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Original Research Article

Phytochemical Screening and Free Radical Scavenging Activities of the Fruits and Leaves *of Allanblackia floribunda* Oliv (Guttiferae)

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Abstract

Purpose: *To compare the phytochemical constituents in the leaves and fruits of Allanblackia floribunda and determine their free radical scavenging activity.*
Methods*: The fruit and leaves of AF collected from the uncultivated farmlands of Okeigbo, Ondo State, Nigeria, were dried, milled and extracted with methanol. Phytochemical screening was carried out according to standard procedures. Free radical scavenging activity was determined by measuring the decrease in the visible absorbance of 2,2-diphenyl-1-picrylhydrazyl (DPPH) on addition of the plant extract. The mean inhibitory concentration (IC_{50}), which is the concentration of extract needed to decrease the initial absorbance of DPPH by 50% was determined graphically. Total phenolic, flavonoids and proanthocyanidin contents were determined by spectro-photometric methods.*
Results*: Alkaloids, anthraquinones, tannins, saponins, steroids, terpenoids, flavonoids and cardiac glycosides were found to be present in both the fruits and leaves. Only AF fruit contained phlobatannins. IC_{50} values of 0.01, 0.02 and 0.1 mg/ml were recorded for Vitamin E, AF leaves and AF fruits respectively. Total phenolic, total flavonoid and proanthocyanidin contents were 65, 0.07 and 2.38 mg/g of powdered plant material for AF fruits, and 12, 51.35, 19.5 mg/g of powdered plant material for AF leaves as gallic acid, rutin and catechin equivalents respectively.*
Conclusion*: AF leaves are five times more potent as a free radical scavenger compared to the fruits though the fruit was found to contain a higher phenolic content.*

Keywords: *Free radical scavenger, phenolic content, proanthocyanidin, flavonoids, DPPH, Allanblankia floribunda, tannins, steroids, alkaloids and anthraquinones.*

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Introduction

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acid, proteins, lipids or DNA and can initiate a variety of disease processes such as cancer, cardiovascular diseases, cataracts, diabetes, asthma, macular degeneration and inflammatory diseases [1-3]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases[4]. Antioxidant effect of a plant is mainly due to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes[5]. Further studies have revealed that some phenolic molecules have anticancer and antimutagenic activities[6].

Allanblankia floribunda is an evergreen tree found in the rainforest. Its fruits are large, up to 30 cm long and 10 cm in diameter containing seeds in a translucent mucilage. The decoction of the bark is taken for dysentery and mouthwash in Gabon. In Congo, it is taken for stomach ache, and a decoction of the bark or the leaves is taken for cough, asthma, bronchitis and other bronchial infections. Decoctions of the whole fruit is used in Ivory Coast to relieve scrotal elephantiasis. The traditional uses indicate possible anti-inflammatory and antimicrobial activity. The decoction of the leaves and fruits have also been reported for use in the treatment of malaria and toothache [7,8]. All parts of the plant are used traditionally in the treatment of smallpox, chickenpox and measles indicating possible antiviral activity. The fatty substance of the seeds is mildly purgative.

Previous studies of the hearthwood and the root bark of *Allanblackia floribunda* reported the isolation of benzophenones, xanthones and some biflavonoids, some of which exhibited a wide range of pharmacological activities such as cytotoxic, anti-inflammatory, antimicrobial and antifungal as well as HIV inhibitory activity[9-12]. A new prenylated xanthones (1.5-dihydroxy-xanthone) was isolated from the stem bark along with some other known compounds[13].

This present study, aims to investigate the free radical scavenging activity of *Allanblankia floribunda*, determine the total phenolic, total flavonoid and proantho-cyanidin contents in AF fruit and leaves. We also aim to investigate the correlation between the free radical scavenging activity of the methanolic extract of AF fruit and leaves and the phenolic, total flavonoid and proanthocyanidin contents of the plant. To the best of our knowledge, similar work has not been carried out to date.

