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TITLE: PROXIMATE, PHYTOCHEMICALS, MINERALS, AND ANTINUTRITIONAL CONTENTS OF
FICUS THONNINGII SEED

AUTHORS: Hamza MUHAMMAD, Omolara OLUWANIYI

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Proximate, Phytochemicals, Minerals, and Antinutritional Contents of *Ficus Thonningii* Seed

Hamza Ibrahim Muhammad^{1*} , Omolara Olusola Oluwaniyi²

¹Federal University of Lafia, Department of Chemistry, Lafia, Nigeria.

²University of Ilorin, Department of Industrial Chemistry, Ilorin, Nigeria.

Abstract: The proximate, phytochemicals, minerals, and anti-nutritional content of *Ficus thonningii* seed obtained from Nasarawa, Nasarawa State, Nigeria were investigated. The proximate analysis revealed that the seed is abundant in ash ($8.36 \pm 0.77\%$), protein ($27.52 \pm 0.17\%$) and carbohydrate (40.02%) but contains low moisture ($8.76 \pm 0.06\%$) and fiber ($2.81 \pm 0.09\%$). The minerals analysis showed very high quantities of Ca (2067.50 mg/100 g), P (1985.40 mg/100 g), Mg (1184.10 mg/100 g), K (918.30 mg/100 g), S (192.50 mg/100 g), Fe (434.10 mg/100 g), Mn (39.30 mg/100 g), Cu (57.40 mg/100 g), and Zn (63.60 mg/100 g), Co (1.2 mg/100 g) and Mo (1.10 mg/100 g). Phytochemicals analysis revealed that it contains more flavonoids ($6.13 \pm 0.02\text{ g/100 g}$) and phenolics ($8.77 \pm 0.01\text{ g/100 g}$) than saponins ($1.73 \pm 0.02\text{ g/100 g}$), tannins ($4.37 \pm 0.06\text{ g/100 g}$) and alkaloids ($0.62 \pm 0.01\text{ g/100 g}$). The study has shown that *Ficus thonningii* seed is highly nutritive with very high mineral content and low quantities of anti-nutrients which make it a good source of food for humans and animals. It also contains biologically active phytochemicals which could have medicinal uses.

Keywords: *Ficus thonningii*, seeds, proximate, minerals, phytochemicals, anti-nutrients.

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***Corresponding author. E-mail:** ibrahim.hamzamuha@science.fulafia.edu.ng.

INTRODUCTION

Global food security and economic growth now depends on a declining number of plant species (1). Nigeria, and Africa at large, is bestowed with seed-bearing plants, which over the years have served various purposes, and yet many of them remain unused (2). The lack of information on the characteristics and usefulness of these many and varied plants is more of a problem than their shortage (3). Underutilized plants can also meet nutritional requirements due to their better nutritional value compared to some optimally utilized plants (1, 4). The seed bearing plants within our environments drop their fruits and large amounts of fruit seeds are being discarded yearly at processing plants. This is not only a waste of potentially valuable resources but also worsens an

already serious waste disposal problem (5). In addition, the medicinal values of plants lie in their phytochemical components, which produce definite physiological results on the human body. Polyphenolics for example appear to play a significant role as antioxidants in the protective effect of plant-derived foods and medicines and have become the focus of nutritional and therapeutic interest in recent years (6).

Ficus thonningii (Moraceae) is a member of the pan tropical genus *Ficus* which contains more than 700 species (7). It is a wild evergreen plant about 30 m in length, with a dense crown that spreads around. It also grows sometimes as a climber by growing on and wrapping itself around other trees. *Ficus thonningii* is characterised by simple, dark green, smooth, slightly elongated leaves and small,

globose, slightly rough-surfaced fruit which is green, greenish-yellow or yellow, depending on maturity and usually in season around October in Africa (8). The seed is simple, spherical, smooth-surfaced, yellow, and about 1-3 mm in diameter.

Ficus thonningii is a neglected and underutilized plant. There is limited information on the chemical composition of its different parts despite the fact that it is a traditionally valuable plant with both nutritional and therapeutic benefits. It is drought resistant, easy to propagate, has high growth rate and has no allelopathy (9). Work has been carried out on its leaf (7, 10-15), bark and twig (9) and its root, leaf, and stem bark (14, 17, 18). This study aims to evaluate the proximate, mineral, phytochemical and antinutritional composition of its seed in order to assess its potential as food, fodder, medicine, and energy source.

