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# Phytochemical, Proximate and Mineral Composition, Antioxidant and Antidiabetic Properties Evaluation and Comparison of Mistletoe Leaves from Moringa and Kolanut Trees

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ARTICLE INFO	A B S T R A C T
Research Article	This study evaluates the chemical properties of mistletoe leaves powder from moringa trees (MLPM) and mistletoe leaves powder from kola nut trees (MLPK). The MLPM and MLPK were subjected to phytochemical analysis, vitamin A determination, lipid peroxidation inhibition activity,
Received : 26/02/2022 Accepted : 19/04/2022	2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) analysis, proximate and mineral composition analysis, alpha-amylase inhibitory activity and alpha-glucosidase inhibitory activity. Total phenol, steroids, and tannins concentrations were higher in MLPK than in MLPM; steroids was higher in MLPK than in MLPM; whereas total flavonoids and saponins concentrations, were equivalents.
<i>Keywords:</i> Botanicals Mistletoes Nutraceuticals Phytobiotics Supplements	MLPK had greater vitamin A concentrations and DPPH than MLPM. The lipid peroxidation inhibition of MLPM and MLPK did not differ significantly. MLPM had higher moisture, crude fibre, and ash content than MLPK. Furthermore, MLPK had larger fat, crude protein, and nitrogenfree extract contents than MLPM. The MLPM and MLPK have similar Zn (0.9 vs. 1.3 mg/kg), Ca (20.1 vs. 13.1 mg/kg), Mg (66.1 vs. 70.2 mg/kg), and P (185.8 vs. 183.5 mg/kg) contents. The alpha-amylase inhibitory action of MLPM (40.40%) and MLPK (35.07%) were similar. MLPK (44.37%), on the other hand, has a stronger alpha-glucose inhibitory action than MLPK (32.47%). Finally, phenol, steroids, tannins, and steroids varied between MLPM and MLPK, with phenol, steroids, and tannins being greater in MLPK. The MLPM had higher vitamin A concentrations and antioxidant activity than MLPK. The MLPM and MLPK inhibited alpha-amylase and alpha-glucosidase activity.

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## Introduction

Botanicals or their preparations are becoming more popular and generally recognised as part of man's and animals' typical diets due to their widespread availability, chemical composition, and medical usefulness (Carratu et al., 2010; Sevindik et al., 2017; Ayodele et al., 2021; Pehlivan et al., 2021). These plant components (e.g., flowers, leaves, root, bark, stem, and so on) are occasionally powdered and used as a food supplement to change, restore, or correct physiological functioning in humans and animals (Carratu et al., 2010; Kına et al., 2021; Oloruntola et al., 2021; Akgül et al., 2022). Botanicals are useful in the prevention, management, and treatment of a variety of ailments in the past (Na-Bangchang et al., 2014; Oloruntola et al., 2021; Uysal et al., 2021).

African mistletoes (*Loranthus micranthus* Hook.f.) is a hemiparasitic plant also known as devil's fuge, Iscador, bird lime, all-heal, afomo, and other names, is a woody parasitic plant that belongs to the Viscaceae and Loranthaceae families. The Loranthaceae family includes the majority of African mistletoe genera (Adesina et al., 2013). Mistletoe can be found on a variety of economically important tree crops, including the neem tree, citrus species, cocoa, moringa, kola nut, and shea butter tree (Begho et al., 2007; Jiofack et al., 2010; Adesina et al., 2013).

African mistletoes is utilized to treat a variety of maladies in practically every culture on the planet, including diabetes, hypertension, cancer, and edematous and non-edematous associated diseases (Jadhav et al., 2010). Some individuals utilize the plant parts to cure and manage a variety of human and animal illnesses, including diarrhoea, hypertension, wounds, and dysentery (Akinmoladun et al., 2007). Mistletoe has also been shown to have antioxidant, anti-diabetic, and anticholesterolemic effects (Deeni and Sadiq, 2002; Iheanacho et al., 2008).

The chemical composition of botanicals may have an impact on their therapeutic properties (Cao and Wang, 2013; Adeyeye et al., 2020; Korkmaz et al., 2021). However, there are few studies on the chemical makeup of mistletoe leaves compared to other botanicals, and the chemical content of this plant might vary from one host plant species to the next (Ishiwu et al., 2013). As a result, the goal of this research is to compare the phytochemical, proximate and mineral composition, antioxidant activity, alpha-amylase and alpha-glucosidase inhibitory properties evaluation and comparison of mistletoe leaves collected from moringa and kola nut trees.