Materials and Method

Collection and identification of plants

Allanblackia floribunda plant materials were collected fresh from forest sources in Oke-igbo, Ondo State, South-West Nigeria in March 2007, identified at the Forestry Research Institute of Nigeria (FRIN), and given a voucher number ((FHI107929). The plant fruits were chopped into bits and dried in the oven at a temperature of 40 ^{o}C for 3 days, while the leaves were air dried in the oven for 3 days at a temperature of 40 ^{o}C.The dry plant materials were blended using a kitchen blender after which the powdered samples were weighed.

Extraction and phytochemical screening of plant

The powdered plant materials (60 g each) were soaked in methanol (Sigma-Aldrich, UK) for 3 days and the crude extracts were filtered and concentrated using a rotary

evaporator. Phytochemical Screening was performed using standard procedures[14-16].

Determination of the free radical scavenging activity (FRSA) of plant extracts

The antioxidant activity of each extract was measured in terms of hydrogen donating or free radical scavenging activity, using the stable radical DPPH[17]. Briefly, to a methanolic solution (1 ml) of extract of various concentrations (0.02 – 0.1 mg/ml) was added 0.5 ml of 1 mM DPPH solution in methanol. A blank solution was prepared containing 1 ml of methanol and 0.5 ml of 1 mM DPPH. The experiments were carried out in triplicates. The test tubes were incubated for 15 min, methanol was used to zero the spectrophotometer and the absorbance was read at 517 nm. The radical scavenging activity was calculated using the following formula[18]:

% inhibition of DPPH = {(A_B – A_A)/A_B} x 100 where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution.

The results are expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC_{50}) determined from a plot of absorbance of DPPH versus concentration of extract[4].

Determination of total phenolic content

Total phenolic content was determined according to a previously described method[18-20]. To 0.5 ml aliquot of various concentrations (0.01 – 0.05 mg/ml) of gallic acid in methanol was added 2.5 ml of a ten-fold diluted Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate. Concentration of 0.1 mg/ml and 1.0 mg/ml of each plant extract in methanol were used as test solutions. The absorbance was read after 30 mins at room temperature at 760 nm spectrophotometrically. All determinations were performed in triplicates. Total phenolic contents obtained for AF leaves and fruit were obtained from the regression equation of the calibration curve of gallic acid *(y = 10.454x = 0.0201, R^2 = 0.97)*, and expressed as gallic acid equivalents (GAE).

Determination of total flavonoid content

Total flavonoid content was determined according to a previously described method [18,20]. To 2 ml of 2% $AlCl_3$ in ethanol was added 2 ml of the test sample. The UV absorption was measured at 420 nm after 1 hr at room temperature. Concentrations of 0.1 mg/ml and 1.0 mg/ml sample solutions were used while rutin concentrations of 0.025 – 0.4 mg/ml were used to obtain a calibration curve.. Determinations were performed in triplicates. Total flavonoid contents were obtained from the regression equation of the calibration curve of rutin *(y = 2.9215x + 0.3292, R^2 = 0.93)*, and expressed as rutin equivalents (RE).

Proanthocyanidin content

Proanthocyanidin content was determined according to a previously described method[18,20]. In brief, 0.5 ml of HCl was added to various concentration of catechin in methanol (0.025 – 0.4 mg/ml) and allowed to stand for 15 min. Concentrations of 0.1 mg/ml and 1.0 mg/ml of the extracts in methanol were used in the assay. The absorbance was taken at 500 nm. All determinations were carried out in triplicates. Proanthocyanidin contents were determined from the regression equation of the calibration curve of catechin (y = 2.1145x + 0.0145, r^2 = 1.0) and expressed as catechin equivalents (CE).

