Assessment of the Nutraceutical Values of the Leaf of Tapinanthus Globiferus Hosted by the Neem Tree

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Abstract
The objective of this study is to evaluate the nutraceutical values of the leaf of Tapinanthus globiferus hosted by the neem tree. The phytochemical composition, antioxidant activities, antidiabetic properties, anti-inflammatory capabilities, and proximate composition of the Tapinanthus globiferus leaf powder (TLP) were examined. The results revealed the phytochemical profiles: phenol (501.51 mg/g), alkaloids (408.25 mg/g), saponins (7.16 mg/g), steroids (7.16 mg/g), flavonoids 933.79 mg/g) and tannins (86.53 mg/g); antioxidant assay results: Ferrous chelating activity (51.17%), hydroxyl radical inhibition (55.96%), ABTS (76.09%), DPPH (71.34%) and lipid oxidation inhibition (66.18%); antidiabetic assay results: α-glucosidase inhibition (75.88%) and α-amylase inhibition (72.14 %); anti-inflammatory assays: antiproteinase activity (72.38%) and albumin denaturation inhibition (44.67%) of TLP. Tapinanthus globiferus leaf powder has nitrogen-free extract content of 36.09%, 18.47% crude protein, 13.27% moisture, 12.78% ash, 9.44% crude fibre, and 9.93% crude fat. These findings indicated that TLP had anti-inflammatory, anti-diabetic, and antioxidant properties.

Introduction
Natural foods known as nutraceuticals are renowned for fusing the lines between food and medicine by helping to tackle some of the most serious health issues, including arthritis, cancer, osteoporosis, diabetes, cardiovascular disease, and cholesterol (Das et al., 2012). In addition, a wide variety of products, including processed cereals, processed drinks, isolated nutrients, plant products, diet supplements, and genetically modified items, are included in the category of nutraceuticals (Padmavathi, 2018). The nutraceutical industry’s fastest-growing subsectors include dietary supplements and herbal products (Hathcock, 2001, Das et al., 2012). Numerous commonly occurring plants, including eucalyptus, red silk cotton, lemon, henna, spearmint, basil, and mandarin orange, have been suggested to have potential as nutraceuticals (Amat-Ur-Rasool et al., 2020).

The current global ban on the use of antibiotic-supplemented diets in animal production, which was brought about by consumers’ growing awareness of the importance of consuming high-quality animal protein, has encouraged the use of medicinal plants or their extracts as a feed supplement in animal production to improve animal performance and play a significant role in potential therapies to improve animal health (Pourhossein et al., 2015; Mahanta et al., 2017). Additionally, nutraceuticals are now acknowledged as a perfect switch to manage a variety of clinical conditions like allergy, blood cholesterol control, inflammation, sleep dysfunction, arthritis, indigestion, depression, hypertension, and malignancies in man as well as human lifestyle disorders like stroke, heart disease, type-2 diabetes, and obesity (Jha et al., 2021).

Tapinanthus globiferus (African mistletoes) is widely distributed (Imarhiagbe, 2021). Birds disperse
the seed of this plant, which they then use to parasitize a variety of hosts, including *Azadirachta indica*, *Citrus sinensis*, *Cola acuminata*, *Vitellaria paradoxa*, and *Combretum glutinosum*, among others (Wang et al., 2022). The plant’s broad pharmaceutical significance is only now becoming realised. African people refer to the plant as an “all-purpose herb” since the Tapinanthus species, in particular, are used to cure inflammation, hypertension, depression, diabetes, cancer, fever, malaria, and other ailments (Wang et al., 2022). The chemical composition of mistletoe leaf has been extensively studied and reported; however, recent reports indicate that phytochemical or botanical variation exists due to factors such as abiotic conditions, season, diurnal rhythm, ontogeny, diurnal rhythm, and abiotic conditions (Lämke and Unsicker, 2018). As a result, regular or periodic examination and characterization of plant parts are needed to determine their effectiveness in producing results when used as a dietary supplement. The majority of therapeutic areas are covered by dietary supplements, including digestion, the prevention of certain cancers, the treatment of colds and coughs, blood pressure, sleep problems, osteoporosis, cholesterol control, diabetes, and depression (Pandey et al., 2010). Therefore, the objective of this study is to evaluate the nutraceutical properties of TLP by exploring the phytochemical profile, antioxidant properties, proximate composition, anti-diabetic, and anti-inflammatory effects of *Tapinanthus globiferus* leaf powder.

