Proximate Composition, Phytochemical Analysis and Elemental Characterization of *Raphia taedigera* Seed

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Abstract

The proximate, qualitative and quantitative phytochemicals, and elemental analysis of *Raphia taedigera* seed for useful bioactive contents were determined. The results showed the seed contained 6.56 %, moisture; 1.96 %, ash; 4.56 %, protein; 7.50 %, crude fibre; 5.30 %, fats and 74.12, % carbohydrate. Qualitative screening of the ethanolic extract of the seed revealed the presence of alkaloids, phenols, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, reducing sugars and amino acid. Quantitatively the seed contained 4.825 mg/100g, alkaloids; 0.418 mg/100g, phenols; 0.319 mg/100g, tannins; 5.190 mg/100g, saponin and 1.651 mg/100g, flavonoid. Elemental analysis using proton-induced x-ray emission showed the seed contained 18.6 ± 2.8, sodium (Na); 2864.7 ± 30.4, magnesium (Mg) 158.9 ± 7.6, aluminium (Al); 885.4 ± 8.1, silicon (Si); 610.8 ±11.8, phosphorus (P); 587.3 ± 8.0, sulphur (S); 1527.9 ± 8.7, chlorine (Cl); 28996.6 ± 31.9, potassium (K); 18070.6 ± 77.7, calcium (Ca); 53.6 ± 8.8, titanium (Ti); 19.9 ± 8.1, chromium (Cr); 112.2 ± 9.7, manganese (Mn); 128.4 ± 6.9, iron (Fe); 9.1 ± 4.0, zinc (Zn) and 51.3 ± 15.2, strontium (Sr). The high content of carbohydrate in the sample shows that

it can serve as an alternative source of energy as feed and for ethanol production. The presence of different ranges of phytochemicals revealed how significant it can be as medicinal use. High concentrations of some of the elements revealed how useful the seed can be to promote various physiological activities in the body.

Keywords: Phytochemicals, PIXE, proximate, Raphia taedigera

Introduction

Biotic community is predominantly of trees, shrubs and other woody vegetation, usually with a closed canopy that exist naturally for millions of years. Forests cover a third of all land on Earth, providing vital organic infrastructure for some of the planet's densest, most diverse collections of life. Arthur (2009) estimated the number of living plants as well above 390,000 with flowering plants as 90%, Conifers as 0.27%, Ferns and horsetails as 3.8% and Mosses 5.85% with at least 250,000 of them have not been assessed for their industrial, medicinal of agricultural potential (Arthur, 2009). Forest resources are required to meet needs of the people and contribute to environmental stability. People began life on this planet as forest dwellers. They were food gatherers and depended on the forest for all their needs: food, clothing, and shelter. They gradually became food growers, clearing a small patch in the forest to grow food. Over time, some plant species became favourites to man owing to their sweetness, beautifying nature, scent and medicinal attribute. In Nigeria, thousands of plant species exist in the forest, many of which yet to be tapped or evaluated for their potential usefulness. The huge potential of the enormous medicinal plant resources to boost rural economy and supplement traditional therapies has remained untapped in this

part of the world, considered to be a treasure trove of medicinal plants. Information on the current status and trend of forest resources is necessary to understand the spatial and temporal dynamics for their sustainable development. Plants, animals, fungi, and microorganism are instrumental in many environmental processes essential to human existence. Humans depend on the contributions of thousands of living species for their survival. Forests are not just a potential source of lumber; the forest provides watersheds, from which we obtain fresh water, control the number and severity of local floods and reduce soil erosion.

On the other hand, *Raphia taedigera* is a cespitous, monoecious, monocarpic species (Moore, 1973) of about 4 – 12 m tall and 25 - 40 cm in diameter when fully matured. It is usually covered by the persistent foiler bases and surrounded at the base by a thick mass of pneumatophores branched. The leaves which are about 1.5 m long unarmed petioles, ascend with curved apex, up to more than 10 m long, with 100 – 200 pairs of lanceolate linear pinnules, up to 1.2m long, slightly dropping, irregularly arranged and on different angles giving the leaf a feathery look (Correia, 1926-1928; Allen, 1965b). *R. taedigera* reproduces by seed taking about a year before germination and grow in swamp forest along streams. The plant is native to Nigeria, Brazil, Cameroon, Costa Rica, Panama and Nicaragua (Bailey, 1935; Allen, 1965 a; Henderson, 1995; Dransfield et al, 2008; Anderson and Mori, 1967). When matured, the tree produces fruits of about 5-7cm long and of 3-4 cm in diameter, covered by imbricate glossy reddish-brown scales containing one seed only. A well dried seed is hard with brown outer part and white shiny inner and with egg-size.

