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# Proximate Composition, Extraction of Oil and Production of Biodiesel from *Chrysophyllum albidum* (African Star Apple) Seeds

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***Abstract***:
*The proximate composition of Chrysophyllum albidum seeds wascarried out using standard analytical methods. The results of the proximate composition was shown to be moisture (14.00±0.22%), ash (4.00±0.10%), fat (25.58±0.51%), fibre (16.00±0.13%), protein (25.76±0.45%) and carbohydrate (14.64±0.01%).The production and characterization of biodiesel from the seeds was done using Standard procedures. Solvent extraction was employed with the yield of 22.70% oil. The extracted oil was characterized and subsequently used for biodiesel production. On characterization of the oil, saponification value was found to be (187.31mgKOH/g), Acid value (7.01mgKOH/g) and Free Fatty Acid (3.10mgKOH/g). The biodiesel was produced using trans-esterification process and was also characterized and shown to have flash point (120$^{o}$C), Cloud point (5.8$^{o}$C), Pour point (4$^{o}$C), density (0.89g/cm$^{3}$) and dark brown in colour.*
*The flash point and density met ASTMD standard while the cloud point and pour point of the biodiesel need further modifications. This study revealed that Chrysophyllum albidum seeds have low moisture content, high fat and oil, fibre, ash and protein content. The result obtained shows the viability of producing biodiesel from Chrysophyllum albidum seed oil.*

***Keywords***: *proximate, composition, solvent extraction, Biodiesel, Chrysophyllum albidum seeds, trans-esterification*

## 1. Introduction

Plants are primary sources of food, medicine, shelter and other materials used by man on daily basis. This has made plants indispensable in man's existence on earth. Today plants have gain prominence in the production of vegetable oil and biofuels and this account for why this research work is tailored towards the production biodiesel from Chrysophyllum albidum seeds oil.

In simple terms, biodiesel is an alternative fuel for diesel engines. It refers to a vegetable oil or animal fat-base diesel fuel consisting of long-chain alkyl(methyl, ethyl or propyl). Its primary advantage is that it is non-toxic and renewable (Meher et al., 2006).

The use of vegetable oils for energy purposes is an age-old phenomenon. However, due to the continued increase in human population which has resulted in the rise in the demand of oils as well as the increase in prices of oils, there is an urgent need to search for alternative unconventional sources of oils particularly for underdeveloped and developing countries. More so, with the global growing concerns for environmental problems cause by fossil fuel exploration and constant emission of atmospheric pollutants, the need for alternative source of energy such as biodiesel that is environmentally friendly cannot be overemphasized.

The production of biodiesel is basically a transesterification reaction (Verma et al., 2005). During the transesterification reaction, fatty acids are removed from glycerol (via methanolysis of the vegetable oil) to create fatty acids methyl esters (fames), which make up biodiesel. Glycerol, a by-product can be cleaned to make detergents, greases and soap (Meher et al., 2006).

The aim of this research work was to carry out proximate composition and to produce biodiesel from the vegetable oil extracted from Chrysophyllum albidum seeds.

## 2. Materials and Methods

*2.1. Sample Collection and Preparation*

Fruits of *Chrysophyllum albidum* were obtained from the botanical garden in the University of Calabar, Calabar Cross River State of Nigeria and was authenticated by an Herbarium, Botany Department of University of Calabar. The seeds were then removed from the epicarp, cracked and place on asbestos pad and dried in an oven at a temperature of 60-70$^0$c. They were brought out and turned over for air circulation. The dried seeds were ground into powdered form and stored in an air tight container for further analysis.

*2.2. Proximate Analysis*

Proximate analysis involves the determination of moisture, ash, crude fibre, crude protein, crude fat and carbohydrates (Verma, 2010). Carbohydrates were calculated while ash, moisture, fats, fibre, and crude protein were determined using methods described by (AOAC, 2000). All determinations were carried out in triplicate. The procedures were as follows:

*2.3. Moisture Content*

The sample was weighed (5g) into pre-weighed beaker and placed in an oven for about six (6) hours at a temperature of 100$^o$C to a constant weight. The loss in weight was expressed as a percentage of the initial weight. Thus the difference in weight indicates the amount of water contained in the sample (AOAC, 2000).