**Material and Methods**

*Tapinanthus globiferus* leaf powder and Reagents

Fresh *Tapinanthus globiferus* leaves were collected from the Federal Polytechnic in Ado Ekiti, Nigeria’s Teaching and Research Farm of the Agricultural Technology Department. The plant was confirmed by a botanist from the Department of Plant and Biotechnology at Adekunle Ajasin University in Akungba Akoko, Nigeria. After being thoroughly cleansed with fresh water, the samples were drained and allowed to dry in the shade for 14 days. After being ground into *Tapinanthus globiferus* leaf powder (TLP), they were stored at 4°C until analysis. The parameters were examined in three repetitions. Three repetitions of analysis for each parameter were performed on the TLP samples. All of the chemicals of the analytical reagent grade used for chemical analysis were purchased from Sigma-Aldrich.

**Quantitative phytochemical analysis of TLP**

The methods for determining phenols, alkaloids, saponins, flavonoids, and tannins were highlighted and reported by Oloruntola (2021), whereas Madhu et al. (2016) reported the methods for determining steroids.

**Phenols**

400 g of TLP received a total of 2000 ml of 70% ethanol, which was then added, shaken for six hours, allowed to stand still for an additional 48 hours, and then filtered through the Whatman No 1 filter paper. A rotary evaporator was employed to vacuum condense the TLP ethanolic extract at 35–40 °C. 200 g of TLP was filtered using Whatman No. 1 filter paper after being immersed in 1000 cc of 70% ethanol and vibrated consistently for 6 hours. The phenolic content of TLP was determined using the Folin-Ciocalteau technique, which Otlles and Yalcin (2012) described. 50 mL of TLP extract or standard solution was combined with 250 mL of the Folin-Ciocalteau reaction. Five minutes were spent letting this mixture sit at room temperature in a dim environment. A 750 microliter solution of 7 percent Na2CO3 was added at the end of this period. With the use of distilled water, the mixture was diluted to 5 mL. The mixture was next given 120 minutes at room temperature and darkness to react. The absorbance of the standards and samples was calculated at 760 nm. In place of the extract, 50 μl of an 80 percent methanol solution was added to the blank solution. The total phenolic content was calculated using a calibration curve and standards that are equivalent to gallic acid.

**Alkaloids**

The gravimetric technique was used to determine the alkaloid content of the TLP sample (Adeniyi et al., 2009). The TLP was combined with 50 ml of an acetic acid solution in ethanol (10% w/v). The mixture was vibrated and left alone for around 240 minutes before being sieved. The filtrate was diluted to one-fourth of its initial volume on a hot plate. The alkaloids were then precipitated by applying drops of highly concentrated ammonium hydroxide. The precipitate was rinsed with a 1 percent solution of ammonium hydroxide after being filtered through filter paper. After being dried in an oven for 30 minutes at 60°C, the precipitate was transferred to desiccators and weighed again until it reached a constant weight. The weight of the alkaloids was calculated as a percentage or proportion of the sample weight.

**Saponins**

The vanillin and concentrated sulfuric acid colourimetric method were used to evaluate saponin content (He et al., 2014). The following ingredients were added to the 0.1 ml of TLP extract: 0.5 ml of 50% ethanol, 4.0 ml of 77% sulfuric acid, and 0.5 ml of freshly prepared vanillin solution. The mixture was allowed to cool to room temperature
before being heated in a water bath for 15 minutes to 60 °C. To quantify the absorbance at 545 nm, a UV/Vis spectrophotometer was used. The total amount of saponin in each sample was determined and represented as mg tea saponin equivalent per g (TSE/g DW) using a tea saponin calibration curve.

