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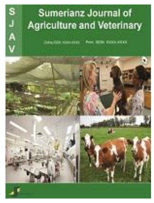

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Nutritional and Phytochemical Composition of *Rothmannia longiflora* Seed

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Abstract

Many plant species, especially less-known and underutilized ones are evaluated for their medicinal application, nutritional profile and energy potentials. This study was aimed at evaluating the nutritional, anti-nutritional, medicinal as well as energy potentials of *Rothmannia longiflora* seeds. The results obtained from proximate analysis seeds were; Moisture content (7.17±0.58%), Ash content (8.67±0.29%), Crude lipid (24.87±0.76%), Crude protein (4.62±0.12%), Crude fiber (2.33±0.29%), Available carbohydrate (59.51±0.02%), Energy value (480.15kcal/100g), Organic matter (84.16±0.59%), Carbon content (48.87±0.14%), C/N (33.20) and Nitrogen content (0.74%). The mineral analysis showed potassium as being predominant with value (180.00±6.25mg/100g) followed by sodium (10.75±0.4mg/100g), Calcium (5.21±1.13mg/100g), Magnesium (4.22±1.33mg/100g) and Phosphorus(0.65±0.002mg/100g) in that order while remaining elements were found in trace concentrations. The anti-nutritional factors analysed were oxalate (0.0045±0.08mg/100g), phytate (4.65±0.082mg/100g), cyanide (0.086±0.00mg/100g), tannins (0.013±0.001mg/100g) and nitrate (1.54±0.001mg/100g). The phytochemical constituents of the seed oil however revealed the presence of terpenoids, flavonoids, saponins, steroids, carotenoids, alkaloids and cardiac glycosides while showing absence of tannins, phlobatannins, phenols and anthraquinones. The results of the study indicated that *Rothmannia longiflora* seeds could be a good source of lipids, mineral elements and could as well find application in energy while the seed oil could also be an important material for traditional medicine and other industrial purposes.

Keywords: *Rothmannia Longiflora* seed nutritional; Phytochemical; Energy.

1. Introduction

There is increasing evidence that consumption of vegetables and fruits is associated with reduced risk of degenerative diseases such as cancer, cardiovascular diseases and cataracts [1]. This association is often attributed to natural antioxidants that are present in fruits and vegetables. These antioxidants include vitamin C, and E, carrotenoids, phenolic acids and flavonoids which prevent free radical damage [2]. Epidemiological studies have established a positive correlation between intake of fruit and vegetables and prevention of diseases like clerosis, cancer, diabetes, arthritis and ageing [3].

The proximate composition of a food substance among others is critical to an understanding of the usefulness of such food materials to human and animal nutrition in order to explore the nutritional content of indigenous plants which are less-known and underutilized species. Such studies are helpful in highlighting their food values and therefore encourage their ultimate utilization in diverse ways. Considering the upsurge in the price of livestock feeds and their increasing demand, this study was conducted to provide information on the nutritional, mineral, anti-nutritional as well as energy potentials of *Rothmannia longiflora* fruit seeds which is often ignored and considered as a waste, so that it can be domesticated for proper utilization as livestock feed or as a supplement in animal food formulation and energy generation.

Rothmannia longifloa is a member of the family *Rubacea*, which is a shrub or small tree of up to 9m in height. The fruit is a globose to ellipsoid shaped berry, 3.5-7cm x 5-6cm, greenish-black; with 10 indistinct ribs, glabrous, many-seeded, calyx persistent, the seed lens-shaped, 6-8mm x 1-1.5mm, brownish-red [4]. The plant is known locally as "Katambiri" in Hausa language, "Aberekamwo" in Igbo land and "Iroro" in Yoruba land [5]. The fruit is commonly used in Africa to make blue-black markings on the hands, face and body, sometimes to imitate tattooing. In Nigeria a dye and an ink-like extract (Katambiri) is made from finely crushed seed [6]. This study is therefore

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aimed at evaluating the nutritional, mineral, anti-nutritional and energy potentials of the fruit seed of *Rothmannia longiflora*in order to establish purposeful utilization of this less-known or underutilized plant.

