



Yield and Quality Assessment of *Trichosanthes cucumerina* Linn. Seed Oil

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ABSTRACT

The biochemical investigations of underutilized seeds as sources of bio-oil for domestic and industrial purposes have increased tremendously. In this study, nutritional composition and physicochemical properties of *Trichosanthes cucumerina* seed oil were examined. The seeds were collected from Ibadan Nigeria. Air-dried, de-hulled and blended. Extraction of the blended seed was done in a Soxhlet extractor using N-hexane as the solvent at 60°C for 9hours. Percentage oil yield, Physico-chemical, Elemental composition, Calorific value, FT-IR of oil were carried out using standard methods. Data obtained were analyzed using descriptive statistics. The result shows that percentage oil yield was 67.05±6.38%. Specific gravity was 0.91, the acid value 5.53, iodine value 91.1, free fatty acid value 2.77. Low concentration of sulphur 0.004% and nitrogen 0.09% were also detected in the oil. The heavy metals detected were low in concentration: Pb (0.0005mg/kg), Ni (0.015mg/kg), Co (0.01mg/kg) and Cd (0.00004mg/kg). The calorific values observed for oil was 38.12MJ/kg. The FT-IR analysis shows the most abundant compounds in the oil are mostly hydrocarbon (Aromatic and aliphatic). These results suggest that *Trichosanthes cucumerina* seed oil possesses suitable characteristics to be used for industrial purposes such as biofuel production.

Keywords: *Trichosanthes cucumerina*. Seed, oil, Physico-chemical and Soxhlet extractor

Introduction

From time immemorial, humans have learn to utilize one form of energy or the other for their survival ranging from coal burning (in steam engines) to petroleum based fuels (fossil fuels). Although there are other renewable energy sources such as solar, wind and many more. But more efforts have been placed world over on petroleum and its derivatives. The discovery of oil (petroleum) in Nigeria especially, has shifted the attention from other energy sources. However, fossil fuels although vary economically very viable but it can be exhausted, hence there is need to investigate under-utilized oil seeds as an alternative source of food and energy (Nzikou *et al.*, 2009; Emil *et al.*, 2009).

The world today is looking for an alternative energy source that is

environmental friendly, economically viable and sustainable renewable for a better adaptation into the climate change era. This project therefore considers it reasonable to bring into the limelight such energy source (mostly of plant origin).

Energy from plant sources such as *Jathropa curcas* and others have been proven by researchers around the world to be very useful as biodiesel and not detrimental to the environment.(Mckendry, 2002; Oseni and Akindahunsi, 2011).Although much has been done on *Jathropa curcas*, yet little is known about *Trichosanthes cucumerina*.

Trichosanthes cucumerina is an under-utilized vegetable that belongs to the cucurbitaceae family and cultivated in gardens in Nigeria. It is well known as snake gourd, viper gourd, snake tomato or



long tomato. *T. cucumerina* fruit is mainly consumed as a vegetable, the seeds are flat, grew-brown, sculptured, and narrow at one end about 1.7cm long. However, the quest to save resources spent on buying oil for domestic and industrial purposes have also created a novel search for using underutilized seeds as sources of oil to complement the already existing traditional sources of oil (Akubugwo and Ugbo, 2007).

Materials and Method

Study Area, Sample Collection and Preparation

The research work was carried out at the Department of Forest Resources



Plate 1: *Trichosanthes cucumerina*.



Plate 2: *Trichosanthes cucumerina* de-hulled



Plate 3: Blended de-hulled.

Management, University of Ibadan. University of Ibadan is located in the Northern part of Ibadan city which is between latitude $7^{\circ}26'34.5''N$ and longitude $3^{\circ}53'54.9''E$ at altitude 700m. This research involves the extraction of oil from the seed of *T. cucumerina*. The sample material which is the seeds of *T. cucumerina* was obtained from a home garden in Apete, Ibadan, Oyo state, Nigeria as shown in Plate 1. The seeds were removed from the placenta of the fruit, air-dried, de-hulled and kept in an air tight bag (Plate 2) and later blended. The blended seed cake was kept in an air tight bag before extraction in the soxhlet apparatus (Plate 3).