*2.4. Ash Content*

5.0g sample in a pre-weighed label crucible was placed in the muffle furnace at a temperature of about 500$^o$C for 20 minutes. The furnace was allowed to cool before removing the crucible with its content. The crucible was later cooled in a desiccator and reweighed to get the ash content (AOAC,2000).

*2.5. Crude Fat Content*

5.0g sample in a soxhlet extractor thimble was wrapped with a filter paper and plugged tightly with a filter paper and with cotton wool. 150ml of petroleum ether (bpt 60 – 80$^o$C) was poured into 300ml round bottom flask containing anti-bombingchips and the soxhlet extractor assembled. The samples were extracted for about 4 hour until the soxhlet become colourless. The extracts were poured into a dried pre-weighed round bottomed flask and the thimble rinsed with a little quantity of petroleum ether into the flask. The flask was heated on a steam bath to recover the solvent. The extracted lipid left in the beaker was dried in desiccators and weighed (AOAC, 2000).

*2.6. Crude Fibre Content*

5.0g sample was put in a pre-weighed beaker. 50ml of 1.25% $H_2SO_4$ solution was added and made up to 200ml with distilled water and stirred. The mixture was heated with continuous stirring for thirty (30) minutes and allowed to cool and settle. Distilled water was added and allowed to settled then decanted, decantation was repeated for six (6) times consecutively to make the mixture acid free. 50ml of 1.25% NaOH was added to 200ml distilled water in a beaker and heated for thirty (30) minutes with continuous stirring. It was cooled and settled. Distilled water was added and decanted for six (6) times consecutively. The mixture was filtered and kept for about forty-five (45) minutes for water to drain completely and the weight taken (AOAC,2000).

*2.7. Carbohydrate Content*

This was obtained by taking each percentage value of protein, fat, fibre and ash content from the total dry matter.

% carbohydrate = 100 – (% fat + % fibre + ash + % protein)

*2.8. Crude Protein (Modified Kjeldahl Method)*

The analysis was carried out in three (3) stages, these were;

- The digestion stage
- The distillation stage
- The titration stage

Digestion Stage: 5g sample was put in a 250ml kjeldahl flask. 2g each of the kjeldahl catalysts (Copper Sulphate and Sodium Sulphate) were weighed into the kjeldahl flasks. Anti-bumping chips were added and 30ml of concentrated Sulphuric acid was also added to the flask. The digestion flask was then placed on the heating mantle for an hour before being transferred to hot plate. The digestion process proceeded with occasional swirling until a clear solution was obtained. The clear solution was transferred into a 100ml standard flask and made up to the mark with distilled water.

Distillation Stage: 10ml of the digest was measured into the micro distillation apparatus. 12.5ml of 1.25% NaOH was also added to the flask. A condenser was connected from the distillation apparatus to a volumetric flask containing 10ml of 5%

boric acid and 2 drops of double indicator (methyl red and methyl blue). The distillate was collected in a flask and then titrated with 0.1M standard hydrochloric acid until a pale pink colour end point was obtained.

% Nitrogen = $\frac{mlofHCl(sample)-mlofHCl(blank)-molarityofHCl}{weightofsample \times mlofdigest \times 1000}$

% Protein = % Nitrogen × Protein factor (AOAC, 2000).

**3. Extraction of Oil from *Chrysophillum albidum* Seed**

The oil content of *Chrysophyllum albidum* seeds were obtained by complete extraction using the soxhlet extractor (Oladeji, 2015). 400g of ground seed was placed in two thimbles (200g each), which were inserted in the centre of the soxhlet apparatus. 250cm$^3$ of the solvent (n-hexane) was poured into the flask, and heated on a heating mantle.