**Steroids**

Following reports from Madhu et al. (2016), the level of steroids in TLP was detected. 1 ml of TLP steroid extract was put into 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5 percent w/v, 2 ml) were then added after the potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 ml). Before being diluted with distilled water to the necessary concentration, the mixture was heated for 30 minutes at 70-20 °C in a water bath with intermittent shaking. The absorbance was calculated at 780 nm and compared to a reagent blank.

**Flavonoids**

The Surana et al. (2016) method was used to determine the amount of flavonoids in TLP. A test tube containing 0.50 ml of TLP extract received 0.1 ml of potassium acetate solution, 1.50 ml of methanol, 0.1 ml of aluminum chloride solution, and 2.8 ml of distilled water. The same method, but with distilled water instead of aluminum chloride solution, was used to make sample blanks for extract and rutin standard dilutions (10-100 g/ml). After that, the solutions were filtered using Whatman filter paper (No. 1). Absorbance ratios were measured at 510 nm in contrast to blanks. The total flavonoid concentration was then discovered to be equal to 1 mg of rutin per gramme of the ethanolic TLP extract.

**Tannins**

The Folin-Ciocalteau method was used to measure the total tannin concentration (Biswas et al., 2020). A volumetric flask was filled with 1 ml of the TLP ethanolic extract, 49 ml of distilled water, 1.7 ml of 75% ethanol, 0.1 ml of metaphosphoric acid, 10 ml of 1.0 mol/ml Na2CO3, and 2.5 ml Folin-Ciocalteu (100 ml). The mixture was thoroughly blended and then let to cool for 15 minutes at room temperature. Then, using a spectrophotometer, the absorbance of the standard solution and TLP extract was determined at 680 nm in comparison to a control. To express the sample’s total tannin content as a reference against the standard curve, tannic acid (TA) mg TA/g dry weight was used (R² = 0.9972).

**Antioxidant activities**

**Ferrous chelating activity**

The techniques employed to determine the ferrous chelating activity of TLP were reported by Ebrahimzadeh et al. (2008). Summarily, 50 l of 2 mM FeCl₂ was added to 1 ml of various dosages of the TLP extract (0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml). The reaction was then started by adding 0.2 ml of a 5 mM ferrozine solution. After thoroughly shaking the mixture, it was allowed to stand at room temperature for 10 minutes. At 562 nm, the solution’s absorbance was then calculated. The positive control was Na₂EDTA.

\[
\% \text{ inhibition} = \left( \frac{\text{The absorbance of control} - \text{The absorbance of TLP extract}}{\text{The absorbance of control}} \right) \times 100
\]

**Hydroxyl radical inhibition**

To evaluate the hydroxyl radical inhibition capacity of TLP extract, the guidelines reported by Tijani et al. (2012) were adhered to. The reaction mixture contained 1.0 ml of reagent (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H₂O₂, 0.1 mM L-Ascorbic acid, and 0.1 mM FeCl₃·6H₂O in 10 mM phosphate buffer, pH 7.4) and various strengths of the extract (50-350 g/ml). 1.0 ml of 1 percent (w/v) TBA (in 0.25 N HCl) and 1.0 ml of 10 percent (w/v) TCA were added after the reaction mixtures had been incubated at 37°C for an hour. The pink chromogen (malondialdehyde-TBA adduct) was extracted into 1.0 ml of butan-1-ol before the absorbance was measured at 532 nm against a reagent blank. The reaction mixtures were heated in a bain-marie of boiling water for 20 minutes at 100 °C.

\[
\% \text{ inhibition} = \left( \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \right) \times 100
\]

