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Phytochemicals and Nutritional Characteristics of Ethanol Extract of the Leaf and Bark *of* Njangsa (*Ricinodendron Heudelotii)* Plant

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ABSTRACT: The phytochemical, proximate and mineral content of leaf and bark of Njangsa (Ricinodendron *Heudelotii)* plant were analysed, using standard procedures described by Harbone, 1973, Sofowora, 1993; Trease and Evans, 1989 and Association of Official Analytical Chemist (AOAC) Official method, 1990; 1984 respectively. The preliminary phytochemical screening revealed the presence of tannins, steroids, terpernoids, alkaloids, flavonoids, cardiac glycosides, reducing sugars and saponins found only for the plant bark. The result of proximate analysis showed that the leaf and bark of the extract contain respectively: moisture content (25.80% and 10%), protein (17.47% and 3.73%), crude fat(1.80% and 2.00%) ash (11.00% and 10.95%), crude fibre (41.00% and 20.50%) carbohydrate (2.93% and 52.82%).The mineral analysis of the plant leaf and bark respectively yielded calcium – 2640.00 mg/kg and 1772 mg/kg, magnesium – 2383 mg/kg and 1605 mg/kg, iron – 25.00 mg/kg and 6.6 mg/kg, zinc – 29.30 mg/kg and 4.4 mg/kg, copper – 14.60 mg/kg and 6 mg/kg and phosphorus – 1012 mg/kg and 305 mg/kg. Manganese was absent in the bark but yielded 1.1 mg/kg in the leaf. Sodium and potassium were found to be absent in the leaf and bark. The presence of some phytochemicals (flavonoids, cardiac glycosides, reducing sugar, tannins and terpernoids) and some essential minerals suggest that it is a potential source of medicine and food.

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Keywords: *Phytochemical, Nutritional Characteristics, Proximate, Mineral, Medicinal, Analysis.*

Phytochemicals are naturally occurring and are believed to be effective in combating or preventing diseases due to their antioxidant effect (Haliwell and Gutteridge, 1992; Ejele *et al*., 2012). A single plant may be used for the treatment of various disease conditions depending on the community. Several ailments including fever, asthma, constipation, esophageal cancer and hypertension have been treated with traditional medicinal plants (Saganuwan, 2010). The plants are applied in different forms such as poultices, concoctions of different plant mixtures, infusions such as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral, nasal (smoking, snuffing or steaming), topical (lotions, oils or creams), bathing or rectal (enemas). Different plant parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Rojas *et al.*, 2001; Rois and Recio, 2005;Adekunle and Adekunle, 2009). Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhea and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal health (Levy, 1998; Van den Bogaard *et al.*, 2000; Smolinski *et al*., 2003). Unfortunately, rapid explosion in human population has made it almost impossible for modern health facilities to meet health demands all over the world, thus putting more demands on the use of natural herbal health remedies. Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *staphylococcus aureus*, *helicobacter pylori*, and *MDR Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties (Voravuthikunchai and Kitpipit, 2003). In addition, the increase in cases of opportunistic infections and the advent of Acquired Immune Deficiency Syndrome (AIDS) patients and individuals on immunosuppressive chemotherapy, toxicity of many antifungal and antiviral drugs has imposed pressure on the scientific community and pharmaceutical

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companies to search for alternative and novel drug sources. Thus objective of this study is to investigate phytochemical constituents and nutritional characteristics of ethanolic extracts of *Ricinodendron Heudelotii*, so as to ascertain suitability of its use as herbal medicine and food.

MATERIALS AND METHODS

Sample collection and identification: The bark and leaves of Njangsa (*Ricinodendron Heudelotii*) were collected from an open area in Benson Idahosa University, Edo State, Nigeria. The plant was identified and authenticated at the department of Pharmacognosy, Faculty of Pharmacy, University of Benin. The plant materials were rinsed in water, and then air dried for about two weeks. The dried leaves and bark was ground using electric blending machine in the Pharmacognosy Department of University of Benin, and the powdery sample was packed into containers prior to extraction and analysis.