MATERIALS AND METHODS

Sample Source and Preparation

Fully mature *Ficus thonningii* fruits were collected fresh from some trees beside Pilot Central Primary School Nasarawa, Nasarawa Local Government Area, Nasarawa State, Nigeria and the fruits were identified at the Herbarium Unit, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The fruits were dried partially before the seeds were manually removed, dried completely under the shed, milled, and stored properly in a polyethylene bag for further analysis.

Proximate Analysis

The proximate composition was determined as described by Ijaritimi *et al.* (19).

Moisture Content

2 g of the defatted seed in pre-weighed crucible was placed in an oven at 105 °C for 24 h, cooled, and reweighed. The percentage moisture was calculated as follows:

$$\text{Moisture \%} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

$$\% \text{ Crude protein} = \frac{14 \times 10^{-5} \times (\text{sample titer} - \text{blank titer}) \times 250 \times 6.25}{\text{weight of sample used} \times 10} \times 100$$

Where 14 is the molecular weight of nitrogen and 6.25 is the nitrogen factor.

Determination of Crude Fiber Content

2 g of sample was weighed into a digestion flask and 200 mL of 1.25% sulfuric acid was added. The sample was connected to a condenser and heated for 30 minutes. The flask was removed and content filtered through linen in a fluted Büchner funnel and washed with hot water until washing was no longer acidic. The residue obtained was further

where W_1 is the weight of the crucible, W_2 is the weight of the crucible and sample before drying at 105 °C, and W_3 is the weight of the crucible and the sample after drying and cooling in airtight desiccators.

Ash Content

2 g of the defatted seed was added into a pre-weighed crucible incinerated in muffle furnace at 550 °C for 5-6 h.

$$\text{Ash (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 is the weight of cleaned, dried, ignited, and cooled crucible, W_2 the weight of the crucible and sample before incinerating at 550 °C, and W_3 the weight of the crucible and sample after cooling in the oven.

Crude Protein Content

Crude protein was determined using semi micro Kjeldahl method. 5 g of sample was separately and accurately weighed and placed into Kjeldahl digestion flask. To the flask, 25 ml conc. H_2SO_4 , 5 g of copper sulfate and 2.5 g of sodium sulfate salt and 0.5 g of selenium dioxide was added. The solution was then digested in a fume hood slowly to avoid undue frothing for at least forty-five minutes until sample became clearer. The obtained sample was allowed to cool and made up to 250 mL with distilled water. About 10 mL of the digest was collected for distillation; 10 mL of 40% NaOH was added to the digest in the distillation flask. The distillate was collected into 2% boric acid solution to absorb the liberated ammonia. This was titrated against 0.01 M HCl using drops of methyl red and bromocresol green indicators and titer value after color change from green to pink was observed and recorded. A blank (without sample) was likewise prepared, distilled, and titrated. The protein content was calculated as:

placed in a flask and 200 mL of 1.25% NaOH was added. The resulting solution was boiled for 30 minutes after which it was removed, filtered, and washed as above. The digested sample was then washed with 25 mL of 1% HCl solution and 25 mL of industrial spirit. The residue collected was put into a pre weighed crucible and dried for about 2 hours at 100 °C in an air oven, cooled in a desiccator, and weighed. The cooled sample was ignited in a muffle furnace at 600 °C for 3 hours, cooled, and weighed. The loss in weight of residue was recorded as the crude fiber content.

$$\% \text{ Crude fiber} = \frac{\text{Loss in wt (g)}}{\text{weight of original sample (g)}} \times 100$$

Carbohydrate Content

The carbohydrate content was determined by difference, that is, addition of all the percentages of moisture, fat, crude protein, ash, and crude fiber was subtracted from 100%. This gave the amount of nitrogen-free extract otherwise known as carbohydrate.

