

Qualitative and Quantitative Phytochemical and Physicochemical Analyses on The Oil and Extracts from *Ricinodendron Heudelotii*

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ABSTRACT

The physicochemical parameters of the oil as well as qualitative and quantitative phytochemical analyses were carried out on *n*-hexane, ethyl acetate, and aqueous extracts of *Ricinodendron heudelotii* (Njangsa) using standard methods. The phyto-compounds were identified and their various concentrations were determined using Gas Chromatography equipped with Flame Ionization Detector (GC-FID). The physicochemical analysis carried out on the oil extract showed that the oil had an iodine value of 24.111g I/100g, peroxide value of 147mL eq / kg, saponification value of 322.58mg/KOH, thiobarbituric acid value of 1.4508mg/kg, density of 0.338g/ml and specific gravity of 0.9699. The results from the phytochemical analysis showed that the extract from all the solvents contained flavonoids (rutin, epicatechin, glycerin, kaempferol, and flavones, with epicatechin having the highest concentrations of 10.2546µg/g and 6.7597µg/g in *n*-hexane and aqueous extracts respectively), tannins (12.5370µg/g). Linamarin a cyanogenic glycoside was found in abundance in the aqueous extract at a concentration of 19.0462µg/g, alkaloids (quinine, 7.8644µg/g, ephedrine 2.7672µg/g) occurred in ethyl acetate extract at appreciable quantity. Resin, cardiac glycoside, saponins, terpenoids and steroids were found in *n*-hexane and ethyl acetate fractions in not very significant concentrations.

Keywords

Ricinodendron Heudelotii, Phytochemical, Quantitative, Qualitative, Gas chromatography-FID.

Introduction

The search for novel plant metabolites is driven by the need for new and effective drugs to tackle the menace of the ever-increasing diseases that plague mankind, super foods to quench the hunger of the world's increasing population as well as industrial raw materials. Nature has been the source of foods, raw materials and therapeutic agents for millions of years of human existence.

In Nigeria, a high proportion of the rural and urban population resort to natural food and food ingredients, particularly because of their availability and affordability. Spices are a large group of such natural food ingredients and they include dried seeds, fruits, roots, rhizomes, barks, leaves, flowers and any other vegetative substances used in a very small quantity as food additives to colour, flavour or preserve foods [1]. Spices are fragrant, aromatic and pleasant-smelling substances used in the preparation or processing

of food. The bulk of the spices consist of carbohydrates such as cellulose, starch, pentosans, mucilage, some amount of protein and minerals [2]. Only very small fractions of dry matter in spices such as the phytochemicals are responsible for the flavouring, colouring and preservatives properties [3].

These phytochemicals are plant metabolites [4] which act as natural defense systems for host plants and provide characteristic colour, aroma and flavour in specific plant parts. They may be non-nutritive compounds but may be biologically active when consumed by humans.

Epidemiological and clinical studies have proven that phytochemicals present in food materials such as cereal, fruits and vegetables are responsible for the reduced incidence of chronic and degenerative diseases among the populations whose diet are high in these foods, majorly as a result of their rich antioxidant contents [5]. Typical phytochemicals with antioxidant activities include phenolic and polyphenolic compounds and their derivatives, phospholipids, ascorbic acids, carotenoids and steroids. Over

the last century, a large number of drugs have been derived from phytochemicals for the treatment and management of various ailments such as quinine an alkaloid for malaria, vincristine and vinblastine also alkaloids for cancer, morphine an analgesic and anesthetic also an alkaloid and lots of others [6]. Studies are ongoing for the search for more of these phytochemicals.