Extraction: 150g of the crushed bark and leaves were measured and placed in containers. Absolute ethanol was used to soak the samples for 72hours and supernatant was filtered using a muslin cloth and the solvent evaporated using a rotary evaporator. The dried extract was weighed and kept in the refrigerator at temperature of -4^{o} C for a period of 48hours.

Qualitative Analysis of Phytochemicals: Phytochemicals are bioactive constituents of medicinal plants which are not nutrients but very useful to the plants. Some bioactive constituents of ethanolic extract were analysed qualitatively for flavonoids, tannins, Cardiac glycosides, Saponin, Steroids, terpenoids, anthraquinones, alkaloids and reducing sugars. Phytochemical screening was carried out on the extracted sample using standard procedure to identify the secondary metabolites (alkaloids, tannins, saponins, glycocides etc) using standard experimental procedure (Harbone, 1973; Sofowora,1993 and Trease and Evans, 1989)

Determination of Flavoniods Content: Two methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973). 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Two drops of 1% aluminum solution were added to portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

Determination of Tannins Content: 0.5g of the dried powered sample was boiled in 20ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green to a blue-black coloration observed.

Determination of Cardiac glycosides Content (Keller-Killani test): 5 ml of each extracts was treated with glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring is formed just gradually throughout the layer.

Determination of Saponins Content: About 2g of powered 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 shaken vigorously for a stable consistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously; emulsion was formed indicating the presence of saponins.

Determination of Steroids Content: 2ml of acetic anhydride was added to 0.5g of ethanolic extract of each sample with 2ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Determination of Terpenoids Content (Salkowski test): 5ml of each extract was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed which is a positive test for the presence of terpenoids.

Determination of Alkaloid Content: 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Determination of Reducing Sugar Content: 1ml of extract (filtrate) was added to boiling fehling's solution A and B in test tube. Color change from blue to green indicated that reducing sugar was present.

Proximate Analysis: The proximate composition (moisture, fat, protein, ash, crude fibre and carbohydrate) of powdery samples of *Ricinodendron Heudelotii* were determined according to standard procedures outlined by the Association of Official analytical Chemist (AOAC, 1984; 1990).

Determination of Moisture Content: 5g of the fresh samples was weighed in a crucible. The crucible was weighed and placed in an oven at 105^0C for 3h, until a constant weight for the samples were gotten.
Moisture percentage was then calculated as follows:

$$moisture\ percentage = \frac{intial\ weight - final\ weight}{weight\ of\ sample\ used} \times 100$$

Determination of Crude Fat Content: The empty round bottom flask was weighed (initial weight).10g of the sample was placed inside a soxhlet extractor and n-Hexane used as extracting solvent. After the extraction process was completed, the round bottom flask was dried in an oven, and then the final weight was measured using a digital weighing balance. The percentage crude fat was calculated as shown below

$$crude\ fat\ percentage = \frac{intial\ weight - final\ weight}{weight\ of\ sample} \times 100$$

Determination of Ash Content: The crucible was weighed and 5g of sample was placed in it, and then later placed in a muffle furnace at 600^0C for 6h. The ash obtained was allowed to cool and then it was weighed. Ash content was then calculated as follows:

Determination of Anthraquinon

$$Percentage\ Ash = \frac{intial\ weight - final\ weight}{weight\ of\ sample} \times 100$$

Determination of Crude Protein Content*:* The crude protein content of the samples was determined using the Microkjeldahl method of AOAC (1984), which involved protein digestion and distillation. The percentage crude protein was calculated from the %nitrogen as follows:

$$\%crude\ protien = \%N \times F$$

Where; F (conversion factor) is equivalent to 6.25.
Determination of Crude Fibre Content: The crude fibre was determined using the method of (AOAC, 1990) (method 14: 020). The percentage crude fibre was calculated as per the formula:

$$Percentage\ crude\ fibre = \frac{weight\ after\ drying}{weight\ of\ sample} \times 100$$

Determination of Mineral Elements Content (AOAC, 1990): Mineral elements estimation indicates the amount of inorganic elements present in the sample. The determination was carried out using standard procedures. During the determination, the sample was first ashed and dissolved in a solvent, and the resultant solution aspirated into air-acetylene flame. The mineral elements determined were; iron (Fe), zinc (Zn), manganese (Mn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), phosphorous (P) and copper (Cu) and this was done by spectrophotometric methods, using flame emission spectrophotometer for sodium (Na) and potassium (K) and atomic absorption for the others.

