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/cm³, 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 mm²/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). Moreover, 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 FHFJ31720). 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
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 chromatograph 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 (RI) was determined using the Perkins formula (1) reported by Babatunde and Bello (2016).

\[ RI = 1.45765 + 0.0001164 IV \quad \ldots \ldots \ldots (1) \]

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:

\[ CN = 46.3 + \frac{54.58}{SV} - (0.225 \times IV) \quad \ldots \ldots \ldots \ldots (2) \]

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:

\[ CV = 4.187 IV - 38.31 SV \left( \frac{KJ}{kg} \right) \quad \ldots \ldots \ldots \ldots (3) \]

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/cm³ 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 mm²/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 (at 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 (C₁₈H₃₂O₂), hexadecanoic acid (C₁₆H₃₂O₂), octadecanoic acid (C₁₉H₃₂O₂), and alpha-linonoic acid (C₁₈H₃₀O₂). 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 | Jatropha curcas | Hura crepitans |
|-----------------------|--------------------|----------------|---------------|
| Colour                | Brown              | Golden         | Pale yellow   |
| Oil class             | Non-drying         |                |               |
| Melting point(°C)     | 76±1.60            |                |               |
| Flash point(°C)       | 80±2.10            | 108            | 152           |
| Specific gravity(g/cm³) | 0.910±0.08        | 0.913          | 0.913         |

¹(Present study), ²(Umaru and Aberuagba, 2012), ³(Otth 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 | Jatropha curcas | Hura crepitans | Reference |
|-------------------------------|--------------------|-----------------|----------------|-----------|
| Refractive index(@ 30°C)      | 1.465±0.07         | 1.47            | 1.36           | 4         |
| Saponification value (mg KOH/g) | 212.96±1.16        | 190             | 220.19         |           |
| Peroxide value (meq/Kg)       | 0.39±0.16          | 2.0             | 20.00          | 0.60(EN rec) |
| Acid value (mgKOH/g)          | 4.24±0.12          | 36.2            | 7.09           |           |
| Iodine value (g/100g)         | 66.77±2.55         | 105             | 149.64         | 120(g/100g) (EN rec) |
| Viscosity value (mm²/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        | C₁₈H₃₂O₂          | 10.80±0.50    |
| 2   | 22.2                 | Hexadecanoic    | C₁₆H₃₂O₂          | 30.01±1.79    |
| 3   | 18.2                 | Octadecanoic    | C₁₉H₃₂O₂          | 59.10±2.22    |
| 4   | 20.2                 | Alpha-linolenic | C₁₈H₃₀O₂          | 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/cm³ was closely consistent with the reported value of 0.913g/cm³ (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/cm³ for biodiesel.
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 Aberuaumba (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 viscosity values (VV) of 2.20±0.12 mm²/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 mm²/s and 40.0 mm²/s were obtained from Hura crepitans and Jatropha curcas as reported by Ottih et al. (2015) and Umaru and Aberuaumba, (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 brasiliensis biodiesel, (Krishnakumar et al., 2013), 52.85 for Ceiba pentandra (Montcho et al.,
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 and 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 10.80±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.

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