**2,2’-Amino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS)**

A technique described by Turkoglu et al. (2010) and Ozgen et al., (2006) was used to perform the modified ABTS experiment assay. Potassium persulfate was used to make ABTS, which was then dissolved in a buffer solution containing 20 mM sodium acetate (pH 4.5) to achieve an absorbance of 0.700. (0.01 at 734 nm). Then, 100 g/ml concentrations of 1 ml of ABTS + solution and 3 ml of TLP extract in ethanol were mixed. The absorbance was measured 30 minutes after mixing, and at each concentration, the radical scavenging % was calculated in comparison to a blank with no scavenger. The degree of decolorization is assessed using the percentage reduction in absorbance. To draw a standard curve, several ABTS + concentrations were used. The scavenging efficiency of the test chemicals was calculated using the formula below:
Absorbance \( = e^{3\alpha}\) as stopped by adding 200 μL V = \( \Delta \) Absorbance

Action was generated = Volic DPPH solution.

\( X = x_100 \)

\( \text{activity of } \alpha \text{-glucosidase and } 50 \text{ L of an } 8 \text{ mg/mL sample solution were added to the phosphate buffer solution (Type I, lyophilized powder, Sigma, EC 3.2.1.20). The solvent control was a } 5 \text{ percent DMSO solution, and the positive control was } 8 \text{ mg/mL of acarbose in each well. The mixes were incubated at } 37^\circ \text{C for } 2 \text{ minutes. } 50 \text{ microlitres of } 4 \text{ mM pNPG were then put into the well. The mixture has to incubate for a further five minutes in the same circumstances. For } 5 \text{ minutes, the pNP was carried out and timed using a microplate reader at } 405 \text{ nm every } 30 \text{ seconds. The following linear relationship equation between absorbance and time was used to calculate the velocity (V).}

\[
\text{Velocity} = \frac{\Delta \text{Absorbance at } 405 \text{ nm}}{\Delta \text{Time}}
\]

Each sample's initial reaction's highest velocity was gathered, and the equation below was used to calculate the percentage of inhibition.

\[
\text{% Inhibition} = \frac{V_{\text{control}} - V_{\text{sample}}}{V_{\text{control}}} \times 100
\]

\( \text{Alpha-amylose inhibitory activity} \)

The \( \alpha \)-amylose inhibition study was carried out using the 3,5-dinitrosalicylic acid (DNSA) method (Wickramaratne et al., 2016). To create concentrations ranging from 10 to 1000 g/mL, the TLP extract was treated with at least 10 percent dimethylsulfoxide and then diluted in buffer (NaCl (0.006 M, Na₂HPO₄/NaH₂PO₄ (0.02 M, at pH 6.9). 200 μL of extract and 200 μL of amylase solution were mixed and incubated at 30 °C for 10 minutes. After that, each tube received 200 μL of the starch solution (1 percent in water (w/v)) and was incubated for 3 minutes. The reaction was stopped by adding 200 μL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-dinitrosalicylic acid solution) to a water bath at 85–90 °C and boiling for 10 minutes. The mix was cooled to room temperature and diluted with 5 mL distilled water before being analysed with a UV-Visible spectrophotometer at 540 nm. By substituting 200 μL of buffer for the plant extract, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was generated using the plant extract at each concentration. As a positive control sample, acarbose (100–200 μg/mL) was employed, and the reaction was conducted in the same manner as the plant extract reaction. Using the equation below, the inhibitory activity of \( \alpha \)-amylose was calculated and reported as a percentage of inhibition. By plotting the percentage of \( \alpha \)-amylose...
inhibition versus the extract concentration, the IC50 values were determined.

\[
\text{% } \alpha - \text{amylase inhibition} = 100 \times \frac{\text{Absorbance 100% Control} \ - \ \text{Absorbance Sample}}{\text{Absorbance 100% Control}}
\]