RESULTS AND DISCUSSION

The phytochemical content of the leaf and bark of *Ricinodendron Heudelotii* are shown below

Table 1 Phytochemical Constituent of Ethanolic Extract of Leaf and Bark of *R. Heudelotii*.

Test	*Ricinodendron* leaf (ethanol)	*Ricinodendron* Bark (ethanol)
Flavonoids	+	+
Tannins	+	++
Cardiac glycosides	+++	+
Saponins	-	+
Steroids	+	++
Terpenoids	++	++
Anthraquinones	-	-
Alkaloids	-	-
Hager's		
Wagner's	-	++
Fehlings A and B	++	+++

KEY: Highly present = +++, moderately present = ++, lightly present = +

Table1 shows that flavonoids, tannins, cardiac glycosides, terpenoids and reducing sugars were present in ethanol extracts of the leaves of *Ricinodendron Heudelotii*. Alkaloids (Wagner's) were absent, while saponins and terpenoids were detected. Flavonoids, tannins, cardiac glycosides, terpenoids and reducing sugars were present in ethanol extracts of the leaves of *Ricinodendron Heudelotii*. Alkaloids (Wagner's) were absent in the ethanol extract of the leaves, while saponins and terpenoids were detected. Anthraquinones and alkaloids (Hager's) were absent in the leaves and bark.

Steroids were detected in the ethanol extract but anthraquinones and alkaloids (Hager's) were found to be absent.

Anthraquinones were found to be absent in ethanol extracts of all the samples. Anthraquinones are known to be insoluble in water and cold organic solvents but soluble in hot organic solvents, it is almost completely insoluble in ethanol near room temperature but 2.25g will dissolve in 100g of boiling ethanol (Macleod and Allen, 1934).

Herbal preparations of the leaves of *Ricinodendron Heudelotii* which are traditionally used for various medicinal purposes (as a disinfectant, in treating tonsillitis, ophthalmic, stomach and back pain and as a poison for arrows) was found to contain flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids, and alkaloids in this study. Plants generally contain chemical compounds called secondary metabolites, which are biologically active (Soetan and Oyewole, 2009). Secondary metabolites may be applied in nutrition and pharmacologically active agent (Soetan and Oyewole, 2009). Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo, 2011; Edeoga et. *al*; 2005). Therefore, *Ricinodendron Heudelotii* leaf is a potential source of drugs. Anibogu (1999) reported that *Ricinodendron Heudelotii* has no known toxicity. Also the bark of *Ricinodendron Heudelotii* are traditionally used to treat pain, rheumatism, diarrhea, stomach problems, edema in children, infertility rashes, mouth sores, chest problems and as a galacticusogogue. These medicinal properties may be due to the presence of flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids and alkaloids

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Table 2: Results Proximate Analysis

Samples	Moisture	Crude Protein	Crude Fat	Ash	Crude Fibre	Carbohydrate
Ricinodendron leaf	4.00	5.00	3.00	4.00	78.22	5.78
Ricinodendron bark	11.89	9.85	4.40	2.60	64.00	7.26

Table 2. shows that leaves of *Ricinodendron Heudelotii* has 4.00% moisture, 5.00% proteins, 3.00% crude fat, 4.00% ash, 78.22% crude fiber and 5.78% carbohydrates. These value for crude fiber is comparable to the value obtained by Adebisi and Oyeleke (2009) (moisture-5.77%, fat-12.21%, carbohydrate-30.93%, and crude fiber-37.3%), while the values for crude fat and carbohydrates are close to the results obtained by Isah, *et al.* (2013) (moisture-74.60%, protein-4.96%, crude fat-0.31%, ash-3.75%, crude fiber-7.51% and carbohydrate-8.87%). The results indicate that the leaves of *Ricinodendron Heudelotii* is a good source of fiber, minerals (ash) and proteins, but not a very good source of energy due to low carbohydrate and fat content.