## **Material and Method**

## **Collection of Mistletoes Leaves and Processing**

On November 2021, fresh mistletoes leaves were collected separately from moringa and kola nut trees within the vicinity of the Federal Polytechnic, Ado Ekiti, Nigeria [annual rainfall:1247 mm; mean annual temperature:  $26.2^{\circ}$ C; location: 437 mm above sea level; latitudes:  $7^{\circ}$  37' N and  $7^{\circ}$  12' N and longitudes:  $5^{\circ}$  11' E and  $5^{\circ}$  31' E] (Oloruntola, 2019).

Fresh mistletoe leaves were cleaned in clean flowing water and divided into species based on their host tree. The leaves were hung on a rack in the shade for 20 days before being blended into mistletoe leaf powders, labelled (MLPM: Mistletoe leaf powder from moringa tree; MLPK: Mistletoe leaf powder from Kola nut trees), and stored for laboratory analysis.

## Chemical analysis

Phytochemical determination

The analytical reagent grade reagents used for chemical analysis were purchased from Sigma-Aldrich.

Tannins

500 g mistletoe leaf powder (MLP) was soaked in 2500 mL 70% ethanol, judder for 360 minutes, then left quiescent for another 2880 minutes before filtering with filter paper (Whatman No 1). The MLP ethanolic extract was vacuum condensed at 35-40°C using a rotary evaporator.

300 g of MLP was soaked in 1500 mL 70% ethanol, vibrated continuously for 360 minutes, then allowed to rest for another 2880 minutes before being filtered using Whatman No 1 filter paper.

Total tannins were determined using the Folin-Ciocalteau method (Biswas et al., 2020). 1 mL of the leaf ethanolic extract was diluted in a volumetric flask (100 mL) with 49 mL distilled water, 1.0 mol/mL Na<sub>2</sub>CO<sub>3</sub> (10 mL), 0.1 mL metaphosphoric acid, 1.7 mL 75% ethanol, and 2.5 mL Folin-Ciocalteu. The mix was well stirred and allowed to rest for 15 minutes at room temperature. The absorbance of the standard solution and leaf extract was then measured against a blank in a spectrophotometer at 680 nm. The standard curve ( $R^2 = 0.9972$ ) was utilised as a reference, and the sample's total tannin content was represented as tannic acid (TA) mg TA/g DW.

Total phenol

The Folin-Ciocalteau method (Otles and Yalcin, 2012) was used. The measure of 250  $\mu$ L of Folin-Ciocalteau reactive was added to 50  $\mu$ L of mistletoe leaf extract or standard solution and kept at room temperature and dark

environment for 5 minutes. Thereafter, a 750  $\mu$ L 7 % Na<sub>2</sub>CO<sub>3</sub> solution was added at the end of the 5 minutes. Then, the mixture was diluted with distilled water to 5 mL and kept at room temperature in a dark environment for 2 hours to react. At 760 nm, the absorbance of the samples and standards were measured. In place of 50 litres of extract, 50  $\mu$ L of 80% methanol solution were added to the blank solution and a calibration curve employing gallic acid equivalent standards was used to quantify the total phenolic concentration.

Total flavonoids

A measure of 1.50 mL methanol, 0.1mL aluminium chloride solution, 0.1 mL potassium acetate solution and 2.8 mL distilled water were added to a 0.50 mL of leaf powder extract-containing test tube. The rutin standard dilutions (10-100 g/mL) sample blanks and leaf extract were made similarly as earlier mentioned, but with distilled water instead of aluminium chloride solution. The solutions were filtered through Whatman filter paper (No. 1) and the absorbance ratios were measured at 510 nm against blanks. The total flavonoid content of the leaf extract was determined to be 1 mg rutin/gram (Surana et al., 2012).

Alkaloids

The gravimetric method (Adeniyi et al., 2009) was used. 5 g of the mistletoes leaf sample was dispensed in 50 ml of acetic acid solution in ethanol (10%, w/v). This mixture was vibrated and kept undisturbed for approximately 240 minutes before being sieved. On a heated plate, the filtrate was reduced to a fraction of its original volume. After that, drops of concentrated ammonium hydroxide were used to precipitate the alkaloids. The precipitate was filtered through filter paper before being rinsed with a 1 percent ammonium hydroxide solution. After that, the precipitate was oven-dried for half an hour at 60°C before being transferred to desiccators and weighed again until it reached a constant weight. The alkaloids' weight was determined as a proportion of the total sample weight.

#### Total saponins

Saponin was measured using the vanillin and concentrated sulfuric acid colourimetric technique (He et al., 2014). The 0.1 mL extract was combined with 4.0 ml sulfuric acid (77 percent w/v), 0.5 mL ethanol (50 percent w/v) and 0.5 mL freshly made vanillin solution (8% w/v). After cooling to ambient temperature, the mixture was heated in a water bath to 60 °C for 15 minutes.

