Qualitative and quantitative evaluation of the phytochemical constituents of three wood species in Ogun state, Nigeria

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Abstract: Studies on the phytochemicals of the stem wood of tropical trees are scarce, despite its importance to plant protection and preservation as most researches focused on their leaves and fruits. This research work aimed to qualitatively and quantitatively analyze the phytochemicals present in the stem wood of *Gmelina arborea*, *Tectona grandis* and *Anogeissus leiocarpus*. Freshly sawn timbers were collected from a local sawmill and then ground into finely powdered wood samples. The powdered wood samples and its extracts were screened for the presence or absence of phytochemicals using standard methodologies. The qualitative screening revealed the presence of various secondary metabolites such as tannin, saponin, steroids, flavonoid, alkaloids and terpene in all the three species. The result also showed that *Tectona grandis* had the highest percentage of Alkaloid (7.5%), Tannin (4.95%), and Flavonoid (4.67%) while *Anogeissus leiocarpus* had the highest percentage of Saponin (3.06%) and Terpene (1.45%). This study established the fact that the three selected species studied have potentials in the industries for medicinal and anti-pathogenic usages.

Keywords: Plant protection - Aqueous extract - Stem wood - Anti-pathogenic - Medicinal.

INTRODUCTION

Phytochemicals are chemical compounds that are produced as a result of the metabolic reaction during plant growth (Taiz & Zeiger 1998). They include alkaloids, flavonoids, coumarins, tannins, terpenes, terpenoids, phenols, gums, polysaccharides, and glycosides (Harborne 1973, Okwu 2004). They possess protective or disease-preventing properties which are useful in plants to defend against attack from insects, fungi, and herbivorous animals (Judith 2000, Sandhya et al. 2006). They are also responsible for the coloration and organoleptic properties in plants (Taiz & Zeiger 1998).

Although, plants produce these chemicals to protect themselves, but recent research demonstrates that they can also protect humans against diseases (Breslin 2017). The medicinal value of a plant lies in the phytochemical (bioactive) constituents of the plant which shows various physiological effects on the human body (Akinmoladun et al. 2007). Therefore, various important compounds which may be used as the bases of modern drugs for curing various diseases can be detected during phytochemical screening (Sheikh et al. 2013).

Despite the widely achieved importance of phytochemicals in plants, only very few tropical trees have been screened (Conrick 2007). As posited by Ezeonu & Ejikeme (2016), phytochemical studies of stem wood used in the Nigerian timber industry are inadequate as most research focused on the leaves and fruits of the trees. Out of the three hundred and fifty (350) timbers identified in Nigeria (Eboatu et al. 1990, Akindele & Lemay 2006), there has been few or no study on their chemical constituents (Ejikeme et al. 2014).

In view of the importance of the phytochemicals to plant protection and preservation and also in pharmaceuticals for human health and safety, investigation of major tropical wood species is imperative. Therefore, the qualitative and quantitative analysis of the phytochemicals of these three species *Gmelina*...
arborea Roxb., Tectona grandis L.f. and Anogeissus leiocarpus (DC.) Guill. & Perr. were carried out to determine the presence or absence of the classes of phytochemicals. It is expected that this information will provide insight into the utilization potentials of these chemical compounds in the protection of wood rather than using non-environmentally friendly chemicals against harmful pathogenic organisms.

MATERIALS AND METHODS
Sample collection and preparation
Freshly sawn timbers were collected from a local sawmill in Camp, Abeokuta, Ogun State, Nigeria and the samples were taken to the Wood Laboratory in the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta for identification and authentication. The wood samples were air-dried for 2 weeks and then crushed into fine particles using a laboratory mechanical grinder. An aqueous extract was prepared by weighing 100 g of the powdered wood samples into 500 ml of distilled water and allowed to soak for 72 hours. After soaking, the extract was filtered using layered muslin cloth into a beaker. The filtrate was evaporated in water bath at 35°C.

