ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research
7(3): 627–633, 2020
DOI: 10.22271/tpr.2020.v7.i3.078

Research article

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

K. M.Ogunjobi*, S. O. Abdulwahab, O. F. Gakenou, O. E. Thompson and O. Olorunfemi

Department of Forestry and Wildlife Management, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria

*Corresponding Author: ogunjobikm@funaab.edu.ng
[Accepted: 02 December 2020]

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 grounded 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, alkaoids 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.

[Cite as: Ogunjobi KM, Abdulwahab SO, Gakenou OF, Thompson OE & Olorunfemi O (2020) Qualitative and quantitative evaluation of the phytochemical constituents of three wood species in Ogun state, Nigeria. *Tropical Plant Research* 7(3): 627–633]

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 plant (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*

Received: 07 December 2019 Published online: 31 December 2020
https://doi.org/10.22271/tpr.2020.v7.i3.078

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 bathe 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 cm^3 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 cm^3 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 cm^3 of distilled water, boiled for 10 minutes in a water bath and filtered using Whatman filter paper No 42 (125 mm). The filtrate (10 cm^3) was mixed with 5 cm^3 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 cm^3 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 cm^3) was added to 5 cm^3 of the ethanoic extract of each sample following with the addition of 2 cm^3 of concentrated tetraoxosulphate (VI) acid. The color changed from violet to blue indicated the presence of steroids.

iv. Test for terpenoid: 30 cm^3 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 cm^3 of each extract was mixed in 2 cm^3 of chloroform and 3 cm^3 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 cm^3 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 cm^3 of 1.0 M dilute ammonia solution was added to 10cm^3 of the aqueous filtrate of each wood extract followed by the addition of 5cm^3 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 cm^3 conical flask and 20 cm^3 of 5% tetraoxosulphate (VI) acid (H_2SO_4) 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 cm^3 of 28% ammonia solution (NH_3). The solution was extracted with equal volume of chloroform (5 cm^3). The chloroform solution was extracted with two 5 cm^3 portion of 1.0 M dilute tetraoxosulphate (VI) acid, the final acid extract was then used to carry out the following test:-

To 2 cm^3 of acid extract 0.5cm^3 of Dragennorff'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 & Kocipai-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_2WO_4) in 37 cm^3 of distilled water, followed by adding 10 g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and 25 cm^3 of orthophosphoric acid (H_3PO_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_2CO_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 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_2CO_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.

$$\text{Tannic acid} = \frac{mg}{100} = \frac{C \times extract\ volume \times 100}{Aliquot\ volume \times weight\ of\ sample} \quad (1)$$

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 in 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:-

$$\%\ \text{Alkaloid} = \frac{weight\ of\ alkaloid}{weight\ of\ sample} \times 100 \quad (2)$$

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 later 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:-

$$\%\ \text{Flavonoid} = \frac{weight\ of\ flavonoid}{weight\ of\ sample} \times 100 \quad (3)$$

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°C. The mixture was filtered and the residues were extracted with another 100 cm^3 of 20% aqueous ethanol, heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was reduced to 40 cm^3 over water bath at a temperature of 90°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:

$$\%\ \text{Saponin} = \frac{weight\ of\ saponin}{weight\ of\ sample} \times 100 \quad (4)$$

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^{a}	3.17^{a}	1.45^{a}	0.67^{a}	3.06^{a}	630^{a}
2	*Gmelina arborea* Roxb	1.36^{b}	2.62^{b}	0.59^{b}	0.83^{b}	0.92^{b}	230^{b}
3	*Tectona grandis* L.f.	4.67^{c}	7.50^{c}	1.15^{c}	0.69^{c}	2.61^{c}	4950^{c}

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

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; *Sacoglottis gabonensis* (Baill.) Urb., *Khaya ivorensis* A. Chev., *Phyllanthus discoideus* Müll. Arg., *Lovoa trichiloides* Harms), *Bridelia micrantha* (Hochst.) Baill., *Bombax brevicuspe* Sprague., *Glyphea brevis* Spreng. and *Monodara 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* Müll. Arg. (9.2%), *Amphimas pterocarpoides* Harms. (9.2%), *Albizia adianthifolia* (Schumach.) W.Wight (7.2%), *Afrormosia laxiflora* (Benth.) Harms (8.0%), *Combretodendron macrocarpum* (P.Beauv) Keay. (9.2%), *Sacoglottis 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, moistening, softening and antiseptic. 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 (Angiosperm), 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 (Molyneux *et al.* 1996). Alkaloids in plants have been extracted to cure asthma, snake bite and skin diseases (Miean & 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. *Anogeisus 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.

ACKNOWLEDGEMENTS

The authors appreciate the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta and the department of Veterinary Virology whose laboratories were used for the experiments.

REFRENCES

Akindele SO & Lemay VM (2006) Development of tree volume equations for common timber species in the Tropical Rain Forest area of Nigeria. *Journal of Forest Ecology and Management* 226: 41–48.

Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM & Farombi EO (2007) Phytochemical constituents and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essay* 2: 163–166.

Amadi BA, Agomuo EN & Ibegbulem CO (2004) *Research Methods in Biochemistry*. Supreme Publishers, Owerri, pp. 90–115.

Bhargava D, Shivapuri JN, Kar S, Pandit BR, Sidhiqie A, Upadhyay A, Thakur S & Mondal KC

(2012) Evaluation of antigonorrhoeal activity of saponins extract of *Sapindus mukorossi* Gaertn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 3: 459–470.

Boham BA & Kocipai AR (1994) Flavonoids and condensed Tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Science* 48: 458–463.

Breslin A (2017) *The Chemical Composition of Green Plants*. Sciencing, Leaf Group Ltd.

Chuarienthong P, Lourith N & Leelapornpisid P (2010) Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. *International Journal of Cosmetic Science* 32: 99–106.

Conrick J (2007) *Neem: The miraculous healing herb*. Beverly Hills: America Inc.

Eboatu AN, Altine AN & Abdulraham FW (1990) Studies on thermal characteristics of some common tropical timbers. *Journal of Applied Polymer Science* 44: 239–242.

Edeoga HO, Okwu DE & Mbaebie BO (2005) Phytochemical constituents of some Nigerian Medical Plants. *Africa Journal of Biotechnology* 4: 685–688.

Ejikeme CM, Ezeonu CS & Eboatu AN (2014) Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. *European Scientific Journal* 10: 247–270.

Ezeonu CS & Ejikeme CM (2016) Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Journal of Science* 35: 89–99.

Harborne JB (1973) *Phytochemical Methods*: a *guide to modern techniques of plant analysis.* Chapman and Hall, New York.

Hikino H, Kiso Y, Wagner H & Fiegi M (1984) Antihepatotoxic actions of Flavonoids of Flavonoligans. *Planta Medica* 5: 248–250.

Judith S (2000) *The Natural History of Medicinal Plants*. Timber Press, 16 p.

Katie EF & Thorington RW (2006) *Squirrels: the Animal Answer Guide*. Johns Hopkins University Press, Baltimore, 91 p.

Koes RE & Quattrocchio F (1994) The flavonoid biosynthetic pathway in plants: Function and evolution. *BioEssays* 16: 123–132.

Malinowska P (2013) Effect of flavonoids content on antioxidant activity of commercial cosmetic plant extracts. *Herba Polonica* 59: 63–75.

Mazid M, Khan TA & Mohammad F (2012) Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine* 3: 232–249.

Middleton EJ (1998) Effect of plant flavonoids on immune and inflammatory cell function. *Advances in Experimental Medicine and Biology* 439: 175–182.

Miean KH & Mohamed S (2001) Flavonoid (Myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry* 49: 3106–3112.

Molyneux RJ, Nash RJ & Asano N (1996) Pelletier SW (ed) *Alkaloids: Chemical and Biological Perspectives, Vol. 11*. Pergamon, Oxford.

Nakatani N (2000) Phenolic antioxidants from herbs and spices. *Biofactors* 13: 141–146.

Obadoni BO & Ochuko PO (2001) Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Homostatic Plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences* 8(2): 203–208.

Okwu DE (2004) Phytochemicals and indigenous spices of South Eastern Nigeria. *Journal of Sustainable*

Agriculture and Environment 6: 30–37.

Römpp CD (1995) In: Falbe J & Regitz M (eds) *CD-RÖMPP Chemie Lexikon*, *9th edition*. Georg Thieme Verlag, Stuttgart.

Sandhya B, Thomas S, Isabel W & Shenbagarathai R (2006) Ethnomedical plants used by the Valaiyan community of Piranmalai Hills (reserved forest), Tamilnadu, India - A pilot study. *African Journal of Traditional, Complementary and Alternative Medicines* 3: 101–114.

Sheikh N, Kumar Y, Misra AK & Pfoze L (2013) Phytochemical screening to validate the Ethno-botanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India. *Journal of Medicinal Plants Studies* 1: 62–69.

Silva BP, Correa, Soares JBR, Souza EP, Palatnikc M, Sousa CBP & Parente JP (2005) Pulcherrimasaponin from the leaves of *Calliandra pulcherrima*, as adjuvant for immunization in the murine model of visceral leishmaniasis. *Vaccine* 23: 1061–1071.

Stalikas CD (2007) Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science* 30: 3268–3295.

Taiz L & Zieger E (1998) *Plant substance, 2nd edition*. Sinauer Associate Inc., Sunderland.

Ullah MF & Khan MW (2008) Food as medicine: Potential therapeutic tendencies of plant derived polyphenolic compounds. *Asian Pacific Journal of Cancer Prevention* 9: 187–195.

Wurdig G & Woller R (1995) *Chemie des Weines*. Eugen Ulmer, Stuttgart, Germany.