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Examining the Phytochemicals in Leaves of Pterocarpus Species in Nigeria

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Abstract

Aim of study: The study carried out a phytochemical analysis for tannin, flavonoid, saponins, phenol, alkaloids, terpenoids and anti-nutrient compounds – Oxalate and phytate.

Materials and method: These were carried out on powdered leaf methanolic extract of five endemic Pterocarpus species viz; *P. osun, P. mildbraedii, P. soyauxii, P. erinaceus* and *P. santaloides* using standard procedure.

Main results: Both qualitative and quantitative analysis indicated that the leaves sample contained similar phytochemical/secondary metabolites with variation in mean concentration value (mg/100g); Tannins (1.06 - 2.89 mg/100g); flavonoid (2.17-5.46 mg/100g); saponins (2.78-9.38 mg/100g), phenol (1.13-2.56 mg/100g), Alkaloids (3.06-6.28 mg/100g), Terpenoids (1.32-2.46 mg/100g); Oxalate (0.34-1.14 mg/100g) and Phytate (0.16-0.46 mg/100g). These differed significantly ($p \geq 0.05$) amongst the examined taxa.

Research highlights: The highest concentration of saponins, phenol, tannin and phytate was found in leaves of *P. mildbraedii* while *P. erinaceus* contained the highest concentration of flavonoid, alkaloid and oxalate. However, terpenoid was found highest in leaves of *P. soyauxii*. Generally, the studied Pterocarpus species contained low level of anti-nutrient compound making them safe for consumption. Thus, the study revealed the rich phytochemical content in leaves of this genus to justify their ethnobotanical usage for leafy vegetable and ethnomedicine. Furthermore, findings provided additional information in support of the taxonomic placement and classification of members of the genus on the basis of their phytochemistry.

Keywords: Phytochemical, Pterocarpus, Ethnomedicine

Nijerya'daki Pterocarpus Türlerinin Yapraklarındaki Fitokimyasalların İncelenmesi

Öz

Çalışmanın amacı: Çalışmanın amacı Pterocarpus türlerinin yapraklarındaki tanen, flavonoid, saponinler, fenol, alkaloitler, terpenoidler ve anti-besin bileşikleri olan oksalat ve fitat açısından fitokimyasal içeriklerini belirlemektir.

Materyal ve yöntem: Analizler, *P. osun, P. mildbraedii, P. soyauxii, P. erinaceus ve P. santaloides* olmak üzere beş endemik Pterocarpus türünün toz halindeki metanol ekstraktları üzerinde standart prosedürler kullanılarak gerçekleştirildi.

Temel sonuçlar: Hem nitel hem de nicel analizler, yaprak örneklerinin ortalama konsantrasyon değerlerinde (mg/100g) değişiklik gösteren benzer fitokimyasal/ikincil metabolitler içerdiğini göstermektedir; tanenler (1.06-2.89 mg/100g); flavonoid (2.17-5.46 mg/100g); saponinler (2.78-9.38 mg/100g), fenol (1.13-2.56 mg/100g), alkaloidler (3.06-6.28 mg/100g), terpenoidler (1.32-2.46 mg/100g); oksalat (0.34-1.14 mg/100g) ve fitat (0.16-046 mg/100g). Elde edilen fotokimyasal değerler incelenen taksonlar arasında önemli ölçüde farklılık göstermiştir ($p \geq 0.05$).

Araştırma vurguları: Saponinler, fenol, tanen ve fitatın en yüksek konsantrasyonu *P. mildbraedii* yapraklarında bulunurken, *P. erinaceus* en yüksek flavonoid, alkaloid ve oksalat konsantrasyonunu içermiştir. Terpenoid oranı en yüksek olarak *P. soyauxii* yapraklarında bulundu. Genel olarak, incelenen Pterocarpus türleri düşük düzeyde anti-besin bileşiği içermiştir ve bu da onları tüketim için güvenli hale getirmektedir. Bu nedenle, çalışma bu cinsin yapraklarındaki zengin fitokimyasal içeriği ortaya koyarak, yapraklı bitkiler ve etnomedikal için etnobotanik kullanımlarını haklı çıkarmaktadır. Ayrıca, bulgular cins üyelerinin fitokimyalarına göre taksonomik yerleşimini ve sınıflandırılmasını destekleyen ek bilgiler sağlamaktadır.

