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Proximate and Mineral Composition, Phytochemical Analysis, and Antioxidant Activity of Fig Trees (*Ficus spp.*) Leaf Powder

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study aims to analyse and characterise the leaf powder of *Ficus carica, Ficus exasperata*, and *Ficus thonningii*.

Methodology: *Ficus carica, Ficus exasperata,* and *Ficus thonningii* leaf powder were analysed quantitatively for proximate, phytochemicals, minerals, and antioxidant activity.

Results: The ash, crude protein, and nitrogen free extract concentration were significantly (P<0.05) higher in *Ficus carica* leaf powder than the rest *Ficus species* under study. However, the crude fiber and crude fat concentrations were higher (P<0.05) in *Ficus thonningii* and *Ficus exasperata* leaf powder, respectively. The concentration of tannins, flavonoids, phenols, and saponins was higher (P<0.05) in *Ficus exasperata* than other *Ficus species* under study. However, the concentration of the alkaloids was higher (P<0.05) in *Ficus carica* leaf powder than *Ficus exasperata* and *Ficus thonningii* leaf powder. The Zn and P were higher (P<0.05) in *Ficus carica* than the rest *F. species* under study. The Fe contents of *F. carica* and *F. exasperata* leaf powders were higher (P<0.05)

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than *F. thonningii* leaf powder. The Ca contents of *F. carica* leaf powder and *F. thonningii* leaf powder were higher (P>0.05) than *F. exasperata* leaf powder. The 2, 2-diphenyl-1-picryl-hydrazyl-hydrate value was higher (P<0.05) in *Fiscus exasperata* leaf powder than *F. carica* and *F. thonningii*. However, the value of vitamin c was higher (P<0.05) in *Ficus carica*, compared to the rest *F. species*.

Conclusion: This study reveals that the leaf powder of *F. carica, F. exasperata*, and *F. thonningii* contains a significant number of minerals and phytochemicals with high antioxidant activity and could be employed as a natural feed supplement in animal nutrition.

Keywords: Characterisation; Ficus spp; phytochemicals; phyto-supplements.

1. INTRODUCTION

With the increasing demand for organic-based food, the quest for plant-based growth promoters and antioxidant agents by health-conscious individuals is rising. Consumers now prefer organically produced substances rather than inorganic substances that are used in production due to residues of the inorganic substances with their attendant health implications [1]. Plants are a good source of a wide range of phytochemicals such as polyphenols, carotenoids, alkaloids, sulphur-containing groups, terpenes and terpenoids, which contains enormous antimicrobial and antioxidant properties [1]. Several studies have been carried out using different plants and their various parts to improve productivity and boost the immunity status of animals and enhance the quality of animal products. Such studies include Ocimum gratissimum leaf powder, Irvingia gabonensis kernel powder [2]; moringa leaf meal, garlic rhizome meal [3]; Syzygium aromaticum leaf meal, Myristica fragrans seed meal [4]; pawpaw, mustard and black cumin seed meal [5]; soursop juice [6]. The inclusion of these phytogenic substances of plant origin in poultry diets has contributed to enhance the performance of animals, improving carcass traits and health status, ameliorating the negative impacts of oxidative stress and conferring positive effect on animal products [2,3,4].

The genus Ficus belongs to the family Moraceae, which comprises over 1000 species and is widely distributed in the tropics and temperate regions [7]. Ficus species are of economic importance due to their medicinal values due to the array of secondary metabolites such as flavonoids stilbenes, triterpenoids and xanthose [8]. Preparation of the combination of different parts such as leaves, barks. latex and fruits has proved effective for the treatment haemorrhoids. diabetes. of

stomachache, skin diseases, diarrhoea, dysentery and ulcers [9]. The preparation has been used as anti-cancer agents, carminative, antioxidant, anti-inflammatory and astringent [9].

