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Comparison of the Presence of Flavonoid and Tannin in the Leaves of Four *Pterocarpus Species* Found in the Southeast Nigeria

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ABSTRACT

In the Southeast Nigeria, four species of the genus *Pterocarpus* have been discovered. The four species are *Pterocarpus mildbraedii, Pterocarpus osun, Pterocarpus santalinoides* and *Pterocarpus sayauxii.* They are all trees with compound imparipinate leaves and entire leaflets. The phytochemical result of the leaves revealed the presence of flavonoid and tannins. Flavonoid was distributed as follows: *Pterocarpus mildbraedii* (0.6 ± 0.2), *Pterocarpus osun* (0.8 ± 0.2), *Pterocarpus santalinoides* (1.0 ± 0.3) and *Pterocarpus soyauxii* (nil), whereas tannin was distributed as follows: *Pterocarpus mildbraedii* (1.8 ± 0.8), *Pterocarpus osun* (4.8 ± 1.2), *Pterocarpus santalinoides*(20.0+ 0.3) and *Pterocarpus sayauxii* (24.0 ± 2.1). Flavonoid is found in trace quantities in the leaves of the four plants to compare with tannin. The highest quantity of flavonoid (1.0+ 0.3) was found in the leaves of *Pterocarpus santalinoides,* and the least quantity (nill) was found in the leaves of *Pterocarpus soyauxii.* For tannin, the highest quantity (24.0 ± 2.1) was found in the leaves of *Pterocarpus sayauxii*, whereas the least quantity (1.8 ± 0.8) was found in the leaves of *Pterocarpus mildbraedii.* This result suggests that the plants the leaves of the fur plants especially those of *Pterocarpus sayauxii* are good materials for the production of tannin-based drugs. The result also supports the idea of placing the four plants in one genus.

INTRODUCTION

Pterocarpus is a pantropical genus of trees in the family Fabaceae. It belongs to the subfamily Faboideae, and was recently assigned to the informal monophyletic *Pterocarpus* clade within the Dalbergieae (Lavin, 2013). The scientific name is Latinized Ancient Greek and means "wing fruit", referring to the unusual shape of the seed pods in this genus. Flavonoids or bioflavonoids (from the Latin word *flavus* meaning yellow, their colour in nature) are a class of plant secondary metabolites. Flavonoids were referred to as Vitamin P probably because of the effect they had on the permeability of vascular capillaries) from the mid-1930s to early 50s, but the term has since fallen out of use. Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. Flavonoids secreted by the root of their host plant help *Rhizobia* in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. Rhizobia living in soil are able to sense the flavonoids and this triggers the secretion of Nod factors, which in turn are recognized by the host plant and can lead to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule. In addition, some flavonoids have inhibitory activity against organisms that cause plant diseases, e.g. *Fusarium oxysporum* (Galeotti *et al.,* 2008).

Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-allergic, (Cho *et al.,* 2023) anti-inflammatory, (Cazarolli *et al.,* 2008) antioxidant, (Cushnie and Lamb 2011) anti-microbial antibacterial, (McGrowder *et al.,* 2020) antifungal (Cushnie and Lamb, 2005) and antiviral (Friedman, 2007)). Anti-cancer, (Schuier *et al.,* 2005) and antidiarrheal activities (Suherman, *et al.,* 2012). Flavonoids have also been shown to inhibit topoisomerase enzymes (Bandele, *et al.,* 2008) and to induce DNA mutations in the mixed-lineage leukemia (*MLL*) gene in *in vitro* studies (Raj *et al.,* 2024). However, in most of the above cases no follow up *in vivo* or clinical research has been performed, leaving it impossible to say if these activities have any beneficial or detrimental effect on human health. Biological and pharmacological activities which have been investigated in greater depth are described below.

Flavonoid-rich grape-seed extract has been shown to have antioxidant activity in *in vivo* studies with rats, protecting their gastrointestinal mucosa against the reactive oxygen species generated by acute and chronic stress (Bagchi *et al.,* 1999). In the absence of any additional *in vivo* data, it is impossible to say if these findings are generalizable to all flavonoids. Also, without any clinical studies, it is impossible to say if the antioxidant activity of grape-seed flavonoids offers any protection against oxidative stress in the human gastrointestinal tract.