Anahtar kelimeler: Fitokimyasal, Pterocarpus, Etnomedikal

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Introduction

Pterocarpus jacq. is a large pantropical genus of mostly trees belonging to the family Fabaceae (sub family: Fabiodeae). Recent phylogenetic studies have assigned the genus to the informal monophyletic Pterocarpus clade within Dalbergieae (Lavin et al., 2001; Cardoso et al., 2013). About 46 species, all members of the genus have been recorded and distributed within the tropics and subtropical regions with the greatest diversity of close to 20 species found in tropical Africa (Allahi et al., 2011; Plantlist, 2020; IPNI, 2024). In Nigeria, available taxonomic literature shows that 6 *Pterocarpus* tree species are endemic in the country. These are; *Pterocarpus osun* Craib (common name: camwood, Igbo: Uhie), *P. erinaceus* Poir (common name: African paduak), *P. mildbraedii* Harms (common name: mildbraedi*i* paduak, Igbo: Oha ocha), *P. santalinoides* L' Heit ex DC (common name: Red sandalwood, Igbo: Nturukpa), *P. lucens* Guill & Peir, *and P. soyauxii* Taub (common name: African paduak, Igbo: Oha oji) (Keay, 1989). *P. lucens* may sometime occur as shrub or small tree within the savanna ecosystem where it is confined.

The folkloric medicinal uses of the component part of *Pterocarpus* species – roots, stem barks and leaves have been reported and are largely ethnic community- and ecosystem specific. The roots have been used for cough remedy (Sandrine, 2006); the bark for tooth and mouth scrapes; bark resin as astringent for serious diarrhea and dysentery while the leaves is used to treat diarrhea, stomach ache, elephantiasis and fever (Okpo et al., 2011; Obi et al., 2019). Generally, the leaves of the members of the genus *Pterocarpus* have been exploited from time immemorial as a common source of treatment for human and animal diseases and nutrition (Ndukwe & Ikpeama, 2013; Olafadehan, 2013; Özgenç et al., 2017; Obi et al., 2019). Specifically, 3 *Pterocarpus* species viz (*P. mildbraedii, P. soyauxii, and P. santaliodes*) are recognized as being an important indigenous leafy vegetable, used for soup making and consumed in the south-eastern region of Nigeria, particularly among the Igbo speaking region (Ekumankama, 2008; Ndukwe & Ikpeama, 2013). In animal nutrition, leaves of *P. erinaceus* are considered, a cheap source of protein and nitrogen in animal diets, improving their overall performance and weight gain (Olafadehan, 2013). Also, the ethno-veterinary usage of leaves of *P. santaloides* have been reported and proven to possess some anti-trypanosomal activity and may be a headway for the manufacture of an efficient alternative anti-trypanosomal drug (Obi et al., 2019).

However, following the recent upsurge in phytomedicinal product for human health, more scientific interest have been shifted into phytochemicals present in plants. Hence, numerous phytochemical studies have focused on the potential therapeutic effects of various secondary metabolites derived from medicinal plants that possess various pharmacological properties, including, flavonoids, saponins, alkaloids, phenols, tannins, and terpenoids (Nunes & Miguel, 2017). These plant metabolites have been widely studied as medicinal agents and differ from one generic group to another. Interestingly, the phytochemicals in leaves of the component species within the genus *Pterocarpus* in Nigeria is too imperfectly known to be fully reported. Various authors differ in the number of species and phyto-constituents studied; 3 (Akinmoladun et al., 2015), 2 (Ekumankama, 2008; Ndukwe & Ikpeama, 2013) and 1 species (Ogbonna & Idumah, 2018) amongst others. Hence, this study was initiated in order to carry out a qualitative and quantitative phytochemical and phytonutrient assessment of leaf samples of *Pterocarpus* species recorded in Nigeria. The data obtained from this study will provide valuable information for phytomedicinal research and contribute to the taxonomic placements and relationship within this generic group.

Material and Methods

Plant Material

The leaves of the 5 *Pterocarpus* species viz *P. osun, P. santaloides, P. mildbraedi, P. soyauxii* and *P. erinaceus* were collected from the Arboretum of Forestry Research Institute of Nigeria outstation located at Humid Forest Research Station, Umuahia (Lat 5°30′48″N and Long 7°31′32″E) of the Greenwich meridian, and Swamp Forest Research

Station, Onne (Lat 4°42′13″N and Long 7°10′36″E) of the Greenwich meridian respectively. The specimen were identified by the senior author and further authenticated at the Forest Herbarium, Ibadan (FHI) listed in Holmgren (1990) following conventional taxonomic practice. The freshly collected leaves (200 kg) from each *Pterocarpus* species were cut into smaller pieces and air-dried separately for 2 weeks.

