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Phytochemical Screening and Physicochemical properties of oil and Biodiesel produced from non-edible *Croton megalocarpus* seeds grown in Huye District

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Abstract

Production of biodiesel from non-edible plantsis regarded as a sustainable alternative to depleted fossil sources mostly because it isrenewable and does not compete with human food. Such plantsmay also have the potential to become suitable sources of bioactive phytochemicals.The purpose of this study wasto identify the phytochemicals presentin*Croton megalocarpus* seeds oiland tocharacterize the physicochemical properties of the oil andbiodiesel produced. The seeds were collected from Huyedistrict in Southern Province, Rwanda.Oil was extracted from the milledseedsbysolvent extraction method using n-hexane andwas converted intobiodiesel via esterification and transesterification process using standard methods. The Phytochemicals in the oiland fuel related properties ofbiodieselwere investigated accordingly and compared with set standards. The yield of oil and biodiesel produced from the seed of *C.megalorcapus* were 38.20 and 82.50% respectively. Alkaloids, Saponins, Flavonoids, Tannins, Steroids, Terpenoidsand Phenols were present in the oil but Cardiac glycosides and anthraquinonewereabsent. All the physicochemical parameters of the biodiesel assessed in this study such as density (890kg/m^3), flash point (120^{0}C) and acid value (0.65mg/KOH/g) were found within standard specifications for biodiesel except cloud point (9^{0}C), viscosity (7.2mm^2/s) and moisture (0.25%) which were above the specifications. This study indicates that *C.megalocarpus* seeds oil has the potential for biodiesel production. However, the development of methods to recover the phytochemicals from the oil prior to biodiesel production may lead to increase in profitability and suitability of the seeds for biofuel.

Keywords: Biodiesel,*Croton megalorcapus*seed, Oil, Phytochemical, Physicochemical, Renewable energy.

1. Introduction

The combustion rate of fossil fuels is increasing on daily basis due to the high demand of energy globally, which has resulted in depletion of fossil fuel resources [1,2]. The rapid consumption rate, rising costs, unreliability and harmful emissions associated with the use of fossils are the main reasons to search for alternative renewable sources. Much emphasis has been placed on searching for alternative fuels lately and significant investigations have been carried out regarding the production of biodieselwith a view to minimize the over dependence on fossil fuels, reduce harmful emissions and improve the rural economy.The major differences between biodiesel and fossil fuels depend on the carbon content and amount of emission they release during combustion.

Biodiesel has been chosen as one of the interesting alternative fuels and has been receiving a lot of attention because it is carbon neutral, renewable, biodegradable, non-toxic and environment-friendly [3]. According to the US Standard Specification (ASTM D6571), biodiesel is defined as a fuel comprised of mono-alkyl esters of long-chain fatty acids derived from transesterification of vegetable oils or animal fats in the presence of a catalyst [4] (**Figure 1**).Biodieselplays a major role in the energy sector due to its similar chemical and combustion properties with petroleum diesel [5],so modification of engine before biodiesel applications is not needed [2].

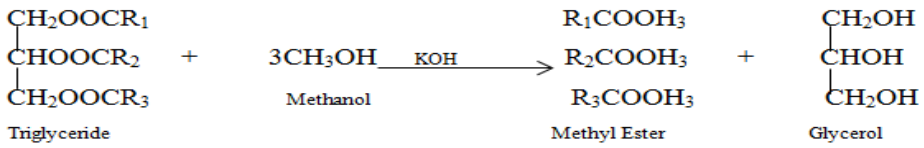

Figure 1: Basic Transesterification reaction

Most crops commonly use for biodiesel production such ascotton seed oil, cashew seed oil,rice bran oil, and palm kernel oil are edible crops, and this practice could compete with human food, soit is not economicallyadvisableto use edible sources of vegetables oils to produce biodiesel.Much attention has beenfocused on the use of low cost and non-edible sources from wild plants to produce biodiesel for fulfillment of energy demand in the future and suchplants have the potential of reclaiming wasteland and do not compete with food [3].

Such non-edible plantswith biofuel properties may also have the potential to be suitable sources of valuable phytochemicals and other bioactive compounds [6].Hence, the production of biodiesel from such multipurpose plants should proceed after recovering the bioactive compounds present in the plantwith the aim of improving the biofuel yield. According to Popp *et al*., [7], some phytochemicals contained in energy plants can influence the efficiency of bioenergy production and it has been shown that extraction of such compounds may be a good method of biomass pretreatment to enhance overall biodieselquality [7,8].