Despite the wide distribution and vast potentials of the *Ficus species*, under-utilisation still exists. In the face of the rising price of inorganic drugs and supplements and the trend of organic drugs and supplements conscious population, it becomes crucial to explore the vast potentials and health benefits of the *Ficus species*. Therefore, this study seeks to characterise the leaf meals from three fig trees: *Ficus carica* (opoto), *Ficus exasperata* (ipin) and *Ficus thonningii* (odan) in order to determine their proximate, phytochemicals, antioxidants and mineral status to determine their relevance and usage as nutraceuticals.

2. MATERIALS AND METHODS

2.1 Plant Materials

Ficus carica, Ficus exasperata, and *Ficus thonningii* fresh leaves were gathered at a farm in Akure, Ondo State. The leaves were identified in the Department of Forestry at the Federal University of Technology in Akure, Nigeria and then air-dried for two weeks at room temperature in the shade to get a consistent weight. The leaves were then powdered using an electric blender and kept in airtight containers.

2.2 Laboratory Analysis

2.2.1 Proximate analysis

The Association of Official Analytical Chemists' established techniques were used for proximate analysis [10].

2.2.2 Tannin

The Folin-Ciocal method was used to determine total tannins [11]. 1 mL of leaf extract was mixed with 49 mL of purified water, 1.7 ml of 75 percent ethanol, 0.1 ml metaphosphoric acid, 1.0 mol/ml Na2CO3 (10 ml), and 2.5 ml Folin-Ciocalteu in a volumetric flask (100 ml). The mixture was thoroughly blended and allowed to rest at room 15 temperature for minutes. In а spectrophotometer set to 680 nm. the absorbance of normal solutions and leaf meal combinations was measured against a blank. The standard curve ($R^2 = 0.9972$) was utilised as a reference, and tannic acid was used to reflect the overall tannin content of the sample (TA) mg TA/q DW.

2.2.3 Flavonoids

Surana et al. [12] procedures were used to assess the flavonoids content of leaf samples. In a test tube, 0.50 mL of leaf powder extracts were carefully measured. The test tube was then filled with a 0.1 mL aluminum chloride solution, 1.50 mL methanol, 0.1 mL potassium acetate solution, and 2.8 mL distilled water mixed. Sample blanks for extract and rutin standard dilutions (10-100 a/ml) were generated in the same method, but with distilled water rather than aluminium chloride solution. The solutions were filtered using Whatman filter paper (No. 1) to determine the absorbance. The absorbance ratios were compared to blanks at 510 nm. 1 mg rutin per gram of extract was used to calculate the overall flavonoid content.

2.2.4 Phenols

The Folin-Ciocalteau method, as developed by Otles and Yalcin [13], was used to determine the total phenolic content of the leaf sample. To 50 µL of nettle extract or standard solution, 250 µL of Folin-Ciocalteau reactive was added. This mixture was kept at room temperature for 5 minutes in a dark environment. At the end of this time, a 750L of 7 percent Na₂CO₃ solution was added. In this approach, the hydroxyl groups in phenolics could deliver H to water. Pure water was used to dilute the mixture to 5 mL. The mixture was then kept at room temperature for 120 minutes in a dark environment to react. At 760 nm, the absorbance of the samples and standards were measured. Instead of the 50L extract, an 80 percent methanol solution was added to the blank solution. Using gallic acid

equivalent standards, a calibration curve was constructed to determine total phenolic content.

2.2.5 Total saponins

The vanillin and concentrated sulfuric acid colourimetric method published by He et al. [14] was used to measure saponin. The 0.1 ml sample was combined with 0.5 ml ethanol (50%), 4.0 ml sulfuric acid (77%) (w/w), and 0.5 ml freshly made vanillin solution (8% w/v), then allowed to settle to ambient temperature before being heated in a water bath to 60°C for 15 minutes. A UV/V is spectrophotometer was used to detect the absorbance at 545 nm. The total saponin content in each sample was measured using a tea saponin calibration curve and represented as mg tea saponin equivalent per g (TSE/g DW).