Preparation of the Extracts

The leave samples were pulverized and 80 g each were extracted with 400 ml of methanol which made up the ratio of 1:5 using Soxhlet apparatus. The extracts were concentrated in a hot air oven at 40°C and the concentration gave dark green paste which were transferred aseptically in a sterile bottle and stored in refrigerator for future use.

Qualitative Analysis of the Plant Extracts

Phytochemical screening were carried out on the extract of the 5 species using standard methods to identify the selected metabolites as described by Trease & Evans (2009) and modified by Chaudhary et al. (2010). The leaves extracts were tested for alkaloids, phenols, flavonoids, saponins, terpenoids, tannins, phytate and oxalate.

Test for Tannins: 2 ml of each extract was measured into a test tube and added to it was drops of 0.1% ferric chloride and observed for brownish green or dark blue coloration.

Test for Saponins: Using the frothing test, 2 ml of each leaf extract was added to 20 ml of distilled water in a graduated cylinder and shaken vigorously for 15 minutes. The observation of 1 cm layer of foam confirmed saponins. 3 drops of olive oil was added to the cylinder to further confirm saponins through the formation of a stable foam.

Test for Terpenoids: 10 ml of methanol was added to 0.8 g of each powdered sample in a test tube and thoroughly shaken. Each solution was filtered using No 42 whatman filter paper. 2 ml of chloroform was mixed with 5 ml of each plant extract in a test tube followed by 3 ml of conc. H_2SO_4 added carefully gradually forming a layer. The interface of a brownish red coloration is formed which indicates that terpenoids metabolite is present.

Test for flavonoids: Three procedures were initiated to test for the existence of flavonoids in the 5 extracted plant samples. Firstly, 5 ml from dilute ammonia solution was added to a ration of the extract of each plant after which 1 ml of concentrated H_2SO_4 was added. A yellow color which disappears when held erect shows the existence of flavonoids in each plant extract. Secondly, Little drops of 1% aluminium solution was added to small portion of each plant extract. Presence of flavonoid is indicated by a yellow coloration. Thirdly, a portion of each plant sample was individually heated using 10 ml ethyl acetate above a steam bath for about 3 minutes and filtered. 4 ml taken from the filtrate was measured and mixed vigorously with 1 ml of diluted ammonia solution. The yellow coloration noticed indicates flavonoids.

Test for Alkaloids: To 5 ml of each leaf extract, 5 ml of 1% HCl was added and boiled above a water bath for 5 mins. To the mix was filtered and 2 ml of the filtrate was treated with 3 drops of Dragendorff's reagent was added. Presence of reddish-brown precipitate confirms alkaloids.

Test for Phenols: To 2 ml of each extract in a test tube, 2 ml of distilled water and 5 drops of 10%

Ferric chloride was slowly added. The appearance of blue or green coloration shows the existence of phenols.

Test for Oxalate (Blue ring test): 1 ml of leaf extract was dispensed into a test tube, 2 to 3 flakes of resorcinol and 1 ml of distilled water was added and the solution heated. Few mils of concentrated H_2SO_4 were added to the test tube sidewalls after the solution had cooled. The presence of oxalic acid is confirmed by the appearance of a blue ring where two layers meet.

Test for Phytate: 1 ml of each leaf extract was dispensed into a test tube, 2 ml of 20% Trichloroacetic acid and 2 ml of distilled water was added and the mix shaken thoroughly. The mixture was filtered and 1 ml of the filtrate was dispensed into a test tube, few drops of bromocresol purple indicator and 1 ml of magnesia mixture reagent was added. The mixture was stirred and neutralized by adding Ammonium Hydroxide dropwise. After the change of colour, 1 ml of Ammonium Hydroxide solution was added

with mixing. A flocculent precipitate which separates completely from the solution, indicates the presence of phytic acid.

Quantitative Determinations of Different Extracts of Pterocarpus genus

The plant extracts were evaluated quantitatively with the following procedures according to AOAC (2005) with some modifications by other authors.