Qualitative Phytochemical screening
Qualitative phytochemicals screening of the wood samples: The qualitative phytochemical screening of the various wood samples was determined by adopting standard methods as described by Harborne (1973), Hikino et al. (1984), Edeoga et al. (2005), modified by Ejikeme et al. (2014) to indicate the presence or absence of the metabolites.

i. Test for tannins: Weighed 0.30 g of each wood powder was boiled in 30 cm³ of distilled water in a water bath for 10 minutes and then filtered using Whatman filter paper No 42 (125 mm). Three drops of 0.1% ferric chloride was added to 5 cm³ of the filtrate and it will observe for brownish green or a blue-black coloration.

ii. Test for saponin: 0.30 g of the wood powder was added to 30 cm³ of distilled water, boiled for 10 minutes in a water bath and filtered using Whatman filter paper No 42 (125 mm). The filtrate (10 cm³) was mixed with 5 cm³ of distilled water and shaken vigorously for a stable persistent froth. The frothing was then mixed with three drops of olive oil and shaken vigorously, and then it was observed for the formation of the emulsion.

iii. Test for steroid: 20 cm³ of ethanol was added to 0.30 g of the wood powder in a beaker, the mixture was allowed to stand for 2 hours. Acetic anhydride (2 cm³) was added to 5 cm³ of the ethanoic extract of each sample following with the addition of 2 cm³ of concentrated tetraoxosulphate (VI) acid. The color changed from violet to blue indicated the presence of steroids.

iv. Test for terpenoids: 30 cm³ of distilled water was added to 0.30 g of each wood powder weighed into a beaker and the mixture was allowed to stand for 2 hours. Measured 5 cm³ of each extract was mixed in 2 cm³ of chloroform and 3 cm³ of concentrated tetraoxosulphate (VI) acid was added to form a layer. A reddish-brown colouration formed at the interface showed a positive result for the presence of terpenoids.

v. Test for flavonoids: 30 cm³ of distilled water was added to 0.30 g of the wood powder weighed into a beaker, the mixture was allowed to stand for 2 hours and filtered using Whatman filter paper No 42 (125 mm). Then, 5 cm³ of 1.0 M dilute ammonia solution was added to 10 cm³ of the aqueous filtrate of each wood extract followed by the addition of 5 cm³ of concentrated tetraoxosulphate (VI) acid. Observation of yellow colouration which disappeared on standing indicates the presence of flavonoid.

vi. Test for alkaloids: 2 g of each wood powder was placed in a 250 cm³ conical flask and 20 cm³ of 5% tetraoxosulphate (VI) acid (H₂SO₄) in 50% ethanol was added. The mixture was boiled for 2 minutes and filtered through Whatman filter paper No 42 (125 mm). The filtrate was placed in a separating funnel and made alkaline with 5 cm³ of 28% ammonia solution (NH₃). The solution was extracted with equal volume of chloroform (5 cm³). The chloroform solution was extracted with two 5 cm³ portion of 1.0 M dilute tetraoxosulphate (VI) acid, the final acid extract was then used to carry out the following test:- To 2 cm³ of acid extract 0.5 cm³ of Dragendorff’s reagent (Bismuth potassium iodide solution) was added and observed for orange-coloured precipitation indicating the presence of alkaloid.

Quantitative Phytochemical screening
Quantitative phytochemicals screening of the wood samples: Quantitative phytochemical analysis was determined by carrying out the chemical test on the aqueous extract and powdered samples using standard methods as described by Harborne (1973), Boham & Kociapai-Abyazan (1994), Obadoni & Ochuko (2001) and Amadi et al. (2004), modified by Ejikeme et al. (2014).

i. Determination of tannin: The Folin-Denis reagent was prepared by dissolving 50 g of sodium tungstate...
(Na$_2$WO$_4$) in 37 cm$^3$ of distilled water, followed by adding 10 g of phosphomolybdic acid (H$_3$PMO$_{12}$O$_{40}$) and 25 cm$^3$ of orthophosphoric acid (H$_3$PO$_4$). The mixture was refluxed for 2 hours, cooled and diluted to 500 cm$^3$ with distilled water. 1 g of each wood powder was weighed into a conical flask and 100 cm$^3$ of distilled water added. This was boiled gently on an electric hot plate for 1 hour and filtered through Whatman filter paper No. 42 (125 mm) into a 100 cm$^3$ volumetric flask. For colour development, 50 cm$^3$ of distilled water and 10 cm$^3$ of diluted extract (aliquot volume) were pipetted into a 100 cm$^3$ conical flask, followed by the addition of 5 cm$^3$ Folin-Denis reagent and 10 cm$^3$ of saturated Na$_2$CO$_3$ solution. After thorough mixing, the solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C. Optical density was measured at 700 nm with the aid of a Spectrum Lab23A spectrophotometer and optical density (absorbance) compared on a standard tannic acid curve. The tannic standard curve was prepared by dissolving 0.20 g of rated ammonium hydroxide drop wise to the filtrate until the solution was transparent. 5 cm$^3$ of tannic acid in distilled water and diluted to 200 cm$^3$ mark (1 mg cm$^{-3}$). Tannic acid solution of varying concentrations (0.2–1.0 mg cm$^{-3}$) were mixed into five different test tubes. 5 cm$^3$ of Folin-Denis reagent and 10 cm$^3$ of saturated Na$_2$CO$_3$ solution were also pipetted into the test tube, and were made up to the 100 cm$^3$ mark with distilled water. The solution was left to stand for 30 minutes in a water bath at a temperature of 25°C. Optical density was measured at 700 nm with the aid of a Spectrum Lab23A spectrophotometer. A plot of optical density (absorbance) versus tannic acid concentration was made.