2.2.6 Alkaloids

The gravimetric technique was used to determine the alkaloid content of the leaf sample [15]. In a 50 mL of acetic acid solution mixed with ethanol, 5 g of the sample was dispersed (10 percent). The mixture was left undisturbed for roughly 240 minutes after being vibrated before being sieved. The filtrate was reduced to a fraction of its original volume on a hot plate. The alkaloids precipitated were then usina drops of concentrated ammonium hydroxide. Before being washed with a 1 percent ammonium hydroxide solution, the precipitate was filtered through filter paper. The precipitate was then oven-dried at 60°C for half an hour before being transferred to desiccator and weighed again until it attained a constant weight. The weight of the alkaloids was calculated as a percentage of the overall sample weight.

2.2.7 Phytate

The amount of phytate in leaf samples was determined using an anion exchange method published by Davies and Reid [16]. The filter (0.2-1.0 ml) was diluted to a final volume of 1.4 ml with distilled water, then 1.0 ml ferric ammonium sulphate solution containing 50 µg Fe was added and thoroughly mixed. The test tubes were then sealed and placed in a boiling water bath for 20 minutes. 5 ml amyl alcohol was added to the test tube once it had cooled to room temperature, followed by 0-1 ml of a 100 g/l ammonium thiocyanate solution. The contents of the test tubes were immediately mixed using inversion and shaking. The colour intensity in the

amyl layer was measured using а spectrophotometer at 465 nm against an amyl alcohol 'blank' exactly 15 minutes after the applied **HN₄CNS** following brief was centrifugation at low speed. The extinction at 465 nm in the amyl layer is inversely related to the phytate anion concentration because, at pH 1-2, ferric ions complexed with phytate cannot combine with the thiocyanate ion to form the pink complex.

2.2.8 2, 2-diphenyl-1-picryl-hydrazyl-hydrate

2-diphenyl-1-picryl-hydrazyl-hydrate The 2, (DPPH) antioxidant activity of the leaf sample was measured using the DPPH radical degradation activity technique described by Otles and Yalcin [13]. The DPPH radical was created using pure methanol at a concentration of 6 x10-5 M daily (molar). 2 microlitres of methanolic DPPH solution were added to 100 microlitres of sample extract or reference solution. For 20 minutes, this mixture was kept in the dark place. The absorbance was measured at 515 nm after this time. As a control, a pure methanol blank solution was employed. Instead of 100 microliters of extract. 100 microliters of clean water were used in the control solution. To examine the antioxidant capabilities of sample extracts, a calibration curve was produced using different concentrations (10-100 ppm) of gallic acid solution.

2.2.9 Vitamin C

The vitamin C content of the Ficus species leaf sample was determined using the Benderitter et al. [17] method. 2 g dinitrophenyl hydrazine, 270 mg copper sulphate (CuSO₄. 5H₂O), 75µl DNPH solution, and 230 mg thiourea in 100 ml of 5 ml/L H₂SO₄) was added to 500 µl extract combination (300 µl extract dilution with 100µl 13.3% trichloroacetic acid and water). The reaction mixture was then incubated at 37°C for 3 hours before adding 0.5 ml of 65 percent H₂SO₄ (v/v) to the medium and measuring the absorbance at 520nm with a UV spectrophotometer. Ascorbic acid was used as a reference component to assess the vitamin C content of the leaf powder.

2.2.10 Mineral composition

The colourimetric approach was used to determine the amount of phosphorus (adapted from AOAC 965.17). In a 100 mL volumetric flask, an aliquot of the leaf sample solution containing 0.2 to 1.5 mg P was placed. Following

that, 20 ml of molybdovanadate reagent was added and carefully mixed after diluting to volume with H_2O . After allowing the solution to stand for 10 minutes, it was read at 400 nm using H_2O as the blank. The concentration of P was calculated using the standard curve. The leaf sample's Zn, Ca, and Mg were analysed using an Atomic Absorption Spectrophotometer (Bulk scientific, USA, model 210 VGP) after wet digestion with a mixture of nitric sulphuric acid and hydrochloric acid.