Determination of Tannins

The value of Tannins was carried out using folin-ciocalteu assay according to (Md. Mahadi et al., 2014).1 g of leaf extract was measured into a 50 ml beaker and 7.5 ml of distilled water, 0.5 ml folin-ciocalteu reagent and 1 ml of 35% sodium carbonate was added. The mixture was shaken and allowed to stand for 30 minutes for the colour to develop. 0 to 0.5 mg/ml of Tannic acid dilutions were used as standard solutions. An UV-VIS spectrophotometer was used to measure the absorbance of the extracts and standard solutions of tannic acid at 725 nm in relation to the blank.

Determination of Alkaloid was Estimated Using

5 g of each sample was measured into a 250ml beaker and 40 ml of 10% acetic acid was added, mixed and covered after which it was permitted to stand for 4hour. The filtrate is concentrated to ¼ of its actual measurements using a water bath for 25 mins and left to concentrate. Concentrated ammonium hydroxide was then added gradually in a drops to the concentrated filtrate till precipitation was finished. The mixture was kept to settle and its precipitate was separated, rinsed using 0.1% dilute ammonium hydroxide, filtered, dried and weighed then expressed in percentage by using the formula (Md. Mahadi et al., 2014);

$$Total\ alkaloids\ (g/100g) = \frac{Weight\ by\ residue}{Weight\ of\ sample\ taken} \times 100 \quad (1)$$

Determination of Flavonoids

At room temperature, 10 g of the samples were extracted many times using 100 ml of methanol. Whatman filter paper NO 42 was then used to filter each of the solutions. After that, the filtrate was put into a crucible, dried out over a water bath, and weighed to ensure it remained constant.

$$Total\ flavonoids\ (g/100g) = \frac{Weight\ of\ residue}{Weight\ of\ sample} \times 100 \quad (2)$$

Determination of Saponins

Quantitative evaluation of Saponins was evaluated using the procedure proposed by Obadoni and Ochuko (2001) with slight modification from Koomson et al. (2018). 5 g of each powdered sample was measured in a 250 cm^3 conical flask and 100 ml of 20% aqueous ethanol was added and kept in a shaker for 30 min. The mixture were boiled above a water bath for 4 h at 55°C. The mixture was then separated with a filter paper and re-extracted using 200 ml of 20% aqueous ethanol. Over a water bath at 90°C, the mixed extracts were reduced to 40 ml. 20 ml of diethyl ether were used to extract the concentrate twice after it had been transferred into a separating funnel. The aqueous layer was kept while the separated ether layer was disposed of. Following the addition of 60 ml of n-butanol, the extract was twice rinsed with 10 ml of 5% aqueous sodium chloride before being vapourized on a water bath. The samples were then dried up in a hot air oven at 40°C to constant weight. Saponin content was evaluated using the formula:

$$Saponin\ (g/100g) = \frac{Final\ weight\ of\ sample}{Initial\ weight\ of\ extracts} \times 100 \quad (3)$$

Determination of Phenols

Using the folin-ciocalteu assay method according to Marinova et al. (2005), 2 g of leaf sample was weighed and defatted using about 100 ml of diethyl ether and a soxhlet apparatus for 24 hours. The defatted sample was then boiled with 50 ml of diethyl ether for the abstraction of the phenolic components for 15 minutes. 5 ml of the extract was pipetted into a 50 ml volumetric flask and 10ml of distilled water was poured into it, accompanied with the addition of 2 ml ammonium hydroxide solution and 5 ml of concentrated amyl alcohol. The sample was filled up to mark with distilled water and left to react for 30 minutes for colour development. The absorbance against reagent blank was

evaluated at 550 nm using an UV-Visible spectrophotometer.

Determination of Total Terpenoid Content

The total terpenoid content was evaluated according to Indumathi et al. (2014) by soaking 1 g of each leaf sample in 9 ml of ethanol for 24 hrs. In a separating funnel, 10 milliliters of petroleum ether were used to filter and extract the mixture. The ether extract was dried after being put into glass vials that had been previously weighed. Ether was vaporized and the yield (%) was measured using the formula;

$$Terpenoid\ (\%) = \frac{\frac{Final\ weight\ of\ sample - initial\ weight\ of\ sample}{Weight\ of\ sample}}{} \times 100 \quad (4)$$

Oxalate Determination (Titrimetric Method) (AOAC, 2005)

This method comprises of 3 parts; Digestion, Oxalate precipitation and Permanganate titration.

Digestion

A 250 ml volumetric flask was filled with 190 ml of distilled water and 2 ml of each sample. The suspension was digested at 100°C for one hour after 10 milliliters of 6M HCl were added. The solution was cooled and filled the volumetric flask to the 250 ml mark before being filtered.