\[
\text{Tannic acid} = \frac{mg}{100} = \frac{C \times \text{extract volume} \times 100}{\text{Aliquot volume} \times \text{weight of sample}}
\]  

(1)

Where, C = concentration of tannic acid.

ii. Determination of alkaloids: 2.50 g of each wood powder was weighed into a 250 cm$^3$ beaker and 200 cm$^3$ of 10% acetic acid in ethanol was added to each wood powder and allowed to rest for 4 hours. It was then filtered and the filtrate was poured into a water bath containing about one - quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the filtrate until the precipitation was complete. The whole mixture was allowed to settle for 3 hours, the supernatant was discarded and the precipitates washed with 20 cm$^3$ of 0.1M of ammonium hydroxide and then filtered using Whatman filter paper No 42 (125 mm). The residue was dried in an oven and weighed using an electronic weighing balance Model B-218. The percentage of alkaloid can be expressed mathematically as:-

\[
\% \text{ Alkaloid} = \frac{\text{weight of alkaloid}}{\text{weight of sample}} \times 100
\]  

(2)

iii. Determination of flavonoid: Each wood powder weighing 2.50 g was placed in a 250 cm$^3$ beaker and 50 cm$^3$ of 80% aqueous methanol added, covered and allowed to stand for 24 hours at room temperature. The supernatant was discarded and the residue re-extracted three times with the same volume of ethanol. The whole solution of each wood sample was filtered through Whatman filter paper No 42 (125 mm). The filtrate of each wood sample was then transferred into a crucible and evaporated to dryness over a water bath. The crucible and its content was cooled in a desiccator and weighed until a constant weight was obtained. The percentage of flavonoid is expressed mathematically as:-

\[
\% \text{ Flavonoid} = \frac{\text{weight of flavonoid}}{\text{weight of sample}} \times 100
\]  

(3)

iv. Determination of saponin: 5 g of each wood powder was poured into a 250 cm$^3$ conical flask and 100 cm$^3$ of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of $55^\circ$C. The mixture was filtered and the residue extracted with another 100 cm$^3$ of 20% aqueous ethanol, heated for 4 hours at a constant temperature of $55^\circ$C with constant stirring. The combined extract was reduced to 40 cm$^3$ over water bath at a temperature of $90^\circ$C. The concentrate was transferred into a 250 cm$^3$ separator funnel and 20 cm$^3$ of diethyl ether was added and shaken vigorously. The ether layer was then discarded. The purification process was repeated twice. 60 cm$^3$ of butanol was added and the butanol extract was washed twice with 10 cm$^3$ of 5% sodium chloride. The sodium chloride layer was discarded and the remaining solution heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

\[
\% \text{ Saponin} = \frac{\text{weight of saponin}}{\text{weight of sample}} \times 100
\]  

(4)

RESULTS

Qualitative phytochemical screening of the wood species

The qualitative determination of phytochemicals as shown in table 1 revealed the presence of various plant...
secondary metabolites in which Flavonoid is heavily present in *Gmelina arborea* and *Tectona grandis* but slightly present in *Anogeissus leiocarpus*. The heavy presence of Alkaloids was recorded for all the three timber species. The presence of Terpene and Steroid was also observed in all the three wood species. It was also observed that Saponin was heavily present in *Anogeissus leiocarpus*, slightly present in *Gmelina arborea* and present in *Tectona grandis*. Tannin was heavily present in *Tectona grandis* and present in both *Anogeissus leiocarpus* and *Gmelina arborea*.

Table 1. Qualitative phytochemical screening for the three species.

| S.N. | Tree species                      | Flavonoid % | Alkaloid % | Terpene % | Steroid % | Saponin % | Tannin % |
|------|-----------------------------------|-------------|------------|-----------|-----------|-----------|----------|
| 1    | *Anogeissus leiocarpus* (DC.) Guill. & Perr. | ++          | +++        | +         | +         | +++       | +        |
| 2    | *Gmelina arborea* Roxb.            | +++         | +++        | +         | +         | ++        | +        |
| 3    | *Tectona grandis* L.f.             | +++         | +++        | +         | +         | +         | +++      |

Note: +++ = Heavily present; ++ = Slightly present; + = Present.