Ricinodendron heudelotii locally known as Njansa is a large, deciduous tree, with an average height of 20-30 m but can grow up to 50 m. It has a straight trunk of average diameter 0.7 m and short buttresses. The crown is broad and broken branches are often observed. The tree's slash is dark red, densely mottled with scattered pits. The bark is grey, becoming scaly with age [6]. The fruit is an indehiscent yellow-greenish drupe, 2 to 3 lobed, generally spherical, 3-5 cm long and 2.5-4 cm width; weight ranges from 19 to 47 g. Fruits smell of overripe apples. The fruit has a fleshy mesocarp and a woody endocarp, and contain 2-3 seeds [7]. Seeds are used for multiple purposes in different countries in Africa, in Sierra Leone, they are used in rattles for bundu dances, while in Cameroon they are used in musical instruments as well as for local games: 'songo' in Cameroon and 'okwe' in Nigeria. The hard endocarp contains seeds which are the edible part of the fruits. Fruit pulp and endocarp are usually discarded after the seeds or kernel have been extracted [7]. Most often kernels are dried and used as a condiment/spices in recipes. Crushed njansa is used to thicken and flavour soups, stews and other dishes [8]. They are also cooked with chicken and vegetables or eaten plain [9-11]. In addition, kernels may be roasted, made into a paste and used for making a sauce similar to peanut sauce [7,12].

The Seeds can be processed to obtain oil. The oil is light yellow, with a pleasant taste resembling that of groundnut oil [8]. The yield of extracted oil from the kernels of *R. heudelotii* were found to be between 49% to 63%. It has been suggested that the high oil content of the seed together with the high proportion of poly-unsaturated fats in the oil, makes it suitable for commercial production of cooking oil and margarine as well as for soaps and pharmaceutical preparations [7]. However, Njansa oil is only traditionally processed and hence, not yet produced in large quantities.

In West Africa, the characteristics of this seed and the oil as well as their use in other areas apart from domestic are hardly known. A sustained effort to search for a better understanding of the chemical composition and physico-chemical properties of seed and its oil is needed to take better advantage of this plant.



Plate 1: The seeds of *Ricinodendron heudelotii*.

In this perspective, this work was carried out in order to determine the phytochemical and physico-chemical components of the seed and the oil extract from *Ricinodendron heudelotii*.

Materials and Methods

Collection and identification of samples

The dried seed of *Ricinodendron heudelotii* were bought from Eke-Awka market in Awka, Anambra State, Nigeria. It was identified at the Botany Department of Nnamdi Azikiwe University, Awka and a specimen kept for proper documentation.

Preparation of samples

The seeds were sorted to remove the bad and broken ones, washed and kept under the shed to dry for seven days and then ground into powdered form using a manual kitchen grinding machine.

Extraction

A Soxhlet extractor was used in the extraction, about. 300.013g of the ground Njansa seed was extracted exhaustively with n-hexane at a temperature of 68°C. The extract was concentrated and the residue allowed to dry in the dessicator for 24 hours, the residue was subsequently extracted with ethyl acetate and then hot water to afford the ethyl acetate and aqueous extracts respectively. These were concentrated, allowed to dry and stored in air tight containers and kept in the refrigerator to be used for the various analyses.

Another 100g of the ground seed were extracted with a sohxlet extractor using 40-60 petroleum ether to afford the oil for the oil analysis and characterization.

Qualitative Phytochemical analysis

Qualitative phytochemical analysis was carried out on all the extracts using the methods of Trease and Evans [13] and Harborne [14] to ascertain the presences of the different phytochemicals in the leaves before quantitative analysis were carried out.

Test for alkaloids

Wagner's reagent test: About 1.0ml of filtrate was added to a test tube using a pipette and then 1.0ml of Wagner's reagent was also added to the test tube and properly mixed to observe the colour change. Then a reddish brown precipitate which was observed indicates the presence of alkaloids.

Meyer's reagent test: About 1.4 g of mercuric chloride was dissolved in 60 ml of distilled water, 4.5g of potassium iodide was also dissolved in 20 ml distilled water, the two solutions were mixed together and made up to 100 ml with distilled water. 1.0 ml of extract was pipetted into a test tube and 1.0 ml was added to the extract and shaken, A cream coloured precipitate indicated the presence of alkaloids.