One of such non-foodwild plant that should be considered for bioenergy production is the seed of *Croton megarlocapus* treebelonging to plant family *Euphorbiaceae.* It is a multipurpose tree widely spread in mountains regions inEast Africa including Rwanda, Tanzanian, Mozambique, Kenya and Uganda [9,10]. The leaves and bark are usedas traditional herbal medicine within this distributional range [10].The seed maturity takes between 5 to 6 months and each punch contains between 70 to 80 seeds. The annual seed yield per tree ranges between 25-40kg [9].

This present study is aimed at identifying important phytochemicals present in the seed oil of *C. megalocarpus* and characterize its biodiesel production potential.Different non-edible oil sources might have beenpreviously studied as a feedstock for biodiesel production [2,11,12]. In this study, *Croton megalocarpus* non-edible seed oil is selected as a potential feedstock for biodiesel production due to its higher oil content and abundance. This would create more awareness on double benefit and comprehensive useofthe plant seed in the form of medicinal phytochemicals and bioenergy production as value added product.

2.0 Materials and Methods

2.1 Plant materials collection

Matured seeds of *C. megalocarpus* were collected from tress at the Arboretum of National University of Rwanda located atRuhande in Huye district, Southern Rwanda, when the seeds are fully matured.The seeds were identified at the Botany department of National University of Rwanda.

2.2 Sample preparation

The seeds were sorted and rinsed with tap waterto remove foreign materials and dirt on the surface. They were sun dried in the open air until the casing splits and sheds the inner seeds which were further dried in oven at 40^0C to constant weight.The dried seeds were grinded into fine powder using electrical grinding machine (**Figure 2**) and stored in an air tight container in a desiccator until used for extractions. All solvents used in this work were obtained from the Chemical and Reagents storage Unit of Department of Chemistry, National University of Rwanda and were of analytical grade.

Figure 2: *C. megalorcapus* seeds processed into powder

2.3 Oil extraction and percentage yield

The oil was extracted from the powdered seeds using Soxhlet extraction method with n-hexane as solvent, according to the method described by William [13]. The percentage oil yield after extraction was determined using the following expression:

$$\text{Oil content (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

2.4 Qualitative Phytochemical screening

Tannins and glycosides were analysed according to Sofowara [14] methods. Anthraqinonesand Phenol were screened according to Harbone [15] method,while the rest of the phytochemicals were analyzed using the method described by Mbatchou and Kossono [16].

2.4.1 Test for Tannins:

5 drops of 0.1% $FeCl_3$was added to 2 mL of the oil extract in a test tube and the appearance of brownish green or blue-black coloration indicates a positive result

2.4.2Test for Saponins:

The 2 mL of the oil extract was diluted with 2 mL of distilled water and agitated in a test tube for 5 min. The formation of about 0.1 cm layer of foam indicates a positive result

2.4.3 Test for Flavonoids:

The 2 mL of 10% NaOHwas added to 2 mL of the oil extract in a test tube. An intense yellow color was formed which turned colorless upon addition of 2 mL of dilute HCl indicating a positive result

2.4.4 Test for Alkaloids:

To 2 mL of the oil extract, 2 mL of 10% hydrochloric acid was added followed by 1 mL of dragendroff's reagent. An orange precipitate indicates a positive result. The test was repeated with Meyer's reagent. An orange precipitate indicates a positive result.

2.4.5 Test for Steroid:

2 mL of the oil extract was dissolved in 10 mL of chloroform, and 10 mL of concentrated H_2SO_4 was added by the side of the test tube. The appearance of red color at the upper layer upper and yellow on the sulphuric acid layer with green fluorescence indicates a positive test

2.4.6 Test for Terpenoids:

The 2 mL of the oil extract was mixed with 2 mL of chloroform and 1 mL of concentrated H_2SO_4 was carefully added to form a layer. A clear upper and lower layer with a reddish brown interphase indicates a positive result.