Before now several accounts on the use of *R. taedigera* have been reported. These include the use of leaves for covers by the Amazonian, stems and petioles for walls in rural construction (Henderson, 1995). Others are the use of petioles for fabricating traps for crustaceans and fishes; oil from the pulp of the fruits is used for soap making and as medicine for frictions against rheumatism. The cleaned and polished seeds are also used as jewellery.

In Nigeria, R. taedigera present in abundance in the southern region and is popularly called "Ope Oguro" among the Yorubas. The tree is popular for the unique taste of the wine (Oguro) obtained from a matured tree and use as drinks for relaxation, requirement for traditional weddings and as medicine for measles by the rural populace. However, despite its popularity among the local people, no research to date to the best of my knowledge addresses the role of *Raphia taedigera* seed in a wider cultural and socioeconomic system and its relevance in the field of food science, medicine, agriculture, materials science and industrial application. Therefore, the aim of the present study is to carry out the proximate analysis, phytochemical (qualitative and quantitative) analysis, and elemental analysis using Proton Induced X-ray Emission (PIXE).

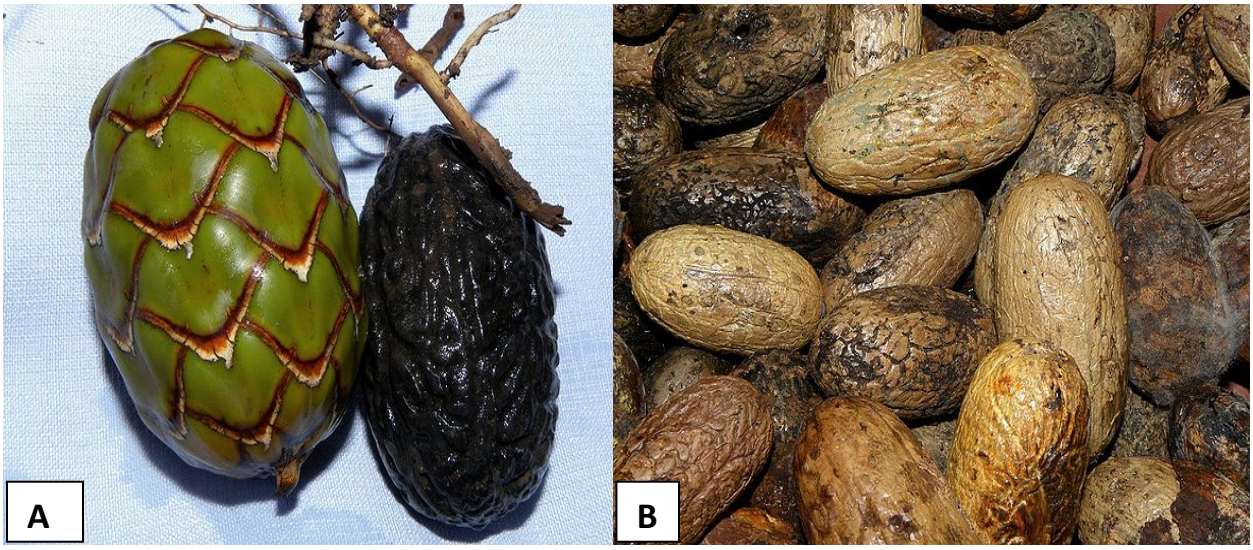

Figure 1: A - Fresh seed of *Raphia taedigera.* B - Dried seed of *Raphia taedigera*

Materials and Method

Sample Collection

Seeds of *Raphia taedigera* were collected from the farmland at Ise-Ekiti, in Ekiti, western part of Nigeria. The seeds were collected in a polythene bag and transported to the laboratory for further analysis.