Extraction Process of Oil

Soxhlet extraction using N-Hexane solvent

Oil was extracted from the sample in five different batches by means of direct solvent extraction, using N-hexane as the solvent in a Soxhlet extraction apparatus (Plate 4). Each batch of the sample was weighed to be 50g and they were wrapped securely in a clean piece of white Muslim cloth and inserted in the thimble of the Soxhlet extractor. The round bottom flask attached to the extractor was filled with 300mL of N-

hexane and the reflux condenser fixed. Therefore, the heat source was adjusted to $60^{\circ}C$. This set-up was then left in this condition for 9hours so that all the fat in the sample was extracted through repeated washing. The oil was concentrated from the oil-solvent mixture by removing the defatted sample from the thimble and distilling off some, but not all of the solvent from the mixture in the flask. The oil rich mixture was then exposed to air for complete evaporation of the residual solvent leaving the oil behind (Plate 5).



Plate 4: Soxhlet apparatus set up for oil extraction



Plate 5: The extracted oil

Percentage Oil Yield

The weighing was carried out using an electrical weighing balance. The blended un-extracted seed cake was first extracted and weighed, the weight of the empty specimen bottle was recorded, the oil was poured in the specimen bottle and the weight also recorded. This was followed by weighing of the defatted seed cake and percentage oil yield was calculated.

The percentage yield was calculated in accordance with Juliu *et al.*, (2013) using the equation as shown below:

$$\% \text{ Oil Yield} = \frac{\text{Weight of Oil}}{\text{Weight of Unextracted Milled Seed}} \times 100 \dots 1$$

Physico-Chemical Analyses of the Oil

The following physical characteristics analysis was determined on the extracted oil:

Determination of smoke point

The smoke point is the temperature at which the sample begins to smoke when tested under specific conditions. Two grams of oil were weighed into a cylindrical metal container, attached with 0-100°C mercury in glass thermometer. The set-up is heated at a controlled rate in a Gallenkamp Oven set at 105°C. The temperature of the point at which a thin continuous stream of bluish smoke when first observed was measured by the thermometer and taken as the smoke point.

Determination of flash point

The flash point is the temperature at which a flash appears at any point on the surface of the sample due to the ignition of volatile gaseous products. In this experiment, 2g of the oil was weighed into a cylindrical metal container attached with 0-100°C mercury in glass thermometer. The set-up is heated at a controlled rate on a Gerhardt Heating Mantle set at 105°C with naked flame from a match stick being passed over the surface of the heated oil at regular interval of 5min. The temperature measured by the thermometer at a point at which a flash appears at any point of the surface of the sample is known as flash point.

Determination of fire point

Fire point is the temperature at which evolution of volatile due to the thermal decomposition of the lipids proceed so quickly that continuous combustion occurs (a fire). This experiment involves the measurement of 50mL of oil into 250mL conical flask with rubber cork containing a Thermometer and placed on a heating mantle. The flask and its content were heated to decompose the oil to point of evolution of the volatiles which proceeds so rapidly that continuous combustion occurred that is, a fire. The temperature at which the continuous combustion occurred was taken as the firing point.

Determination of relative density (Specific Gravity)

This is the ratio of the weight of the oil sample in grams to the equal volume of



water. A density bottle with its stopper was weighed (W_1), filled with the oil, covered with its stopper and re-weighed (W_2). The density bottle was then washed, drained, filled with water and weighed (W_3).

$$\text{Specific gravity} = \frac{W_2 - W_1}{W_3 - W_1} \dots\dots\dots 2$$

Determination of acid value

About 1g of the extracted oil was weighed into 250mL conical flask. 25mL of 95% alcohol was then added followed by 1mL of phenolphthalein indicator and the solution was titrated with 0.1M potassium hydroxide (KOH). It was shaken vigorously until there is a change from colourless to pink.

$$\text{Acid value} \left(\frac{\text{mgKOH}}{\text{g}} \right) = \frac{\text{Titre value} \times 0.1 \text{M alkali} \times 56.1}{\text{Weight of sample used (g)}} \dots\dots 3$$

Determination of Free Fatty Acid (FFA) Value

The titre values obtained from the acid value determination was used for the determination of free fatty acid for the sample. 1ml 0.1M NaOH=0.282g oleic acid.