2.4.7 Test for Glycosides:

The 2 mL of acetic acid was added to 2 mL of the oil extract. The mixture was cooled in cold water bath. The 2 mL of concentrated H_2SO_4 was added and color development from blue to bluish green indicates the presence of glycosides

2.4.8 Test for Anthraqinones:

2 mL of the oil extract was boiled with 5 mL of 10% hydrochloric acid for 3 min followed by addition of 5 mL of chloroform and 5 drops of 10% ammonia. A rose pink coloration indicates a positive result.

2.4.9 Test for Phenols:

The 2 mL of the oil sample was mixed with 2 mL of 1% ferric chloride. The formation of deep blue or blue-black coloration is an indication of a positive result.

2.5 Biodiesel production

The biodiesel production from the extracted oil was in two steps, according to the method described byFolaranmi, [2]. Firstly, the free fatty acid contained in the oil was reduced by heating the oil to 60^0C in a conical flask followed by the addition of mixture of concentrated H_2SO_4 (1% w/w) with methanol (13% v/v). The resulting mixture was stirred for an hour and allowed to settle. For transesterification process, the oil was heated to 50 °C followed by the gradual addition of solution of sodium hydroxide (5.0 g) in methanol (6:1 molar ratio of methanol:oil) into the reaction. The reaction temperature was set at 60 °C and was allowed to proceed for 2 h. After which the mixture was transferred into a separating funnel and left for 24 h, and then the biodiesel was separated from the byproduct. The biodiesel was then washed with hot deionized water (50 °C) five times to remove the glycerol, catalyst, and other impurities [17]and was placed on a hot plate for 10 min to eliminate humidity [18].The percentage yield of biodiesel was calculated as;

$$\text{Yield of biodiesel (\%)} = \frac{\text{Mass of biodiesel}}{\text{Mass of oil used}} \times 100$$

2.6 Physicochemical analysis of oil and biodiesel

Both raw and refined oils were analyzed immediately for saponification value, peroxide value, iodine value and pHfollowing the methods described by American oil Chemist Society [19]and Nkafamiya*et al*., [20]. The result

was placed with the corresponding limit set for ASTMD6751 and EN41211 for biodiesel standards. The methods arestated brieflybelow;

2.6.1 Determination of peroxide value

5 g of sample (raw and refined oil) was dissolved in 30 mL of glacial acetic acid:chloroform (3:2, v/v). 0.5 mL of saturated KI was added and I_2 was liberated by the reaction with the peroxide. The solution was then titrated with standardized $Na_2S_2O_3$ using starch indicator. The peroxide value was determined from equation;

$$\text{Peroxide value (meq/Kg)} = \frac{(X-A) \times M \times 1000}{\text{Sample weight (g)}}$$

Where X = Titre value; A = Blank titre value; M = Molarity of $Na_2S_2O_3$.

2.6.2Determination of iodine value

0.1 M iodine monochloride in acetic acid was added to 0.2 g of sample dissolved in cyclohexane. The mixture was allowed to stand for 10 minutes. 0.1 M of KI solution was added to reduce excess iodine monochloride to free iodine. The liberated iodine was titrated with a standardized solution of 0.1 M sodium thiosulphate ($Na_2S_2O_{3)}$ using starch indicator. The iodine value was calculated from equation:

$$\text{Iodine value} = \frac{(B-S) \times M \times 12.69}{\text{Sample weight (g)}}$$

Where B = blank titre value; S = sample titre value; M = Molarity of $Na_2S_2O_3$; 12.69 = Conversion factor

2.6.3 Determination of Moisture Content

Few quantity of sample was weighed and the mass taken as (M_1), then dried in the oven and the weight after drying was taken as (M_2). The percentage moisture in the oil was then calculated using the formula below:

$$\% \text{ moisture content} = \frac{M_1 - M_2}{M_1} \times 100$$

Where: $M_1$is the weight of sample before drying (g) and $M_2$is the weight of oil sample after drying (g)

2.6.4Determination of saponification value (SV)

2 g of each sample was added to excess alcoholic KOH and the solution was heated for two minutes to saponify the oil. The unreacted KOH was back - titrated with standardized 0.1 M HClusing phenolphthalein as indicator. The SV was calculated from equationas follows;

$$SV = \frac{(V-B) \times M \times 56.1}{\text{Sample weight (g)}}$$

Where; V = Sample titre value; B = Blank titre value; M = Molarity of the HCL; 56.1 = Molecular weight of KOH

2.6.5Determination of Acid value

2 g of the sample was dissolved in 30 mL ethyl alcohol and the mixture was boiled in a water bath for 2 min and then titrated with a 0.1N KOH solution in the presence of phenolphthalein as an indicator. Acid value is expressed as mgKOH/g of oil.