The extraction process was carried out for 3 to 4 hours and it was done in correspondence to the temperature of the solvent used (hexane at 60$^o$C). The extract was reflux for 20 to 30 minutes to recover the solvent from the oil. The oil obtained was weighed and the percentage oil content calculated.

**4. Characterization of Chrysophyllum albidum seed oil**

*4.1. Acid Value and Free Fatty Acid (FFA)*

2g of the extracted oil was measured into a conical flask. 25ml of petroleum ether was mixed with 25ml of ethanol and four (4) drops of phenolphthalein indicator was added to the oil sample in the conical flask and titrated with aqueous 0.1M NaOH. A pink colour which persisted for 20 seconds was taken as the end point (Oladeji, 2015).

*4.2. Saponification Value*

25ml of alcoholic potassium hydroxide solution was measured into a conical flask and 1g of the oil sample added. The flask was sealed with cork having long tube to act as condenser and heated in an oven for 5minutes at 105$^0$C. 1ml of phenolphthalein solution was added and excess alkali was then titrated with 0.5M HCl when hot. A blank was also carried out (that is without the oil sample).(Oladeji, 2015).

**5. Production of Biodiesel from Chrysophyllum Seed Oil**

1% of $H_2SO_4$ (Sulphuric acid) was added with 30ml of methanol and transferred to a conical flask containing 120ml of oil sample. The mixture was stirred vigorously for 30 minutes using a magnetic stirrer. Then poured into a separatory funnel and allowed to separate for about 24 hours. The impurities formed a top layer while the treated oil formed a layer below and was collected in a beaker and oven dried for 2 hours at 70$^o$C to remove the excess methanol (Oladeji, 2015).

*5.1. Transesterification Process*

- Mixing of Alcohol and Catalyst: 0.5g of Sodium hydroxide pellet was mixed with 30 ml of methanol inside a strong heat resistant Pyrex glass beaker. The mixture was heated gently at temperature lower than the boiling point of methanol thereby obtaining a chemical solution known as sodium methoxide ($NaOCH_3$)(Oladeji, 2015).
- The Methyl Ester Mixture:
  The sodium methoxide solution was mixed in a beaker containing 120ml of the treated *Chysophyllum albidum* seed oil and poured into a rubber container and was shaken vigorously for 1 hour in order to obtain a homogeneous mixture.
- Separation of the Biodiesel from Glycerine:
  After shaking, the mixture was poured into a separating funnel and was allowed to stand for 24 hours. Once the separation is complete two major products exist which are glycerine and biodiesel and was washed with distilled water to remove some traces of soap and other contaminants and the water was allowed to settle down before removing it by draining. The washed biodiesel was collected into a beaker and gently heated in an oven, at 150$^o$C to evaporate the excess water and methanol in the biodiesel. The biodiesel yield was 114mls at the end of the purification process. (Oladeji, 2015).

**6. Physico-Chemical Analysis**

The physico-chemical properties of biodiesel produced were determined as follows;

*6.1. Flash Point of the Biodiesel*

The flash point of the biodiesel was determined by the method of ASTMD-93, using the pensky-martens closed cup tester. The determination of the flash point of the biodiesel was done within the temperature range of 60$^o$C to 190$^o$C by an automated pensky-martens closed cup apparatus according to the standard method of testing flash point. This was done by heating a sample of the fuel in a stirred container and passing flame over the surface of the liquid, when the temperature was at or above the flash point, the vapour was ignited and an easily detected flash point was observed. The flash point has produced sufficient vapour to maintain a continuous flame (Bagby, *etal., 1987).*

*6.2. Density of the Biodiesel*

The density was determined using a density bottle and was estimated as shown,

$$Density = \frac{mass of oil}{volume of biodiesel}$$

*6.3. Cloud Point*

The oil sample was poured into a test tube to a certain level with a thermometer inserted and sealed alongside with the test tube. The setup was placed in a freezer and monitored at intervals. The temperature at which some traces of cloudy or hazy suspension appeared in the test tube was taken as the cloud point of the biodiesel sample.