Research at the Linus Pauling Institute and the European Food Safety Authority shows that flavonoids are poorly absorbed in the human body (less than 5%), with most of what is absorbed being quickly metabolized and excreted(Lotito and Frei, 2006)). These findings suggest that flavonoids have negligible systemic antioxidant activity, and that the increase in antioxidant capacity of blood seen after consumption of flavonoid-rich foods is not caused directly by flavonoids, but due to increased production of uric acid resulting from excretion of flavonoids from the body (Ravishankar *et al.,* 2013).

Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds. Flavonoids have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase, and to modify intracellular signaling pathways in immune cells (Izzi *et al.,* 2012)).

Procyanidins, a class of flavonoids, have been shown in preliminary research to have anti-inflammatory mechanisms including modulation of the arachidonic acid pathway, inhibition of gene transcription, protein expression and activity of inflammatory enzymes, as well as secretion of anti-inflammatory mediators (Martinez-Micaelo *et al.,* 2012)).

Clinical studies investigating the relationship between flavonoid consumption and cancer prevention/development are conflicting for most types of cancer, probably because most studies are retrospective in design and use a small sample size (Romagnolo and Selmin 2012)). Two apparent exceptions are gastric carcinoma and smoking-related cancers. Dietary flavonoid intake is associated with reduced gastric carcinoma risk in women and reduced aerodigestive tract cancer risk in smokers (Woo and Kim 2013)).

Flavonoids have been shown to have (a) direct antibacterial activity, (b) synergistic activity with antibiotics, and (c) the ability to suppress bacterial virulence factors in numerous *in vitro* and a limited number of *in vivo* studies. Noteworthy among the *in vivo* studies (Van *et al.,* 2013) is the finding that oral quercetin protects guinea pigs against the Group 1 carcinogen *Helicobacter pylori*. Researchers from the European Prospective Investigation into Cancer and Nutrition have speculated this may be one reason why dietary flavonoid intake is associated with reduced gastric carcinoma risk in European women. Additional *in vivo* and clinical research is needed to determine if flavonoids could be used as pharmaceutical drugs for the treatment of bacterial infection, or whether dietary flavonoid intake offers any protection against infection (Rasmussen, 2013).

Tannin (also known as *vegetable tannin*, *natural organic tannins* or sometimes *tannoid*, i.e. a type of biomolecule, as opposed to modern synthetic tannin) is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.

The term tannin (from *tanna*, an Old High German word for oak or fir tree, as in Tannenbaum) refers to the use of wood tannins from oak in tanning animal hides into leather; hence the words "tan" and "tanning" for the treatment of leather. However, the term "tannin" by extension is widely applied to any large polyphenolic

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compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with various macromolecules.

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Katie *et al.*, 2006)). The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine (Harold, 2004). Likewise, the destruction or modification of tannins with time plays an important role in the ripening of fruit and the aging of wine.

Tannins are distributed in species throughout the plant kingdom. They are commonly found in both gymnosperms as well as angiosperms. Simon. (1993) studied the distribution of tannin in 180 families of dicotyledons and 44 families of monocotyledons (Cronquist). Most families of dicot contain tannin-free species (tested by their ability to precipitate proteins).

The best known families of which all species tested contain tannin are: Aceraceae, Actinidiaceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae, Dipterocarpaceae, Ericaceae, Grossulariaceae, Myricaceae for dicot and Najadaceae and Typhaceae in Monocot. To the family of the oak, Fagaceae, 73% of the species tested (N = 22) contain tannin. For those of acacias, Mimosaceae, only 39% of the species tested (N = 28) contain tannin, among Solanaceae rate drops to 6% and 4% for the Asteraceae. Some families like the Boraginaceae, Cucurbitaceae, Papaveraceae contain no tannin-rich species.

The most abundant polyphenols are the condensed tannins, found in virtually all families of plants, and comprising up to 50% of the dry weight of leaves. Tannins of tropical woods tend to be of a cathetic nature rather than of the gallic type present in temperate woods. There may be a loss in the bio-availability of still other tannins in plants due to birds, pests, and other pathogens (Kadam *et al.*, 1990)).

