80±0.50
2	22.2	Hexadecanoic	$C_{16}H_{32}0_2$	30.01±1.79
3	18.2	Octadecanoic	$C_{19}H_{32}0_2$	59.10±2.22
4	20.2	Alpha-linonenic	$C_{18}H_{30}0_2$	0.09±0.02
		Four compounds		Total 100

Mean values ± standard deviation for n = 3

Discussion

Physical Properties of Crude Seed oil Extract of Azadirachta indica

The crude seed oil extract of *A. indica* gave a dark brown colour which is the characteristics as reported by Ungo-kore et al. (2019), who obtained light and dark brown colour for the crude oil extract of *A. indica*, based on Soxhlet and Cold maceration methods of extraction respectively. Umaru and Aberuagba (2012), reported a golden colour for *Jatropha curcas* Oil, while a pale yellow coloration was obtained from oil extract of *Hura crepitans* (Ottih et al., 2015). The melting point of 76±1.60.°C and a flash point of 80±2.10°C were obtained for crude seed oil extract of *A. indica,* while 108°C and 152°C were reported for *Jatropha curcas* seed oil (Umaru and Aberuagba, 2012) and *Hura crepitans* seed oil (Ottih et al., 2015). The value of the specific gravity of 0.910±0.08g/cm^3 was closely consistent with the reported value of 0.913g/cm^3 (Umaru and Aberuagba, 2012; Ottih et al., 2015). According to Ibeto et al. (2012), the specific gravity of a good oil should be close to the accepted range of 0.87–0.90 g/cm^3 for biodiesel

(Odjobo and Umar., 2019) . They opined that these values must be maintained within a moderate range for optimal auto engine performance, as higher density oil or its mixture impedes the combustion process..

Biodiesel Properties of Crude Seed Oil Extract of Azadirachta indica

The crude seed oil extract of *A. indica* recorded a refractive index (RI) value of 1.465±0.07 at 30°C, similar to 1.47 of *Jatropha curcas* Oil (Umaru and Aberuagba, 2012) and higher than 1.36 of *Hura crepitans* (Ottih et al., 2015). Ungo-kore et al. (2019), indicated similar range of 1.464-1.465 from neem, while Manji et al. (2013), reported a value of 1.478 from crude *B. aegyptiaca* oil, Abeer et al. (2020), reported RI of 1.485 from *Ocimum basilicum* seed oil. They opined that RI varies with temperature, wavelength and unsaturation as well as the chain length of fatty acids. This supported documented claims of Kadam et al. (2012).

The acid value (AV) of 2.49±0.12mgKOH/g obtained from the crude seed oil extract of *Azadirachta indica* was higher than the 0.6(EN) standard value reported by Sidohounde et al. (2018). Other researchers have reported AVs of 6.171-6.520 mgKOH/g, 7.09 mgKOH/g and 36.2 mgKOH/g from neem (Ungo-kore et al., 2019), *Hura crepitans* (Ottih et al., 2015) and *Jatropha curcas* (Umaru and Aberuagba, 2012), respectively. The AVs determine the edibility, shelf life and extent of industrial applications of such oils. Oils with Low AVs (<4.0 mg KOH/g) which are considered non-poisonous to human and livestock, maintain constituent integrity for a long time without becoming rancid (Sunmonu et al., 2017; Nwe et al., 2019). According to Umaru and Aberuagba (2012), oils with very low acid values are indications of good biodiesel potential.

A value of 0.39±0.16 meq/kg as peroxide value (PV) of the crude seed oil extract of *A. indica* was slightly above the range of 0.07-.0.28meq/kg as reported by Ungo-kore et al. (2019). Umaru and Aberuagba, (2012) and Ottih et al., (2015) indicated 2.0meq/kg and 20.0meq/kg as PVs from *Jatropha curcas* and *Hura crepitans,* respectively. Manji et al. (2013), pointed out that oils with low PVs have long shelf life and are not susceptible to rancidifying agents. While higher PVs of 20 - 40 meq/kg or more, promote rancidity, precipitation of polymeric compounds formed as a result of incomplete combustion, leading to blockage of the filters of auto-engines (Montcho et al., 2018).

The crude seed oil extract of *A. indica* gave an iodine value (IV) of 66.77±2.55 g/100g, lower than 105 g/100g (*Jatropha curcas*) and 149.64 g/100g (*Hura crepitans*), reported by Umaru and Aberuagba, (2012) and Ottih et al. (2015). Adewuyi et al. (2014) reported a standard (EN) value of 120 g/100g. According to Jauro and Adams (2011), the iodine value (IV) of oil extracts determined their rating. Oils with IV less than 100 g/100g are classified as non-drying while those with values between 100-130 g/100g and >130 g/100g are referred to as drying and semi-drying, respectively. In this regard, the neem seed crude oil extract is classified as non-drying (Table 2). Low IV suggests high stability against oxidation agents and suitability for biodiesel production (Nwe et al., 2019).

The saponification value (SV) of 212.96±1.16 mgKOH/g recorded for the crude seed oil extract of *A. indica* was found to be higher and lower than 190.00 mgKOH/g (*Jatropha curcas*), 211.78-214.52 mgKOH/g (*Azadirachta indica*) and 220.19 mgKOH/g (*Hura crepitans*) as reported by Umaru and Aberuagba, (2012), Ungo-kore et al., (2019) and Ottih et al. (2015). Higher SV implies higher molecular mass and long C-C chain fatty acids (Ferhat et al., 2014). Oils with higher SV are considered appropriate for manufacturing of biodiesel, due to their lubricating enhancement tendency, which prolongs and promotes engine functional life (Ejilah et al., 2012).