$$\text{FfA}(\text{mgKOH/g}) = \frac{\text{Titre value} \times 0.282}{\text{Weight of sample used (g)}} \dots\dots\dots 4$$

FfA= Free fatty Acid value (mgKOH / g)

Calorific value

It is the quality of heat that was obtained from the combustion of the oil. The gross calorific/energy value of oil was determined using a CAL 2K-ECO bomb calorimeter. 0.5g of the sample was weighed into the steel capsule. A 10cm cotton thread was attached to the thermocouple to touch the capsule. The bomb was closed and charged in with oxygen up to 3000kpa. The Bomb was fixed up by depression into the measuring chamber. This was followed by the firing phase which involves the igniting of the samples to burn in excess oxygen. After a successful firing phase, the main

phase sets in where its duration of 10minutes before the final reading was taken. The maximum temperature rise in the bomb was measured with the thermocouple and galvanometer system and reading was recorded as the calorific value for the samples.

Chemical Composition

Heavy metals

The mineral nutrients and heavy metal analyses of the oil was determined according to the method of ASTM 3174-76 (2009) and the use of the atomic absorption spectrophotometer (AAS) to determine elements such as Pb, Cd, Ni, Co and S. For the samples, 0.5g of sample was weighed into a beaker and 10mL of an acid solution (Nitric/perchloric acid) in ratio 1:2 was added to the sample in the beaker. Then it was allowed to undergo heating on a hot plate at 105°C for an hour. The colour change while heating was observed to be from brown to clear colourless. The digest at this point was allowed to cool. The digest was then read on the atomic absorption spectrophotometer to determine the metals.

Elemental analysis

Determination of Carbon and Hydrogen

The following procedure was used to determine the essential elements except for sulphur 2g of each sample was weighed into platinum crucible and placed in a Leibig-pregle chamber containing Magnesium percolate and sodium hydroxide while the water was absorbed by magnesium percolate. The amount of C, H, N were calculated using the formular below while O was calculated by finding their difference.

$$\%C = \frac{a \times 0.2727}{\text{Weight of unextracted milled seed}} \times 100 \dots\dots\dots 5$$

$$\%H = \frac{b \times 0.117}{\text{Weight of unextracted milled seed}} \times 100 \dots\dots\dots 6$$

$$\%N = \frac{(S - B) \times 0.014 \times D}{\text{Weight of milled seed} \times V} \times 100 \dots\dots\dots 7$$



Where

S= Sample titration reading

B= Blank titration reading

N= Normality of HCl

D= Dilution of sample after digestion

V= Volume taken for distillation

0.014= Millilitre equivalent weight of Nitrogen.

$$\%O = 100 - (\%C + \%H + \%N) \dots\dots\dots 8$$

Determination of Sulphur from the Digest

5mL of the digest was pipette into a 100mL beaker and 10mL of distilled water was added and mixed thoroughly to a complete homogenized solution. Then 1mL of 1% Barium gelatin solution was added to precipitate the sulphur. Working standard of sulphur was prepared from range 0-50mg/l and treated similarly like sample. Absorbance of standard as well as sample was read on a spectronic 21D spectrophotometer at a wavelength of 420nm. Sulphur in mg/100g was calculated using formula:

$$S = \text{Absorbance} \times \text{Gradient factor} \times \text{Dilution factor} \dots\dots\dots 9$$

Spectrophotometric Analysis (FT-IR)

This analysis was performed on the oil using FT-IR to determine the possible functional group present in the samples. The

response of the functional groups were characterized by observing the transmission of infrared radiations and comparing it with known standards in order to identify the type and the nature of functional groups present in the samples.

Statistical Analysis

Data obtained from the seed oil yield, physico-chemical analysis and elemental analysis, were subjected to descriptive statistics.

Result and Discussion

Percentage Oil Yield

Percentage oil yield of the selected oil samples were; 54.88%, 76.44 and 69.84% for three batches respectively (Table 1). The average oil yield was 67.05%. The value of the oil yield is higher than the reported range for commercial vegetable oils such as cotton seed (19.50%), soybeans seed (19.0%), palm oil (48.65) and groundnut (49.0%) (Matchet, 1963; Dawodu, 2009). Although the yield compares well with the result of Adeniyi and Isiaka (2013) who recorded a 63.33% yield from the seed of *Trichosanthes cucumerina*. However, it has been reported in the literature that oil-bearing seeds that yield up to 30% oil are to be considered suitable for commercial and industrial applications.