Oxalate Precipitation

Replica portions of the filtrate was measured into 250 ml beakers and 4 drops of methyl red indicator is added. Ammonium Hydroxide solution was added dropwise, until the mixture turned faint yellow with pH between 4.0 – 4.5. Each part of the mixture is heated at 90ºC, left to cool and filtered to get rid of precipitate containing ferrous ion. The filtrate was heated again to 90ºC and 10 ml of 5 % $CaCl_2$ solution was added with consistent stirring. The solution was heated and left over night at 25ºC. It was centrifuged at 2500 rpm for 5 minutes. 10 ml of a 20% (v/v) H_2SO_4 solution were used to fully dissolve the precipitate after the supernatant was decanted.

Permanganate Titration

The total volume of the filtrate from the digestion was made up to 300 ml. After heating a 125 ml aliquot of filtrate almost to boiling, it was titrated against a 0.05 M standardized $KMnO_4$ solution until it turned a light pink.

$$Oxalate\ (g/100g) = \frac{Titre\ value * DF}{ME * Mf * 1} \times 100 \quad (5)$$

Where,
T: Titre value of $KMnO_4$ (ml)
Df: Dilution factor = Vt/A, where Vt is the total volume of the filtrate (300 ml) and A is the aliquot used (250 ml).
ME: Molar equivalent of $KMnO_4$ in oxalate.
Mf: weight of sample

Phytate Determination (Titrimetric method) (Lucas & Markakas, 1975)

A 250 ml conical flask was filled with 2 g of each leaf sample, which was then steeped for three hours in 100 ml of 20% concentrated HCl. Whatman No. 42 filter paper was used to filter the sample. A 250 ml beaker was filled with 50 ml of the filtrate, 100 ml of distilled water, and 10 ml of an indicator solution containing 0.3% ammonium thiocyanate. After shaking, the solution was titrated against a reference solution of iron 111 chloride, which has 0.00195 iron per milliliter. The final point produced a brownish color that continued for five minutes.

$$Phytate\ (g/100g) = \frac{Titre\ value * 0.00195 * 1.19}{Weight\ of\ sample} \times 100 \quad (6)$$

Data Analysis

Analysis of Variance (ANOVA) and mean separation was conducted with the aid of turkey method.

Results

The results obtained for qualitative phytochemical analysis of crude extract of leaves of Pterocarpus species as shown in Table 1 revealed the presence of secondary metabolites, such as alkaloids, saponins, tannins, flavonoids, phenols, and terpenoids while anti-nutrient compounds such as oxalates and phytic acids were also detected. There was high presence of alkaloids and flavonoids in *P. erinaceus*, saponins in *P.*

mildbraedii, P. osun, P. santaloides and phenol in *P. mildbraedii.* Anti-nutrient compounds – phytate was absent in *P. santaloides and P. soyauxii* while oxalate was absent in *P. soyauxii* but was shown to be slightly present in leaf samples of other species.

Table 1. Qualitative phytochemical analysis of leaves extract of *Pterocarpus* species

Phyto - constituents	*P. santalinoides*	*P. erinaceus*	*P. osun*	*P. mildbraedii*	*P. soyauxii*
Saponin	+++	++	+++	+++	++
Alkaloids	++	+++	++	++	+
Phenol	++	++	++	+++	++
Flavonoid	++	+++	+	++	++
Terpenoid	++	+	+	++	++
Tannin	+	++	++	+	++
Phytate	-	+	+	+	-
Oxalate	+	+	+	+	-

*** (-) = Absent; (+) = Slightly present; (++) = Moderately present; (+++) = Highly present

Quantitative phytochemical analysis of the methanol crude extract of *Pterocarpus* leaves is shown in Table 2. The result indicated that the leaf extracts of *P. mildbraedii,* contains 9.38±0.08 mg/100 g of saponin, 2.56±0.06 mg/100 g of phenol and 2.89±0.03 mg/100 g of tannin. This was the highest value obtained amongst the studied species. While the least content, 2.78±0.02 mg/100 g of saponin, 1.13±0.01 mg/100 g of phenol in *P. soyauxii* and 1.06±0.02 mg/100 g of tannin in *P. santalinoides* was recorded. The highest concentration of alkaloid (6.28±0.03 mg/100 g) and flavonoid (5.46±0.02 mg/100 g) was recorded in *P. erinaceus* while showing the least concentration in *P. soyauxii* (3.06±0.03 mg/100 g) and *P. osun* (2.17±0.07 mg/100 g) respectively. The estimates of terpenoid however, showed highest concentration in *P. soyauxii* (2.46±0.03 mg/100 g) and the least in *P. osun* (1.32±0.04 mg/100 g).