The viscosity values (VV) of 2.20±0.12 mm^2/s obtained from the crude seed oil of *Azadirachta indica* compares with the standard EN range of 3.5-5.9 mm2/s (Adewuyi et al., 2014). Higher values of 5.01 mm^2/s and 40.0 mm^2/s were obtained from *Hura crepitans* and *Jatropha curcas* as reported by Ottih et al. (2015) and Umaru and Aberuagba, (2012), respectively. Viscosity has been shown to affect fuel injection operation, diesel injector and, in fuel pump flow, triglycerides constituent and other chemical properties (Azuaga et al*.,* 2018). They opined that the nature of the C-C triglyceride chains of oils varies proportionally with viscosity and inversely with density.

Cetane Number and Calorific Value of Crude Seed Oil Extract of Azadirachta indica

The crude seed oil extract of *Azadirachta indica* gave a cetane number (CN) of 56.91±2.19, higher than the 51.0 EN standard value (Adewuyi et al., 2014) and 45.62 of *Hura crepitans* (Ottih et al., (2015). Previous works have indicated varying CN values of 50.0 for *Heavea brassilensis* biodiesel, (Krishnakumar et al., 2013), 52.85 for *Ceiba pentandra* (Montcho et al., 2018), 53.0 for neem diesel, (Banik et al., 2018), 59.01-60.47 for Cyperus esculentus (Sidohounde et al., 2018). According to Aligrot (1994), the ability of any fuel to ignite is a measure of its CN. According to Montcho et al. (2018), higher cetane number reduces the ignition delay time, thereby promoting combustion efficiency. Ejilah (2012), indicated oils with more saturated C molecules have better combustion efficiency due to higher CN.

The calorific value (CV) measures the unit of energy released per kilogram of fuel combusted (Montcho et al., 2018). The CV of 39.21±1.11 MJ/Kg was higher than 39.10 MJ/Kg (*H. crepitans*, Ottih et al., 2015) and 35.00 MJ/Kg EN standard value (Sidohounde et al., 2018), and lower than 42.0 MJ/Kg (*Jatropha curcas*, Umaru and Aberuagba, 2012). This suggests a good potential for biofuel production (Ofoefule et al., 2013).

Flash Point (FP) of Crude Seed Oil of Azadirachta indica

According to Jauro and Adams (2011), the flash point measures the overall flammability of an oil, such that higher values indicate a less likelihood to ignite accidentally. The FP of 80±2.1°C indicated from the current study was lower than 152°C and 108°C recorded for *Hura crepitans* and *Jatropha curcas*, as described by Ottih et al. (2015) and Umaru and Aberuagba, (2012), respectively. This was also below the ASTM standard of 100°C. Raja et al. (2011) posited that fuels with flash points above 66°C are considered safe and suitable for all climatic conditions.

Percentage Free Fatty Acids (FFA) and Fatty Acids Composition of Crude Seed Oil Extract of Azadirachta indica

Azuaga et al. (2018), described the percentage of free fatty acid in an oil as an important variable determining the quality of oils, suggesting that oils with lower FFA indicate better quality for edibility (≤ 10) and 2.0% maximum limit for high-grade (Codex Alimentairus Commission, 1993). The %FFA of 2.13±0.05 obtained in this study was lower than 4.61% and 18.1% obtained for *Hura crepitans* and *Jatropha curcas* as reported by Ottih et al. (2015) and Umaru and Aberuagba (2012), respectively. However, the EN**EN** 14214 /ASTM D6751 acid value was 0.5 (mg KOH/g)".

The free fatty acids composition of seed oil of *Azadirachta indica* revealed the presence of linoleic, hexadecanoic, octadecanoic and alpha linolenic acids, with retention time and % composition of 18.2 min and 10.80±0.50%, 22.2 min and 30.01±1.79%, 18.2 min and 59.10±2.22%, and 20.2 min andd 0.09±0.02% respectively. These are similar to the findings of Aransiola et al. (2012). Linolenic contents have been reported as being the least % composition (Ejilah et al., 2012; Aransiola et al., 2012), which corroborated the present findings. The fatty acids composition of extracted crude oils are affected by some other inherent oil properties such as cetane number, viscosity, oxidation stability, and others (Sidohounde et al., 2018). The unsaturated fatty acids constituents of neem seed oil extract promote oil efficiency and shelf life.

Conclusion and Recommendation

The study has shown the presence of some important physical properties such as specific gravity, flash and melting points respectively. Chemical properties such as iodine value, refractive index, saponification value, peroxide value, acid value, viscosity value, cetane number, calorific value and free fatty acids were found to be within the standard range. Fatty acids composition of crude seed oil of *Azadirachta indica* included linoleic, hexadecanoic, octadecanoic and alpha linolenic acids, with retention time and % composition of 18.2 min and 10.8±0.50%, 22.2 min and 30.01±1.79%, 18.2 min and 59.10±2.22%, and 20.2 min and 0.09±0.02% respectively. These preliminary findings depict the biodiesel potential of the crude seed oil of *Azadirachta indica*. This, when transesterified with further treatment, could be incorporated as proximate blends in auto-engines. This therefore would necessitate intensive afforestation efforts of the plant species for sustainable utilization.

Acknowledgements

The authors are grateful to the laboratory staff of the National Research Institute for Chemical Technology (NARICT), Zaria - Kaduna State. We also express our thanks to the staff of the Herbarium Unit of the Federal College of Forestry, Jos, for their assistance and support during the field work.

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