Table 1: Percentage Oil Yield from *Trichosanthes cucumerina*

Extraction Batches	Percentage Oil Yield (%)
A	54.88
B	76.44
C	69.84
Average	67.05±6.38



Physicochemical Analysis

Specific Gravity

The specific gravity of the oil sample as shown in the Table 2 was 0.91. This falls within the range of most popular plant oils which have specific gravity ranging from

0.91-0.94 and specific gravity of 0.91 is considered a pretty good number for any cooking oil (Minzangi *et al.*, 2011). It also falls within the standard given by ASTM 6751-02 (2002)(0.87-0.92) for biodiesel production.

Table 2: Physicochemical Analysis of the Oil

Parameters	Mean
Specific gravity (g/cm ³)	0.907±0.013
Flash point (°C)	268.3±3.774
Fire point (°C)	346.7±21.911
Smoke point (°C)	366.2±25.275
Iodine value	91.117±13.429
Acid value (%)	5.532±0.550
FFA value (%)	2.767±0.275
Calorific value (MJ/kg)	38.12±0.519

Values are mean±standard error of 6 replicate values.

Flash, Fire and Smoke Point

Table 2 shows that the flash point of the oil samples is 268.3°C. Flash point is the temperature at which the decomposition products formed from frying oils can be ignited (AOCS Method). This temperature ranges from 265°C to 330°C for different oils and fats and this oil falls within that range. The fire point of the oil sample shown in Table 2 has a value of 346.7±21.91°C. Fire point is a measure of the tendency of the test fuel to support combustion. The test for fire point is usually applicable to all petroleum products with flash points above 79°C and below 400°C and this oil sample falls within that range. Smoke point is the temperature at which a fat or oil produces a continuous wisp of smoke when heated. It is usually above 200°C for frying oil and the value of smoke point for this oil sample according to Table 2 is 366°C.

Iodine Value

Table 2 shows the iodine value of the oil sample to be 91.12±13.43. According to B100 Research (2002), most oils and fats

have an iodine value of between 44 and 75 and the standard given by Europe's EN 14214 (2008) as cited by Son *et al.*, (2010) is 120. A lower iodine value indicates that oil samples have lower degree of unsaturation and higher cloud point but good oxidative stability and vice versa.

Acid Value/ Free fatty acid Value

Acid value and percentage free fatty acid value are used as indicator of the edibility of oil. These two parameters determine the use of oil for either edible or industrial utility. Acid value of the oil suitable for edible purpose should not exceed 4mg KOH/g according to Oladele and Oshodi (2008) and the oil sample according to Table 2 is 5.53mg KOH/g and hence the oil is not suitable for edible purpose. The low percentage FFA content of *T. cucumerina* (2.77%) seed oil indicates that the oil can be stored for a long time without spoilage through oxidative rancidity.

Calorific Value

The calorific or heating value of any fuel is said to be the largest factor and an important property in the fuel economy and power



deliverability. It defines energy density of fuels (Oghenejoboh and Umukoro, 2011). Table 2 shows that calorific value of *T. cucumerina* seed oil is 38.12MJ/kg. This falls within the ranges of the calorific values of oil-bearing species and this indicates a good combustion property. The calorific value of some oil-bearing species are; corn oil and cottonseed oil (39.5MJ/kg), rape seed oil (39.7MJ/kg), rubber seed oil (37.5MJ/kg), soyabean oil and sunflower oil (39.6MJ/kg), crambe oil (40.5MJ/kg) and the petroleum diesel (43.8MJ/kg) (SEA, 1996).

Chemical Analysis

Heavy metal

Table 3 shows the heavy metals present in the seed oil. The heavy metals tested for in these samples are; Pb, Co, Ni and Cd. Lead (Pb) content in the oil sample has the lowest value which is 0.0005mg/Kg. Cobalt (Co) in the same table was also seen to be 0.01mg/Kg. The content of Nickel (Ni) is 0.01498mg/Kg. Cadmium value in the seed oil is 0.00004mg/Kg. It shows that the concentration of heavy metals found in the seed oil falls within the permitted range (0.002-0.3mg/Kg) in the environment according to (USEPA 1992). This gives a basis for exploitation of the seed oil for biofuel because it is environmentally.

