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Phytochemical screening and antioxidant activity of *Pseudocedrela kotschyi* Schweinf Harms (Meliaceae) and *Strophanthus sarmentosus* DC (Apocynaceae)

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Abstract
Medicinal plants often have secondary metabolites that possess some antioxidant properties, which could provide protection for living organisms from damage caused by reactive oxygen species (ROS), with concomitant lipid peroxidation, and DNA strand breaking because of their redox properties. The aim of this work was the phytochemical screening and evaluation of antioxidant activity of *Pseudocedrela kotschyi* (PK) Schweinf Harms and *Strophanthus sarmentosus* (SS) DC. Plants were collected, identified and authenticated using standard procedures. The phytochemical screening of the medicinal plants was carried out using the standard official methods. The antioxidant activities were determined by DPPH free radical scavenging method as described by Brand-Williams and co-workers with a slight modification. The probit analysis graph pad prism 7 software was used for statistical analysis. The results showed that flavonoids, tannins and saponins are more abundant in PK-L (leaf) than PK-B (bark) whereas alkaloids and cardiac glycosides are more in SS-R (root) and SS-L. The IC_{50} of the extracts were 7.94, 14.96, 50.11 and 251.20 µg/ml for (PK-B > PK-L>SS-L>SS-R) respectively, thus indicating activity resides more in the bark and leaf of *Pseudocedrela kotschyi* than the *Strophanthus sarmentosus.* Furthermore, the IC_{50} of PK-B (7.94 µg/ml) has a higher activity compared to the standard, rutin (10.00 µg/ml). Thus, *Pseudocedrela kotschyi* is a potential source of active constituents that could be used in further drug development.

Keywords: Medicinal plants; Phytochemicals screening; Antioxidant; Free radical scavenging; Tarok people.

INTRODUCTION

Medicinal plants are the most ready sources of drugs for curing chronic/infections of human and animal diseases. The contribution of medicinal plants in the healthcare delivery system remains very relevant throughout the world and notably the tropics [1]. Medicinal plants also provide raw materials for the pharmaceutical industries for producing new potential medicine [2]. They form substantial part/sources of naturally occurring substances available for treatment of diseases. Statistics shows that more than 80% population of world depends on traditional medicine for their primary health care needs [3]. Indeed, about 25% of prescription drugs dispensed in the United States contain at least one active ingredient

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derived from a plant material. Some are made from plant extract; others are synthesized to mimic a natural compound [4].

Some degenerative diseases such as atherosclerosis, cancer, cirrhosis and diabetes have been reported to be linked to Reactive oxygen species (ROS) [5-8] and also in wound healing [9]. Naturally occurring plants are known to have some metabolites that possess some antioxidant properties i.e. tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins which could delay or provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage, and DNA strand breaking [9] because of their redox properties, which allow them to act as hydrogen donors, reducing agents, free radical scavengers. Antioxidants are believed to terminate or slow down the oxidation processes by scavenging free radicals. Another study also reported that the ingestion of natural antioxidants have been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing [10].

EXPERIMENTAL

Collection. *Pseudocedrela kotschyi* and *Strophanthus sarmentosus* were collected from Langtang North Local Government Area of Plateau State, Nigeria in October 2013 and were identified and authenticated at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria and the voucher specimen were deposited.

Extraction of plant material. About 100 g of the crude powdered leaves, root and bark of the plants were individually and or separately macerated in 500 mL of methanol for 18 h in a conical flask at room temperature and shaken with mechanical shaker. Each extract was filtered and concentrated on a heating water bath and kept in the refrigerator, to be used when needed.

Preliminary phytochemical screening. The methanol extract of the leaves, bark and the root were separately subjected to preliminary phytochemical screening using the standard techniques to determine the presence or absence of plant metabolites such as alkaloids, cardiac glycosides, tannins, anthraquinones, steroids, carbohydrates, flavonoids and saponins [11-14].