2.3 Statistical Analysis

The study's findings were derived from the averages of triplicate values. The SPSS version 20 statistical software tool was used to analyse the acquired data [18]. The substantial differences in mean values were investigated using a one-way ANOVA.

3. RESULTS

The proximate composition of *Ficus carica, Ficus exasperata* and *Ficus thonningii* are shown in Table 1. All the parameters were significantly (P<0.05) different across the three *Ficus species* leaf powder under study. The moisture contents of the leaf powder were significantly (P<0.05) higher in the *Ficus exasperata* and *Ficus thonningii* leaf powder than *Ficus carica* leaf powder. The ash, crude protein, and nitrogenfree extract concentration were significantly (P<0.05) higher in *Ficus carica* leaf powder than the rest *Ficus species* under study. However, the crude fibre and crude fat concentrations were higher (P<0.05) in *Ficus thonningii* and *Ficus exasperata* leaf powder, respectively.

The concentration of the phytochemicals varies (P<0.05) among the *Ficus species* under study, except (P>0.05) for phytate (Table 2). The concentration of tannins, flavonoids, phenols, and saponins was higher (P<0.05) in *Ficus exasperata* than other *Ficus species* under study. However, the concentration of the alkaloids was higher (P<0.05) in *Ficus carica* leaf powder than *Ficus exasperata* and *Ficus thonningii* leaf powder.

Fig. 1 shows that the antioxidant properties vary across the *Ficus species* under study. The DPPH value was higher (P<0.05) in *Fiscus exasperata* leaf powder than *F. carica* and *F. thonningii*. However, the value of vitamin c was higher (P<0.05) in *Ficus carica* compared to the rest *F. species*.

As shown in Fig. 2, the Zn and P were higher (P<0.05) in *Ficus carica* than the rest *F. species* under study. However, the Fe contents were similar (P>0.05) in *F. carica* leaf powder and *F. exasperata* leaf powder but higher (P<0.05) than *F. thonningii* leaf powder. The Ca contents of *F. carica* leaf powder, and *F. thonningii* leaf powder was similar (P>0.05) and higher (P>0.05) than that of *F. exasperata* leaf powder.

4. DISCUSSION

The proximate compositions of the three *Ficus species* were presented in Table 1. The range of moisture contents (8.69- 9.87%) recorded for *F. carica, F. exasperata,* and *F. thonningii* was lower than the 8.25% reported for *F. exasperate* leaf powder [19]. The value of the moisture contents of a



Fig. 1. The antioxidant properties of Ficus spp. leaf meals



Fig. 2. The mineral compositon of Ficus spp. leaf meals

Fig tree leaf species	Moisture	Ash	Crude fibre	Crude fat	Crude protein	Nitrogen free extract
Ficus carica	8.69±0.09 ^b	12.97±0.13 ^ª	17.36±0.21 ^b	4.59±0.23 ^c	15.76±0.00 ^ª	40.61±0.01 ^a
Ficus exasperata	9.50±0.21 ^ª	11.41±0.18 ^b	17.26±0.24 ^b	9.61±0.18 ^ª	15.01±0.08 ^b	37.20±0.11 [°]
Ficus thonningii	9.87±0.09 ^a	11.91±0.21 ^b	22.61±0.09 ^a	8.51±0.01 ^b	7.63±0.07 ^c	39.46±0.15 ^b
P value	0.00	0.00	0.00	0.00	0.00	0.00

Table 1. The proximate composition (%) of fig tree species leaf powder

Means on the same column with different superscripts are significant (P<0.05)