The absorbance at 545 nm was measured using a UV/Vis spectrophotometer. A tea saponin calibration curve was used to quantify the total saponin content in each sample, which was expressed as mg tea saponin equivalent per g (TSE/g DW).

Steroids

Procedures described by Madhu et al., (2016) was used to determine the steroids content of the leaf samples. 1 mL of steroid solution test extract was put into 10 mL volumetric flasks. Iron (III) chloride (0.5 percent w/v, 2 mL) and Sulphuric acid (4N, 2 mL) were added, then potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 mL) was added. The mix was cooked in a water bath at 70°C for 30 minutes, stirring occasionally, and then diluted to the desired concentration using distilled water. At 780 nm, the absorbance was measured against a reagent blank.

#### Vitamin A

Procedures described by Speek et al., (1986) for determining vitamin A was used in this study. High-performance liquid chromatography was used to determine vitamin A as alpha-carotene. Following that, the absorbance was measured at 445 nm and the following equation was calculated: Vitamin A = 1/6 (alpha-carotene) +1/12 (remaining carotenoids).

#### Lipid Peroxidation Inhibition

A procedure previously reported by Bajpai et al., (2015) was used to determine the lipid peroxidation inhibition of mistletoe leaf extracts. The reaction mixture containing 1 mM FeCl<sub>3</sub>, 50  $\mu$ l of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer with a final volume of 330 $\mu$ l was incubated at 37°C for 60 minutes in the absence and presence of mistletoe leaf extract (50–250 g/mL) or reference compound. The hydroxyl radicals produced in the generation of malondialdehyde (MDA), which was quantified by the 2-thiobarbituric acid (TBA) reaction. The inhibitory activity was determined as a percentage.

Percent inhibition (%)=
$$\frac{(A \text{ control-A test})}{(A \text{ control})} \times 100$$

A control: Absorbance of the control reaction A test: Absorbance of a test reaction.

# 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH)

The DPPH antioxidant activity of the MLP was determined using the DPPH radical degradation activity method (Otles and Yalcin, 2012). To make the DPPH radical, pure methanol ( $6x10^{-5}$  M), sample extract or standard solution (100litre), and methanolic DPPH solution (2 litres) were added. For 20 minutes, the DPPH radical was held in the dark. At 515 nm, the sample absorbance was measured. A pure methanol blank solution was used as a control. In the control solution, 100 microliters of clean water were used instead of 100 microliters of extract. A calibration curve was created with varying concentrations (10–100 ppm) of gallic acid solution to test the antioxidant properties of sample extracts.

#### **Proximate and Mineral Composition Determination**

The AOAC (2010) method was used to examine AOLP for ash, crude fibre, crude fat, crude protein, and nitrogenfree extract. The amount of phosphorus was determined using the colourimetric method (adapted from AOAC 965.17). An aliquot of the leaf sample solution containing 0.2 to 1.5 mg P was put in a 100mL volumetric flask. Then, after diluting to volume with distilled water, 20 mL of molybdovanadate reagent was added and well agitated. The absorbance was measured at 400 nm using H<sub>2</sub>O as the blank after allowing the solution to stand for 10 minutes. The standard curve was used to calculate the concentration of P.

After wet digestion with a mixture of nitric acid, sulphuric acid, and hydrochloric acid, the leaf sample's magnesium, calcium, and zinc were measured using an Atomic Absorption Spectrophotometer (Bulk Scientific, USA, model 210 VGP).

#### Alpha-amylase Inhibitory Activity

The 3,5-dinitrosalicylic acid (DNSA) technique was used to perform the  $\alpha$  -amylase inhibition study (Wickramaratne et al., 2016). The MLP extract was treated with at least 10% Dimethylsulfoxide and then diluted in buffer ((NaCl (0.006 M, Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> (0.02 M, at pH 6.9) to provide concentrations ranging from 10 to 1000  $\mu g/mL$ . 200  $\mu L$  of  $\alpha$  -amylase solution (2 units/mL) was combined with 200 µL of extract 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 а 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. Acarbose (100 µg/mL-200 µg/mL) was used as a positive control sample, and the reaction was carried out in the same way as the plant extract reaction. The  $\alpha$ -amylase inhibitory activity was determined using the equation below and expressed as percent inhibition. The IC50 values were calculated by plotting the percent  $\alpha$ -amylase inhibition (α-AI%) against the extract concentration.

 $\alpha$ -AI% =100 ×  $\frac{\text{Abs 100\% Control-Abs Sample}}{\text{Abs100\% Control}}$ 

## Alpha-glucosidase inhibitory Activity

Dej-adisai and Pitakbut described an assay for determining  $\alpha$ -glucosidase inhibitory activity (2015). The yellow product, p-nitrophenol (pNP), formed by the glucosidase enzyme from the substrate, p-nitrophenol-D-glucopyranoside (pNPG), is used to determine the  $\alpha$ -glucosidase reaction. In a well plate, 50 µL of 10 mM phosphate buffer solution (pH 7) was added, which contained 2 mg/mL bovine serum albumin and 0.2 mg/mL sodium azide.