Table 3: Heavy metal present in Seed Oil

Parameters (%)	Mean
Pb(mg/kg)	0.0005±0.0001
Co(mg/kg)	0.0100±0.5192
Ni(mg/kg)	0.01498±0.0033
Cd(mg/kg)	0.00004±0.00002

Data shown are Means±Standard errors of replicate values of seed oil values; Significant at p-value<0.05

Elemental

Figure 1 shows the percentage proportion of Carbon, Hydrogen, Nitrogen, Oxygen and Sulphur found in the seed oil of *Trichosanthes cucumerina*. Elemental analysis, according to ASTM D6751, is a test that forms an important measure of quantifying the product quality in the oil industry as well as the biofuel sector. The test is required to ensure regulated parameters that will enhance general quality of biofuel that does not cause environmental damages. Higher values of these elements emitted during combustion form greenhouse gases or compounds that will not easily be absorbed back, thereby causing global warming.

In Fig. 1, the percentage carbon in the oil of the seed is 29.97%, percentage hydrogen is

5.22%, Nitrogen content is 0.09%, and Oxygen is 64.8% while Sulphur is 0.004%. The low Nitrogen content of the oil makes it more suitable to be used as bio-oil because oil samples with high nitrogen are considered to be low quality but are easily biodegraded. The high oxygen content in the oil is the cause of low calorific value obtained. This is because high oxygen lowers the Heating Value (Kiky *et al.*, 2015). On the other hand, according to Bardalai and Mahanta (2015), the amount of oxygen content in a bio-oil is as a result of the water content present in it. The low sulphur content of seed oil falls below ASTM Standard D4294-08 (2008) which implies that the oil is of good quality and good for use as a biofuel and could be environmentally friendly.

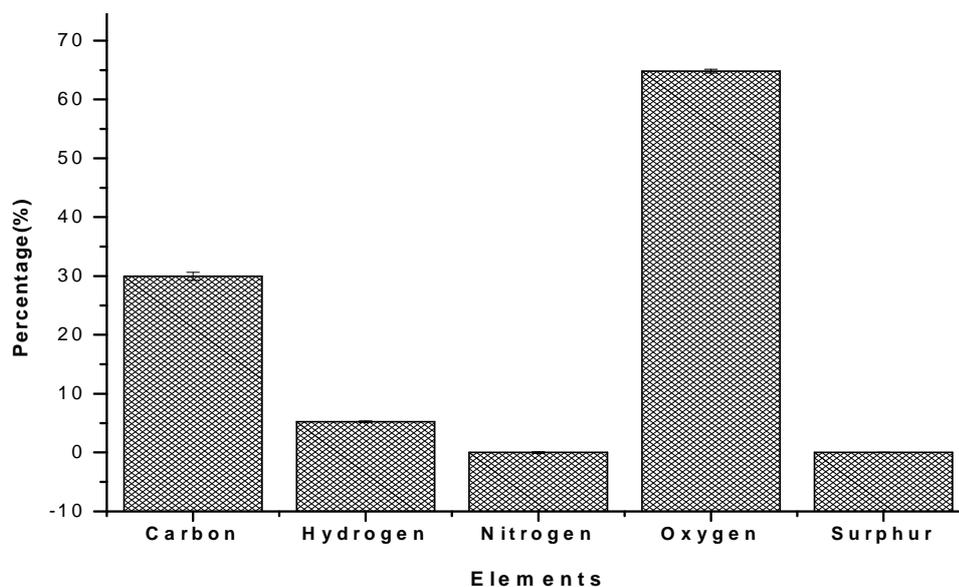


Figure 1: Elemental analysis of seed oil

Spectra Analysis (FT-IR)

The FT-IR in Fig. 2 show a clear spectra which according to Ma *et al.*, 1997 gave an excellent potential in providing qualitative

and quantitative data for fuels. It is used extensively as a quantitative analytical procedure for assessing edible oil quality parameter.