*6.4. Pour point*

The sample was kept in a test jar and allowed to cool in a bath to allow the formation of paraffin wax crystals. At every 3$^{o}$C, the test jar was removed to check for surface movement. It was observed that when the sample was tilted, it did not flow. When the jar was held horizontally for 5 seconds, it still did not flow; the temperature at which it flows on being tilted was now recorded as the pour point (Onyema et. al., 2014).

**7. Result and Discussion**

| S/N | Parameters | Results (%) |
|---|---|---|
| 1 | Moisture content | 14.00±0.22 |
| 2 | Ash content | 4.00±0.10 |
| 3 | Crude fat content | 25.58±0.51 |
| 4 | Crud fibre content | 16.00±0.13 |
| 5 | Protein content | 25.75±0.45 |
| 6 | Total carbohydrate content | 14.64±0.01 |

*Table 1: Results of proximate composition of Chrysophyllum albidum seed (Adopted from our previous work)*
*Data are: mean ± standard deviation of triplicate determination*

| Properties | Chrysophyllum albidum oil | ASTMD standard |
|---|---|---|
| Free fatty acid (mgKOH/g) | 3.10 | 25max |
| Saponification value (mgKOH/g) | 187.31 | 189-198 |
| Acid value (mgKOH/g) | 7.01 | 10 |
| Oil yield (%) | 22.70 | ------- |

*Table 2: Physicochemical properties of Chrysophyllum albidum oil*

| Properties | Biodiesel | ASTMD 93 | ASTMD 975 |
|---|---|---|---|
| Flashpoint (close cup) ($^{o}$C) | 120 | 130 | 107 |
| Cloud point ($^{o}$C) | 5.8 | ----- | -15 to -5 |
| Pour point ($^{o}$C) | 4 | ----- | -35 to -15 |
| Density (g/cm$^{3}$) | 0.89 | 0.860-0.900 | 0.82-0.0845 |
| Colour | dark brown | ----- | ----- |

*Table 3: Properties of biodiesel produced from Chrysophyllum albidum seed*

Table 1 shows the proximate composition of *Chrysophyllum albidum* seed. From the result obtained, moisture content was found to be 14.00±0.22%. This value is lower than 56.04±1.64 for *Chrysophyllum cainito* seed (Oranusi et al., 2015). This connote that this seed will have relatively long shelf life. This is because the moisture content of seeds, fruits and vegetable is indicative of their shelf life. The higher the moisture content, the more susceptible the seed, fruits and vegetable is to microbial attack. Ash was found to be 4.00±0.10%. This is higher than 2.6% for African oil bean (Odoemelam, 2005), 2.48±0.01 for both cowpea and watermelon (Inobeme et al., 2014) (Anthony and Egbuonu 2015). This evidently shows that Chrysophyllum albidum seed has a high mineral content. Crude fat was found to be 25.58±0.51%. This is quite high. Seeds with high lipid content are usually compared with those of soybean, locust bean and cotton seeds. These are commercially exploited and are classified as oil seeds (Ayodele et al., 2000). This makes *Chrysophyllum albidum* seed a good source of lipid for both commercial and industrial use. The fibre content of the seed was found to be 16.00±0.13%. This value is higher than 2.37±0.00 for both locust bean seed and water melon seed (Anthony and Egbuonu 2015) (Inobeme et al., 2014). Fibre helps in the maintenance of human health and has been known to reduce cholesterol and sugar level in the blood. The protein content was found to be 25.76±0.45%. This value is higher than 21.46±0.04 for cowpea (Inobeme et al., 2014) and 21.46±0.04 for watermelon seed (Inobeme et al., 2014). The results however, suggest that *Chrysophyllum albidum* seed could be used as an

alternative source of protein supplement. More so, carbohydrate was found to be 28.66±0.01%. This is lower than 78.49±0.04 for *Chrysophyllum cainato* (Oranusi et al., 2015). However, this seed is not a good source of carbohydrate.