Table 2. Quantitative phytochemical analysis of leaf extract of *Pterocarpus* species in mg/100 g

Phyto-Constituents	*P. santalinoides*	*P. erinaceus*	*P. osun*	*P. mildbraedii*	*P. soyauxii*
Saponin	5.04±0.04	4.87±0.04	6.31±0.03	9.38±0.08	2.78±0.02
Alkaloids	3.77±0.03	6.28±0.03	4.84±0.04	4.14±0.03	3.06±0.03
Phenol	1.23±0.03	2.36±0.03	1.25±0.03	2.56±0.06	1.13±0.01
Flavonoid	4.12±0.02	5.46±0.02	2.17±0.07	3.80±0.03	3.05±0.05
Terpenoid	2.15±0.02	1.72±0.04	1.32±0.04	2.04±0.03	2.46±0.03
Tannin	1.06±0.02	2.33±0.04	1.82±0.03	2.89±0.03	1.26±0.02
Phytate	0.16±0.04	0.37±0.03	0.29±0.02	0.46±0.03	0.25±0.03
Oxalate	0.53±0.03	1.14±0.03	1.05±0.04	0.85±0.03	0.34±0.03

Results from the anti-nutrients analyses revealed a higher concentration of phytate (0.46±0.03 mg/100 g) in *P. mildbraedii,* as compared to the lowest value (0.16±0.04 mg/100 g) in *P. osun.* Similarly, oxalate level was found to be highest in *P. erinaceus* (1.14±0.03 mg/100 g) with least value in *P. soyauxii* (0.34±0.03 mg/100 g). From Table 3, the statistical analysis showed that all the secondary metabolites of the examined species varied significantly ($p \geq 0.05$).

Table 3. Mean separation of phytochemical variation between *Pterocarpus* species

Phyto-Constituents	*P. santalinoides*	*P. erinaceus*	*P. osun*	*P. mildbraedii*	*P. soyauxii*
Saponin	5.04^{bc}	4.87^{c}	6.31^{b}	9.38^{a}	2.78^{e}
Alkaloids	3.77^{ce}	6.28^{a}	4.84^{b}	4.14^{bc}	3.06^{e}
Phenol	1.23^{bc}	2.36^{b}	1.25^{bc}	2.56^{a}	1.13^{c}
Flavonoid	4.12^{b}	5.46^{a}	2.17^{d}	3.80^{bc}	3.05^{cd}
Terpenoid	2.15^{b}	1.72^{c}	1.32^{d}	2.04^{bc}	2.46^{a}
Tannin	1.06^{d}	2.33^{b}	1.82^{bc}	2.89^{a}	1.26^{cd}
Phytate	0.16^{c}	0.37^{ab}	0.29^{b}	0.46^{a}	0.25^{bc}
Oxalate	0.53^{bc}	1.14^{a}	1.05^{ab}	0.85^{b}	0.34^{cd}

**Means with same letter are not significantly different ($p \geq 0.05$)

Discussion

Phytochemicals present in plant have been proven to be useful for nutritive and medicinal purposes. Their presence or absence and their biosynthetic pathways responsible for their production have been employed in establishing the taxonomic relationship amongst plant taxa (Özkan et al., 2018; Okeke et al., 2015; Akpabio, 1998; Domingues et al, 1998). The present study showed that the powdered leaves of all examined Pterocarpus species contained tannins, saponins, terpenoids, alkaloids, flavonoids and phenol at varying mean concentration from one species to another. The variation may be credited to genetic and ecological condition of the growing trees (Wadood et al., 2013).