**Anti-inflammatory activities**

**Albumin denaturation inhibition**

The assay was carried out as outlined by Osman et al., (2016). Ibuprofen and diclofenac, two positive standards, were produced at a concentration of 0.1 percent each (1.0 mg/ml), along with the TLP extracts. Each mixture's reaction vessel was made up of 1000 µl of the test extract, 1400 µl of phosphate-buffered saline, and 200 µl of egg albumin. As a negative control, distilled water was utilised in place of the extracts. The mixtures were then heated for 5 minutes at 70°C after 15 minutes of incubation at 37°C. Their absorbances at 660 nm were measured after cooling. This formula was used to determine the protein denaturation inhibition percentage:

\[
\text{% Denaturation inhibition} = \left( 1 - \frac{\text{Absorbance reading of the test sample}}{\text{Absorbance reading without test sample (ve control)}} \right) \times 100\%
\]

**Antiproteinase activity**

The test was performed as outlined by Rajesh et al., (2019). 1 ml of 20 mM Tris-HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 ml of the test sample with varying concentrations (100–500 g/ml) were all included in the reaction mixture (2 ml). For five minutes, the mixture was kept heated at 37°C. 1 ml of 0.8 percent (w/v) casein was then added to the mixture. A further 20 minutes were spent keeping the mixture heated. To stop the process, 2 ml of 70% perchloric acid was added to the mixture. The murky suspension was then centrifuged after that. The supernatant's absorbance was then measured at 210 nm using a buffer as a blank. Three times the experiment was conducted. The following formula was used to calculate the % inhibition of proteinase inhibitory activity:

\[
\text{% inhibition} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100\%
\]

**The TLP proximate composition analysis**

The TLP was analyzed for moisture, crude fat, crude protein, ash, and nitrogen-free extract using the AOAC method (AOAC, 2010).

**Statistical analysis**

The average mean was the statistical method used in this study. Each assay was performed three times, and the results' average mean was reported. To better comprehend the average mean, bar graphs were made in Excel.

**Results**

The secondary metabolites, or phytochemicals, that plants synthesize as a defence against reactive oxygen species play a part in determining the phytogens' nutraceutical qualities. The phytoconstituents analysis as shown in Figure 1 reveals phytochemical profiles of TLP: phenol (501.51

![Figure 1. Phytoconstituents of Tapinanthus globiferus leaf powder](image-url)
mg/g), saponins (7.16 mg/g), and tannins (86.53 mg/g). This shows that TLP might have a place in nutraceuticals. TLP’s content of phenol and flavonoids, in particular, may influence cellular functions like apoptosis, gene expression, and intracellular signalling that may have anti-atherogenic and anti-carcinogenic effects (Duthie et al., 2003).

Additionally, the substantial amounts of saponins and tannins in TLP also demonstrate the phytogens’ nutraceutical properties because the aforementioned phytochemicals have been identified as significant bioactive plant components with anticancer, antibacterial, antioxidative, and antimicrobial properties (Yildirim and Kutlu, 2015). The results of this study’s phytochemical examination of TLP hosted by neem trees are different from those for total phenol (155.01 mg/g), flavonoids (15.22 mg/g), alkaloids (6.75 mg/g), saponins (220.83 mg/g) and tannins (10.10 mg/g) recorded for the leaf of mistletoe hosted by kola nut trees being reported by Oloruntola and Ayodele (2022). These variances may result from various plant sources, including host plant species and various processing techniques (Ishiwu et al., 2003; Oloruntola, 2022).