Table 2. The phytochemical composition (mg/g) of fig tree species leaf powder

Fig tree leaf species	Tannins	Flavonoids	Phenols	Saponins	Alkaloids	Phytate	
Ficus carica	3.01±0.00 ^b	90.34±3.86 ^b	20.89±0.21 ^b	60.17±0.09 ^b	103.00±1.15 ^ª	3.27±0.01	
Ficus exasperata	3.33±0.05 ^ª	165.28±22.01 ^ª	56.52±3.48 ^a	80.72±0.22 ^a	81.00±1.15 ^b	4.20±0.42	
Ficus thonningii	2.69±0.02 ^c	81.55±1.21 ^b	15.69±0.01 ^b	51.42±0.62 ^c	59.50±0.86 ^c	4.17±0.02	
P value	0.00	0.00	0.00	0.00	0.00	0.06	

Means on the same column with different superscript are significant (P<0.05)

typical feed ingredient is one of the most critical factors controlling the deterioration rate of food samples and their nutrients' concentration [20]. The relatively low moisture content recorded in Ficus carica leaf powder could confers immense benefits on the leaf powder and implies that the shelves live is guaranteed against spoilage by micro-organisms [20]. The ash content is a measure of the mineral contents present in any given sample. The relatively high ash content recorded in F. carica implies it could provide more dietary minerals needed when used as a feed supplement or ingredient than the rest leaf powders under study. However, the ash content range (11.41-12.97 %) recorded for F. carica, F. exasperata, and F. thonningii in this study is similar to 12.5% reported for F. exasperata [19]. Therefore, the three Ficus species could be a good source of minerals for healthy human and animal nutrition. Earlier, it was reported that the minerals contained in plants could play a vital role in human and animal nutrition [21].

The relatively low crude protein content of F. thonningii leaf powder, compared to the rest F. spp under study, implies that F. thonningii leaf is inferior when being considered a dietary protein source in animal feed formulation. The crude protein content (7.63-15.76 %) for F. carica. F. exasperata and F. thonningii in this study was found to be lower than 27.13% reported for F. exasperate by Osagie and Aguebor-Ogie [19] and 17.9 % reported by Nkafamiy et al. [22] for F. sycomorus; but higher than 6.92% crude protein reported by Bello et al., [23] for F. exasperate leaves. Various studies have suggested leaf meals from tropical vegetables and herbs as protein sources in animal nutrition [2, 24, 25]. This shows that F. carica and F. exasperate could be used as a dietary protein source in animal nutrition.

Studies have shown that fibers have potentials of reducing the rate of glucose absorption as well as reducing insulin secretion, which is of great importance in diabetic patients [26, 27]. The crude fiber content of F. thonningii which is higher than those of F. carica and F. exasperata in this study could be of great value in animal and human nutrition. This agrees with the findings of Bello et al. [28]. They reported that high dietary fibre in vegetables is advantageous for their roles in the regulation of intestinal transit, increasing dietary bulk and increasing faeces consistency due to their ability to absorb water.

The crude fat contents i.e. 4.59±0.23, 9.61±0.18 and 8.51±0.01 reported for F. carica, F. exasperata, and F. thonningii, respectively, were higher than the 2.11g/100g reported for Moringa oleifera [29]. Bello et al. [28] reported that leaves generally are poor sources of lipid, which is typical of most leafy vegetables. The utilisation of these leaf powders will thus not add to the accumulation of fat in the body, which is a precursor for cardiovascular disorders [30]. The Nitrogen free extract (NFE) estimates the nonfibrous carbohydrates such as sugar and starch [31]. In this study, the NFE value range (37.20-40.61%) recorded for F. carica, F. exasperata, and F. thonningii shows that their leaf powder could contribute to the dietary energy required for the normal functioning of the body.