2.6.6Determination of Specific Gravity (Density)

The density of the samples was determined by a mass over volume measurement at 20 °C. A clean dry bottle of 25 mL was weighed (M_0) and then filled with the sample and reweighed to give (M_1). After washing and drying the bottle, the sample was substituted with water and re-weighed to give M_2. The calculation was s follows:

$$\text{Specific gravity} = \frac{M_1 - M_0}{M_2 - M_0}$$

2.6.7 Determination of Ash

The ash content was determined using the ignition method by burning the sample in a muffle furnace at 550 °Cfor 6 hours. The percentage ash content was calculated using the formula below.

$$\text{Ash (\%)} = \frac{\text{Mass of ash}}{\text{Mass of sample}} \times 100$$

2.6.8Determination of cloud point:

10 mL of biodiesel was measured and transferred in test tube and placed in the fridge for 20minutes. We removed the sample from fridge and immediately the temperature was recorded by using thermometer.

2.6.9 Determination of Refractive index (RI).

The refractive index of the oil or biodiesel sample was determined according to the AOAC method [21]with the aid of arefractometer

2.7Water and sediment content

We filled 10 mL of produced biodiesel in graduated test tube and placed in centrifuge for at 2000rpm for 10 mints. Water and sediment deposited on the bottom of graduated test tube were measured.

3. Statistical Analysis

All the experiments were done in triplicate and the data here presented represents the mean of the three different replicates.

4. Results and Discussion

The percentage yield of the extracted oil and biodiesel was 38.2% and 82.5%respectively. This is considerably similar with the report of Aliyu*et al*.,[9] and Kufuku*et al*., [22]. The seeds have higher oil yield compared with most of the known oil seeds [23]. Generally, the yield of biodiesel produced from vegetable oil depends on many factors which include the molar ratio of methanol to oil used, percentage of free fatty acid in the oil and catalyst used [2,38]. Differences in the oil percentage yield may be attributed to the differences in climate, ripening stage of the seed, the harvesting time and method of oil extraction. The oilyield of the *C. megalorcapus*seeds show that further processing of the oil for industrial purpose can be of economic importance to the community.The result of the qualitative phytochemical analysis of the oil extract of *C. megalorcapus* seed is shown in **Table 1**.Phytochemicals in plants are commonly used for medicinal purposes against several diseases[24].

Table 1: Phytochemical constituents in*C. megalorcapus*seed oil

Phytochemical	**Result**
Flavonoids	++
Alkaloids	++
Tannins	+
Saponins	+
Steriods	+++
Phenols	++
Terpeniods	+++
Glycosides	—
Anthraqinones	—

+: slightly present, ++: moderately present; +++: highly, --: Absent

Tannin has antibacterial activity [25], so its presence in the seed oil shows that the oil possesses some anti-bacterial effects.Terpenoids and Steroids are both present in very high concentration which may be due to their non-polar nature which obviously favors their increased concentration in the oil [26].Steroidal compounds are of importance and interest in pharmacy due to their relationship with sex hormones [27]. The presence of steroids in the oil is an indication that it contains compounds that are related to sex hormones improvement.The absence of cardiac glycosides in the oil may be related to their insoluble nature in lipid [26]. The presence of Phenols, Alkaloids, Saponins and Flavonoids in the oil indicates that *C. megalocarpus*seed oilwill have pharmacological properties because phenols and flavonoids are biological antioxidants. Also, alkaloids have anesthetics properties [28] while Saponin serves as anticancer [29] and also as an antioxidant.These particular phytochemical compositions of *C. megalorcapus*seed oil was not found in the literature for comparison purpose with our results.

The results of the physicochemical properties of the raw and refined oil of *C. megalorcapus* seed are presented in **Table 2** togetherwith the corresponding limits (ASTMD6751 and EN14214) set for biodiesel standard [4,30]. The acid value describes the amount of potassium hydroxide required to neutralize the free fatty acids present in a mass unit of biodiesel which is important to test the quality of a particular biodiesel. A high acid number affects engine fuel injection system and increases corrosion of engine components [31]. The acid value of the biodiesel (0.65mgKOH/g) iswithin the specification by ASTMD6571 (0.8 max) but slightly above the EN14214 specifications (0.5 max). Another important factor that determines oil quality is the pH.If the pH of the biodiesel is too high, more glycerin will be formed at the end of biodiesel production which will affect the yield and quality [11]. From our result, the pH of the biodiesel is close to neutral.