Results

The amount of the methanol extract obtained from the extraction was 11.65 g (19.4% w/w) for the leaves and 7.39 g (12.3% w/w) for the fruits. Phytochemical screening of the two plant parts revealed the presence of anthraquinones, alkaloids, tannins, cardiac glycosides, flavonoids, steroids, saponins and terpenoids in both the fruit and the

Table 1: IC_{50} values, total phenolic, total flavonoid and proanthocyanidin contents for *A. floribunda* leaves and fruit methanol extract

Plant part	IC_{50} (DPPH inhibition) mg/ml	Total Phenolic Content (GAE) mg/g powdered plant material	Total flavonoid content (RE) mg/g powdered plant material	Total Proanthocyanidin content (CE) mg/g powdered plant material
A.floribunda leaves	0.02	12	51.35	19.5
A.floribunda fruit	0.1	65	0.07	2.38

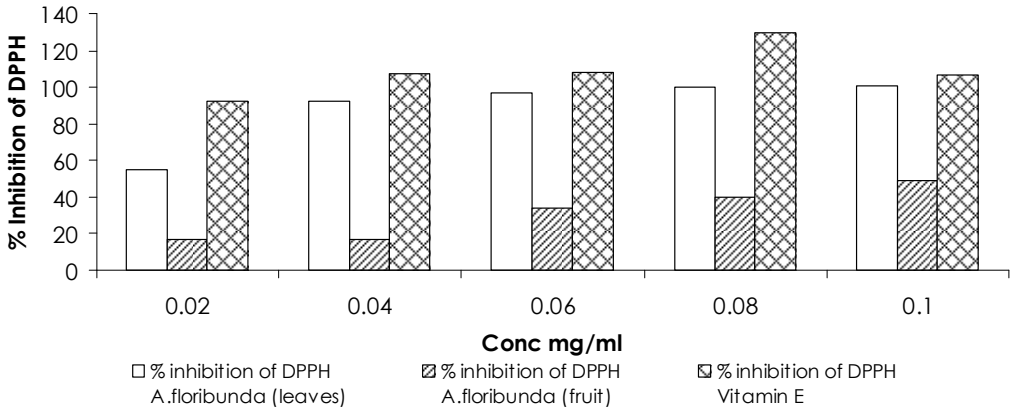

Figure 1: Inhibition (%) of DPPH against concentration of extracts of *A. floribunda* leaves and fruits, and Vitamin E

leaves. Phlobatannins were present in the fruits and not in the leaves of *A. floribunda*.

Percentage inhibition of DPPH and IC_{50} are parameters widely used to measure antioxidant/free radical scavenging power[4,21-23]. The IC_{50} value obtained for DPPH inhibition were 0.01, 0.02 and 0.1 mg/ml for vitamin E, AF leaves, and fruit respectively (Figure 1). Results from the DPPH inhibition shows that Vitamin E is twice as potent as AF leaves and ten times more potent than AF fruit as a free radical scavenger in the DPPH test. Total phenolic contents were 12 and 65 mg/g for AF leaves and fruit respectively (Table 1). A correlation of 0.66 and 0.94 were obtained between the data for phenolic content and DPPH inhibition for AF leaves and fruits respectively. Total flavonoids contents were 51.35 and 0.7 g/g of powdered plant for AF leaves and fruits respectively. A correlation of 0.66 and 0.94 were obtained between the data for total phenolic content and percentage DPPH inhibition for AF leaves and fruits respectively. Proanthocyanidin contents were 19.5 and 2.38 mg/g powdered plant material for the leaves and fruits respectively (Table 1). A correlation of 0.66 and 0.94 were obtained between the data for proanthocyanidin content and % inhibition of

DPPH. The purpose of the correlation was to establish if there is a relationship between the free radical scavenging activity, total phenolic, flavonoid or proanthocyanidin contents.

Discussion

The extraction procedure appeared to be more efficient for the leaves (19.4% yield) compared to the fruits (12.3% yield). Phytochemical screening revealed a slight difference between the constituents of the fruits and leaves. AF fruits were found to contain phlobatanins but not the leaves.