The phosphate buffer solution was combined with 50  $\mu$ L of 8 mg/mL sample solution and 1 unit/mL of  $\alpha$ -glucosidase from Saccharomyces cerevisiae (Type I, lyophilized powder, Sigma, EC 3.2.1.20). A 5 percent DMSO solution was utilised as the solvent control, while the positive control was replaced with 8 mg/mL of acarbose in each well. For 2 minutes, the mixtures were incubated at 37° C. Finally, 50  $\mu$ L of 4 mM pNPG was poured into the well. The combination has to be incubated for another 5 minutes under the same conditions. The pNP was done and monitored by a microplate reader at 405 nm every 30 seconds for 5 minutes. The velocity (V) was calculated using the following linear relationship equation between absorbance and time.

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

The highest velocity from each sample's initial reaction was collected, and the percentage of inhibition was computed using the equation shown below.

% Inhibition=
$$\frac{V \text{ control-V sample}}{V \text{ control}} \times 100$$

## Statistical Analysis

All experiments were repeated three times, and the results were presented as mean standard deviation. SPSS software was used for statistical analysis (version 20). One-way analysis of variance was used to compare mean values, followed by a Dunkan post hoc test, with a p-value of <0.05 considered significant.

## Results

The phytochemical content of Mistletoe leaves from Moringa and Kolanut trees are shown in Table 1. Total phenol, steroids, and tannins concentrations were higher (P<0.05) in Mistletoe K than in Mistletoe M; steroids was higher (P<0.05) in mistletoe K than in mistletoe M;

whereas total flavonoids and saponins concentrations, were equivalents (P>0.05).

Mistletoe K had considerably greater vitamin A concentrations and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl hydrate) than (P<0.05) Mistletoe M. The lipid peroxidation inhibition of Mistletoe M and Mistletoe K did not differ (P>0.05) significantly (Table 2).

Figure 1 depicts the proximate composition of Mistletoe leaves from Moringa and Kolanut trees. Mistletoe M had higher moisture, crude fibre, and ash content (P<0.05) than Mistletoe K. Furthermore, Mistletoe K had larger fat, crude protein, and nitrogen-free extract contents than (P<0.05) Mistletoe M.

Mistletoe M and mistletoe K have similar (P>0.05) Zn (0.9 vs. 1.3 mg/kg), Ca (20.1 vs. 13.1 mg/kg), Mg (66.1 vs. 70.2 mg/kg), and P (185.8 vs. 183.5 mg/kg) contents (Figure 2).

The alpha-amylase inhibitory action of Mistletoe M (40.40%) and mistletoe K (35.07%) were similar (P>0.05), as shown in Figure 3. Mistletoe K (44.37%), on the other hand, has a stronger (P<0.05) alpha-glucose inhibitory action than Mistletoe M (32.47%).

Table 1. The phytochemical composition of Mistletoe leaves from Moringa and Kolanut trees

			U			
Leaves	Total phenol	Total flavonoids	Alkaloids	Saponins	Steroids	Tannins
	(mg/g)	(mg/g)	(%)	(mg/g)	(mg/g)	(mg/g)
Mistletoe M	83.32 <sup>b</sup>	12.55	8.58 <sup>a</sup>	200.13	15.98 <sup>b</sup>	5.39 <sup>b</sup>
Mistletoe K	155.01 <sup>a</sup>	15.22	6.75 <sup>b</sup>	220.83	17.75 <sup>a</sup>	10.10 <sup>a</sup>
SEM	17.94	0.91	0.41	5.78	0.41	1.08
P value	0.01	0.16	0.00	0.05	0.01	0.01

Means on the same column with different superscripts are significant (P<0.05); SEM: Standard error of the mean; Mistletoe M: Mistletoe leaf from Moringa tree; Mistletoe K: Mistletoe leaf from Kolanut tree

Table 2. The vitamin A	A concentration and	antioxidant act	ivities of Mistleto	e leaves from l	Moringa and Kolanut trees
					0

Leaves	Vitamin A (UI)	Lipid peroxidation inhibition (%)	2,2-diphenyl-1-picrylhydrazyl hydrate (%)
Mistletoe M	1654.91 <sup>b</sup>	56.16	49.31 <sup>b</sup>
Mistletoe K	1750.35ª	58.28	57.11 <sup>a</sup>
SEM	24.67	0.77	1.80
P value	0.02	0.19	0.01

Means on the same column with different superscripts are significant (P<0.05); SEM: Standard error of the mean; Mistletoe M: Mistletoe leaf from Moringa tree; Mistletoe K: Mistletoe leaf from Kolanut tree



Figure 1. Proximate composition of Mistletoe leaves; Mistletoe M: Mistletoe leaf from Moringa tree; Mistletoe K: Mistletoe leaf from Kolanut tree.