The physicochemical properties of Chrysophyllum albidum oil is shown in Table 2. From the result obtained, saponification value was found to be 187.31mgKOH/g. This value is within the range of ASTMD standard for saponification value (189-198). Saponification value is an indication of the molecular weight of fat or oil. Hence, the smaller the saponification value, the higher the molecular weight or longer the side chains of the fatty acids making up the triglyceride molecule and vice versa. Saponification values >200mgKOH/g indicate the presence of fatty acids of low or fairly low molecular weight, while values <190mgKOH/g indicate higher molecular weight fatty acids(Onyema et al., 2014). Therefore, oil produced from Chrysophyllum albidum seed contain predominantly high molecular weight fatty acids. The Free Fatty Acid value of 3.10mgKOH/g is lower than that of cashew nut 5.4mgKOH/g (Akinhanmi et al., 2008) and the value of 2.25mgKOH/g for previous work on Chrysophyllum albidum seed (Adebayo et al., 2012). Oils with high Free Fatty Acid values will chemically react with alkali catalyst producing soaps which will further create an impediment for the conversion reaction to biodiesel and make the washing of the finished product more difficult. Acid Value was found to be 7.01mgKOH/g. This value is lesser than 10.096mgKOH/g for watermelon seed oil (Oladeji, 2015) and 10mgKOH/g for ASTMD standard. However, fuels with high acid value have strong solvent tendency effect on number seals and hoses in engines. It can cause failure and may also leave deposits. Hence, Chrysophyllum albidum oil has high acidic concentration and should be blended with other diesel with a low acid value or neutralized with a base.

Table 3 shows the properties of biodiesel produced from Chrysophyllum albidum seed oil. From the result obtain, Flash point of the biodiesel was found to be 120$^0$C. This value is higher than 107$^0$C for watermelon oil biodiesel (Oladeji, 2015) but differ from 204.6$^0$C for cashew nut oil biodiesel (Onyema et al., 2014). These values are higher than that of high speed diesels (60-80$^0$C). However, Flash point for non-edible based seeds are higher than fossil diesel (Pramanik, 2003). The cloud point and pour point was found to be 5.8$^0$C and 4$^0$C respectively. This is in the range of that of cashew nut oil biodiesel with cloud point of 12$^0$C and pour point of 7.8$^o$C (Onyema et al., 2014) but far higher than that of watermelon oil biodiesel with cloud point and pour point of -1$^o$C and -3$^o$C respectively (Oladeji, 2015). The percentage yield of biodiesel (21.1%) from Chrysophyllum albidum seed oil is relatively high compared to 49% for watermelon seed oil (Oladeji, 2015). And lower than 75.3% for Jatropha curcas oil (Omotoso, 2011). The density of the Chrysophyllum albidum biodiesel was found to be 0.89g/cm$^3$. This value is within the range of ASTM standard (0.860-0.900g/cm$^3$).

## 8. Conclusion

Chrysophyllum albidum seed contains a high amount of fat, oil, fibre and protein which are essential body materials. The research work has also shown the prospects in utilizing Chrysophyllum albidum oil in the production of biodiesel which will serve as a good alternative biofuel to fossil fuels. However, there is a need for modification of the pour point and cloud point which do not fall within ASTMD standard.

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## APPENDIX

**CALCULATIONS.**

**Determination of Moisture Content**:
The moisture content is calculated thus:
% moisture = $\frac{loseinweightondrying}{initialsampleweight} \times \frac{100}{1}$
Weight of empty beaker ($a_1$) = 24.4g
Weight of empty beaker + sample ($a_2$) = 29.4g
Weight of empty beaker + dry sample ($a_3$) = 25.1g
% moisture content = $\frac{a_3 - a_1}{a_2 - a_1} \times \frac{100}{1}$
$= \frac{25.1 - 24.4}{29.4 - 24.4} \times \frac{100}{1} = \frac{0.7}{5} \times \frac{100}{1} = 0.14 \times 100 = 14\%$