DPPH radical scavenging activity. The antioxidant activity (free radical scavenging activity) of the extract on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the methods described in Brand-Williams *et al*. [15], with a slight modification. The following concentrations of extract were prepared 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg/mL. All the solutions were prepared with methanol. 2 mL of each prepared concentration was mixed with 4 mL of 50 µM DPPH solution in methanol. Experiment was done in triplicates. The mixture was vortex for 10 s to homogenise the mixture and test tubes were incubated for 30min at room temperature in the dark, after 30min of incubation the absorbance was measured at 515 nm using UV-vis spectrophotometer (Shimadzu 1620 Japan). Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Gallic acid, ascorbic acid and rutin were used as standards with the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195 µM. Blank solution was prepared by mixing 2mL of methanol with 4mL of 50 µM DPPH solution in methanol. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The ability to scavenge the DPPH radical was calculated by using the following equation.

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL).

Data analysis. The probit analysis graph pad prism 7 software was used to analyse the data.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening are shown in Table 1. The results of antioxidant evaluation using the test for free radical scavenging activity are as shown in Tables 2-4.

The preliminary phytochemical screening of the different plants parts in Table 1 revealed the presence of abundant flavonoids, tannins, and saponins in the leaf and bark of *Pseudocedrela kotschyi* Schweinf Harms and in the leaves of *Strophanthus sarmentosus* DC. Alkaloids and cardiac glycosides are also in abundance in both the root and leaf of *Strophanthus sarmentosus* while alkaloids, steroids, are less in *Pseudocedrela kotschyi*. Anthraquinone is absent in both the different parts of the plants. Cardiac glycosides are however absent in *Pseudocedrela kotschyi*. The presence of flavonoids, tannins and other secondary metabolites like steroids and saponins in PK-L and PK-B could suggest its use as anti-inflammatory and anticancer by the Tarok speaking people of Plateau state Nigeria.

Table 1: Summary of preliminary phytochemical screening of methanol extract

Constituents	PK-L	PK-B	SS-R	SS-L
Alkaloids	+	+	+	+
Cardiac glycosides	-	-	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Anthraquinones	-	-	-	-

N.B. + Presence - Absence

PK- *Pseudocedrela kotschyi* (L- Leaf; B - Bark); SS- *Strophanthus sarmentosus* (R – Root, L – Leaf)

Table 2: Percentage inhibition of the samples in DPPH assay

Concentration		Log of	% Inhibition			
(µg/ml)	(g/mL)	conc(g/mL)	SS L	PKB	SS R	PK L
500	0.0005	-3.30	87.9	90.3	68.2	96.3
250	0.00025	-3.60	86.8	89.6	47.3	96.2
125	0.000125	-3.90	77.0	88.5	38.8	96.2
62.5	6.25E-05	-4.20	52.6	87.9	36.8	95.3
31.25	3.13E-05	-4.51	46.5	85.6	36.4	92.8
15.62	1.56E-05	-4.81	39.5	64.9	35.0	73.1
7.8125	7.81E-06	-5.11	34.1	50.6	34.7	55.7
3.91	3.91E-06	-5.41	32.6	45.0	33.6	47.2
1.95	1.95E-06	-5.71	29.8	40.8	33.6	42.2
0.98	9.8E-07	-6.01	28.9	37.4	33.1	38.2

Table 3: Percentage inhibition of the standards in DPPH assay

Concentration		Log of	% Inhibition		
µM	M	conc (M)	Rutin	Gallic acid	Vit. C
100	0.0001	-4.00	89.27	92.12	75.2
50	0.00005	-4.30	82.20	91.93	81
25	0.000025	-4.60	60.41	92.49	83.6
12.5	1.25E-05	-4.90	52.43	92.02	85.5
6.25	6.25E-06	-5.20	46.55	71.58	86.1
3.125	3.13E-06	-5.51	45.63	53.97	86.6
1.5625	1.56E-06	-5.81	43.47	48.39	86.7
0.7812	7.81E-07	-6.11	44.12	43.67	73.4
0.391	3.91E-07	-6.41	44.50	40.56	50.7
0.195	1.95E-07	-6.71	45.04	39.57	42

Key: E= Exponential

Table 4: IC_{50} of the samples and the standards.