Figure 2. The mineral composition of Mistletoe leaves; Mistletoe M: Mistletoe leaf from Moringa tree; Mistletoe K: Mistletoe leaf from Kolanut tree.



Figure 3. The Alpha-amylase inhibition and Alpha-glucosidse inhibition capacity of Mistletoe leaves; Mistletoe M: Mistletoe leaf from Moringa tree; Mistletoe K: Mistletoe leaf from Kolanut tree

# Discussion

Plants or botanicals have medical potential because of bioactive phytochemical elements that have а physiological effect on humans and animals (Aborisade et al., 2017; Korkmaz et al., 2021). Because of changes in the concentration of various phytochemical ingredients between Mistletoe K and Mistletoe K, the potential for these botanicals to produce physiological effects on a biological organism may differ. The discovery of total phenol, total flavonoids, alkaloids, saponins, steroids, and tannins in Mistletoe M and Mistletoe K by quantitative analysis in this study demonstrates their pharmacological potentials and the potential function they could play in human and animal health. The phenolic compound, a secondary metabolite, is a well-known antioxidant that has been shown to aid in the treatment and prevention of a varietv of diseases in humans and animals (Tungmunnithum et al., 2018); antimicrobial, antiparasitic, and antiviral properties of alkaloids result in a variety of psychopharmacological, physiological, and behavioural consequences in people and animals (Bouayad et al., 2012); steroids have anti-inflammatory and immunemodulating characteristics (Ericson-Neilsen and Kaye, 2014); while tannins can have biological effects as an unabsorbable, complex structure with binding properties that can have local effects (antioxidant, radical scavenging, antimicrobial, antiviral, antimutagenic, and antinutrient) in the gastrointestinal tract or as absorbable tannins and absorbable tannin metabolites that can have systemic effects in various organs (Sieniawska, 2015; Kina et al., 2021; Korkmaz et al., 2022). In this study, increased total phenol, alkaloids, steroids, and tannins concentrations were found in Mistletoe K compared to Mistletoe M, indicating that these botanicals may have different efficacy and effectiveness in human and animal health.

Plants produce carotenoids (vitamin A precursors), which can be transformed into real vitamin A by a

specialised enzyme found in the intestinal walls of mammals. Vitamin A's physiological functions in reproduction, eyesight, development, epithelium and bone construction make plants with measurable vitamin A valuable in animal and human diet (McDowell, 2021). The considerable difference in vitamin A concentration in Mistletoe leaves from different host trees found in this study points to Mistletoe K's nutritional superiority over Mistletoe M. Furthermore, because several botanicals include antioxidant phytochemicals, their consumption has been linked to a lower risk of developing a variety of chronic diseases (Zhang et al., 2015). The differences in the levels of 2,2-diphenyl-1-picrylhydrazyl hydrate in the two botanicals under investigation reveal that Mistletoe K has superior antioxidant properties to Mistletoe Mand by implication, Mistletoe K may be of more pharmacological and nutritional value than mistletoe M (Pietrzak and Nowak, 2021). The stronger antioxidant activity of mistletoe K may be due to its higher phenol concentration than mistletoe M. Phenols are phytochemicals that have potent antioxidant activity (Kumar et al., 2014).

The food business is interested in the proximate composition of foods for product creation, quality control, and regulatory purposes (MdNoh et al., 2020). The amount of moisture in food can have a big impact on factors like texture, shape, flavour, appearance, and weight. Moisture also has consequences for food labelling, food quality measurement, food or food product shelf life, and food processing processes (Isengard, 2001). The lower moisture content of mistletoe K, compared to mistletoe M suggests that mistletoe M may have a better quality and shelf life (Isengard, 2001). The higher fat, crude protein and nitrogen-free extract concentration of mistletoe K, over mistletoe M in this study reveals mistletoe M as a better contributor of dietary energy and protein in human or animal nutrition. Because dietary fibre consumption is linked to general metabolic health as well as some other diseases such as colonic health, cardiovascular disease, gut motility, and the risk of colorectal cancer, the higher crude fibre content of mistletoe M compared to mistletoe K in this study is of nutritional and health significance. Dietary fibre aids the gut microbiota in metabolic activities, appetite management, and chronic inflammatory pathways, all of which are supported by gut bacteria (Barber et al., 2020). The entire mineral content of foods is represented by ash content (Harris and Marshall, 2017). As a result, mistletoe M appears to be a stronger supplier of dietary minerals in human and animal nutrition than mistletoe K, even though both botanicals have identical amounts of Zn, Ca, Mg, and P.