2. Materials and Methods

The fruits were collected from different branches of selected trees at Wanke bush along Lungu road in Shagari Local Government area of Sokoto State of Nigeria, using methods adopted by Ayaz, *et al.* [7], Asaolu-Barko and Asaolu [8]. The fresh fruit samples were authenticated at the Herbarium unit of the biological sciences department of Usmanu Danfodiyo University, Sokoto. The seeds were separated manually from the peel, then air-dried and mechanically grounded into fine powder using a blender. The powder was stored in a covered plastic container for analysis.

2.1. Proximate Analysis

The moisture content was determined at 105^{0}C in an oven, ash content was determined at 550^{0}C. crude protein, lipid and fiber were determined in accordance with AOAC [9] procedures. Crude nitrogen was determined using the Kjeldahl procedure and crude protein value was obtained by multiplying nitrogen value by a factor of 6.25, while available carbohydrate was estimated by difference in accordance with the equation below:

$$CHO = 100 - (\%Ash \pm \%\,crude\ protein \pm \%\,crude\ lipid \pm \%\,crude\ fiber)$$

$$Energy\ value\ (Kcal) = [(\%\,CHO \times 4) \pm (\%\,crude\ protein \times 4) \pm (\%crude\ lipid \times 9)]$$

(Hassan, *et al*; 2009)

2.2. Mineral Analysis

A sample of 0.5g was put into Kjeldahl digestion flask to which 24cm^3 of a mixture of concentrated nitric acid (HNO_3), Conc H_2SO_4 and 60% $HClO_4$ (9:2:1 V/V) was added. The flask was allowed to stand overnight to prevent excess foaming [10]. The flask was put on a heating block and digested to a clear solution, cooled and the content filtered into 50cm^3 volumetric flask. The solution was then diluted. Blank solution was prepared in a similar manner without the sample being added. The solution was used for mineral analysis. The mineral content (calcium, magnesium, iron, zinc, copper, manganese, lead, chromium, cobalt and cadmium) were determined using atomic absorption spectrometry (A.A.S). Sodium and potassium content were determined using atomic emission spectrometry while phosphorous was determined by colorimetry using Vanadium molybdate (blue) method [9]

2.3. Anti-Nutritional Analysis

The method of Ola and Obah [11] was adopted for determination of phytate. Hydrocyanic acid was determined by AOAC [12] method. Oxalate and nitrate were determined by the method of Krishna and Ranjhan [13]. For determination of tannins the method of Van-Burden and Robinson [14] was employed

2.4. Phytochemical Analysis of Extracted Oil

The phytochemical constituents of crude oil such as tannins, phlobatannins and steroids were determined in accordance with procedures described by Safowora [15], while anthraquinones, flavonoids ,cardiac glycosides were determined by methods of Haborne [16]. Phenols, cumarins and carotenoids were however determined by the approach of Sani, *et al.* [17], Also terpenoids and saponins determination followed the approach of Mbatchon and Koosono [18], then alkaloids using methods described by El-Olemy , *et al.* [19].

2.5. Determination of Nitrogen Content

Two grams (2g) of the dried sample was weighed into Kjeldahl digestion flask and 0.5g of Kjeldahl tablet was added, followed by addition of 10 cm^3 of concentrated tetraoxosulphate(VI) acid. The content was then heated in Kjeldahl digestion unit until the digest became clear after approximately 2 hours. After the digest had been completed, the flask was cooled, diluted with 10cm^3 distilled water and filtered with a Whatman No1 filter paper in a

100cm^3 volumetric flask and made up to the mark with distilled water. 10cm^3 of homogenous aliquot solution was pipetted into the distillation flask and 20cm^3 of 45% NaOH solution was added. The content was then diluted to about 200cm^3 with distilled water and distilled using micro-kjeldahl distillation apparatus. The distillate was collected in a receiving flask containing 10cm^3 boric acid indicator solutions. After the distillation, the distillate was titrated with standardized 0.01M HCl to the end point. Blank was determined using all the reagents in the same quantities as described above. The process was carried out in triplicate and the crude protein (CP) calculated using the formula [20] Thus:

$$\%N = \frac{(S - B) \times 0.1M\ HCl \times 0.014 \times D \times 100}{\text{weight of sample } \times V}$$

2.6. Determination of Organic Matter

The organic matter (volatile solid) was determined by subtracting the percentage of moisture and ash content from 100% in accordance with Garba [21].