Table 2: Physicochemical properties of the *C. megalorcapuss*seed crude oil and biodiesel

Property	Oil	Biodiesel	ASTM6751	EN14214
Viscosity @40^0 C (mm^2/s)	27.23	7.2	1.9-6.0	3.5-5.0
Iodine value (gI_2/100g)	117	115.8	130 max	120 max
Density@15^{0}C (kg/m^3)	920	890	850—900	860-900
pH	5.14	6.78	--	--
Moisture (%)	0.8	0.25	0.05max	--
Ash (%)	2.4	0.01	<0.02	--
SV (mg/KOH/g)	188	145	--	--
Acid value (mg/KOH/g)	2.52	0.65	0.8 max	0.50 max
Refractive Index @25^O C	1.54	1.49	--	--
Peroxide Index (meq/Kg)	10.29	1.73	--	--
Cloud point ^{0}C	--	9.0	-2 to -12	--
Flash point^{0}C	--	120	>93	>101
Water sediment (%)	--	0.02	0.05 max	--
Visual Color	Yellow	Brown	--	--

*SV= saponification value

Cloud point is a criterion used for low temperature performance of fuel and a higher cloud point can affect the engine performance and emission adversely under cold climatic conditions [2].The cloud point of the biodiesel produced in this study(9.^{o}C) is higherthan the specificationsfor biodiesel as seen in **Table 2**.The presence of high cloud point may limit the use of the biodiesel under low temperature.The water sediments content is within the range, indicating that water washing process was well performed.One of the most important characteristics of any fuel is the flash point which is defined as the lowest temperature at which it can vaporize to form an ignitable mixture in air [2]. It is a property that must be considered when assessing the overall flammability hazard of a material.The flash point is between the specifications range for biodiesel. This makes the produced biodiesel in this study safe for use and storage.

Another veryimportant property of fuel is the viscosity. It is a measure of resistance of a liquid to flow due to internal friction of one part of a fluid moving over another. Fuels with high viscosity has higher tendency to cause problems in the engine [2,32]. The viscosity of the biodiesel produced ($7.2mm^2/s$) is above the standards (1.9-6.0) as given by ASTM for biodiesel.Again, temperatureaffects the viscosity of biodiesel such that anincrease in temperature reduces viscosity and causes the fuel to flow more easily which is favorable to fuel injection efficiency and atomization [2].The density of a fuel is also an important factor for good engine performance. The higher the density, the more difficult it becomes to pump the fuel [2]. Thedensity of the biodiesel produced from *C. megalorcapus* seed oil is 890 kg/m^3which is within the acceptable range according to ASTM [4] and EN14214 [30]. Density of biodiesel depends upon the raw materials used for biodiesel production and the methyl ester profile [33].

The saponification value (SV) is used for measuring the average molecular weight of oil. A high SV in oil indicates a higher proportion of low molecular weight fatty acids in the oil or vice versa [34].The iodine valueis a measure of the degree of unsaturation of the biodiesel which is a useful parameter in studying oxidative rancidity and chemical stability properties of different oils.No much change of biodiesel iodine value was observed in comparison with the raw oil and the value obtained is within the specifications. The lower the iodine value, the better the fuel will be as a biodiesel [34]. The refractive index (RI) is a parameter that relates to molecular weight, fatty acid chain length and degree of unsaturation in oil [5]. It is a significant parameter to evaluate the state of a biodiesel.The refractive index (RI) of biodiesel produced from *C. megalorcapus* seed oil is 1.49. This result is considerably in agreement with those of Ismail and Ali, [5], Dominguese*t al*., [35] and Ullah*et al.*, [36] who reported that refractive index of pure biodiesel is in the average of 1.45.