Alpha-amylase is a well-known enzyme present in pancreatic juice and saliva that breaks down large insoluble starch molecules into absorbable ones (Afifi et al., 2008). The last phase of digestion of starch and disaccharides, which are common in the human diet, is catalysed by mammalian alpha-glucosidase in the mucosal brush border of the small intestine (Manohar et al., 2002). Alphaamylase and alpha-glucosidase inhibitors slow the breakdown of carbohydrates in the small intestine and lower postprandial blood glucose levels (Kwon et al., 2007). In this study, the alpha-amylase inhibition and alpha-glucosidase properties exhibited by mistletoe M and mistletoe K suggest that these botanicals have antidiabetic properties. The active components in the botanicals under investigation are likely competing with the substrate for binding to the enzyme's active site, limiting the conversion of oligosaccharides to disaccharides (Matsuda et al., 2002). The presence of phytochemicals such as flavonoids, saponins, and tannins in mistletoe M and mistletoe K may be responsible for their inhibitory effects (Kazeem et al., 2013). This result is consistent with a previous study that found alpha-amylase inhibition and alpha-glucosidase inhibition activity in aqueous extracts of mistletoe leaves from the moringa and almond host plants (Oyeniran et al., 2021). In addition, the higher alpha-glucosidase inhibition activity demonstrated by mistletoe K over mistletoe M in this study suggests the former botanical has higher antidiabetic property.

# Conclusion

Finally, phytochemical concentrations such as phenol, steroids, tannins, and steroids varied between mistletoe leaves obtained from moringa and kola nut trees, with phenol, steroids, and tannins being greater in mistletoe K. Mistletoe K had higher vitamin A concentrations and antioxidant activity. While the composition of Zn, Ca, Mg, and P was not changed between mistletoe M and mistletoe K, mistletoe K may be a greater donor of dietary protein and energy. The two botanicals under investigation inhibited alpha-amylase and alpha-glucosidase activity.

#### References

- Aborisade AB, Adetutu A, Owoade A O 2017. Phytochemical and Proximate Analysis of Some Medicinal Leaves. Clinical Medicine and Research,6(6): 209-214.
- Adeniyi SA, Orjiekwe CL, Ehiagbonare J E. 2009. Determination of alkaloids and oxalates in some selected food samples in Nigeria. African Journal of Biotechnology, 8(1):110-112.
- Adesina SK, Illoh H C, Johnny II, Jacobs I E. 2013. African mistletoes (Loranthaceae); ethnopharmacology, chemistry and medicinal values: an update. African Journal of Traditional Complementary and Alternative Medicine, 10(4):161–170.
- Adeyeye SA, Oloruntola OD, Ayodele SO, Falowo AB, Agbede J O. 2020.Wild sunflower and goat weed composite-mix supplementation in broiler chicken: effects on performance, health status and meat. Acta Fytotechnica et Zootechnica, 23(4): 205-212.
- Afifi A F, Kamel EM, Khalil AA, Foaad MA, Fawziand EM, Houseny M. 2008. "Purification and characterization of aamylase from penicillium olsonii under the effect of some antioxidant vitamins," Global Journal of Biochemistry and Biotechnology, 3(1): 14–12.
- Akgül H, Mohammed FS, Kına E, Uysal İ, Sevindik M, Doğan M. 2022. Total Antioxidant and Oxidant Status and DPPH Free radical activity of *Euphorbia eriophora*. Turkish Journal of Agriculture-Food Science and Technology 10(2): 272-275.
- Akinmoladun AC, Akinrinlola BL, Fraambi EO. 2007. Chemical constituents and antioxidant activity of mistletoe. African Journal of Biotechnology, 6 (10):1197-1201.
- AOAC. 2020. Official Methods of Analysis of Association of Offical Analytical Chemists. 18th Edition, Washington DC.
- Ayodele SO, Oloruntola OD, Ayeyeye S A, Jimoh OA, Falowo A B, Omoniyi I S. 2021. Supplementation value of Mucuna seed powder on performance, antioxidant enzymes, meat cholesterol and peroxidation, and serum metabolites of broiler chickens. Malaysian Journal of Animal Science, 24(1):11-22.