$$Organic\ matter = 100\% - (\%Ash + \%Moisture\ content)$$

2.7. Determination of Percentage Carbon

The percentage carbon was estimated using equation as adopted by Abba, *et al.* [22]

$$\%C = 0.5 \times organic\ matter$$

2.8. Determination of Carbon-Nitrogen Ratio

The carbon to nitrogen ratio was evaluated by calculating the ratio of organic carbon content to that of nitrogen content [23]

$$C{:}N = \frac{\%\ Organic\ carbon\ in\ the\ sample}{\%\ Nitrogen\ in\ the\ sample}$$

Abba, *et al.* [22]

3. Results of Analysis

Table-1. Proximate Composition of *Rothmannia longiflora* Seeds (Dry Weight)

Parameters	Concentration (%)
Moisture(%wet weight)	7.17±0.58
Ash	8.67±0.29
Crude protein	4.62±0.12
Crude lipid	24.83±0.76
Crude fibre	2.33±0.29
Available carbohydrate	59.51±0.02
Energy Kcal/100g	480.15
Organic matter	84.16±0.57
Carbon content	48.87±0.14
C:N	33.20
N	0.74

The data are mean ± standard deviation of triplicate results.

Table-2. Mineral Composition of *Rothmannia longiflora* Seeds

Mineral elements	Concentration in DW (mg/100g)
Sodium	10.75±0.41
Potassium	180±6.29
Phosphorous	0.65±0.002
Calcium	5.21±1.18
Magnesium	4.22±1.33
Zinc	0.04±0.02
Iron	0.12±0.37
Copper	0.02±0.1
Chromium	0.03±0.02
Manganese	0.04±0.01
Cadmium	0.002±0.00
Lead	ND
Nickel	ND

ND = Not Detected.

The average moisture content of *Rothmannia longiflora* seed was 7.17% this result compares favourably with 7.00% for *Moringa oleifera* seed [24], but higher than 6.0% for *Tammarindus indica* Seed [25] and 4.25% for melon seed [26]. Also the results obtained was found to be lower than 11.7% for Bambara groundnut [27] and 9.40% for *Dioclea reflexa* [28]. The low moisture content of the seeds would help to enhance its storage life span.

The ash content was 8.67% which is lower than 9.4% for cotton seed [25] but higher than 6.70% reported for melon seed [29], 5.78% for *sphenostylis* [30], 5.00% for *Moringa oleifera* [24]. The high ash content is an indication of high concentration of various mineral elements which speed up metabolic processes and improves growth and development [31, 32]. The ash contents of seeds and tubers should be in the range of 1.5 to 3.5% in order to be suitable for animal feeds. In this case, the ash content was slightly out of the range but it may find application for use in formulating animal feeds by applying appropriate proportions.

The crude protein content of the seed was 4.60% which is comparable to 4.33% for tiger nut as reported by Adel, *et al.* [33]. This result is lower than 6.86% for *gardenia Sokotensis* seed [34]. However, the bioavailability of these proteins to individual subjects is determined by other factors such as genetic and antinutritional contents of the plant food. Obasi, *et al.* [26]. The plant seeds could be incorporated in human and animal nutrition to supplement protein intake.

Thus, according to Kukeera, *et al.* [35], any seed that can give up to 17% of oil yield is considered and as an oil seed plant, as such *Rothmannia longiflora* could be considered as oil seed plant and has potential of suitability to be processed industrially. Since the seeds are higher in crude lipid, it could be a good source of edible vegetable oil if well harnessed and could supplement the conventional sources. Lipid provide body with maximum energy, approximately twice higher than that of protein and carbohydrate and facilitate intestinal absorption and transportation of solute vitamin A,D,E and K. On the other hand, the seeds may find application for use in biodiesel production.

The crude fiber content of 2.33±0.29% obtained from *Rothmannia longiflora* seeds was found to be comparable to 2.41±0.12% for Brebra seeds [36], 2.00±0.2% for *Dioclea reflaxa* [28], but lower than 2.68±0.02% for *Voadzeia subterranean* and 3.85±0.02% for *Spherostylis stenocapa* as reported by Chinedu and Nwinyi [30]. The value obtained is however lower than 3.33±0.62% for *Strychnos innocus Del* reported by Abdulmumin, *et al.* [34]. It is believed that adequate intake of dietary fiber can lower cholesterol level risks constipation, diabetes, colon, and breast cancer [37].