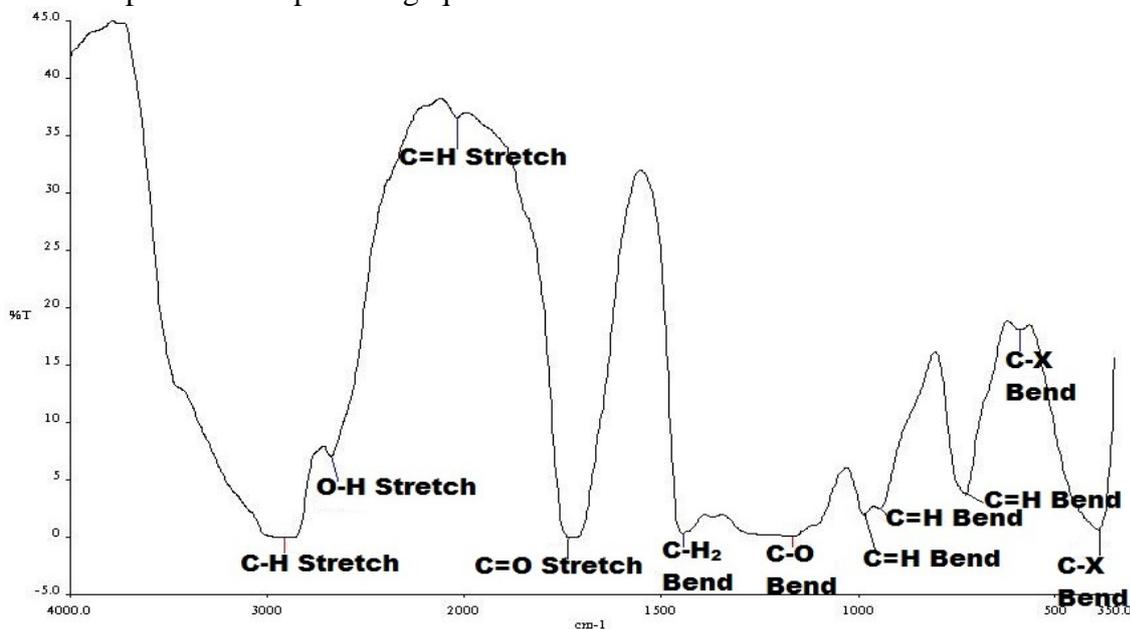


Figure 2: FT-IR spectral analysis for Seed oil

In Figure 2, the compounds observed from FT-IR analysis of the oil sample were Alkanes, Alcohols, Alkynes, Alkenes, Esters and Alkyl halides and the type of bond common were both single and double bonds. The most abundant group is the

Alkane group which is a single bond and this suggests that the oil has more of saturated fatty acid compounds. The presence of alkane group indicate prevalence of properties such as pour and cloud points that affect the performance of



biodiesel during cold weather engine operation (Younis *et al.*, 2009). However, the presence of groups with double bonds (C=H) which are also abundant can cause the fuel to remain in liquid state but may be liable to possible oxidation during storage. In the region of 1733 and 945 cm^{-1} , different absorption bands were associated with various constituents and those vibration modes were because of the derivatives of carbohydrates and lignin (Siengchum *et al.*, 2013). It could be concluded that the presence of different functional group of compounds in the oil indicate the presence of hydrocarbons in the fuel while the O-H broad peak indicate the presence of water contents in the bio-oil (Zeban *et al.*, 2016).

Conclusion

In view of the results obtained in this study, the following conclusions were drawn

The oil yields was observed to be high in comparison to other oil bearing trees, an indication that *T. cucumerina* is an oil-bearing seed suitable for both domestic and industrial usage. The oil obtained could be described as heavy oil based on its high specific gravity. The values obtained for the free fatty acid value and acid value indicates that the oil is of good quality for biodiesel production but not edible because of the high acid value.

Also, calorific value was observed to be high; therefore the combustion rate will be high. Sulphur content of the oil was quite low this makes the oil environmentally friendly. FT-IR analysis shows that the most abundant compounds are hydrocarbon (aromatic and aliphatic) alkanes, alcohols and alkyenes.

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