Moisture is the quantity of water present in the sample. The moisture content of the biodiesel (0.25%) was higher than the ASTM specifications for biodiesel. Generally, moisture content in biodiesel is always a problem as it promotes severe damage onfuel-injection equipment and contributes to corrosion of the engine [38]. Ash content indicates the amount of mineral matter left after biodiesel is completely burn to ash. The result obtained in this study is within the ASTM D6751 standard specification.The peroxide valueis another quality factor of biodiesel which determines the presence of oxidants and level of rancidity in the biodiesel. The oxidants usually found in biodiesel are hydro-peroxides formed when oxygen reacts with fatty esters [5], which is the first step in the pathway of oxidative degradation of biodiesel [38] which affects quality.

5.0 Conclusion

The current study suggests that *C.megalorcapus*non-edible seed is a good source of oilwith important phytochemicals and has the potentialfor being a large scale resource for biodiesel production which does not

affect food security. The development of methods for the extraction and recovery of valuable bioactive phytochemicals from the seed oil prior to the biofuel usage may lead to increase in its profitability by providing simultaneously, a double benefitand also improve the properties of final biodiesel. The bioactive compounds when extracted can find applications in pharmaceuticals, feedstuff, cosmetics or agriculture. Further studies to quantify thebioactive phytochemicalsand determine other properties of this biodiesel such as cetane number, free and total glycerine and sulfur contents as required by ASTM are imperative.

6.0 Conflicts of Interest

The authors declare that there is no conflict of interest related to this work.

7.0 Acknowledgement

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8.0 Reference

1. Balat M, Balat H. A critical review of bio-diesel as a vehicular fuel.*Energ. Convers Manage*; 2008;49:2727–2741.
2. Folaranmi J. Production of biodiesel (B100) from jatropha oil using sodium hydroxide as catalyst. *Journal of petroleum Engineering*, 2013; 956479, 1-6
3. Basumatary S, Barua P, Deka DC. *Gmelinaarborea* and *Taberneace Montana divariacata* seed oils as non-edible feed stocks for biodiesel production *Int. J. ChemTech Res*, 2014;***6*** (2), 1440-1445
4. American Society for Testing and Materials, ASTM D6751-08: Standard specification for biodiesel fuel (B100) blend stock for distillate fuels.*Annual Book of ASTM 2003 Standards* (West Conshohocken, PA: ASTM International)
5. Ismail SA, Ali RF. Physicochemical properties of biodiesel manufactured from waste frying oil using domestic adsorbent. *Sci. Technol. Adv. Mater*. 2015, 034602, 1-9
6. Oleszek M, Kowalska I, Oleszek W. Phytochemicals in bioenergy crops. *Phytochem Rev*, 2019; (18), 893-927
7. Popp D, Harms H, Strauber H. The alkaloid gramine in the anaerobic digestion process inhibition and adaptation of the methanogenic community. *ApplMicrobiol Biot100*, 2016;7311–7322
8. Attard TM, Theeuwes E, Gomez LD *et al*., Supercritical extraction as an effective first-step in a maize stoverbiorefinery. *RSC Adv*, 2015; 5:43831–43838
9. Aliyu B, Agnew B, Douglas S. Croton *megalocarpus* (Musine) seeds as a potential source of bio-diesel. *Biomass Bioenergy*, 2010; 34: 1495-1499.
10. Maroyi A. Croton megalocarpus Hutch in tropical Africa: phytochemistry, pharmacology and medicinal potential. *Res. J. Med. Plants*, 2017, (11), 124-133
11. Marutani M, Soria JA, Martinez MA. Characterization of crude and biodesiel oils of Jatrophacurcas and Calophylluminophyllum in Guam. *Micronesica*2018-01:1-15
12. Oleszek M, Krol A, Tys J *et al*., Comparison of biogas production from wild and cultivated varieties of reed canary grass. *BioresourTechnol*, 2014; 156:303–306
13. William, J. The origin of the Soxhlet apparatus. *J. Chem. Educ*.2007, 84: 1913-1918