The carbohydrate value of the sample was found to be 59.51±1.02% which is lower than 67.68±0.31% for *Pearsea Americana* mill seed [38] and 98.21±1.15% for pear fruits [39]. However, the value obtained is higher than 39.54±0.06% and 21.51±0.06% for *Punica granatun* fruit seeds from Nigeria and Saudi Arabia as reported by Dangoggo, *et al.* [40]. Similarly, the result obtained is also higher than 50.72% for *Termarindus indica L* seeds [25] and 56.31±0.52% for *Strychnos innocus Del* [34]. These results indicate that, the seed could contribute large energy value as a result of higher lipid contents of the seeds.

The seed was also found to contain 84.16% organic matter which is lower than 93.94% for castor cake as reported by Baladhiya and Joshia [41]. The results for percentage carbon, nitrogen and C/N ratio content of the seeds were also found to be 48.81%, 1.47 and 33.20 respectively. Higher carbon content influences combustion processes and calorific value of solid fuels and low nitrogen content of the seeds could be attributable to the protein nature of the seeds.

Table 2 shows the mineral composition of the seed of *Rothmannia longiflora*, The constituent elements were sodium, potassium, magnesium, calcium, zinc, iron, copper, manganese, lead and phosphorous. Potassium concentration of the seed (180±6.298mg/100g) was higher as compared with 4.941±0.004mg/100g for melon seed [29], but lower than 383.33±12.47mg/100g for *Strychnos innocus Del* [34] and 356.4mg/100g for *Annona diversifolia kanel* [42]. Potassium is an essential nutrient and plays an important role in the synthesis of amino acid and proteins. Potassium plays an important role in the physiological function of the body and helps in maintaining normal water balance in the body [43]. The result obtained is within the recommended daily intake of potassium which is not expected to exceeded 2000mg/day for both men and women and therefore, this plant seed could serve as potential supplement for human potassium requirements. The sodium content in the sample was 10.75±0.41mg/100g which is higher when compared with 0.21±0.005mg/100g for melon seed [29] and 8.31±3.87 for pear fruit [39]. The value obtained is lower than 23.15±0.21mg/100g for wild melon [44] and 35.5mg/100g for *Annona diversifolia Kanel* [42]. Sodium plays a vital role in regulating blood pressure and for proper functioning of muscles and nerves. The recommended daily intake for sodium should not exceed 3300mg/day for health adult and not more than 1500mg/day for individuals with high blood pressure [45]. The plant seed is therefore considered of low sodium content and so could serve as supplement. The phosphorous content of 0.651±0.002mg/100g is relatively higher than 0.27±0.07mg/100g for wild melon seed [44]. However, the value obtained is lower than 2.10±0.00mg/100g for *Strychnos innocus* Del [34] and 5.77±0.007mg/100g for melon seed [29]. Phosphorous functions as a constituent of bones, teeth, adenosine triphosphate (ATP). Phosphorous is an essential macronutrient for plants and one of the three nutrients generally added to the soil in fertilizer application because of its vital role of energy transfer in living organisms and in plants. Calcium concentration in the seed was found to be 5.21±1.18mg/100g. The value is much lower than 30.5±1.6mg/100g for Bambara groundnut [27] and 23.8±0.06mg/100g and 44.00±0.06mg/100g for *Punica granatum* fruit seed from Nigeria and Saudi Arabia respectively [40]. The results was also found to be higher than 0.10±0.002mg/100g melon seeds [29], but comparable to 4.67±0.47mg/100g for *Strychnos innocus* Del [34]. Calcium is a constituent of bones and helps the body to contract correctly, blood to clot and the nerves to convey messages. Calcium is essential for disease prevention and control, and may therefore contribute to medicinal influence of plants [46]. The magnesium content was found to be 4.22±1.33mg/100g in the sample. This result obtained is comparable to 4.5%mg/100g for *Annona diversifolia* seeds but much lower than 15.4±0.75mg/100g for pear fruits [39] and 20.45±0.00mg/100g for melon seeds [29]. Magnesium is beneficial for regulating blood pressure and helps to prevent sudden heart attacks, cardiac arrests and stroke. Like calcium, magnesium is an important component of bones and contributes to its structural development. Zinc in the sample seed was 0.04±0.02mg/100g which is quite lower than other researches as reported for different plants seeds such as 1.9±0.1mg/100g for