The presence of flavonoid in all leaf samples is indicative of their antioxidant properties. Flavonoid have been reported to function as transformers, altering the metabolic responses of the body to allergens, viruses and carcinogens. They also possess anti-inflammatory, anti-bacteria and UV radiation protective tendencies in addition to protecting DNA, from oxidative damage (Malinowska, 2013). The high concentration of flavonoid in *P. erinaceus* justify its use in animal nutrition (Olafadehan, 2013). The leaf alkaloid content was more in *P. erinaceus* compared to other members of the genus. However, the mean concentration values obtained falls lower than the maximum recommended daily intake (10/100g) for human consumption (Calabro et al., 2015). Alkaloid have been reported to be physiologically active with analgesic and sedative properties used in the management of malaria, depression and stress (Finotti et al., 2006). However, large intake of alkaloids can be toxic because of their stimulatory properties which can cause excitation linked to nerve and cell problems (Obochi, 2006).

Terpenoid contents in *Pterocarpus* leaves is an indication of its anticancer properties. Shohba et al. (2014) reported that several terpenoids displayed cytotoxicity to counter a range of tumor cells and also indicated cancer prevention as well as anticancer efficiency in preclinical animal models. The phenolic content obtained in this study was slightly higher than that of Ndukwe and Ikpeama, (2013) for *P. soyauxii* and *P. santaloides*. This may be due to genetic difference or source of plant materials (Wadood et al., 2013). Phenolic are known to be among the most abundant and they are used to remove bacteria and as a poison to eradicate parasites (Khanam et al., 2012).

The main concentration of saponins in leaves of Pterocarpus species was within the range obtained for *P. santaloides* by Ogbonna and Idumah, (2008). The author confirmed that the highest secondary metabolites for *P. santaloides* leaves is saponin. This was in contrast to our study as *P. mildbraedii* contained the largest amount of saponins amongst the species. Generally, saponins functions as an adjuvant and enhance immune response, they are widely used in animal vaccination. A diet rich in saponins can be used to treat hypercalciuria in humans, prevent acute lead poisoning and decrease dental decay and platelet aggregation (Shi et al., 2004). Just like alkaloids, leaves rich in saponins have bitter taste and astringency thereby reducing their intake. The level of tannins found in this study is lower than the acceptable daily intake for vegetables (560 mg/100 g) reported by Anonymous (1973). Haslam (1996) reported that tannins is one of the major ingredient in phytomedicine and it

is used as antioxidants in beverages as well as retaining antibacterial, antiviral and antitumor activities. Due to its antiviral activities, it has been reported to distinctively hinder HIV duplication (Kashiwada et al., 1992).

The anti-nutrient levels of phytate and oxalate in the *Pterocapus* species are appreciably low. Just as all anti-nutrient, phytate forms mineral-phytic acid complexes thereby reducing mineral and protein availability in the body. It is often associated with nutritional diseases like osteomalacia in adults and rickets in children. An intake of phytate at the toxic level, is said to minimize iron absorption by 4-5 folds (Bushway et al., 1998). The phytate levels were higher in *P. mildbraedii and P. erinaceus* which are edible vegetables for humans and animals respectively. However, it is important to note that, ruminant animals (cows, goat, sheep etc), chew first, swallow, then later vomit their food which is chewed a second time. Due to the rumen in its first stomach chamber, they can easily separate, and digest the minerals in phytate unlike human being and non-ruminant animal (Weaver & Kannan, 2002).

Phytate mean concentration in leaves of *Pterocarpus* species recorded in this study is very low compared to the toxic level of 200 mg in consumed vegetables (Hurell, 2004). Also, the quantity of oxalate content in the leaves of the species is below the toxic level of 50 mg/100 g reported by Abdi et al. (2022). The range of values reported in this study were comparable to studies reported for *P. santaloides and soyauxii*, two leafy vegetables of the *Pterocarpus* species (Nwachukwu et al., 2015; Ndukwe et al., 2013). High concentration of oxalates are known to cause adsorptive poisoning, reduce mineral availability and form divalent metallic complexes with calcium, iron, magnesium and zinc forming crystals of the oxalates thereby meddling with their utilization and could cause kidney stones (Sango et al., 2016). From this study, the quantity of anti-nutrient compounds found are far too low to cause a problem based on their level of toxicity.

Conclusion

The study provided valuable information on the rich phytochemical content in leaves of endemic *Pterocarpus* species in Nigeria. The low concentration of anti-nutrient compound found further revealed that all examined leaf samples are safe for human and animal consumption. However, there is urgent need for more collaborative pharmacological research studies on the component part of members of the genus. This is to adequately harness the rich therapeutic phytomedicinal properties present for human health. In addition, proposal for ex-situ conservation of *Pterocarpus* species in gene banks or medicinal plant gardens is advocated.

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Conflict of Interest

The Authors declare that there is no competing interests.

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