Test for flavonoids

Ferric chloride test for phenolics: 1.0 ml of extract was pipetted

into a test tube and 1.0 ml of 10% ferric chloride was added to the test tube and properly mixed. A greenish black precipitate was observed which indicated the presence of phenolic nucleus.

Lead Acetate Test: 1.0 ml of extract was added to a test tube and then 1.0ml of 10% lead acetate solution was also added to the same test tube and mixed properly to observe colour change or precipitate which indicates presence of flavonoids.

Test for tannins

Acid Test: Procedure: 3.0ml of extract was added to 2.0ml of 1% HCl, the presence of red coloured precipitate indicate the presence of phlobotannins was observed.

Lead acetate test: 2.0 ml of extract was added to a test tube using a pipette and 3 drops of 5% extract solution added, a dark precipitate was observed indicating the presence of phlobotannins.

Test for cardiac glycosides: 1ml of the extract was added to 10cm³ of 50% H₂SO₄. The solution was heated in boiling H₂O for 5 minutes. 10cm³ of fehling's solution (5cm³ of each solution A and B) was added and boiled. A brick red precipitate indicating presence of glycosides was observed.

Test for steroids: To a 0.5ml of the chloroform extract in a test tube, 1ml of concentrated H₂SO₄ was carefully added to form a lower layer. Then, the colour at the interface was observed and recorded.

Test for resins: About 0.2g of the oil sample was extracted with 15ml of 90% ethanol. The alcoholic extract was then poured into 20ml distilled water in a beaker. A precipitate occurring indicates the presence of resins.

Test for saponins: About 2g of the oil sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and was agitated vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and was agitated vigorously, then observed for the formation of emulsion.

Test for terpenoids: Salkowski test: 5ml of each extract was mixed in 2ml of chloroform and concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown colouration of the inner face was formed to show positive results for the presence of terpenoids.

Quantitative phytochemical analysis

Extraction of phytochemicals: 1g of sample was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50% m/v potassium hydroxide was added. The contents of the test tube was allowed to react in a water bath at 60°C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. This extracts were

combined and washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent evaporated. The sample was solubilized in 1000µl of pyridine of which 200µl was transferred to a vial for analysis.

Quantification by GC-FID

The quantitative analysis of phytochemicals was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250µm x 0.15µm) was used. The injector temperature was 280°C with split injection of 2µl of sample and a linear velocity of 30cms⁻¹, Helium 5.0 pa.s was the carrier gas with a flow rate of 40 mLmin⁻¹. The oven operated initially at 200°C, it was later heated to 330°C at a rate of 3°C min⁻¹ (this temperature was kept for 5min). The detector operated at a temperature of 320°C.

Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expressed in µg/g.

Physicochemical analysis of oils

Iodine value

Preparation of wiji's solution: 8g of iodine trichloride was dissolved in 200ml glacial acetic acid. 9g of iodine was also dissolved in 300ml carbon tetrachloride. The two solutions were mixed and diluted to 1000ml with glacial acetic acid.

Approximately 1.0g weight in gram of the oil was taken and calculated by dividing by 20, the highest expected iodine value.

10ml of carbon tetrachloride was added to the oil and dissolved. 20ml of wiji's solution was added and then the stopper inserted (previously moistened with potassium iodine solution) and allowed to stand in the dark for 30 minutes. About 15ml of potassium iodide solution (10%) was added and made up to 100ml with water mixed and then titrated with 0.1M thiosulphate solution using starch as indicator just before the end-point (titration = a ml).

A blank was added at the same time commenced with 10ml of carbon tetrachloride (titration = b ml).

$$\text{Iodine content} = \frac{(b - a) \times 1.269}{\text{Weight of sample (g)}}$$

Note: if (b-a) is greater than b/2 the test must be repeated.