Plants containing tannins have been successfully used as astringents, as diuretics, against stomach and duodenal tumours [32], while plants that contain flavonoids provide health benefits such as antioxidants and anti-inflammatory effects [33]. The tannins and flavonoids found in F. carica, F. exasperata, and F. thonningii support its potential as an anti-microbial, antiinflammatory, and anti-tumour agent and a means of removing harmful free radicals and reactive oxygen species [34]. Koche et al. [35] reported that the antioxidant capabilities of phenolics are crucial in their role as protective agents against free radical-mediated disease processes. The phenols contents (15.69-56.52 mg/g) as reported for F. carica, F. exasperata, and F. thonningii leaf powders implies that they could be a good source of phenols when supplemented in dietary intake of human and livestock. Saponins are essentially dietary supplements as well as exhibiting anti-microbial properties. They are beneficial in modulating blood lipids, reducing cancer risk, improving response and antioxidant blood glucose properties [36]. The leaf powder of F. carica, F. exasperata and F. thonningii contains an appreciable amount of saponins and is possessing suspected of anti-microbial potentials. The presence of alkaloids in F. carica, F. exasperata, and F. thonningii shows that the leaf powder has various pharmacological qualities. including antihypertensive. antiarrhythmic. anti-malaria, and anti-cancer activities, which is consistent with Saxena et al., [32] findings. Phytate is an anti-carcinogenic that protects against colon cancer, as well as a possible antioxidant that inhibits Fenton processes that lead to lipid peroxidation and polyphenol oxidase inhibition [37]. Therefore,

phytate concentrations (3.27- 4.20 mg/g) reported for *F. carica, F. exasperata,* and *F. thonningii,* respectively, could be of benefit. These benefits, however, are contingent on several circumstances, including the level of dietary inclusion, the animal species that consume the feed, and the feed composition [38].

Zinc is required for the normal functioning of the body's defensive (immune) system. It is also involved in cell division, cell development, wound healing, and carbohydrate breakdown. The senses of smell and taste require zinc as well [39, 40]. Phosphorus is primarily involved in the production of bones and teeth. It has a significant impact on how the body utilises carbohydrates and lipids. Protein is also required to grow, maintain, and repair cells and tissues in the body [41]. The zinc and phosphorus content being higher in F. carica than F. carica and F. thonningii in this study suggests F. carica is a better source of those minerals mentioned above. Iron is required for various metabolic processes, including oxygen transport, DNA synthesis, and electron transport. It is also necessary for the body's synthesis of red blood cells [42]. According to Thomas and Krishnakumari [43], iron facilitates the oxidation of biomolecules that control overweight, a precursor that disposes individuals to various diseases. The concentration of iron (6.63-10.14 mg/g) recorded in the Ficus carica, F. exasperata, and F. thonningii in this study further supports the use of several Ficus species as blood tonic in traditional medicine due to their blood-boosting properties [44]. Calcium is required for blood clotting, bone and tooth development, and as a cofactor in the catalysis of several enzymes [40]. The presence of calcium (11.35- 12.51mg/g) in Ficus carica, F. exasperata, and F. thonningii leaf powder in this study suggests that these leaves could be a good dietary source of Ca in animal and human nutrition.

Vitamin C is necessary for immune response activation. wound healing, osteogenesis, detoxification, iron absorption, collagen production, blood vessel clotting prevention, and many other metabolic functions [45]. The vitamin C contents (7.15 -5.91mg/100g) of F. carica, F. exasperata and F. thonningii, respectively is an indication that the leaf powder of the Ficus species analysed can be a good source of vitamin C, which is water-soluble, a nonenzymatic natural antioxidant and can be used

as an alternative to synthetic antioxidant [46]. The DPPH values (67.32-87.15 %) of the leaf powder of *F. carica, F. exasperata* and *F. thonningii* was higher than the 46.64% reported for blueberry fruit [47]. It was reported that DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreases significantly on exposure to radical proton scavengers. The concentration of DPPH in the *Ficus species* indicates that they can be a good substitute for inorganic based antioxidants.

5. CONCLUSION

The results of this study reveal that the leaf powder of *F. carica, F. exasperata,* and *F. thonningii* contains a significant number of minerals and phytochemicals with high antioxidant activity. As a result, the leaf powder of *F. carica, F. exasperata,* and *F. thonningii* could be employed as a natural feed supplement in animal nutrition. More research is needed to assess the efficacy of *F. carica, F. exasperata,* and *F. thonningii* leaf powder as feed supplements on animal performance and health.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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