It is established that consuming phytochemicals with high antioxidant phytochemical concentrations raises the serum’s antioxidant capacity (Zhang et al., 2015). The outcomes of the TLP antioxidant assays in this study: Ferrous chelating activity (51.17%), hydroxyl radical inhibition (55.96%), ABTS (76.09%), DPPH (71.34%) and lipid oxidation inhibition (66.18%) (Figure 2) further unveils the antioxidant properties of TLP. The scavenging activity, iron-reducing capacity, iron binding ability, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, and OH scavenging activity are the most widely used antioxidant tests for determining the antioxidant activity of food or feed (Adjimani and Asare, 2015). The phytogen is a potential candidate for an antioxidant dietary supplement based on its ferrous chelating activity, hydroxyl radical inhibition, ABTS, DPPH, and lipid oxidation inhibition properties. When compared to Justicia carnea leaf powder, TLP’s levels in several antioxidant assays are higher: hydroxyl radical inhibition (55.96% vs. 42.81%), ABTS (76.09% vs. 28.49%), DPPH (71.34% vs. 54.35%), and lipid oxidation inhibition (66.18% vs. 62.21%) (Oloruntola et al., 2022). The antioxidant activities of TLP are beneficial because consuming antioxidants is thought to lessen the harm that oxidative stress causes, such as cancer, ageing, atherosclerosis, and vision loss (Salminen et al., 2014).

The α-glucosidase inhibition (75.88%) and α-amylase inhibition (72.14 %) activities of TLP (Figure 3) in this study suggest the phytogens could be a potential antidiabetic feed or food supplement. These antidiabetic properties could be due to the phytoconstituents of TLP, particularly the polyphenols (e.g. phenol and alkaloids) concentrations (Sekhon-Loodu and Rupasinghe, 2019) of TLP. In addition to their antioxidant benefits, dietary polyphenols have been shown to exhibit anti-hyperglycemic properties through their binding to glucose transporters and competitive suppression of digestion enzymes (Lacroix and Li-Chan, 2014). Dietary starch is broken down by the hydrolyzing enzymes α-amylase and α-glucosidase into glucose, causing a postprandial glucose spike. Therefore, one of the main methods to treat hyperglycemic situations is to limit the activities of α-amylase and α-glucosidase (Sekhon-Loodu and Rupasinghe, 2019). It is known that the polyphenols interact with the enzyme by non-specific binding, which inhibits enzyme activity (Wang et al., 2013). The inhibition of α-glucosidase and α-amylase recorded for TLP in this study is greater than the corresponding inhibitions of 65.82% and 65.96% recorded for Jacobinia leaf powder (Oloruntola et al., 2022).

Figure 4 depicts the anti-inflammatory properties of Tapinanthus globiferus leaf powder. The
phytochemical constituents aid in the expression of botanicals' anti-inflammatory properties (Leyva-Jimenez et al., 2020). According to the percentages of antiprotease activity (72.38%) and albumin denaturation inhibition (44.67%) that were measured for TLP in this study, the phytogens may have anti-inflammatory effects when used as dietary supplements. This is of health benefit because, with an anti-inflammatory diet, the risk of heart disease, inflammatory bowel illnesses, arthritis, Alzheimer’s, psoriasis, and other conditions could be decreased (Stromsnes et al., 2021). The antiprotease activity and albumin denaturation inhibition properties of TLP could be due to its phenolic content because correlations were reported to exist between the anti-inflammatory activities of phytogens and their phenolic contents (Naz et al., 2017). Phenolic chemicals can reduce inflammation by either inhibiting the generation of pro-inflammatory mediators or their activity (Ambriz-Perez et al., 2016). The percentage of antiprotease activity and albumin denaturation inhibition recorded for TLP are lower than 79.17% and 62.71%, respectively in the Juglans regia kernel (Oloruntola, 2022).

The proximate composition influences the suitability of food and supplements for dietary inclusion (Oloruntola et al., 2022). TLP has a nitrogen-free extract content of 36.09%, 18.47% crude protein, 13.27% moisture, 12.78% ash, 9.44% crude fibre, and 9.93% crude fat, according to Figure 5. The proximate profile of TLP indicates that it may serve as a source of protein and dietary minerals when used as a feed ingredient, dietary supplement, or additive in man and animal nutrition.

Conclusions

TLP may therefore have anti-inflammatory, anti-diabetic, and antioxidant effects. Therefore, TLP is advised for use in feeding studies using an animal model as a dietary phytogenic and nutraceutical supplement.

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References


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