Peroxide value

Approximately 1g of oil was weighed into a clean dry boiling tube and while it was still in liquid form, 1g of powdered potassium iodide was added and also 20ml of solvent mixture (2 vol glacial acetic acid + 1 vol chloroform). The tube was placed in boiling water allowing the liquid to boil within 30 seconds (it was not allowed to boil for more than 30 seconds). The contents were quickly washed into a flask containing 20ml of potassium iodide solution (5%), and then the tube washed twice with 25ml water

and titrated with 0.002M sodium thiosulphate solution using starch. A blank was performed at the same time.

The peroxide value is reported as the number ml of 0.002N (M) sodium thiosulphate per g of sample. If the value so obtained is multiplied by 2, the figure then equals milliequivalent of peroxide oxygen per g of sample (ml) M per g), which has greater international recognition.

Saponification value

Procedure: 2g of the oil or fat was weighed into a conical flask and exactly 25ml of the alcoholic potassium hydroxide solution was added. A reflux condenser was attached and the flask heated in boiling water for 1hr, with frequent shaking. 1ml of phenolphthalein (1%) solution was added and the hot excess alkali titrated with 0.5M hydrochloric acid (titration = a ml). A blank was carried out at the same time (titration = b ml).

Calculation:

$$\text{Saponification value} = \frac{(b - a) \times 28.05}{\text{weight of sample(g)}}$$

Determination of free fatty acids (FFA)

Procedure: 25ml of diethyl ether was mixed with 25ml of alcohol and 1ml phenolphthalein solution (1%). it was then carefully neutralized with 0.1M NaOH. 2g of the oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1M NaOH with constant shaking until a pink colour which persisted for 15 seconds was obtained.

Calculation:

$$\text{Acid value} = \frac{\text{Titre (ml)} \times 5.61}{\text{Weight of sample used}}$$

The FFA figure is usually calculated as oleic acid (1ml 0.1M) Sodium hydroxide = 0.0282g oleic acid), in which case the acid value = 2x FFA.

For most oils, acidity is noticeable to the palate when FFA calculated as oleic acid is about 0.5 – 1.5%.

Density

Procedure: A 50ml pycnometer bottle was thoroughly washed with detergent water and petroleum ether and then it was dried and weighed. The bottle was then filled with the sample and weighed. After the bottle had dried it was filled with the oil sample and weighed.

Specific gravity

Procedure: A 50ml pycnometer bottle was thoroughly washed with detergent, water, and petroleum ether and then it was dried and weighed. The bottle was then filled with water and weighed. After the bottle had dried, it was filled with the oil sample and weighed.

Calculation:

$$\text{Specific gravity} = \frac{\text{Weight of Xml of the oil}}{\text{Weight of Xml of water}}$$

Thiobarbituric acid number or value (TBA)

Procedure: 10g of fatty food was macerated with 50ml of water for 2minutes and washed into a distillation flask with 47.5ml water. 2.5ml of hydrochloric acid was added to bring the PH to 1.5, followed by an antifoam preparation and a few glass beads. The flask was heated by means of an electric mantle so that 50ml distillate was collected in 10 minutes from the time boiling commenced. 5ml of distillate was added to a glass-stopper tube using a pipette and 5ml of TBA reagent was added (0.2883g/100ml of 90% glacial acetic acid) and agitated then heated in boiling water for 35minutes. A blank was prepared similarly using 5ml water reagent. Then the tubes were cooled in water for 10 minutes and the absorbance (D) was measured against the blank at 538nm using 1cm cells. TBA no. (as mg malonaldehyde per kg sample) = 7.8D.