Carbohydrate% = 100 - (Moisture% + Fat% + Ash% + Crude fiber% + Crude protein%)

Calorific Value

The energy content of the seed was calculated from the formula:

Total Energy (kcal/kg) = (4 x Carbohydrate%) + (9 x Fat%) + (4 x Crude protein%)

Minerals Analysis

The minerals content of the seed cake was analyzed using the Skyray Instrument EDX3600B X-ray fluorescence spectrometer.

Quantification of Phytochemicals and Anti-Nutrients

The phytochemical and antinutritional analysis was carried out according to the methods described by Oluwaniyi *et al.*; and Oluwaniyi and Bazambo (20, 21).

Quantitative Determination of Saponins

5 g of the defatted seed was dispersed in 100 mL of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55 °C and filtered using Whatman No 1 filter paper. The residue was re-extracted with another 100 mL of 20% ethanol. The combined extract was reduced to 40 mL over a water bath at about 90 °C. The concentrate was transferred into 250 mL separating funnel and extracted using diethyl ether by adding 20 mL of diethyl ether, shaken vigorously and then allowed to settle and then discard the ether layer. The purification process was repeated. The aqueous layer was extracted twice with n-butanol and washed twice with 10 mL of 5% aqueous sodium chloride. The solution was then evaporated over a water bath and dried to a constant weight. (20)

Quantitative Determination of Tannins

5 g of the defatted seed was weighed into a conical flask and 100 mL of 2 M HCl was added. The content was boiled on a water bath for 30 minutes. The extract was cooled and filtered using Whatman No.1 filter paper. The filtrate was taken twice in diethyl ether with two 40 mL portions. The ether extract was heated to dryness and weighed (20).

Quantitative Determination of Alkaloids

5 g of defatted seed was weighed into a 250 mL beaker, 200 mL of 20% acetic acid in ethanol was added and allowed to stand for 4 hours. This was then filtered and the extract was concentrated using a water bath to evaporate about a quarter of the original volume. Concentrated ammonia was added drop-wise to the extract until precipitation was completed. The entire solution was allowed to settle and the precipitate collected by filtration and weighed. (20)

Quantitative Determination of Total Phenolics

2 g of the defatted seed was soaked in 100 mL of n-hexane for 4 hours to remove all fats. The residue was extracted with 50 mL of diethyl ether, and the ether extract was extracted into 50 mL of 10% NaOH solution. The aqueous layer was acidified to pH 4.0 with 10% HCl solution and then extracted into 5 mL of dichloromethane (DCM). The organic layer was finally collected, dried, and weighed (21).

Quantitative Determination of Flavonoids

10 g of the defatted seed was extracted with 100 mL of 80% aqueous methanol repeatedly at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm) and the filtrate evaporated to dryness over a water bath, dried and weighed (21).

Quantitative Determination of Cyanide

4 g of the defatted seed was soaked in a mixture containing 40 mL of distilled water and 2 mL of orthophosphoric acid, mixed stoppered and left overnight at room temperature to free bound hydrocyanic acid. 5 mL of the resulting mixture was distilled into 40 mL of distilled water containing 0.1 g of NaOH pellets. The distillate was made up to 50 mL with distilled water and 20 mL of this was titrated against 0.01 M silver nitrate solution using 1.0 mL of 5% potassium iodide solution to an end point indicated by a faint but permanent turbidity (21).

Quantitative Determination of Oxalates

75 mL of 0.3 M H₂SO₄ was added to 1 g of the defatted seed stirred and filtered. 25 mL of the filtrates (extract) was titrated still hot (80-90 °C) against 0.05 M KMnO₄ solution to the point when pink color appeared that persisted for at least 30 seconds. (21)

$$\% \text{ oxalate (mg/100 g)} = \frac{T \times [Vme] [DF] \times 2.4 \times 100}{ME \times Mf}$$

Where T = titer of KMnO_4

V_m = Volume-mass equivalent (i.e 1 mL of 0.05 M KMnO_4 solution is equivalent to 0.00225 g of anhydrous oxalic acid)

DF = Dilution factor, VT/A

VT = Total volume of filtrate (75 mL), A = Aliquot used (25 mL)

ME = Molar equivalent of KMnO_4

Mf = Mass of sample used.