Bambara groundnut [27], 16.2±0.06mg/100g and 5.70±0.10mg/100g for *Punica gratum* fruit seeds from Nigeria and Saudi Arabia [40]. Zinc boosts the health of human hair, plays a role in metabolism and also assists in metabolism of vitamin A from its storage site in the liver and also facilitates the synthesis of DNA and RNA that are necessary for cell production [47]. The concentration of iron in the sample was 0.12±0.1mg/100g. The value obtained from the sample was found to be much lower than 7.80±0.01mg/100g for *Cucurbita maxima* [48] and 8.8±0.6mg/100g for Bambara groundnut [27]. Iron helps in the formation of blood and in the transfer of oxygen and carbon dioxide from one tissue to another. Iron deficiency results in impaired hearing and behavioural problems in children and also engenders anemia [49]. The copper content of 0.02±0.1mg/100g for *Rothmannia longiflora* seeds is below the recommended daily allowance of 1.5-3.0mg/day for adult male and female. This result is found to be lower than 0.5±0.00mg/100g for Bambara groundnut seed [27]. 2.23±0.09mg/100g for *Strychnos innocus Del* [34] and 2.53±0.002mg/100g for melon seed [29]. Copper is also necessary for bone growth and nerve functioning. Deficiency of copper may result in anemia and osteoporosis. The results of manganese concentration in the sample was 0.04±0.01mg/100g which is much lower than 1.86±0.07mg/100g for *Cucurbita maxima* [48], 3.67±0.06mg/100g for *strycnos innocus* Del [34] and 19.2mg/100g for *Annona diversifolia* seed [42]. Manganese plays an important role in the transfer of oxygen from the lungs to the cells and activation of enzyme reaction concerned with carbohydrate, fat and protein metabolism. Element deficiency can result in stunted growth and skeletal disorder [47]. The result was however below the recommended daily intake of 5mg [50]. The concentration of chromium and cadmium was found to be 0.03±0.02mg/100g and 0.002±0.00 mg/100g respectively which are lower than 0.40±0.00mg/100g and 3.47±0.05mg/100g for *Strychnos innocus Del* as reported by Abdulmumin, *et al.* [34]. Besides high content the seeds contain reasonable concentrations of potassium, sodium, calcium and magnesium and as such, it has a potential to supply mineral for consumers and microbial media for microorganisms. The results of analysis of *Rothmannia longiflora* seed therefore shows that the plant part can be consumed alongside other foods to provide the mineral elements requirements of the human body.

Table-3. Anti-nutritional Properties of *Rothmannia longiflora* Seeds

Parameter	Concentration (mg/100g)
Oxalate	0.0045±0.082
Phytate	4.65±0.082
Cyanide	0.086±0.00
Tannins	0.013±0.001
Nitrate	1.54±0.001

The results of anti-nutritional composition of seeds of *Rothmannia longiflora* are presented in table 3. The oxalate content of the seeds was 0.0045±0.082mg/100g which is very much lower in comparison with 0.43±0.02mg/100g for *Cucurbita maxima* [48], 0.05±0.00mg/100g for water melon [51] and 0.26±0.01mg/100g for *Nephelium lappaceum* [52]. Oxalate combine with calcium present in food substances thereby rendering calcium unavailable for physiological and biochemical roles such as the maintenance of strong bones and teeth, as cofactor in enzyme reaction, in nerve impulse transmission and as a clotting factor in the blood [53]. High value in human diet can increase the risk of renal calcium absorption and has been implicated as a cause of kidney stone. Chai and Liebman [54]. The value obtained for this fruit seed was below established toxic levels.