Results and Discussions

| Parameters | Aqueous | N-Hexane | Ethyl acetate |
|--------------------|---------|----------|---------------|
| Alkaloids | ++ | + | ++ |
| Phenols | - | + | ++ |
| Flavonoids | ++ | + | +++ |
| Tannins | ++ | + | ++ |
| Resins | - | ++ | + |
| Saponins | + | ++ | + |
| Cardiac glycosides | + | ++ | ++ |
| Terpenoids | ++ | +++ | +++ |
| Steroids | - | +++ | +++ |

Table 1: Qualitative phytochemical analysis

Key: + + + Strongly present

+ + Moderately present

+ Scantly Present

- Not present

From table 1, the qualitative phytochemical analysis of the extracts showed the presence alkaloids, phenol, tannins, flavonoids, cardiac glycosides, steroids, terpenoids, resin and saponins. A plant containing all these constituents according to Sofowara [4] is known to exhibit medicinal and physiological activities.

| Component | Aqueous extract (µg/g) | N- Hexane (µg/g) | Ethyl acetate(µg/g) |
|--------------|------------------------|------------------|---------------------|
| Rutin | 1.2149 | 0.3170 | 6.0554 |
| Epicatechin | 6.7597 | 10.2546 | 6.6293 |
| Naringin | 3.3857 | - | 2.3544 |
| Glycetein | - | 3.8567 | 4.8613 |
| Sapogenin | - | 2.4955 | 2.8209 |
| Lunamarin | 19.0462 | 3.8640 | 6.3456 |
| Kaempferol | 1.4172 | - | 1.0744 |
| Naringenin | - | 3.6559 | 2.5140 |
| Ribalinidine | 16.0646 | 3.1606 | 3.0035 |
| Flavone | 2.9905 | 1.3153 | 1.0386 |
| Epihedrine | - | - | 2.7672 |

| | | | |
|---------------|----------|---------|---------|
| Quinine | 7.8644 | 6.7559 | 6.0895 |
| Tannin | 12.5370 | 5.3746 | 5.3510 |
| Anthocyanines | 0.4044 | - | 0.4571 |
| Sparteine | - | - | 10.2722 |
| Catechin | - | 2.2158 | - |
| Anthocyanin | 15.7066 | 3.8567 | - |
| Oxalate | 12.0940 | - | - |
| Phytate | 9.1734 | - | - |
| Total | 108.6586 | 49.6066 | 61.634 |

Table 2: Quantitative phytochemical analysis of *Ricinodendron heudelottii* using FID Gas chromatography.

The result of quantitative analysis of the identified compounds by GC–FID shown in Table 2 above, indicated that tannins were present in high concentration in all the extracts at 12.5370, 5.3746 and 5.3510 µg/g for aqueous, n-hexane and ethyl acetate extract respectively. It has been noted that tannins possess a natural defense mechanism against microbial infection and exert other physiological effects such as blood pressure reduction, decrease in serum lipid level, liver necrosis production, rapid blood clotting and immune response modulation [15].

Appreciable concentration of alkaloids (quinine) 7.8644, 6.7559 and 6.0895 µg/g and for aqueous, n-hexane and ethyl acetate respectively and flavonoids rutin, epicatechin and flavones, were seen in all the extracts. Their content in plant materials have severally been reported to be associated with anti-oxidant and anti-inflammatory and activities. Alkaloids has been used medicinally as antiarrhythmic, anticholinergic, stimulant, analgesic, antimalarial and antihypertensive [16].

Saponins though not quantified were also found to be present in *Ricinodendron heudelottii* and has been reported to be used in the treatment of wounds, cough, asthma, hay fever and generally as antimicrobial and antiviral compound. Saponins have also been used as adjuvants in vaccines to boost immune response by production of cytotoxic T- Lymphocytes against exogenous antigens [17].

| Test parameters | Average values |
|-----------------------------------|----------------|
| Iodine Value | 24.11 |
| Peroxide Value (meq/kg) | 147.00 |
| Saponification Value (mg/KOH) | 322.58 |
| Free fatty acid (%) | 6.32 |
| Density (g/ml) | 0.33 |
| Specific gravity | 0.96 |
| Thiobarbituric acid value (mg/kg) | 1.45 |
| Viscosity (Pa.s) | 1.73 |

Table 3: Physicochemical characteristics of the oils from *Ricinodendron heudelottii* extracts.