Tannins are found in leaf, bud, seed, root, and stem tissues. An example of the location of the tannins in stem tissue is that they are often found in the growth areas of trees, such as the secondary phloem and xylem and the layer between the cortex and epidermis. Tannins may help regulate the growth of these tissues.

In all vascular plants studied so far, tannins are manufactured by a chloroplast-derived organelle, the tannosome. Tannins are mainly physically located in the vacuoles or surface wax of plants. These storage sites keep tannins active against plant predators, but also keep some tannins from affecting plant metabolism while the plant tissue is alive; it is only after cell breakdown and death that the tannins are active in metabolic effects.

Tannins are classified as ergastic substances, i.e., non-protoplasm materials found in cells. Tannins, by definition, precipitate proteins. In this condition, they must be stored in organelles able to withstand the protein precipitation process. Idioblasts are isolated plant cells which differ from neighboring tissues and contain non-living substances. They have various functions such as storage of reserves, excretory materials, pigments, and minerals. They could contain oil, latex, gum, resin or pigments etc. They also can contain tannins. In Japanese persimmon (*Diospyros kaki*) fruits, tannin is accumulated in the vacuole of tannin cells, which are idioblasts of parenchyma cells in the flesh.

The convergent evolution of tannin-rich plant communities has occurred on nutrient-poor acidic soils throughout the world. Tannins were once believed to function as anti-herbivore defenses, but more and more ecologists now recognize them as important controllers of decomposition and nitrogen cycling processes. As concern grows about global warming, there is great interest to better understand the role of polyphenols as regulators of carbon cycling, in particular in northern boreal forests.

Leaf litter and other decaying parts of a kauri (*Agathis australis*), a tree species found in New Zealand, decompose much more slowly than those of most other species. Besides its acidity, the plant also bears substances such as waxes and phenols, most notably tannins, (Bajaj, 1988) that are harmful to microorganisms. The leaching of highly water soluble tannins from decaying vegetation and leaves along a stream may produce what is known as a blackwater river. Water flowing out of bogs has a characteristic brown color from dissolved peat tannins. The presence of tannins (or humic acid) in well water can make it smell bad or taste bitter, but this does not make it unsafe to drink (Takashi and Akira 1987)).

Tannins leaching from an unprepared driftwood decoration in an aquarium can cause pH lowering and coloring of the water to a tea-like tinge. A way to avoid this is to boil the wood in water several times, discarding the water each time. Using peat as an aquarium substrate can have the same effect. Many hours of boiling the driftwood may need to be followed by many weeks or months of constant soaking and many water changes before the water will stay clear. Adding baking soda to the water to raise its pH level will accelerate the process of leaching, as the more alkaline solution can draw out tannic acid from the wood faster than the pH-neutral water. (Calvi *et al.*, 1995) .

Softwoods, while in general much lower in tannins than hardwoods, are usually not recommended for use in an aquarium so using a hardwood with a very light color, indicating a low tannin content, can be an easy way to avoid tannins. Tannic acid is brown in color, so in general white woods have a low tannin content. Woods with a lot of yellow, red, or brown coloration to them (like southern yellow pine, cedar, redwood, red oak, etc.) tend to contain a lot of tannin (Souza, S. M. C. *et al.*; Aquino, L. C.; Milach Jr, A. C.; Bandeira, M. A.; Nobre, M. E.; Viana, G. S. (2006)).

Tannins have traditionally been considered antinutritional but it is now known that their beneficial or antinutritional properties depend upon their chemical structure and dosage. The new technologies used to analyze molecular and chemical structures have shown that a division into condensed and hydrolyzable tannins is far too simplistic. Recent studies have demonstrated that products containing chestnut tannins included at low dosages (0.15–0.2%) in the diet of chickens may be beneficial. Some studies suggest that chestnut tannins have been shown to have positive effects on silage quality in the round bale silages, in particular reducing NPNs (non protein nitrogen) in the lowest wilting level. Improved fermentability of soya meal nitrogen in the rumen has also been reported by Mathieu and Jouany (1993). Studies by S. Gonzalez *et al.* (2002) on *in vitro* ammonia release and dry matter degradation of soybean meal comparing three different types of tannins (quebracho, acacia and chestnut) demonstrated that chestnut tannins are more efficient in protecting soybean meal from *in vitro* degradation by rumen bacteria.