**Quantitative phytochemical screening of the wood species**

The result from this study showed that *Tectona grandis* had the highest percentage of Flavonoid (4.67%), Alkaloid (7.5%) and Tannin (4950 mg / 100 g) while *Anogeissus leiocarpus* had the highest percentage of Terpene (1.45%) and Saponin (3.06%). *Gmelina arborea* had the lowest percentage of most of the phytochemicals except for Steroid (0.83%) which is the highest of the three species. As revealed in table 2, a significant difference (P < 0.05) existed in the phytochemicals of the three wood species.

**DISCUSSION**

**Tannin**

The tannin content recorded in *Tectona grandis* (4950 mg / 100 g) showed highest value compared to the value that was observed for *Anogeissus leiocarpus* (630 mg / 100 g) and *Gmelina arborea* (230 mg / 100 g) as shown in table 2. The value recorded for *Tectona grandis* in this study is higher than values observed in the fourteen tropical indigenous timbers reported by Ejikeme *et al.* (2014) which ranged between 620 to 1180 mg / 100 g and also the twenty-four indigenous Nigerian softwoods with values ranging from 690 to 1240 mg / 100 g reported by Ezeonu & Ejikeme (2016). Both the result for the qualitative and quantitative screening revealed that tannin can be extracted from *Tectona grandis* for industrial and medicinal usage. Tannin are useful in plant growth regulation and plant protection (Katie & Thorington 2006). The defensive properties of tannins are generally attributed to their ability to bind proteins (Mazid *et al.* 2011). Tannins serves as caustics for cationic dyes (tannin dyes) used in the dyestuff industry as well as in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulant in rubber production (Römpp 1995). Other uses of tannin are for wine, fruit juice, and beer clarification in food industries (Wurdig & Woller 1989). They are also used as a constituent to reduce the viscosity of drilling mud for oil wells, and in boiler water to prevent scale formation. The styptic and astringent properties of tannin makes it useful in treating tonsillitis, pharyngitis, haemorrhoids, and skin eruptions; it has been administered internally to check diarrhea and intestinal bleeding and as an antidote for metallic, alkaloidal, and glycosidic poisons, with which it forms insoluble precipitates (Ejikeme *et al.* 2014).

**Saponin**

From the result in table 2, it was observed that *Anogeissus leiocarpus* had the highest source of Saponin content (3.06%), which is comparably lower to the value recorded by Ezeonu & Ejikeme (2016) for the same species (12.5%), followed by *Tectona grandis* (2.61%) while *Gmelina arborea* showed the lowest source of Saponin (0.92%). The Saponin content observed in *Gmelina arborea* (0.92%) is lower than (2.8 to 12%) observed by Ejikeme *et al.* (2014) and (1.6 to 12.5%) reported by Ezeonu & Ejikeme (2016). While, *Anogeissus leiocarpus* and *Tectona grandis* falls within the range of these appreciable quantities. Saponins are phytochemicals which are found in most of the herbs, beans and vegetables. They protect plants from

Table 2. Quantitative phytochemical analysis for the three species.

| S.N. | Tree species                      | Flavonoid % | Alkaloid % | Terpene % | Steroid % | Saponin % | Tannin (mg/100g) |
|------|-----------------------------------|-------------|------------|-----------|-----------|-----------|-----------------|
| 1    | *Anogeissus leiocarpus* (DC.) Guill. & Perr. | 1.87<sup>a</sup> | 3.17<sup>b</sup> | 1.45<sup>b</sup> | 0.67<sup>b</sup> | 3.06<sup>b</sup> | 630<sup>b</sup> |
| 2    | *Gmelina arborea* Roxb.            | 1.36<sup>b</sup> | 2.62<sup>b</sup> | 0.59<sup>b</sup> | 0.83<sup>b</sup> | 0.92<sup>b</sup> | 230<sup>b</sup> |
| 3    | *Tectona grandis* L.f.             | 4.67<sup>c</sup> | 7.50<sup>c</sup> | 1.15<sup>c</sup> | 0.69<sup>c</sup> | 2.61<sup>c</sup> | 4950<sup>c</sup> |

Note: Means in column with different superscripts denotes significant difference (P<0.05) level.

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phytopathogenic microorganisms, insects and phytophagous mammalian (Silva et al. 2005), this is due to their ability to produce alteration in the feeding behavior, molting process, interaction with hormones that regulate the growth and causing death in the different stages of development. Industrially, saponins are used in the preparation of soaps, detergents, fire extinguishers, shampoos, beer and cosmetic (Bhargava et al. 2006).