Sample Preparation

The seeds were washed under running water and air-dried. Observations such as colour, shape and weight were made on the sample and noted. The dried samples were taken to the department of mineral resources engineering where they were crushed using 911MPE-24 laboratory crusher. The sample was further made into powder form using blender and sieved through a 100-mm mesh Tyler sieve. The pulverized sample was stored in an airtight plastic container.

Methods

Proximate Analysis

Moisture content, ash content, ether extract, crude fibre and protein content were determined using methods described by AOAC (AOAC, 1994). The carbohydrate content was determined by difference using equation 1 below:

$$\%\text{Carbohydrate} = 100 - \%(\text{Moisture content} + \text{ash content} + \text{ether extract} + \text{crude fibre} + \text{protein content}) \quad (1)$$

Phytochemical Analysis

Phytochemical screening of the sample was carried out by the methods reported by Abegunde and Ayodele-Oduola (2015) and summarized below.

Table 1: Methods of Qualitative analyses of some Phytochemical Parameters

Phytochemical	**Method**	**Observatory Colour**
Alkaloids	Wagner's Test	Formation of reddish brown precipitate.
Reducing Sugar	Molish's Test	Formation of a red or dull violet colour at the interphase of the two layers.
Cardiac Glycosides	Keller Kelliani's Test	A brown ring at the interface indicated the presence.
Flavonoid	Alkaline reagent Test	Formation of intense yellow colour, which becomes colourless on the addition of dilute hydrochloric acid
Phenols	Ferric chloride Test	Formation of deep blue or black colour.
Amino Acid	%Ninhydrin solution in Acetone	Formation of purple colour

Saponins	Foam Test	Formation of persistent foam
Sterols	Liebermann- Burchard Test	Formation of dark pink or red colour.
Tannins	Braymer's Test	Formation of blue or greenish colour solution
Terpernoids	Salkowki's Test	A reddish brown precipitate

Phytochemical Quantification

Parameters quantified are phenol, tannin, alkaloid, flavonoid, and saponin.

Determination of Flavonoid

10 g of the pulverized seed sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature for about 30 minutes. The resulting solution was filtered using Whatman filter paper No 45. The filtrate was transferred quantitatively into a crucible and evaporated to dryness on a water bath and the weight was determined (Bohn and Kocipai-Abyazan, 1994).

Determination of Tannins

0.1 g of the seed sample was weighed and transferred into a 250 ml conical flask. 100 ml of distilled water was added and boiled for about 1 hour. The boiled solution was diluted with distilled water to 100 ml and filtered. 5.0 ml was measured out of the filtrate and added to 10 ml of freshly prepared 17% sodium carbonate. 2.5 ml of Folin Denis reagent was added to the solution in a test tube. The resulting solution was allowed to stand for 20 min for colour development. Thereafter, the absorbance was taken at 520 nm using JENWAY 6305 UV

spectrophotometer. The blank and standard solutions of tannic acid without the sample material were also prepared. The standard tannic acid curve was prepared and used to determine the amount of tannin in the sample (Harborne and Williams, 2000).

Determination of Alkaloids

Alkaloids were determined quantitatively using the method reported by (Harborne and Williams, 2000). 200 mL of 10 % acetic acid in ethanol was added to 5 g of the sample, covered and allowed to stand for about 4 hours, then filtered. The filtrate was concentrated on a water bath to about one-fourth of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the formation of precipitate was completed and the solution was allowed to settle. The precipitates obtained were washed with a dilute ammonium hydroxide solution and filtered. The residue (precipitate) was dried and weighed.

The alkaloid content was determined using equation 2.

$$\% \text{ alkaloid} = \frac{\text{final weight of the sample}}{\text{initial weight of the extract}} \text{ x } 100 \qquad (2)$$

Determination of Saponins

Quantitative determination of saponins was carried out using the method described by (Obadoni and Ochuko, 2001). 2 g powdered seed sample was added to 100 ml of 20 % aqueous ethanol and shaked on a shaker for 30 min. The sample was then heated over a water bath at 55^{o}C for 4 hours. The mixture was filtered and, the residue re-extracted with 200 ml of 20% aqueous ethanol. The combined extract was reduced to about 40 ml on a water bath at 90°C. The concentrate was transferred into a separatory funnel (250 ml) and extracted twice with 20 ml diethyl ether. The ether layer was discarded while 60 ml *n*-butanol was added to the aqueous

layer. The *n*-butanol extract was washed twice with 10 ml 5% aqueous sodium chloride. The remaining solution was heated over a water bath. After the evaporation process, the sample was dried in an oven at 40°C to a constant weight.

