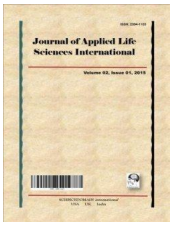

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Phytochemical Analysis and Antioxidant Properties of the Ethanolic Extract from *Tetracarpidium conophorum* (African Walnut) and *Pterocarpus soyauxii* (oha) Leaf

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCCM designed the study, wrote the protocol and read the first draft; author CEU managed the literature search and wrote the first draft of the manuscript; authors ANO and PNO read the first draft; authors CCD and COO did the statistical analysis and read the first draft; author ACN carried out the laboratory analysis and managed the data. All authors read and approved the final manuscript.

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ABSTRACT

The study investigated the phytochemical and antioxidant vitamins constituents of the leaves of *Pterocarpus soyansii* (oha) and the nuts of *Tertracarpidium conophorum* (African Walnut). The phytochemicals and antioxidant vitamins were determined on the ethanolic extracts using standard methods. The results of the phytochemical studies revealed the presence of the tested

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phytoconstituents in both plants using ethanol extraction medium. *Tetracaarpidium conophorium* contained terpenoids, alkaloids, tannins, phlobatanins, flavonoids and saponins while *Pterocarpus soyansii* contained terpenoids, alkaloids, tannins, flavonoids and saponins. The two plants extract contained copious amounts of antioxidant vitamins A, C and E. African Walnut possessed higher values of vitamin A(1283.30±30 mg/100 g) followed by vitamin C(14.80±2.43 mg/100 g) and vitamin E(0.22±0.02 mg/100 g). The *Pterocarpus soyansii* leaves exhibited the highest values of vitamin A(48.00±0.01 mg/100 g) followed by vitamin E(0.29±0.01) and vitamin C(0.32±0.02). The results show that the antioxidant vitamins A and C were significantly higher in *Tertracarpidium conophorum* than in *Pterocarpus soyansii.* The results suggested and showed that both African Walnut and 'oha' leaves possess copious amounts of the antioxidant vitamins and can serve as good nutritional supplements. These substances may be responsible for the medicinal and nutritional properties of the plants.

Keywords: Phytochemical; antioxidant; Tetracaarpidium conophorium; Pterocarpus soyauxii.

1. INTRODUCTION

Natural products with pharmacological potential can be of therapeutic importance in treating different disease states. Natural products are therefore the active constituents of most traditional and orthodox medicine. *Tetracarpidium conophorum* Mull. (Arg) commonly called African walnut belonging to the family *Euphobiaceae* is a flowering plant useful for the nutritive value of its nuts [1-4]. According to Ayoola et al. [4], *Tetracarpidium conophorum* is planted primarily for its nuts which are cooked and eaten as snacks. The extracts and fractions of the seed have been reported to have the antimicrobial potential [3]. Oxalate, phytate and tannins have been reported in the raw *Tetracarpidium conophorum* nuts [5] while Nwokolo [6] studied the effect of traditional processing methods on the nutritive value of the nut. Ayoola et al. [4] studied the walnut root and reported the presence of thiamine, ascorbic acid, riboflavin, niacin, cyanocobalamine and some phytoconstituents.

Pterocarpus soyauxii is a tropical plant belonging to the family *Leguminosae* [7]. The leaves are popularly used in preparing 'oha' soup in South Eastern Nigeria. Okerulu et al. [8] reported that the leaves contain vitamin A and C while the phytochemical analysis of the different solvent extract showed the presence of alkaloids, saponins, glycosides and tannins. The crude extract of the leaves has been reported to significantly improve the haematological profile in albino rats [9]. The plant has been reported to have many pharmacological potentials [10]. Keeping in view the medicinal properties already noted in these plants, their phytochemical and antioxidant properties were evaluated.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh samples of *Tetracarpidium conophorum* (TC) pods were purchased from Awka Anambra State while fresh samples of *Pterocarpus soyauxii* (PS) leaves were purchased from Ahiaeke market in Umuahia, Abia State. They were wrapped in a fresh nylon bags and taken to the Biochemistry Laboratory, Michael Okpara University of Agriculture Umudike Abia State, Nigeria where they were refrigerated till further use. For TC, the pods were shelled and four seeds recovered from each pod. The seeds were cracked to remove the hard shells. The inner portion which looked like mesocarp/cotyledons were washed with distilled water and air-dried at room temperature. Thereafter, the dried mesocarps were ground into fine powder using pestle and mortar and stored in an air-tight glass jar at 4^{O}C prior to use. The leaves of *Pterocarpus soyauxii* were hand-picked, washed with distilled water and cut into small pieces and air-dried. Thereafter, the dried leaves were ground with a hand grinder and the powdered sample was placed in a container and stored at room temperature until use.

