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Short communication

Phytochemical Analysis and Antioxidant Activity of Stem Bark Extract and Fractions of *Pycnanthus angolensis* Myristicaceae (Welw) Warb

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ABSTRACT

Pycnanthus angolensis is used extensively in African traditional medicine for treating stress-related ailments. Antioxidants play an essential role in scavenging and inhibiting free radicals and hence protecting human beings against infection and related degenerative diseases. Modern research is directed toward natural antioxidants from medicinal plants due to their safety as therapeutic agents. The present study is aimed at evaluating the phytochemical constituents and antioxidant activity of the stem bark extract and fractions of *Pycnanthus angolensis*. The phytochemical investigation was performed by using standard protocols. The extract and fractions were investigated for the antioxidant activity with ascorbic acid as the reference standard by using the 2,2-diphenyl1-1-picrylhydrazyl (DPPH) *in-vitro* model. Phytochemical analysis of the methanol extract and solvent fractions of *P. angolensis* stem bark showed the presence of alkaloids, flavonoids, tannin, saponin, terpenoids and steroids. The crude methanol extract (ME), hexane fraction (HF), ethyl acetate fraction (EF) and methanol fraction (MF) showed *in vitro* antioxidant IC_{50} of 9.44 ± 1.40 µg/mL, 5.47 ± 0.18 µg/mL, 3.86 ± 1.60 µg/mL, 1.09 ± 2.27 µg/mL, 6.89 ± 3.96 µg/mL and 31.33 ± 2.75 µg/mL respectively. The MF, with an IC_{50} and ARP of 1.09 µg/ml and 91.7% respectively, appeared to provide a starting point for the discovery of potent antioxidant lead compounds from *Pycnanthus angolensis*.

***Keywords*:** Antioxidants, Phytochemicals, *Pycnanthus angolensis*, 2,2-diphenyl1-1-picrylhydrazyl.

Introduction

Oxidative mechanisms in the body generate reactive oxygen species (ROS) which lead to disease conditions such as coronary heart disease, diabetes mellitus, cancer, autoimmune disease, neurodegenerative ailments and aging.[1] Some of the ROS include hydrogen peroxide (H_2O_2), superoxide anion radical (O^2), highly reactive hydroxyl radical (HO^-) and singlet oxygen (O_2).[2]
Antioxidants, which protect the human body from these ROS, can be enzymatic (superoxide dismutase, glutathione reductase catalase, glutathione peroxidase) or non-enzymatic (polyphenols, carotenoids, vitamins, flavonoid and minerals).[3] Antioxidants exhibit this important action by a breakdown of free radicals, electron donation or prevention of transition metal interaction with hydrogen peroxide and superoxides.[4] Some plant contains antioxidants which protect the cells from the oxidative stress and cellular damaging effect of ROS.[5]
Pycnanthus angolensis (Myristicaceae), commonly called African nutmeg is an important medicinal plant found in tropical Africa. It is widely used in ethnomedicine as an anti-aging agent.[6] The leaves are used to prevent miscarriage in pregnant women. Its bark serves as an appetizer, mouth wash, and anti-emetic and it is also used by lactating mothers to stimulate and purify their breast milk.[7] The sap possesses anti-hemorrhagic properties and the bark is a poison antidote and treatment of leprosy, infertility, anemia, gonorrhea and malaria.[8]

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The potential antioxidants from plants such as tannin, terpenoid, saponin, polar flavonoid, phenol, non-polar flavonoid, ascorbic acid, and others possess the ability to scavenge free radicals.[9] Their wide range of effects on the biological system has put forward many experimental works. Presently, more research is going on to find out the potential of phytochemical antioxidants as health promoters and benefactors. This is because of their ability to neutralize the ROS or free radicals or oxidants that are responsible for the onset of and gradual cell damage. It has been found that synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyaniline (BHA), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) are toxic and/or mutagenic to human health.[10] Attention has been shifted to naturally occurring antioxidants. It has been reported that the seeds of *Pycnanthus angolensis* possess free radical quenching/antioxidant activity.[11] It is also known that different morphological parts of the *P. angolensis* plant are used traditionally to manage certain autoimmune diseases and neurodegenerative ailments.[12-14] However, the antioxidant property of the stem bark has not been reported. Studies have also shown that plants with flavonoids and other phenolic compounds are reputed as scavengers and inhibitors of lipid peroxidation.[15] This study, therefore, investigated the phytochemical constituents and antioxidant activity of extract and fractions of *P. angolensis* stem bark by the DPPH model.

Materials and Methods

Materials
Equipment, Chemicals and reagents
Methanol, n-hexane, and ethyl acetate (EtOAc) and standard drug (ascorbic acid) were produced from SRL, Mumbai, India. The 2,2-diphenyl1-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, Germany. All chemicals and solvents used were of analytical grade. A double-beam ultra-violet/visible spectrophotometer was used for the antioxidant assays.