Condensed tannins, i.e. quebracho tannin, and hydrolyzable tannins, i.e., chestnut tannin, appear to be able to substitute a high proportion of synthetic phenol in phenol-formaldehyde resins for wood particleboard. Tannins can be used for production of anti-corrosive primer, sold under brand name-Nox Primer for treatment of rusted steel surfaces prior to painting, rust converter to transform oxidized steel into a smooth sealed surface and rust inhibitor. The use of resins made of tannins has been investigated to remove mercury and methylmercury from solution. Immobilized tannins have been tested to recover uranium from seawater (Kolodziej and Kiderlen 2005).

Genus *Pterocarpus* has many species that occur throughout the tropics (Keay *et al.*, 1964). Accordin to Uju *et al*, (1992), the genus is a pantropical genus of family Fabaceae. Most species of this genus yield valuable timer traded as ‘padauk’; other common names are *mukwa or nara or oha.* The scientific name is latinised ancient Greek word which means ‘win fruit’, referring to the unusual shape of the seed pods in this genus.

In general, all Nigerian species of *Pterocarpus* are trees with bright yellow flowers and usually with alternation of leaflets, except *Pterocarpus erinacius* which has lower pair opposite leaflets. There are no stipules, though, *P. osun* shows vague stipule-like projection at the base of leaflet stalk. The stem-bark oozes blood-like exudates when slashed and the wood is often red and very hard, being source of camwood. It has bizarre roots (Hutchinson, 1926).

The winged, more or less circular, one seeded fruits are highly distributive and are the boot character for distinguishing various species. The fruit body is roughly knobby, smooth, or bristly and the wing is usually membraneous glabrous and hairy and covered with network of veins. Most of the Nigerian species have seven to fifteen leaflets decreasing in size downwards, with thin, sometimes numerous, up-curving. lateral nerves looping vaguely near the margin and a very close network of veins in between. The flowers are borne in

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racemes. They have cup-like calyx with district teeth, broad, long petal and narrow keel shorter than the petal, 10 stamens and a very short style (Keay *et al.*, 1964).

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Pterocarpus mildbraedii, Pterocarpus osun, Pterocarpus santalinoides* and *Pterocarpus sayauxii* were collected from Uto forest in Ugwuto community, Nsude, Enugu state, Nigeria. The specimen were authenticated by Prof. Clement Okeke, a plant Taxonomist in the department of Botany, Nnamdi Azikiwe University, Awka and the voucher specimen were deposited at the herbarium of department of Botany, Nnamdi Azikiwe University, Awka.

Preparation of plant material

The leaves of *Pterocarpus mildbraedii, Pterocarpus osun, Pterocarpus santalinoides* and *Pterocarpus sayauxii* were washed with distilled water and dried in the lab at room temperature. The dried parts were ground to fine powder with mortar and pestle. The dried and powdered samples were used for the analyses. A.O.A.C (1990) method was used for the test for flavonoid and Tannin.

RESULTS AND DISCUSSIONS

Table 1: Qualitative Phytochemical Content of the *Pterocarpus* species

Constituents	*Pterocarpus mildbraedii*	*Pterocarpus osun*	*Pterocarpus santalinoides*	*Pterocarpus sayauxii*
Flavonoid	+	+	+	-
Tannin	+	+	+	+

+ = presence

- = absence.

The result indicates that flavonoid is present in the leaves of Pterocarpus mildbraedii, Pterocarpus osun and Pterocarpus santalinoides but absent in the leaves of Pterocarpus sayauxii, whereas tannin is present in the four plants.

Flavonoids are a diverse group of phytonutrients found in many plants, known for their antioxidant properties and potential health benefits, including anti-inflammatory and anti-carcinogenic effects (Middleton *et al.*, 2000). The presence of flavonoids in Pterocarpus mildbraedii, Pterocarpus osun, and Pterocarpus santalinoides suggests that these species might possess significant antioxidant capacities, which could be beneficial in preventing oxidative stress-related diseases. However, the absence of flavonoids in Pterocarpus soyauxii indicates that this particular species might lack some of the protective antioxidant effects attributed to flavonoids.