2.1.1 Preparation of ethanolic extracts of *Tetracarpidium conophorum* and *Pterocarpus soyauxii*

Fifteen (15) grams of each powdered samples of TC and PS were soaked in 200 ml of absolute ethanol separately and allowed to stand for 24hours at room temperature for extraction. Thereafter, each extract solution was filtered with Whatman filter paper and the filtrates stored in a refrigerator until use.

2.1.2 Qualitative phytochemical screening of extracts of *Tetracarpidium conophorum* and *Pterocarpus soyauxii*

Terpenoids, alkaloids, tannins, phlobatanins, flavonoids, steroids and saponin were determined in the two extracts as described by Dutta et al. [11].

2.1.2.1 Test for terpenoid

Five millilitres of ethanol leaves extract was mixed with 2 ml of chloroform. Three millilitres of concentrated H_2SO_4 was added to the solution slowly along the wall of the test tube. Care was taken not to stir the solution of the test tube. Reddish brown colouration formed at the junction of two liquid phases indicates the presence of terpenoid.

2.1.2.2 Test for alkaloid

Two millilitres of ethanol filtrate was taken in a test tube and 2 ml of 2N HCl was added to it. The solution was shaken vigorously to mix and kept aside for 5 min. The aqueous phase was separated from the two liquid phase and a few drops of Mayer's reagent ($HgCl_2$+ KI in water) was added to it and shaken to observe the formation of creamy coloured precipitation.

2.1.2.3 Test for tannin

Ten millilitres of the ethanol extract was taken in the test tube and few drops of 0.1% $FeCl_3$ solution was added to it. Formation of blue-black precipitation indicates the presence of tannin.

2.1.2.4 Test for phlobatannin

Ten millilitres of the extract was taken in a test tube and allowed to boil. Two millilitres of concentrated HCl was added in the test tube with proper caution so that the solution dose not bumped out. The mixture was boiled for 1 minute. Deposition of red precipitate indicates the presence of phlobatannin.

2.1.2.5 Test for flavonoid

Two grams of crude powdered extract was heated with 10 ml of ethyl acetate in a hot water bath for a period of 5 min. The solution was filtrated using Whatman filter paper. The ethanol filtrate (1.4 ml) was mixed with 10% dilute ammonia solution and shaken vigorously. Yellow colouration of the solution indicates the presence of flavonoid.

2.1.2.6 Test for steroid

Five millilitres of ethanol filtrate was treated with 0.5 ml of anhydrous CH_3COOH and was cooled in an ice bath for 15 min. Five hundred µl of chloroform was added to the solution. One millilitre of concentrated H_2SO_4 was poured along the walls of the test tube very carefully. At the separation level of two liquids, a radish brown ring was formed, as an indication of the presence of steroids.

2.1.2.7 Test for saponin

Crude powder of leave (0.5 g) was boiled with 15 ml of double distilled water in a hot water bath. Intensive forth formation indicates the presence of saponin.

2.2 *In vitro* Antioxidant Vitamin Tests

2.2.1 Determination of vitamin A

Vitamin A content of the extracts was determined as described by Kirk and Sawyer [12] as reported by Ageing et al. [13]. The sample (2 g) was weighed into a flat bottom reflux. Distilled water (10 ml) was added and shaken carefully to form a paste; 25 ml of alcoholic potassium hydroxide solution was added and a reflux condenser attached. The mixture was heated in a boiling water bath for one hour with frequent shaking, allowed to cool and 30 ml f of distilled water was added. The hydrolyzed product obtained was transferred into a separator funnel and the solution was extracted three times with 20 ml of ether; 20 g of anhydrous Na_2SO_4 was added to the extract to remove any traces of water. The mixture was then filtered into a 100 ml volumetric flask and made up to the mark with ether. Standard solution of β-carotene of range 0-50 µg/ml was dissolved in 100ml of ether. The gradients of different standard solutions were determined with reference to their absorbance from which the average gradient was taken to calculate β-carotene in µg/ 100 g. The absorbance of sample and standards were read on a spectrophotometer at 328 nm. The protocol was repeated for *Pterocarpus soyauxii.* The actual beta-carotene value was calculated using the formula,

Vitamin A(mg/100 g)= Absorbance of sample × Dilution factor × gradient factor/weight of the sample.