Determination of the Total phenolic

Quantitative determination of total phenolic contents was done using the method of (Singleton & Rossi, 1965). 200 µl (1-5 mg/ml) of the powdered seed and each concentration standard (50-200 mg/L) were added in ten milliliters of 1:10 Folin-Ciocalteau reagent. The mixture was mixed thoroughly and incubated for 5 min. 7 ml of 0.115 mg/ml Na_2CO_3 was added to each of the solutions. The resulting solution was incubated further for about 2 hours before absorbance was taken at 765 nm. Calibration curve from different concentrations of gallic acid was prepared. Results were expressed as mg gallic acid/g dry plant material.

Elemental Analysis

Elemental analysis of the seed was carried out using Proton Induced X-ray Emission technique described by (Agaja and Obiajunwa, 2012).

Results and Discussion

Table 2: Result of proximate analysis of *Raphia taedigera* seed

Parameter	Moisture Content	Ash Content	Protein Content	Crude fibre Content	Fat Content	Carbohydrate Content
Percentage (%)	6.56	1.96	4.56	7.50	5.30	74.12

Table 3: Result of qualitative and quantitative phytochemical analysis ethanolic *Raphia taedigera* seed extract.

Parameters	Phytochemical Screening	Quantitative Phytochemical (mg/100g)
Alkaloids	✓	4.825
Phenols	✓	0.418
Tannins	✓	0.319
Saponins	✓	5.190
Flavonoids	✓	1.651
Sterols	X	ND
Terpenoids	✓	ND
Cardiac glycosides	✓	ND
Reducing sugar	✓	ND
Amino Acid	✓	ND

✓ indicates present, X indicates absent, ND = Not determined

Table 4: Result of Elemental Analysis using Proton Induced X-ray Emission

Element	Conc (ppm)
Na	18.6 ± 2.8
Mg	2864.7 ± 30.4
Al	158.9 ± 7.6
Si	885.4 ± 8.1

P	610.8 ±11.8
S	587.3 ± 8.0
Cl	1527.9 ± 8.7
K	28996.6 ± 31.9
Ca	18070.6 ± 77.7
Ti	53.6 ± 8.8
Cr	19.9 ± 8.1
Mn	112.2 ± 9.7
Fe	128.4 ± 6.9
Zn	9.1 ± 4.0
Sr	51.3 ± 15.2

Values±SD represents the mean of triplicate determinations ± standard deviation

Discussion

The results of proximate analysis of dried *Raphia taedigera* seed are shown in Table 2. The moisture content of the dried seed was 6.56 %, which is considered to be moderate. Moisture content is an important parameter in food analysis. It plays an important role during food storage and also in industrial processes such as drying and milling (Lubatti and Bunday, 1960). Low moisture content in food helps to minimize damage to the sample components and maintain seed viability and therefore beneficial for prolonging the shelf life of the seeds. In this present study, the ash content was computed as 1.96 %. Ash content in seed becomes important in order to determine the minerals present in the seed sample. The protein content of the seed was determined to be 4.56 %. This protein content shows that the seed cannot be totally depended on

as a source of protein. The percentage of crude fibre in the dried seed was 7.50%. This level of crude fibre showed the sample can be used as both human and animal feed. Crude fibre measures the quantity of indigestible cellulose, pentosans and lignin in the sample. The fat content of the seed was determined to be 5.30%. This is relatively low as the plant seed cannot be regarded as oil seed. The seed contained a significant amount of carbohydrate, which was determined to be 78.12% of the sample. This showed *Raphia taedigera* will be a good source of energy for the body. C*arbohydrate* helps to provide energy, as they are the body's main source of fuel, needed for brain function, operation of the organs and physical activity. Therefore, it can be a good option for feed formulation in both humans and animal feed. Also with high carbohydrate content, the seed can be a good raw material for production of ethanol.