The DPPH test shows the ability of the test compound to act as a free radical scavenger. DPPH is a free radical and it produces a strong absorption band at 517 nm, in the visible region of the electromagnetic radiation. The colour turns from purple to yellow as the molar absorptivity of the DPPH reduces from 9660 to 1640 at 517 nm when the odd electron of DPPH becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H[21-23]. The higher the % inhibition of DPPH absorbance the higher the FRSA and the lower the IC_{50} value the higher the FRSA/antioxidant power. Hence from this study, methanol extract of AF leaves has a higher FRSA compared to AF fruits but a lower FRSA compared to vitamin E. A higher percentage inhibition of DPPH was recorded for vitamin E at a concentration of 0.08 mg/ml compared to the value at 0.1 mg/ml. The reason for this is yet unclear.

Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers. Therefore, it was reasonable to determine the total phenolic content in the plant extract. The result shows that the phenolic content of AF fruit is higher than that of AF fruit and the radical scavenging activity (RSA) is likely to be due to the phenolics however, phenols may not be solely responsible for the FRSA in the case of AF leaves due to a low correlation of 0.66 between the phenolic content and % inhibition of DPPH.

Flavonoids are a ubiquitous group of polyphenolic substances which are present in most plants. Therefore it was also reasonable to determine the total flavonoid content in the plant materials. The total flavonoid content in AF leaves is considerably higher than that in AF fruit. This may contribute to the difference in antioxidant activity as AF leaves is 5 times more potent than AF fruit as a free radical scavenger. However, there was a good correlation between the total flavonoids content and the DPPH assay (r^2 = 0.94), for AF fruit indicating that flavonoids were contributory to the free radical scavengeing activity of the fruit extract, but a low correlation of 0.66 obtained for the leaf extract implies that flavonoids are not likely to be solely responsible for the antioxidant activity of AF leaves. It is also known that only flavonoids of a certain structure and particular hydroxyl position in the molecule determine antioxidant properties. This property depends on the ability to donate hydrogen or electron to a free radical[18].

Proanthocyanidins are a type of bioflavonoid that has been shown to have very potent antioxidant activity. Proanthocyanidin content in AF leaf was greater than that in AF fruits. The difference may account for the higher potency of AF fruit as a free radical scavenger. There was a good correlation between the proanthocyanidin content and the DPPH assay (R^2 = 0.94) for AF fruit, but a low correlation of 0.66 was obtained in the case of AF leaves. This indicates that proanthocyanidins present in the extract are involved in the free-radical scavenging activity of the AF fruit extract, but other phytochemicals may also be responsible in the case of AF leaves.

Flavonoids have been shown to have anti-bacterial, anti-inflamatory, antiallergic, antineoplastic, antiviral, anti-thrombotic and vasodilatory activities[1]. The potent antioxidant activities of flavonoids have been suggested to be responsible for many of the

above actions as oxidative damage is implicated in most disease processes. Indeed laboratory research on flavonoids and other antioxidants suggest their use in the prevention and treatment of a number of these diseases. Hence both AF leaf and fruit extracts can be exploited in the treatment of the various diseased conditions mentioned above. Traditional uses of AF for asthma, bronchitis and toothache suggest possible anti-inflammatory properties, which are in line with some of the properties of antioxidants.

Conclusion

Methanol extracts of *A. floribunda* leaves and fruit both showed potent free radical scavenging activity against DPPH. AF leaves was 5 times more potent compared to AF fruits and half as potent compared to Vitamin E as a free radical scavenger. The phenolic content of AF fruit was found to be greater than in AF leaves, however the total flavonoids content and proanthocyanidin contents of AF leaves were much greater than that of AF fruit. Phlobatannins were present in the fruits but not in the leaves. Efforts are now been made in the isolation and characterization of the phytochemicals in *A.floribunda* fruits and leaves for a more detailed investigation of their antioxidant properties.

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