**Flavonoid**

The result of the phytochemical analysis revealed that the highest source of flavonoid in this study is *Tectona grandis* (4.67%) which is also heavily present in the species. The value obtained is lower than these indigenous tropical timbers; *Sacogottitis gabonensis* (Bail.) Urb., *Khaya ivorensis* A. Chev., *Phyllanthus discoideus* Mull. Arg., *Lovoa trichiloides* Harms), *Bridelia micrantha* (Hochst.) Baill., *Bombax brevicaule* Sprague., *Glyphee brevis* Spreng. and *Monodora tenuifolia* Benth. which were above 6% but higher than *Rhizophora racemosa* G.Mey. (2%) and *Cola laurifolia* Mast. (3%) reported by Ejikeme et al. (2014), also comparably lower than these indigenous Nigerian softwoods reported by Ezeonu & Ejikeme (2016); *Monodora tenuifolia* Benth. (7.4%), *Moringa oleifera* Lam. (12.2%), *Barteria nigritiana* Hook. f. (14.2), *Glyphaea brevis* (Spreng.) Monach. (7.2%), *Uapaca guineensis* Mull. Arg. (9.2%), *Amphimas pterocarpoides* Harms. (9.2%), *Albizia adianthifolia* (Schumach.) W.Wight (7.2%), *Afrotrissia laxiflora* (Benth.) Harms (8.0%), *Combretodendron macrocarpum* (P.Beauv) Keay. (9.2%), *Sacogottitis gabonensis* (8.0%). Flavonoids belong to a group of natural substances found in fruit, vegetables, grains barks, roots, stems, flowers, tea and wine (Middleton 1998). They are reported as the most abundant plant pigment along with chlorophyll and carotenoids, also providing fragrance and taste to fruits, flowers and seeds which makes them attractive to other organisms (Koes & Quattrocchio 1994, Stalikas 2007). The biological activities of flavonoid includes protection of the skin from UV light exposure, protect DNA from damage, anti-inflammatory effect, moisturizing, softening and anti-septic. The presence of this phytochemical implies that it can be extracted as ingredients in the preparation of cosmetics and pharmaceutical products (Chuarienthong et al. 2010, Malinowska 2013).

**Alkaloid**

Alkaloids are produced by a large variety of organisms which includes bacteria, fungi, plants and animals. The different concentrations of alkaloids observed in the stem of three species studied revealed that *Tectona grandis* had the highest (7.50%) source of alkaloids, followed by *Anogeissus leiocarpus* (3.17%), while the least is *Gmelina arborea* (2.62%). These values falls within the range of alkaloid content reported in the study of Ejikeme et al. (2014). Alkaloids are mainly found in flowering plants (Angiospermi), this further explains the heavily presence of alkaloids in the three species studied. They are extremely toxic; they protect plants against micro-organisms due to their antibacterial and antifungal activities, insects, and herbivores (feeding deterrence) and also against other plants by means of allelopathically active chemicals (Molynex et al. 1996). Alkaloids in plants have been extracted to cure asthma, snake bite and skin diseases (Mien & Mohamed 2001). This has led to its industrial usage in the production of powerful pain killer medicine and anesthetics agents (Nakatani 2000, Ullah & Khan 2008).

**CONCLUSION**

Tropical tree species apart from their timber uses are also potential for traditional medicine. There have been well-known usages of most of their foliage parts such as barks, seeds and leaves in traditional medicine, but the stem has had less usage in the phytochemical application in industries. This research result has established through the investigation of their photochemistry that all three species studied have potentials in the industries for medicinal and anti-pathogen usages. *Tectona grandis* contain the highest amount of flavonoids, alkaloids and tannin and thus has more potentials usage compared to the other species in the treatment of hypertension, reducing the risk of heart disease and cancer, as an inflammatory agent and other associated uses of flavonoids, alkaloids and tannin. *Anogeissus leiocarpus* contains the highest amount of saponin and because of the toxic nature of saponin it may serve as a useful agent in the production of pesticides, insecticides to protect the wood from fungi, bacterial and other harmful pathogens.

Consequently, the presence of high secondary metabolites in the wood are good indication that if the wood is subjected to further research such as identification and characterization of wood, bioactive compounds with strong biological activities may be isolated and novel compounds may also be identified. The phytochemicals should be extracted and exposed to fungi and other pathogens to check their efficacy on this pathogen and more research should be carried out on the phytochemistry of tropical timber and not just on the leaves, bark and fruits of these tropical species.
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