Quantitative Determination of Phytates

4.0 g of the defatted seed was soaked in 100 mL of 2% HCl for 3 hours and 25 mL of the filtrate was titrated with a standard iron(III) chloride solution using 0.3% ammonium thiocyanate as indicator until a brownish yellow color appeared which persisted for 5 mins. (21)

RESULTS AND DISCUSSION

Proximate Analysis

Table 1: Proximate content of the seed.

Parameter	Mean \pm SD
Fats (%)	12.53 \pm 0.85
Protein (%)	27.52 \pm 0.17
Ash (%)	8.36 \pm 0.77
Fibre (%)	2.81 \pm 0.09
Moisture (%)	8.76 \pm 0.06
Carbohydrate (%)	40.02
Calorific Value (kcal/kg)	382.93

Values are means \pm s.d of triplicate determinations.

The seed has low fats content (12.53 \pm 0.85 %). This is much lower than 31.52 % reported for *Ficus sycomorus* seed (22), 34% found in sweet orange seed (23) and 44% reported for pumpkin seeds (24). This implies that *Ficus thonningii* seed is not suitable as a source of fats for applications that require large quantity of fats. The protein content (27.52 \pm 0.17 %) is higher than the 23.4% and 21.8% reported for watermelon seeds (25,26), 9.23% found in *Ficus sycomorus* seed (22), and 13.5% found in *Dendrocalamus strictus* (27) but lower than 31.44% for *Mucuna pruriens* (28). This indicates that the seed can be used as protein supplement in animal feeds. The seed has high percent ash content (8.36 \pm 0.77 %) which is close to 7.24% reported for *Ficus sycomorus* seed (22) but higher than 4.11% for *Mucuna pruriens* (28). Hence, it can be an excellent source of minerals. The percent fiber content (2.81 \pm 0.09 %) is lower than 3.02% in *Ficus sycomorus* seed (22), 5.35% found in bambara groundnut (28), 10.89% reported

for okra (30) and 4.61% found in mung bean (31). The moisture content of a seed determines its susceptibility to microbial activity and its shelf life. The moisture content (8.76 \pm 0.06 %) is lower than 9.65% reported for *Ficus sycomorus* seed (22), 10.39% reported for cowpea and 8.30% found in mung bean (31) but higher than 6.45% for bambara groundnut (29), 6.43% found in sweets orange seed (23) and 7.731% for pumpkin seed (24). The percent carbohydrate content (40.02%) is high. It is however close to 39.34% reported for *Ficus sycomorus* seed (22). The energy content of the seed is 382.93 kcal/kg.

Minerals Composition

The minerals present in the seed cake are shown in Table 2 below. The seeds cake contains 2067.50 mg/100 g of Ca. This is much higher than the reported 390.77 mg/100 g for *Ficus sycamore* seed, 309 mg/100 g for *Icacina senegalensis* seed and 128.33 mg/100 g for *Moringa oleifera* seed (22, 19). The phosphorus content is 1985.40 mg/100 g. This is also much higher than 119.14 mg/100 g for *Ficus sycamore* seed, 380.24 mg/100 g for *Icacina senegalensis* seed and 103.33 mg/100 g for *Moringa oleifera* seed (22, 19). Especially for children, high intake of calcium and phosphorus are required for bone and teeth formation (32). The seed contains 1184.10 mg/100 g of Mg. This is higher than 300.67 mg/100 g reported for *Ficus sycamore* seed and 268 mg/100 g for *Tamarindus indica* seed (22, 33). Mg is important in protein synthesis, membrane integrity, nervous tissue conduction, muscle contraction and hormone secretion in animals (34) while potassium contributes to the bioelectrical potential of the body (35). The potassium content (918.30 mg/100 g) is also higher than 398.41 mg/100 g and 2.84 mg/100 g reported for lima beans seed coat (6, 22). The seed contains 192.50 mg/100 g of S and 236.60 mg/100 g of Al. Sulfur is a part of cysteine and methionine, both of which are key metabolic intermediates in biosynthesis of glutathione and other sulfur metabolites (36). The microelements: Fe (434.10 mg/100 g), Mn (39.30 mg/100 g), Cu (57.40 mg/100 g), and Zn (63.60 mg/100 g) are so much higher than those reported for *Ficus sycamore* (11.64 mg/100 g Fe, 1.52 mg/100 g Cu and 9.56 mg/100 g Zn) (22) as well as those reported for African locust bean seed (0.60 mg/100 g Mn, 0.08 mg/100 g Cu, and 0.10 mg/100 g Fe) (37). Other micronutrients in this seed include Co (1.2 mg/100 g) and Mo (1.10 mg/100 g). Fe is important in cellular oxygen transport and use, Mn activates enzymes transfer ATP to ADP, Zn promotes DNA and RNA replication and foetal growth, Cu helps melanin pigment formation, and Mo catalyzes reduction of nitrogen to ammonia (38).