Plant material
The stem bark of *Pynanthus angolensis* was collected from Orba, Enugu State, Nigeria in July and August 2019. The plant was identified and authenticated by Mr. Alfred Ozioko, a taxonomist with the Bioresource Development and Conservation Program Centre (BDCP), Nsukka. A voucher specimen (Voucher number: INTERCEDD/09/18) of the plant was stored at the herbarium of the centre.

Methods
Preparation of extract
The extraction was performed by the cold maceration method. A 1.0 kg of the powdered stem bark of *Pycnanthus angolensis* was macerated in 5 L of methanol for 48 h. The mixture was agitated at regular intervals to ensure good and complete extraction. Thereafter, the mixture was pre-filtered using a muslin cloth and then the filtrate was re-filtered using Whatman filter paper (No, 1). The filtrate was dried using a rotary evaporator at 40°C.

Fractionation of extract
A 20.0 g of methanol extract (ME) dispersed in 10 %v/v methanol and partitioned successively in a separating funnel with an equal volume of n-hexane, methanol and ethyl acetate in increasing order of polarity to obtain n-hexane (HF), methanol (MF) and ethyl acetate (EF) fractions respectively.

Phytochemical analysis
Phytochemical screening of ME and the fractions (HF, EF and MF) was carried out according to standard methods.[14] The extract was tested for the presence of alkaloids, flavonoids. saponins, steroids, tannins, terpenoids, glycosides and phenolic compounds.

Assessment of antioxidant activity
Free radical scavenging (FRS) activity by DPPH assay
The free radical scavenging (FRS) activity of ME and fractions (HF, EF and MF) based on the scavenging activity of the stable DPPH (deep purple colour) free radical was determined by the standard method described in previous reports.[2-5] The stock solutions of ME and fractions were prepared by carefully dissolving a known amount of ME and fractions in methanol. The working solutions of ME and fractions (HF, EF and MF) used (15.63, 31.25, 62.50, 125.25, 500, and 1000 µg/mL) were obtained from the stock solutions using suitable dilution. The diluted working solutions of ME and fractions (HF, EF and MF) were prepared in methanol. Ascorbic acid was used as a standard in 15.63, 31.25, 62.50, 125.250, 500, and 1000 µg/mL solution. A 1 mL of each fraction solution (15.63, 31.25, 62.50, 125, 250, 500 and, 1000 µg/mL) was added to 1 mL of a DPPH solution (0.065 mM in methanol). After 30 minutes of reaction at 25ºC, the absorbances (A) of the solution were measured at a wavelength of 517 nm. The free radical scavenging (FRS) activity of ME and each fraction was determined by comparing its absorbance (A) with that of a blank solution. The ability to inhibit the DPPH radical was calculated using the following equation:

$$\%\ Inhibition = \frac{Ao - As}{Ao} \times 100$$

Where Ao is the absorbance of DPPH alone (control) and As is the absorbance of the sample. All the measurements were performed in triplicate.

Data analysis
The yields of the extraction and fractionation were expressed as percentage yields to the amount of powdered sample. The qualitative phytochemical results were denoted with subjective keys such as + (present) and – (absent) expressing the level of abundance of each phytochemical constituent tested. The Free radical scavenging (FRS) activity by DPPH assay was expressed as Mean ± SEM; significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ were compared to the standard (ascorbic acid). The IC_{50} and antiradical power (ARP) of the extract and fractions of *P. angolensis* were determined by $(1/IC_{50}) \times 100$.

Results and Discussion

Extraction of P. angolensis
The extraction of *P. angolensis* using methanol and the subsequent fractionation of the extract yielded 2.3, 0.002, 0.004 and 0.007% (w/w) of the crude extract, HF, EF and MF respectively relative to the weight of the powdered *P. angolensis* sample. The higher concentration of MF suggested that the methanol extract of *P. angolensis* is rich in polar phytochemicals such as phenols, flavonoids and tannins.

Phytochemical constituents of P. angolensis
The phytochemical analysis of ME and fractions (HF, EF and MF) showed that the plant is rich in different phytochemical compounds. Flavonoids, alkaloid saponin, tannin, steroids, terpenoids, and glycosides were present in ME (Table 1). The constituents were found to be widely distributed in the solvent fractions. Steroids and terpenoids were detected in HF, but they were absent in MF. The presence of flavonoids, alkaloid, saponins, tannins, and glycosides was detected in MF while saponins, terpenoids and phenolic compounds were not detected in EF. The medicinal value of plants including *P. angolensis* is based on these phytoconstituents which have definite pharmacological actions in the human body.[15] Phytochemicals such as flavonoids provide anti-inflammatory, anti-allergic effects, analgesic and antioxidant properties. Alkaloids are known to possess antimycotic activity and are used in the treatment of stomach aches. Saponins exhibit anti-carcinogenic, cholesterol-reducing and anti-inflammatory activities and tannins are anti-inflammatory and control gastritis and irritating bowel disorders. Tannins also contribute to antimicrobial power which heals wounds and stops bleeding. Terpenoids are associated with anti-cancer, antibacterial and other pharmaceutical functions.