2.2.2 Determination of vitamin C

The vitamin C content in the extract of *Tetracarpidium conophorum* was determined as reported by Ageing et al. [13]. To 2 g of dry sample extract, 20 ml of each of 1% acetic acid and 1% oxalic acid were added. The mixture was allowed to stand for two hours before it was filtered. Then, 10ml of the filtrate was pipetted into a conical flask and titrated with 2,4-dinitrophenol-hydrazine and the volume noted. A portion of 10 ml standard ascorbic acid of analytical grade was also titrated with indophenol dye solution. The weight of ascorbic acid oxidized by 1 ml of the dye was calculated in mg. A blank titration was also done in the same manner without the sample. The protocol was repeated for *Pterocarpus soyauxii.* The value obtained was used to calculate the concentration of vitamin C using the formula:

$$\text{Vitamin C(mg/100 g)} = \frac{100 \times V_T (V_1 - V_2)}{W\text{-}V_3 (V_T - V_0)}$$

Where,

W = weight of the sample
V1 = value for the standard ascorbic acid
V2= value for the sample
V0 = value for blank
V1 = volume of extract and for titration

2.2.3 Determination of vitamin E

The vitamin E content of the extract of *Tetracarpidium conophorum* was determined as described by Agiang et al. [13]. One gram (1 g) of the extract was weighted into a 250 ml conical flask containing 10 ml of absolute alcohol and 20 ml of 1 M alcoholic sulphuric acid. The condenser and flask were wrapped in aluminium foil and refluxed for 45 minutes and then cooled for 15 minutes. A volume of 50ml distilled water was added to the mixture and transferred to a 250 ml separating funnel covered with aluminium foil. The unsaponifiable matter in the mixture was extracted with 30 ml of dimethyl ether. The combined extracts were washed free of acid and dry-evaporated at a low temperature and the residues obtained, immediately dissolved in 10 ml of absolute alcohol. Aliquots of solutions of each sample and standards (0.3-3.0 mg vitamin E) were transferred into a 20 ml volumetric flask and 5ml absolute alcohol added, followed by a careful addition of 1 ml concentrated HNO_3. The flask was allowed to cool rapidly under running water and volume was adjusted with absolute alcohol. The absorbance was read at 470 nm against a blank solution containing 5 ml absolute alcohol and 1 ml HNO_3 treated in a similar manner. The protocol was repeated for *Pterocarpus soyauxii.* The vitamin E content was calculated thus:

Vitamin E(mg/100 g) = Absorbance of sample × Dilution factor × gradient factor/weight of sample.

2.3 Statistical Analysis

Results obtained were subjected to analysis using SPSS version 20.0. The results were presented as mean ± SD and difference in mean were compared using ANOVA at a probability threshold P=0.05.

3. RESULTS

Table 1 represents the results of the phytochemical screening on the ethanolic extracts of *Tetracarpidium conophorum* and *Pterocarpus soyauxii*. The preliminary results showed the presence of the tested phytoconstituents in both plants using ethanolic extraction medium. *Tetracarpidium conophorum* showed the presence of terpenoids alkaloids, tannins, phlobatannins, flavonoids and saponins while *Pterocarpus soyauxii* exhibited the presence of terpenoids, alkaloids, tannins, flavonoids and saponins. The antioxidant vitamin contents of *Tetracarpidium conophorum* and *Pterocarpus soyauxii* are shown in table 2. As indicated, *Tetracarpidium conophorum* contained higher values of the two antioxidant vitamins than *Pterocarpus soyauxii* leaves. *Tetracarpidium conophorum* contained highest values of vitamin A followed by vitamin C and and vitamin E. *Pterocarpus soyauxii* contained highest value of vitamin A followed by vitamin E and vitamin C. The results suggested and showed both nuts of *Tetracarpidium conophorum* and leaves of *Pterocarpus soyauxii* leaves contained copious amounts of the antioxidant vitamins and can serve as good nutritional supplements.

4. DISCUSSION

The study has used phytochemical antioxidant indices to evaluate some nutritional benefits in *Pterocarpus soyauxii* leaves and the nuts of *Tetracarpidium conophorum.* Interest in medicinal plants revived in recent times because of their efficacy in providing cost-effective therapy to several diseases due to the presence of secondary metabolites in their body parts. These compounds otherwise known as

phytochemicals have been found to be responsible for the antioxidant properties of plants. The findings or results from the analysis of the different samples revealed fascinating observations. The results of the phytochemical analysis revealed the presence of terpenoids, flavonoids, alkaloids, tannins, phlobatannins, and saponins in both plants. These constituents were known to be bioactive agents as was reported by Ojiako et al. [14]. Saponins are naturally phytic glycoside compound [15]. Antifungal activities of saponins have been subject of much research primarily for pharmaceutical and agricultural applications. Many saponin-rich extracts had been reported by Obute and Osuji [16] to show antifungal activities.