- Bajpai VK, Park Y, Agrawal P. 2015.Studies on phytochemical analysis, antioxidant and lipid peroxidation inhibitory effects of a medicinal plants, *Coleus forskohlii*. Frontiers in Life Science, 8(2): 139-147.
- Barber TM, Kabisch S, Pfeiffer A, Weickert MO. 2020. The Health Benefits of Dietary Fibre. Nutrients, 12(10), 3209.
- Begho E R, Omokhafe KO, Omo-Ikerodah E E, Akpaja,EO. 2007. Some observations on the fruit set and incidence of Mistletoes on rubber trees in Nigeria. American-Eurasian Journal of Sustainable Agriculture, 1(1):13–18.
- Biswas A, Dey S, Li D, Yiu L, Zhang J, Huang S, Pan G, Deng Y. 2020. Comparison of Phytochemical Profile, Mineral Content, and In Vitro Antioxidant Activities of *Corchorus capsularis* and *Corchorus olitorius* Leaf Extracts from Different Populations. Journal of Food Quality, 2020:2020(9).
- Bouayad N, Rharrabe K, Lamhamdi M, Nourouti NG, Sayah F. 2012. Dietary effects of harmine, a β-carboline alkaloid, on development, energy reserves and α-amylase activity of Plodia interpunctella Hübner (Lepidoptera: Pyralidae). Saudi Journal of Biological Science, 19(1):73-80.
- Cao J, Wang Y. 2013. Relationship between chemical constituents and herbs properties of relative plant herbs. Zhongguo Zhong Yao Za Zhi, 38(3):453-458.
- Carratu B, Federici E, Gallo F R, Geraci A, Guidotti M, Multari G, Palazzino G, Sanzini E. 2010. Plants and parts of plants used in food supplements: an approach to their safety assessment. Ann Ist Super Sanita, 46(4): 370-388.
- Deeni Y Y, Sadiq N M. 2002. Antimicrobial properties and phytochemical constituents of leaves of African mistletoe (*Tanipanthus dodoneifolius* DC danser) lorathecceae): an ethnomedicinal plant of Hausa land. Northern Journal of Ethnopharmacology, 83: 235-240.
- Dej-adisai S, Pitakbut T. 2015. Determination of α-glucosidase inhibitory activity from selected Fabaceae plants. Pakistan Journal of Pharmacological Science, 28(5): 1679-1683.
- Ericson-Neilsen W, Kaye AD. 2014. Steroids: pharmacology, complications, and practice delivery issues. The Ochsner Journal, 14(2): 203–207.
- Harris G K, Marshall M R. 2017. Ash Analysis. In: Nielsen S.S. (eds) Food Analysis. Food Science Text Series. 2017.Springer, Cham. https://doi.org/10.1007/978-3-319-45776-5\_16
- He J, Wu ZY, Zhang S, Zhou Y, Zhao F, Peng Z Q, Hu ZW. 2014. Optimisation of microwave-assisted extraction of tea saponin and its application on cleaning of historic silks. Journal of Surfactants and Detergents, 17(5):919-28.
- Iheanacho KM E, Ihegboro GO, Udebuani AC. 2008. Phytochemical and effect of aqueous leaf extract of mistletoe on serum glucose and cholesterol concentration in rats. Nigerian Journal of Biochemistry and Molecular Biology, 23 (2):35-39.
- Isengard H. 2001. Water content, one of the most important properties of food. Food Control, 12(7): 395-400.
- Ishiwu C N, Obiegbun, J E, Aniagolu N M. 2013. Evaluation of chemical properties of mistletoe leaves from three different trees (Avocado, African Oil Bean and Kola). Nigerian Food Journal, 31(2): 1-7. https://doi.org/10.1016/S0189-7241(15)30070-9.
- Jadhav N, Patil CR, Chaudhari KB, Wagh JP, Surana SJ, Jadhav R B. 2010. Diuretic and natriuretic activity of two mistletoe species in rats. Pharmacognosy Research, 2(1): 50-57.
- Jiofack R T, Dondjang JP, Nkongmeneck B A. 2010. The Loranthaceae of the Bafou area in Cameroon; identification, distribution, biology and eradication strategies. In: Burgt X van der, Maesen J van der, Onana J M., editors. Systematics and Conservation of African Plants; Proceedings of the 18th AETFAT Congress, Yaounde, Cameroon; 26th February– 2nd March 2007; 2010. pp. 229–235