The oxalate which is regarded as anti-nutritional was found in low concentration as excess of oxalate poses a risk of kidney stone [18]. Also, Phytate has been reported to have antioxidative properties and anti-carcinogenic activities even though like

oxalate, they are regarded as anti-nutritional [19]. The presence of these phytochemicals in the plant *Ricinodendron heudelottii* suggests its potential therapeutically and its use in pharmaceutical industries [20-24].

The physicochemical properties of *R. heudelottii* seed oil as presented in Table 3 showed that the free acidity value of 6.32%. Free fatty acid is an important quality factor and has been extensively used as a traditional criterion for classifying oils in various commercial grades. The free fatty acidity value of *R. heudelottii* seed oil is consistent with the acceptable standard range (1.61–13.06%) as stated by Ramadan et al. [25] This acidity is higher than that found in some edible oil such as linseed oil and sunflower oil. An acid value of 12.6465% was obtained from this seed and this is lower than that of olive oil at 17% [26], and higher than that of shea butter nut 10.3% [27]. High acid value of oil has a great advantage in soap making. The low acid value of oil suggests that it can be used in paint making. The lower the acid value of oil, the fewer free fatty acids it contains and the less exposed to the phenomenon of rancidity [28]. The oxidative rancidity of oils is determined using the peroxide value. The peroxide value of oil is a valuable index to determine oil quality. Peroxide value is also used as a measure of the extent to which rancidity reactions have occurred upon storage. As seen in Table 3, the value of Peroxide value is 147ml/kg (1.47meqO₂/kg) which falls within the range of 0-10meq/kg stipulated for freshly prepared oil [29]. The low peroxide value of the sample shows that *Ricinodendron heudelottii* seed oil has high resistance to lipolytic hydrolysis and oxidative deterioration [30]. This means that the oil possess a longer shelf life, since oils become rancid when the peroxide value ranges from 20 to 40 meqO₂/Kg oil [31]. Peroxide value of *R. heudelottii* seed oil is significantly lower than that of some seeds oils like linseed oil (11.28 meqO₂/Kg) and sunflower oil (12.87 meqO₂/Kg) [32].

The iodine value of the sample is 24.111 gI₂/100g (less than 100) was obtained, which shows that the oil belongs to the class of non-drying oils which are edible oils. Certain researchers in literature [33-35] reported that oils with iodine values greater than 100, possess the property of absorbing oxygen on exposure to the atmosphere and can form a hard dry hard film. Hence *R. heudelottii* is a good source of edible oil. The Specific gravity of *R. heudelottii* (0.96) shows that it is less dense than water. Since viscosity is the measure of a fluid's resistance to flow. The viscosity value of *R. heudelottii* (1.739 Pa.s) being low, shows its flow rate will be fast and should serve as a good nutritional oil not capable of being stored in the blood vessels.

Saponification value is inversely proportional to the mean molecular weight of the glycerides in the oil. The saponification value of *R. heudelottii* was observed to be high (322.58mg/KOH) compared to neem seed (213mgKOH/g) and coconut oil (253.2mg/KOH). High saponification value justifies the usage of fats and oil for soap production [36].

Conclusion

This work revealed the presence of medicinally active constituent

of the seeds of *Ricinodendron heudelotii*. The phytochemical compounds identified have earlier been proved to be bioactive. Hence, the presence of some of these compounds in this seed could be harnessed as a possible source for their use as dietary supplements as well as possible medicinal purposes. The compounds identified are equally none toxic as none of the phyto-compounds identified are known to be toxic.

Furthermore, the results showed that physicochemical properties of the oils were within the range for edible oils, it is therefore suggested that the oils be extracted in large quantities for commercialization as this will help boost and diversify the Nigerian economy since the seeds are readily available and currently not being utilized except as food spice.

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