The phytate content of the seed was found to be 4.65±0.082mg/100g. This result is high than 0.35±0.00mg/100g for *Cucurbita maxima* seeds [48], 0.23±0.01 mg/100g for water melon seed [51] and 2.00±0.31mg/100g for baobab seeds [55]. The result is also comparable with 5.48±0.60mg/100g for pear fruits seed [39] but lower than 12.87±0.02 mg/100g for *Persea Americana* seed [56]. The problem with phytate in food substances is that it combines with essential mineral nutrients in the digestive tract and result in mineral deficiencies. Phytate composition of the fruit seed is lower and might not pose any health threat when compared with a phytate diet of 10-60mg/100g which if consumed over a long period of time could decrease bioavailability of minerals in monogastric animals [40].

The cyanide content of the seed was found to be 0.086±0.00mg/100g which is favourably comparable with 0.08±0.06 mg/100g [40]. The value obtained was also found to be lower than 0.96±0.02 mg/100g for *Strychnos innocus* Del [34], 0.49±0.01mg/100g for *Cucurbita maxima* [48] and 1.56±0.01mg/100g for melon seed [29]. This value is within acceptable levels of human consumption. Plant with more than 20mg of hydrocyanic acid equivalent per 100mg fresh weight is considered dangerous for human consumption [57]. The value for tannins was 0.013±0.00mg/100g which is lower than 0.40±0.01 for water melon [51], 0.15±0.00 mg/100g for *Nephelium lappaceum* [52], 2.84±0.30 mg/100g for baobab seed [55]. The result obtained was also very much lower than 11.29±0.11mg/100g for *Persea Americana* mill seed [56]. 13.66±0.06mg/100g and 23.1±0.10mg/100g for *Punica grantum* fruit seed from Nigeria and Saudi Arabia respectively as reported by Dangoggo, *et al.* [40]. Many studies have shown that tannins is carcinogenic and excessive ingestion of tannin from one or more sources over a prolonged period is detrimental to health [40]. The result of this study however shows that the sample contains very much low concentration of tannin and therefore has very low probability to cause any health risk in human beings and animals.

The nitrate content of the seed is 1.54±0.01 mg/100g which is higher than 0.39±0.04 mg/100g for *Strychnos innocus Del* [34] and quite near to 2.36±0.06mg/100g and 2.52±0.10mg/100g for *Punica granatum* fruit seed from Nigeria and Saudi Arabia respectively as reported by Dangoggo, *et al.* [40]. The value is lower than 13.50mg/100g for *Hasta la pasta* [58] and 14.17±1.94 mg/100g for pear fruit [39]. The value observed is low when compared with the recommended daily intake level of 3.7mg/kg body weight equivalent to 220mg for 60kg person [59]. Nitrate when reduced to nitrite plays an important role in the body as it provides a host defense against numerous microorganisms [59]. These results suggest that levels of anti-nutrition as determined in the sample are all below recommended toxic levels which are cause by the presence of anti-nutrient factors.

Table-4. Phytochemical Screening of *Rothmannia longiflora* Seed Oil

Parameters	**Screening**
Terpenoids	+++
Flavonoids	++
Tannins	-
Phlobatannins	-
Saponins	++
Steroids	+++
Carotenoids	+++
Anthraquinones	-
Phenols	-
Alkaloids	++
Cardiac glycosides	++
Coumarin	++

Key: + = Present, ++= More present, +++ = appreciably present, - = Absent

Preliminary qualitative phytochemical analysis of extracted oil revealed appreciable presence of bioactive constituents such as flavonoids, terpenoids, saponins, steroids, carotenoids, alkaloids, coumarins and cardiac glycoside as shown in table 4 and 5. The quantitative analysis of the oil gave a significant concentration of saponnins, flavonoids, alkaloids with flavonoids being predominant. The results therefore indicates that , both qualitative and quantitative phytochemical constituent presence in the oil are generally moderate in concentration and has the advantage of inference to pharmacological attribute which may find application in pharmaceutical industries for drug formulation.

4. Conclusion

The results obtained from the study shows that *Rothmannia longiflora* seed is a good source of carbohydrate, energy value and lipids. It is also highly rich in potassium and can supplement other elements for human. The low ash and fibre content make the seed suitable for animal feed formulation supported by low anti-nutrient composition. Again, the low protein, high C/N , volatile matter, organic carbon and high lipid content make the plant seeds to find alternative application in bio-energy generation while the phytochemical composition of the seed oil has revealed its possible application in traditional medicine. Both the seeds and seed oil may therefore have nutritional, medicinal and energy potentials.

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