- Kazeem M I, Adamson J O, Ogunwande I.A. 2013. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. BioMedical Research International, 2013:1-6. https://doi.org/10.1155/2013/527570
- Kına E, Uysal İ, Mohammed FS, Doğan M, Sevindik M. 2021. In-vitro antioxidant and oxidant properties of *Centaurea rigida*. Turkish Journal of Agriculture-Food Science and Technology 9(10): 1905-1907.
- Korkmaz N, Dayangaç A, Sevindik M. 2021. Antioxidant, antimicrobial and antiproliferative activities of *Galium aparine*. Journal of Faculty of Pharmacy of Ankara University 45(3): 554-564.
- Kumar S, Sandhir R, Ojha S. 2014. Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC Research Notes, 7: 560. https://doi.org/10.1186/1756-0500-7-560
- Kwon T I, Apostolidis E, Shetty K. 2016. "Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension," Journal of Food Biochemistry, 31(3): 370– 385.
- Madhu M, Sailaja V, Satyadev TNVSS, Satyanarayana MV. 2016. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvent. Journal of Pharmacognosy and Phytochemistry, 5(2): 25-29.
- Manohar V, Talpur NA, Echard BW, Lieberman S, Preuss H G. 2022."Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice," Diabetes, Obesity and Metabolism, 4(1): 43–48.
- Matsuda H, Morikawa T, Yoshikawa M, 2002. "Antidiabetogenic constituents from several natural medicines," Pure and Applied Chemistry, 74(7): 1301–1308.
- McDowell LR. Vitamins in Animal and Human Nutrition. 2021.2nd Edition, Iowa State University Press, USA. Pp. 762.
- MdNoh M, Gunasegavan RD, Khalid NM, Balasubramaniam V, Mustar S, Rashed AA. 2020. Recent techniques in nutrient analysis for food composition database. Molecules, 25(19):4567; https://doi.org/10.3390/molecules25194567
- Na-Bangchang K, Karbwang J. 2014. Traditional herbal medicine for the control of tropical diseases. Tropical Medicine and Health, 42(2 Suppl): 3–13. https://doi.org/10.2149/tmh.2014-S01
- Oloruntola D A, Dada EO, Oladunmoye MK. 2021. Trypanocidal function of *Terminalia catappa* extract in Albino rat. Acta Fytotechnica et Zootechnia, 24(4): 326-333.
- Oloruntola OD. 2019. Effect of pawpaw leaf and seed meal composite mix dietary supplementation on haematological indices, carcass traits, and histological studies of broiler chicken. Bulletin of National Research Centre, 43: 129. https://doi.org/10.1186/s42269-019-0172-0.
- Otles S, Yalcin B. 2012. Phenolic compounds analysis of root, stalk, and leaves of nettle. Scientific World Journal. 2012, 564367. doi: 10.1100/2012/564367.
- Oyeniran O H, Ademiluyi AO, Oboh G. 2021. Host-parasite relationship modulates the effect of African mistletoe leaves on the cholinergic, monoaminergic and carbohydrate hydrolyzing enzymes in fruit fly. Journal of Basic and Clinical Physiology and Pharmacology, 2021 Jun 22. doi: 10.1515/jbcpp-2020-0298.
- Pehlivan M, Mohammed FS, Şabik AE, Kına E, Dogan M, Yumrutaş Ö, Sevindik M. 2021. Some Biological activities of ethanol extract of *Marrubium globosum*. Turkish Journal of Agriculture-Food Science and Technology 9(6): 1129-1132.
- Pietrzak W, Nowak R. 2021. Impact of harvest condition and hos tree species on chemical composition and antioxidant activity of extracts from *Viscum album* L. Molecules, 26: 3741. https://doi.org/10.3390/molecules26123741
- Sevindik M, Akgul H, Pehlivan M, Selamoglu Z. 2017. Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresen Environ Bull 26(7): 4757-4763.

- Sieniawska E. 2015. Activities of Tannins- From in-vitro studies of clinical trials. *Natural Product Communications*, 10(11): 1877-1884.
- Speek AJ., Temalilwa C R, Schrijver J. 1986. Determination of beta-carotene content and Vitamin A activity of vegetables by high-performance liquid chromatography and spectrophotometry. Food Chemistry, 19:65–74.
- Surana AR, Kumbhare MR, Wagh RD. 2016. Estimation of total phenolic and total flavonoid content and assessment of in vitro antioxidant activity of extracts of Hamelia patens Jacq. stems. Research Journal of Phytochemistry, 10(2): 67-74.
- Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. 2018. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines* (Basel, Switzerland), 5(3): 93. https://doi.org/10.3390/medicines5030093
- Uysal İ, Mohammed FS, Şabik AE, Kına E, Sevindik M. 2021. Antioxidant and Oxidant status of medicinal plant *Echium italicum* collected from different regions. Turkish Journal of Agriculture-Food Science and Technology 9(10): 1902-1904.
- Wickramaratne MN, Punchihewa J C, Wickramaratne DB. 2016. *In-vitro* alpha amylase inhibitory activity of the leaf extracts of Adenanthera pavonina. BMC Complementary Alternative Medicine, 16(1): 466. https://doi.org/10.1186/s12906-016-1452-y
- Zhang Y J, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li H. B. 2015. Antioxidant Phytochemicals for the prevention and treatment of chronic diseases. Molecules (Basel, Switzerland), 20(12): 21138–21156. https://doi.org/10.3390/molecules201219753