Free radical scavenging (FRS) activity of P. angolensis
The DPPH scavenging ability (Table 2) of ME and fractions (HF, EF and MF) was found to be concentration-dependent. At 250 µg/mL, the highest percentage inhibition for HF and MF was obtained. The highest inhibition for EF and ME was elicited at concentrations of 31.25 and 125 µg/mL respectively. The DPPH free radical scavenging activity was evaluated by IC_{50} and the result is shown in Table 3. The highest radical scavenging activity was obtained in MF followed by EF. Importantly, the MF (ARP 91.7%) possesses higher antioxidant activity than the control (20.0%). The radical scavenging activity of the *P. angolensis* stem barks ME and fractions (HF, EF and MF) was tested using stable free radical DPPH (deep purple colour), as DPPH has the advantage of being unaffected by certain side reactions. The results showed that the DPPH radical scavenging activity of *P. angolensis* stem bark extract was IC_{50} = 9.441µg/mL compared to the ascorbic acid standard with the IC_{50} value of 4.989 µg/mL. The IC_{50} values of *P. angolensis* methanol extract fractions (IC_{50} for HF = 5.470 µg/mL), (IC_{50} for EF = 3.864µg/mL), (IC_{50} for MF = 1.09 µg/mL) showed that the IC_{50} values of MF and EF were less than the standard ascorbic acid (IC_{50} = 4.989µg/mL).

Table 1: Phytochemical composition of *P. angolensis*

Phytochemical Constituents	Methanol extract	HF	EF	MF
Alkaloids	+	-	+	+
Saponins	+	-	-	+
Flavonoids	+	-	+	+
Steroids	+	+	+	-
Terpenoids	+	+	-	-
Tannins	+	-	+	+
Glycosides	+	-	+	+
Phenolics	+	-	-	+

= absent, + = present

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Table 2: Antioxidant activity of *P. angolensis*

Conc. (ppm)	ME	HF	EF	MF	Ascorbic acid
15.63	91.67 ± 0.84	78.47 ± 10.43	89.49 ± 0.91	66.25 ± 1.31**	91.11 ± 0.65
31.25	95.00 ± 0.21	91.94 ± 2.98	92.03 ± 0.47	72.73 ± 1.37**	91.20 ± 0.54
62.50	96.99 ± 0.65	91.94 ± 2.23	90.51 ± 0.44	78.06 ± 0.63***	91.57 ± 0.86
125.00	97.08 ± 0.08***	93.29 ± 0.52	88.57 ± 0.33*	84.63 ± 0.93***	91.06 ± 0.12
250.00	91.48 ± 0.81	93.93 ± 0.12*	82.78 ± 0.92***	84.77 ± 0.28**	89.81 ± 2.02
500.00	83.84 ± 0.44**	90.93 ± 0.99	74.63 ± 2.39***	77.32 ± 2.03***	91.62 ± 0.32
1000.00	70.79 ± 1.67**	92.73 ± 0.33	62.70 ± 0.08***	62.04 ± 2.28***	90.37 ± 1.02

Data are expressed as Mean ± SEM; n = 5; *, **, and *** means Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively compared to the standard (ascorbic acid).

Table 3: Antioxidant parameters of *P. angolensis*

Sample	IC_{50} (ppm)	ARP
ME	9.441	10.59
HF	5.470	18.28
EF	3.864	25.88
MF	1.090	91.74
Ascorbic acid	4.989	20.00

The observed free radical scavenging effects can be attributed to the presence of the flavonoids and tannins in the plant's phytoconstituent.[17,18] Flavonoids and tannins being phenolic compounds accounts for the primary antioxidants or free radical scavengers since they are plant phenolic.[19,20] Thus the antioxidant activity recorded in the extract and fractions quenches the DPPH free radicals (by providing hydrogen atom (H) or by electron transfer, conceivably through a free radical attack on the DPPH molecule) and converts them to a colourless product (2, 2-diphenyl-1-picryl hydrazyl, or substituted analogous hydrazine) resulting in a decreasing absorbance at the 517 nm. Several mechanisms of antioxidant activity of polyphenolic compounds and their synergistic activity with other phytochemicals have been postulated. Flavonoids react with free radicals to form intramolecular hydrogen bonds. Polyphenols also produce synergistic antioxidant effects with carotenoids, polysaccharides, vitamins C and E and extracts of plant materials.[9,20,21] Other phytochemical constituents of *P. angolensis* identified in this study such as alkaloids, glycosides, saponins and tannins have been reported to possess antioxidant activity.[22-24]

Conclusion

Pycnanthus angolensis extract and fractions are rich in flavonoids, alkaloid saponin, tannin and glycosides. These phytochemicals act synergistically to attenuate oxidative stress through their antioxidant properties. The methanol fraction which has higher antiradical power compared with ascorbic acid is a potential source of antioxidant lead compounds. Further isolation of the antioxidant principles is currently ongoing.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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