Sample/extract	IC_{50} (µg/ml)
SS-L	50.11
SS-R	251.20
PK-L	14.96
PK-B	7.94
Ascorbic acid	0.398
Rutin	10.00
Gallic acid	1.78

Antioxidant (DPPH radical scavenging) activity. The extracts and the standards, rutin, gallic acid and ascorbic acid (vitamin C) exhibited concentration dependent inhibition of oxidation. At a concentration of 0.98µg/mL, the extracts, PK-B, PK-L, SS-L and SS-R produced a percentage inhibition of 37.4, 38.2, 28.9 and 33.1 respectively, which gradually increased with corresponding increase in concentrations up to 500 µg/mL, which produced 90.3, 96.3, 87.9 and 68.2 % inhibition (Table 2). At the lowest concentration of 0.195 µM, rutin, gallic acid and vitamin c produced percentage inhibitions of 45.04, 39.57 and 42.0 respectively. All these also increased remarkably with increase in the concentrations of up to 100 µM, which produced 89.27, 92.12 and 75.20 % respectively (Table 3).

The antioxidant activity (table 2) carried out in this study for the samples showed good activity, which further confirmed report of its use in the traditional management of inflammation and cancer by the Tarok speaking people of Plateau state, Nigeria [16]. Some studies have also reported that the leaves and stem bark of the plant, *Pseudocedrela kotschyi* possess analgesic and anti-inflammatory activities [17,18]. The plant, *Pseudocedrela kotschyi* (PK) has a better antioxidant activity than *Strophanthus sarmentosus* (SS) with higher activity in the stem bark. In Table 3, the antioxidant activity of the standards presented as percentage inhibition revealed ascorbic acid as having the best activity than gallic acid and rutin.

The results in Table 4 gives the summary of the minimum inhibitory concentration (IC_{50}) produced by the samples and standards with better estimate antioxidant activity. The lower the IC_{50}, the higher the activity that is to say decrease in IC_{50} is equivalent to increase activity. IC_{50} is defined as the concentration sufficient to obtain 50%

of a maximum effect estimate in 100%. SD = Standard deviation [19]. Generally, for an IC_{50} value less than 56, it is considered good. For the standards, ascorbic acid (vitamin c) is the most active antioxidant followed by gallic acid and rutin whereas for the samples/extracts, the *Pseudocedrela kotschyi* bark is the most active followed by its leaf, (PK-B and PK-L) as compared to the leaf and root of *Strophanthus sarmentosus* coded as SS-L and SS-R. Furthermore, the IC_{50} of PK-B (7.94 µg/ml) was observed to have a better activity than the standard, rutin (10.00 µg/ml). The antioxidant activity of PK-B and PK-L could be attributed to the presence of the bioactive constituents, flavonoids, tannins, and saponins. The antioxidant activity of the extract decreases in the order; Ascorbic acid >Gallic acid > PK-B > Rutin > PK-L > SS-L > SSF-R.

It can be inferred that the free radical scavenging activity shown by the extracts may be due to the presence of the polar poly-phenolic compounds (tannins and flavonoids) which are good scavengers of free radicals [20]. The free radical scavenging activity of the extract is indirect proportion with their anti-oxidant activity. This study is also in agreement with other studies that have also shown that many of these antioxidant compounds possess anti-inflammatory, antitumor, anticarcinogenic, antimutagenic, antiatherosclerotic, antiviral and antibacterial activities [21, 22].

In conclusion, the results of the phytochemical and antioxidant of the two plants showed that both the leaves and the stem bark of PK-B and PK-L justified the widespread usage of the plant by the Tarok people traditionally. It could therefore be concluded that the PK leaf and bark is a potential source of active constituents that could be used in further drug development.

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