Proximate analysis of the bark of *Ricinodendron Heudelotii* yielded 11.89% - moisture, 9.85%-protein, 4.40%-crude fat, 2.60%-ash, 64.00%-crude fiber, and 7.26%-carbohydrate. These values obtained show that the bark of *Ricinodendron Heudelotii* is a good source of minerals (ash), crude fiber and carbohydrate, but a poor source of protein and crude fat. Results gotten from the leaves and bark extracts showed that, the leaves of *Ricinodendron Heudelotii* are a better source of proteins than the bark; whereas the bark is a better source of carbohydrates than the leaves.*R.Heudelotii* is a source of many nutrients and biologically active compounds that include omega -3- fatty acids, essential amino acids, minerals and antioxidant vitamins (Besong et al; 2011).The leaves are used as important source of high quality folder for sheep and goat in dry season, the values of crude protein determined in this study (5.0% for the leave and 9.9% for the bark) is relatively lower than 16.0% obtained for green foliage of *R. heudelotii* (Anigbogu, 1996). Manga et. al (2000) reported a total fat content of *R. heudeloltii Vernels* from various locations in Cameroon ranging from 50.0 to 65.2%; being by far higher than 1.8% and 2.0 % determined in this study for the leaves and the bark of the *R. heudeloltii* respectively. However, it is generally understood that fat content of seed is higher than that of leaf of a particular plant.

The results reveal that *R. heudSelotii* is rich in fibre; yielding crude fibre content of 78.22% for the leaf and 64.0% for the bark.

However, the study reveals that R. heudololtis leaf and bark could be a good source of crude fibre because the value determined (41.00% for the leave and 20.50% for the bark) is by far more than values determined by other authors for plants, even vegetables. The crude fibre for twelve (12) common vegetables consumed in Edo State Nigeria, ranged between 0.6 -25.5g/dry weight (Mensah *et al*; 2008). Shankutala and Shadaksharaswamy(1987) found the fibre content of common table fruits (banana, guava, mango, orange, pawpaw and pineapple) to range between 0.2-5.2g/100g while the value for the wild plants (*A. digitata, liafzelli and M. africana*) ranged between 27.33-29.48g/100g (Uzoekwe and Agatemor, 2010). The high fibre content of the analyzed plant leave and bark suggest that the sample contains high amount of polysaccharides such as cellulose and hemicellulose. Thus the leaves and bark of the plant could be useful in prevention of cancer of the colon (WCRF,1997; Block *et al*; 1992).

Table 3: Result of Mineral Composition (mg/100g)

Parameters	Ricinodendron Leaves	Ricinodendron Bark
Calcium	5932	4409
Magnesium	1994	2431.20
Sodium	0.00	0.00
Potassium	0.00	0.00
Iron	32.30	15.60
Zinc	18.60	11.50
Copper	22.70	19
Manganese	41.30	27.70
Phosphorous	688	249

Table 3 depicts that *Ricinodendron Heudelotii* leaves were found to have relatively higher quantities of calcium, magnesium and phosphorus. Iron, zinc, copper and manganese were present but not in very high quantities. Sodium and potassium however, were absent. Calcium, magnesium and manganese which are present in high concentrations are essential minerals for life; they are important in the formation of bones and teeth as a cofactor for enzymes and a component of ATP, DNA, RNA and cell membranes respectively. The minerals present in low concentrations (iron, zinc, copper and manganese) perform various important functions in humans like the formation of hemoglobin, growth and sexual maturation, facilitating iron intake, as cofactor for enzymes and so many other functions (Mensah et al, 2008).The bark of the plant was found to contain all the minerals analyzed with calcium and magnesium present in the highest concentrations.

Conclusion: The high content of crude fibre, protein, calcium, iron, potassium in *Ricinodendron Heudelotii* makes it rich in nutrients and it can serve as potential nutritious food supplement to improve the health status of its consumers. Also, the presence of phytochemicals in the plants is evidence that it has high medicinal properties.

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