Table 1. Phytochemical screening of the extracts of *Tetracarpidium conophorum* and *Pterocarpus soyauxii*

Test	*Tetracarpidium conophorum*	*Pterocarpus soyauxii*
Terpenoids	++	+
Alkaloids	+	+
Tannins	+	++
Phlobatannins	+	-
Flavonoids	+	++
Steroids	-	-
Saponins	+	+

Key: += light colure, ++= intense colure,- =negative

Table 2. Antioxidant vitamins content of the extracts of *Tetracarpidium conophorum* and *Pterocarpus soyauxii*

Antioxidant vitamins	*Tetracarpidium conophorum*	*Pterocarpus soyauxii*
Vitamin A(mg/100g)	1283.30±1.18[a]	48.00±0.01[b]
Vitamin C(mg/100g)	14.80 ±2.43[a]	0.29±0.01[b]
Vitamin E(mg/100g)	0.22 ±0.02[a]	0.32±0.02[a]

Results are mean ± SD of triplicate readings. Values with different superscript in a row are significant (P=0.05)

Saponins are known to exhibit cytotoxic effect and are responsible for the bitter taste of the plant. Saponins have been suggested to have oxytocin related activity [4,17]. The presence of saponins in the leaf extract of *Pterocarpus soyauxii* and *Tetracarpidium conophorum* suggests that the extracts from both plants may be used in the treatment of hormone-dependent disorders including cancer and post-menopausal symptoms [18]. Ayoola et al. [4] has reported the presence of saponin in the root extract of *Tetracarpidium conophorum.*

The study has shown that flavonoids have an effect in age-related conditions [19] and do protect against allergies and inflammation. Flavonoids are planted phenolics that give spices and vegetables their flavouring taste [17,20]. Because flavonoids are free radical super antioxidants and with strong anti-cancer property [21], they also serve as an anti-inflammatory agent for their antioxidant effects [22,23]. This could suggest the use of these plants in the treatment of intestinal disturbances in herbal medicine.

Pure alkaloids are used therapeutically and also as predator and insect repellant. According to the report of Okaka et al. [24] alkaloids when ingested by animals affect thyroid function and some mammalian enzyme functions. As pure alkaloids and the synthetic derivatives exhibit analgesic antispasmodic and antibacterial activities they can be used as therapeutic agents [25,26]. Ndukwe and Ikpeama [7] and Okerulu et al. [8] both reported the presence of alkaloids in the leaf of *Pterocarpus soyauxii* thus confirming our results.

As shown in Table 1, the preliminary phytochemical analysis of the extracts revealed the presence of secondary metabolites present in them. The identified constituents are reported to exhibit strong antioxidant scavenging activity for radicals involved in the lipid peroxidation [27]. Polyphenol compounds have an essential role in preventing lipid peroxidation and are also involved in antioxidant activity [28]. Phytochemicals such as flavonoids, phenols, alkaloids and tannins are well known free radicals scavengers and possessing multiple biological activities including antioxidant activity [29]. Vitamins A, C, and E are antioxidant vitamins due to their roles in scavenging free radicals. They are used as a standard in antioxidant assay because of their roles as antioxidants. The results of this study suggest that these extracts of *Pterocarpus soyansii* leaves and *Tetracarpidium conophorum* nuts possess phytochemicals that are capable of donating hydrogen to a free radical.

The presence of tannins in *Tetracarpidium conophorum* can support its strong use for the healing of haemorrhoids, frost and varicose ulcers in herbal medicine [4]. Tannins are reported to be involved in wound healing and have antimicrobial properties which may be the

reason for using leaves that have it in the treatment of diarrhoea and dysentery [23,30]. This study showed the high presence of tannins in both plants extracts supporting the reports of Ndukwe and Ikeama [7] and Okerulu et al. [8].

The presence of vitamin C in the nut and leaf of the plants implies that they can be used to improve good health, prevent ascorbic acid deficiency conditions and used for the treatment of common cold [31,32]. Both *Pterocarpus soyauxii* leaves and *Tetracarpidium conophorum* nuts contained the antioxidant vitamins A, C and E but *Tetracarpidium conophorum* having significantly higher amounts of vitamins A and C. This result implies that *Tetracarpidium conophorum* could elicit more antioxidant activity than *Pterocarpus soyansii* leaves. Antioxidants repair free radical damages to the cells [32]. The presence of antioxidant molecules suggests that both plants can be used as vitamin supplement probably during oxidative stressed conditions. The results of the study showed that both plants have high nutritive values which could attenuate physiological oxidative stress due to their high concentrations of vitamins A. C and E as well as flavonoids contents. The presence of vitamin C in the extracts of both plants may confirm the reported wound healing property of these plants [33].

5. CONCLUSION

This current study, therefore, provides some scientific justification for the utilization of these plants in traditional medicines. The results of the qualitative phytochemical study of the leaves of *Pterocarpus soyauxii* leaves and *Tetracarpidium conophorum* showed the presence of alkaloids, tannins, saponins, terpenoids and flavonoids. The extensive study of these phytochemicals and establishment of good correlation among the plant phytochemicals is essential for ensuring efficiency and quality of the herbal medicine. The result of the antioxidant vitamins content clearly shows that both plants have high nutritional value. These plants can, therefore, be said to be highly medicinal. However, adequate and proper care of these plants during processing and storage will ensure the conservation of their usefulness.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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