14. Sofowara, A. Screening for bioactive agents. In:*Medicinal Plants and Traditional Medicine in Africa,*1993*;*2nd ed. Spectrum Books Limited: Ibadan, Nigeria, pp. 134-156.
15. Harborne, J. B. Phytochemical methods. In: *A Guide to Modern Techniques of Plant Analysis,*1998*;*3rd ed. Chapman and Hall publishing: London, United Kingdom, p. 67
16. Mbatchou VC, Kosoono I. Aphrodisiac activity of oils from *Anacardiumoccidentale*L. seeds. *Phytopharmacology- Intern. J. Phytother. Bioact. Nat. Prod.*2012,2: 81-91
17. Saengprachum N; Poothongkam J, Pengprecha S. Glycerin removal in biodiesel purification process by adsorbent from rice husk. *Int. J. Sci. Eng. Technol*., 2013; 2, 474-478
18. Meng X, Chen G, Wang Y. Biodiesel production from waste cooking oil via alkali catalyst and its engine test. *Fuel Process Technol.* 2008; 89, 851–857
19. AOCS, Official and tentative methods of American Oil ChemistsSociety, 1973; 3rd ed. Champion IL USA.
20. Nkafamiya II, Aliyu BA, Manji AJ, Moddibo UU. Degradation properties of *Adansoniadigitata*(Boabo) and *Prosopsis Africana* (Lughu) oils on storage. *Afr. J. Biotechnol*., 2007b; 6(6): 751-755.
21. AOAC InternationalOfficial Methods of Analysis of AOAC International, 2000; 17th edn (Gaithersburg, MD: AOAC International)
22. Kafuku G,Mbarawa M. Biodiesel production from *Croton megalocarpus* oil and its process optimization. *Fuel*, 2010; 89: 2556-2560
23. Hwang, L. S. Vegetable oils. In: *Bailey's Industrial Oil and Fat Products,*2005*;*6th ed, Fereidoon, S., (Ed.); John Wiley & Sons incorporated, p. 1178.
24. Abbas M, Shahid M, Iqbal M, et al. Antitermitic activity and phytochemical analysis of fifteen medical plants seeds. *Journal of Medicinal plants Research*; Vol.7(22), 1608-1617
25. Liu SW, JiangSB, Wu SG.Tannin inhibits HIV-1 entry by targeting gp41. *Acta Pharm. Sin*., 2004;25: 213-218.
26. Njoku OU, Boniface JAE, Obitte NC, Odimegwu DC, OgbuHI.Somenutriceutical potential of beniseed oil. *Int. J. Appl. Res. Nat. Prod.,*2010*;*2(4): 11-19.
27. Okwu, D. E. Evaluation of the chemical composition of indigenous spices and flavoring agents. *Glob. J. Pur. Appl. Sci*., 2001;7(3): 455-459.
28. Heikens H, Fliers E, Endert E, Ackermans M, VanMont FG. Liquorice-induced hypertension: A new understanding of an old disease. *J. Med.*, 1995;5: 230-234.
29. Philips KM, Ruggio DM, Ashraf-khorassani M. Phytosterol composition of nuts and seeds commonly consumed in the United States. *J. Agric. Food Chem.,*2005*;*53: 9436-9445.
30. European Committee for Standardization 2008 *European Standard EN 14214 CEN* (Belgium: Brussels)
31. Kivevele TT, Mbarawa MM. Comprehensive analysis of fuel properties of biodiesel from *Croton megalocarpus*oil. *Energ Fuel*. 2010;24(11):6151– 6155.
32. Refaat AA. Correlation between the chemical structure of biodiesel and its physical properties. *Int. J. Environ. Sci. Tech*. 2009; 6, 677–694
33. Blangino E, Riveros AF, Romano SD. Numerical expressions for viscosity, surface tension and density of biodiesel: analysis and experimental validation, 2008; 46 (5); 527-547
34. Diwakar BT, Dutta PK, Lokesh BR, Naidu KA. Physicochemical properties of garden cress (*Lepidiumsativum* L) seed oil. *Journal of the American oil Chemists' society*, 2009; 87 (5), 539-543
35. Domínguez LÁA. Biofuels: Use of Vegetable Oils as Renewable Energy, 1996, (Madrid: Ministry of Agriculture,Fisheries and Food)
36. Ullah F, Bano A, Ali S. Optimization of protocol for biodiesel production of linseed (*Linumusitatissium L.*) oil. *Journal of Chemical Technology*, 2013, 15, (1), 74-77
37. Frankel E N. Lipid Oxidation. 2005;2nd edn P J Barnes and Associates (Bridgewater: The Oily Press)
38. Bhandare P, Naik GR. Physicochemical properties of biodiesel produced from Neem oil. *International letters of Chemistry, Physics and Astronomy*, 2015; 8, 41-48