**Determination of Ash Content**:
The ash content is calculated as follows:
Weight of empty beaker ($b_1$) = 30.1g
Weight of empty beaker + sample ($b_2$) = 35.1g
Weight of empty beaker + dry ignited sample ($b_3$) = 30.3g
% Ash = $\frac{b_3 - b_1}{b_2 - b_1} \times \frac{100}{1}$
$= \frac{30.3 - 30.1}{35.1 - 30.1} \times \frac{100}{1} = \frac{0.2}{5} \times \frac{100}{1} = 0.04 \times 100 = 4\%$

**Determination of Crude Fat Content:**
The percentage crude fat was calculated as follows;
Weight of empty round bottom flask ($c_1$) = 24.1g
Weight of round bottom flask + sample ($c_2$) = 29.1g
Weight of round bottom flask + (dry fat), lipid ($c_3$) = 25.2g
% crude fat = $\frac{c_3 - c_1}{c_2 - c_1} \times \frac{100}{1}$
$= \frac{25.2 - 24.1}{29.1 - 24.1} \times \frac{100}{1} = \frac{1.1}{4.3} \times \frac{100}{1} = 0.2558 \times 100 = 25.58\%$

**Determination of Crude Fibre Content**:
The percentage crude fibre content is calculated thus;
Weight of empty beaker ($d_1$) = 97.7g
Weight of empty beaker ($d_2$) = 102.7g
Weight of empty beaker + dry fibre ($d_3$) = 98.5g
% crude fibre = $\frac{d_3 - d_1}{d_2 - d_1} \times \frac{100}{1}$
$= \frac{98.5 - 97.7}{102.7 - 97.7} \times \frac{100}{1} = \frac{0.8}{5} \times \frac{100}{1} = 0.16 \times 100 = 16\%$

**Determination of Carbohydrate Content:**
The total carbohydrate content is calculated as shown below:
Protein = 25.76
Fat = 25.58
Fibre = 16.00
Ash = 4.00
71.38
% carbohydrate content= 100 – 71.38= 28.66%

**Determination of Crude Protein Content:**
The crude protein content obtained from the analysis is calculated thus;
% Nitrogen = $\frac{mlofHCl(sample) - mlofHCl(blank) \times molarityofHCl \times 100 \times 100 \times 14}{weightofsample \times mlofdigest \times 1000}$
% Protein = % Nitrogen × Protein factor = 4.121 × 6.25 = 25.76%

**Extraction of Oil from *Chrysophyllum albidum* Seed**

Initial weight of ground sample =600g

Final weight of extracted sample = 90.80g

% oil yield = $\frac{weight\,of\,practical\,yield}{weight\,of\,initial\,ground\,sample} \times \frac{100}{1}$

= $\frac{90.80}{400} \times \frac{100}{1}$

= 0.227 × 100 = 22.70%

**Density of Biodiesel**

$Density = \frac{mass\,of\,oil}{volume\,of\,biodiesel}$

Mass of bottle = 24.00g

Mass of bottle + $H_2O$ = 74.40g

Volume of water = 50.40g

Mass of biodiesel = 44.90g

$Density = \frac{44.90g}{50.40cm^3} = 0.891g/cm^3$

$Density \simeq 0.89g/cm^3$.

**Acid Value and Free Fatty Acid:**

Acid value and free fatty acid is given by

Acid Value = Titre (ml) XmX56.10/weight of sample (W) used (g)

Where; Titre = 2.51 (ml), m = 0.1M, W = 2g

Acid Value = 2.51X0.1X56.10/2

Acid Value = 7.04mgKOH/g

Free Fatty Acid (FFA) = Acid Value/2

FFA = 7.04/2 = 3.52mgKOH/g

**Saponification Value**

Saponification Value = (B—S) X M X 56.10/Weight(W) of the sample

Where; B = blank titre, S = sample titre value

B =14.28ml, S = 5.64ml, m = 0.5M

Saponification Value = 6.64 X 0.5 X 56.10/1 = 186.25mgKOH/g.