Table 2: Minerals Content of the seed.

Minerals	Quantity (mg/100 g)
Mg	1184.10
P	1985.40
S	192.50
K	918.30
Ca	2067.50
Mn	39.30
Co	1.20
Fe	434.10
Cu	57.40
Zn	63.60
Mo	1.10

Phytochemicals and Anti-Nutrients

The phytochemical contents of *Ficus thonningii* seeds are shown in Table 3. Saponins produce foams when in aqueous solutions precipitate, coagulate red blood cells and bind cholesterol (39). The saponin content (1.73 ± 0.02 g/100 g) is similar to 1.75% reported for *Ficus sycomorus* seed (22). The tannin content (4.37 ± 0.06 g/100 g) is close to 4.03% found in *Ficus sycomorus* seed (22). The alkaloid content (0.62 ± 0.01 g/100 g) is lower than 5.65% reported for *Ficus sycomorus* seed (22). The Flavonoid content (6.13 ± 0.02 g/100 g) is higher than 3.63% reported for *Ficus sycomorus* seed (22). Flavonoids have health-promoting properties which include the prevention of allergies and ulcers and their antioxidant effects (40). Their powerful antioxidant property is the reason for the recent interest in flavonoids as health-promoting compounds. The total phenolics content (8.77 ± 0.01 g/100 g) is high. Phenolics have antioxidant properties and have been found to prevent the formation of oxidized low-density lipoprotein by inhibiting the autoxidation of unsaturated fats which causes cardiovascular disease (41). The phytate content (0.97 ± 0.05 mg/100 g) is lower than 1.98% reported for *Ficus sycomorus* seed (22). Phytates have been known to possess anticancer and antioxidant properties beside their ability to chelate divalent minerals which results in precipitation of such minerals, making them unavailable for absorption in the intestines (42). Oxalate content (0.13 ± 0.01 mg/100 g) is lower than 2.85% found in *Ficus sycomorus* seed (22). The cyanide content (0.04 ± 0.01 mg/100 g) is below the recommended limit of 10 mg HCN Eq/Kg (43) thus making it safe for consumption.

Table 3: Phytochemical and antinutritional content of *Ficus thonningii* seed.

Phytochemicals	Mean \pm sd
Saponins (g/100 g)	1.73 ± 0.02
Tannins (g/100 g)	4.37 ± 0.06
Alkaloids (g/100 g)	0.62 ± 0.01
Flavonoids (g/100 g)	6.13 ± 0.02
Total phenolics (g/100 g)	8.77 ± 0.01
Phytates (mg/100 g)	0.97 ± 0.05
Oxalates (mg/100 g)	0.13 ± 0.01
Cyanides (mg/100 g)	0.04 ± 0.01

Values are means \pm s.d of triplicate determinations.

CONCLUSION

The search, identification, and study of new plant resources is a vital and unending endeavor with the alarming increase in human population and depletion of natural resources. The *Ficus thonningii* seed is a very good source of dietary nutrients. The high proportions of carbohydrate and protein give it a good nutritional value. The high ash content as proved by the incredible quantities of dietary minerals present make it a great minerals supplement and suitable component in feed formulation where high minerals source is required. The flavonoid and phenolic contents make the seed an excellent and natural antioxidant with health-promoting properties. The seed contains very little quantities of anti-nutrients. Plant materials are exploited for their nutritional, therapeutic and antimicrobial properties. *Ficus thonningii* is an underutilized plant whose seed have the potential for food, fodder, energy, and industrial uses.

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