Tannins are polyphenolic compounds that contribute to the astringency and bitter taste in plants and are known for their ability to precipitate proteins (Haslam, 1996). They have been associated with various health benefits, including antimicrobial, anti-inflammatory, and antioxidant activities (Chung *et al.*, 1998). The consistent presence of tannins across all four *Pterocarpus* species examined-*Pterocarpus mildbraedii, Pterocarpus osun, Pterocarpus santalinoides,* and *Pterocarpus soyauxii*- suggests that these plants may have similar health benefits related to their tannin content. This ubiquitous presence also implies that tannins could be a key phytochemical marker within the Pterocarpus genus.

Both tannins and flavonoids are present in *Pterocarpus mildbraedii, Pterocarpus osun,* and *Pterocarpus santalinoides*, indicating that these species might share similar therapeutic properties related to these compounds. *Pterocarpus soyauxii* differs from the other three species by lacking flavonoids. This unique

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phytochemical profile may influence its potential medicinal applications, possibly making it less effective in roles where flavonoid activity is beneficial.

The presence of both flavonoids and tannins in *Pterocarpus mildbraedii*, *Pterocarpus osun*, and *Pterocarpus santalinoides* suggests that these species might be particularly useful in traditional medicine and for developing natural antioxidant and antimicrobial agents. The medicinal uses of these plants in various cultures can be linked to their phytochemical content, supporting their roles in treating ailments such as infections, inflammation, and oxidative stress-related conditions.

Table 2: Qualitative Phytochemical Content (%) of the *Pterocarpus* species

Constituents	*Pterocarpus mildbraedii*	*Pterocarpus osun*	*Pterocarpus santalinoides*	*Pterocarpus sayauxii*
Flavonoid	0.6 ± 0.2	0.8 ± 0.2	1.0 ± 0.3	NIL
Tannin	1.8 ± 0.8	4.8 ± 1.2	20.0 ± 0.3	24.0.0 ± 2.1

Table 2 provides an overview of the concentration of two important phytochemical constituents—flavonoids and tannins—across four different species of the *Pterocarpus genus: Pterocarpus mildbraedii, Pterocarpus osun, Pterocarpus santalinoides*, and *Pterocarpus soyauxii*.

Pterocarpus santalinoides has the highest flavonoid content at 1.0 ± 0.3%, which might suggest a higher potential for antioxidant benefits compared to the other species. The absence of flavonoids in Pterocarpus soyauxii is notable and could influence its utility in applications that benefit from these compounds. Tannins are polyphenolic compounds that have astringent properties and can be beneficial for their anti-microbial and anti-inflammatory activities (Okuda, 2005). The tannin content among the species shows significant variation

Pterocarpus soyauxii exhibits the highest tannin content at 24.0 ± 2.1%, followed closely by *Pterocarpus santalinoides* at 20.0 ± 0.3%. These high levels could imply a stronger astringent property and potential uses in food preservation and medicinal applications where tannins are beneficial. The significantly lower tannin content in *Pterocarpus mildbraedii and Pterocarpus osun* suggests they may be less effective for such applications but could be preferred in cases where a lower astringency is desired.

The comparative analysis reveals that *Pterocarpus santalinoides* stands out with high levels of both flavonoids and tannins, which could make it particularly valuable in both medicinal and nutritional applications. In contrast, *Pterocarpus soyauxii*, despite its high tannin content, lacks flavonoids entirely, potentially limiting its use in areas where the benefits of flavonoids are desired.

CONCLUSION

This comparative phytochemical study highlights the potential health benefits and therapeutic applications of different *Pterocarpus* species. The presence of flavonoids and tannins in most species suggests significant antioxidant and antimicrobial properties. The unique absence of flavonoids in *Pterocarpus soyauxii* suggests that this species may have different or less potent medicinal properties compared to the other species studied. Further quantitative analysis and bioactivity studies are recommended to better understand the specific health benefits and to validate the traditional uses of these plants. The variation in phytochemical content across the *Pterocarpus* species highlights the diversity within this genus and suggests that specific species can be targeted for different applications based on their phytochemical profiles. This data can guide the selection of *Pterocarpus* species for research and development in pharmaceuticals, nutraceuticals, and other industries that utilize plant-based compounds.

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