The results of qualitative and quantitative phytochemical analysis of the ethanolic extract of *R. taegedera* seed are contained in table 3. Phytochemical screening of the ethanolic extract of the seed revealed the presence of alkaloids, phenols, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, reducing sugar and amino acid. The concentrations of phytochemicals assessed were in order saponin > alkaloids > flavonoids > phenols > tannin. Presence of various phytochemicals of therapeutic values in plant has been given as reasons for curing human and animal diseases (Abegunde and Ayodele-Oduola, 2015). Tannins help to protect body cells against oxidative damage caused by free radicals. Saponins function as anti-inflammatory with expectorant effect. It also enhances the immune response in the body where it acts as adjuvant and prevents cancer cells from multiplying. Phenols prevent blood clotting and exhibits anti-cancer properties. Alkaloids confer antibacterial, antimalarial, antipyretic, antifungal, and antitumoric effects. Terpenoids exhibits antitumoric property, it acts as potent inhibitor of cell division and inhibits the formation of solid tumours in the body. Phenols also act as

anticarcinogenic and the activity has been regarded as being caused by stimulation of detoxification enzymes, ability to block carcinogens from damaging cellular DNA, antioxidant activities, protection against mutagenicity, inhibition of tumour initiation, and delay in tumour promotion (Chung et al, 1998; Mukhtar and Ahmad, 2000; Skene and Philip 2006).

The result of elemental analysis using Proton induced X-ray emission was presented in table 4. The results revealed the presence of elements at appreciable amount. The concentrations of elements quantified were in order K > Ca > Mg > Cl > Si > P > S > Al > Fe > Mn >Ti > Sr > Cr > Na > Zn. The presence of these elements in the plant seed is quite important to serve as valuable feed and medicinal plant. The results suggested that the plant seed is extremely rich in Mg, P, K, Ca, Mn, Fe and Zn. These elements are very significant to human nutrition. Mg, K and Ca are necessary in diet in the body for the repair of worn out cells, formation of strong teeth and bones in man and also necessary in building of red blood cells (WHO, 1996). K and Ca are also useful for prevention and control of diseases. Mn, Fe, S and Zn, are essential in the metabolism of enzymes. Mn plays a vital role in the control of diabetes mellitus (Korc, 1988). Fe forms important complex with oxygen in haemoglobin which plays a vital role in biological activities. Fe deficiency has been reported to be the cause of Plummer-Viinson (Paterson-Kelly) syndrome (Larsson, 1975; Wynder et al, 1957). Zn is a constituent of over 200 enzymes. It plays an important role in the metabolism of nucleic acid, replication of cells and repair of tissues (Pohl, 2013). Na with a well-controlled concentration helps to control blood pressure and regulates the activities of nerves and muscles (Hambidge et al, 1986). Sulphur is an important component of protein. It helps to resist bacteria, cleanses blood and protects protoplasm of cells. Titanium, due to its biocompatibility and bioactive surface, is gaining more popularity in medical sciences. It finds application in neurosurgery, bone conduction, hearing aids, and toe implant and in bone

and joint replacement (Ulbricht, 2012). Chlorine as chloride acts as catalyst manufacturing of hormones, being electrolyte it helps proper functioning of muscles, balances acid and base in blood and combine with potassium to form chloride which produces hydrochloride acid in the body to aid the digestion of protein.

CONCLUSION

In this research, seed of *Raphia taedigera* has been successfully evaluated for proximate, phytochemical and elemental analyses using standard methods. The results of proximate analysis showed that the plant seed will constituent a good component of animal feed to supply energy. The Presence of various phytochemicals in the seed pointed to the direction that it has therapeutic values if the toxic nutrients are selectively and carefully removed. The high concentrations of alkaloids and saponins indicated that the seed can be used as anti-inflammatory, to enhance immune response, prevents cancer cells from multiplying, antibacterial, antimalarial, antipyretic, antifungal, and antitumor. Elemental composition of the seed revealed that the seed possessed numerous useful minerals useful for various human physiological activities. With the result of this analysis, it can be inferred that *Raphia taedigera* offers a range of potential for food and feed, therapeutic values and materials science.

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