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Full Length Research Paper

Phytochemical and antioxidant screening of some plants of apocynaceae from South West Nigeria

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The phytochemical and antioxidant properties of *Strophantus hispidus* (stem and root), *Voacanga africana* (stem and leaf) and *Thevetia neriifolia* (stem and leaf) were investigated. The parts of the plants used were based on those used locally. All the plants tested positive for the presence of tannins, flavonoids and cardiac glycosides, all except *S. hispidus* (stem) tested positive for the presence of anthraquinone. Only *V. africana* (stem and leaves) and *Thevetia neriifolia* (leaves) tested positive for the presence of saponins, all except *S. hispidus* (root) tested positive for the presence of terpenoids. Only *V. africana* leaves and *T. neriifolia* tested positive for the presence of phlobatannins. The free radical scavenging activities were investigated based on the presence of flavonoids and tannins in all the plant materials. Preliminary screening of the free radical scavenging activity of the methanolic extracts of the plants with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) using thin layer chromatography only tested positive for *V. africana* leaves. IC_{50} values (concentration of sample required for 50% inhibition of DPPH radical scavenging activity) for the inhibition of DPPH were 0.048 and 0.054 mg/ml for *V. africana* and Vitamin C respectively. The phenolic content, total flavonoid content and proanthocyanidin contents of *V. africana* were determined as, 124, 30 and 90 mg as gallic acid, rutin and catechin equivalents per gram of extract respectively. A good correlation was observed between radical scavenging capacity of *V. africana* and total phenolic, total flavonoid and proanthocyanidin content (R^2 = 0.96 in all cases), showing that flavonoids are likely to be responsible for antioxidant activity.

Keywords: Antioxidants, free radicals, phenolic content, proanthocyanidin content, total flavonoid, DPPH, *Strophantus hispidus*, *Voacanga africana*, *Thevetia neriifolia*.

INTRODUCTION

Phytochemicals are compounds found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. In fact, some people claim that many of the diseases afflicting human beings are the result of lack of phytonutrients in their diet. Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, anti-diabetic and antihypertensive effects to mention but a few. The phytochemical constituent of a plant will often determine the physiological action on the human body (Pamplona-Roger, 1998).

Antioxidants protect cells against damage caused by molecules known as free radicals. The antioxidant effects in plants are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Polterait, 1997). Oxidative damage is implicated in most disease processes such as cardiovascular disease, cancer, inflammatory conditions, asthma, liver disease and macular degeneration (Willcox et al., 2004). Epidemiological, clinical and laboratory research on flavonoids and other antioxidants suggest their use in the prevention and treatment of a number of these disorders. *Strophanthus hispidus*, *Voacanga africana* and *Thevetia neriifolia* are plants from the Apocynaceae family. *S. hispidus* is a climbing shrub, reaching 16m long, of the open savanna woodland and occurs widely throughout West African countries. *V. africana* is a tree, that reaches up to 11 m high, with low branching and

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Table 1. Traditional uses of *S. hispidus*, *V. africana* and *T. neriifoli*a.

Name	Local Name	Common name	Parts used	Medicinal use(s) against
Stropanthus hispidus	Sagere, isagere, isagira	Stropanthus, arrow poison plant	Root, stem bark	Arthritis, stroke, heart failure, rheumatism.
Voacanga africana	Ako dodo, sinrin-pin.	Papaku, Voacanga africana	Latex, stem and root bark.	Fever, toothache, cardiactonic, sores, carious tooth, hyper-tension, improves mental alertness.
Thevetia neriifolia	Olomiojo	Bush milk, yellow oleander, still tree	Bark, kernel, leaves	Cardiac disorders, fever, ringworms, wasp stings, measles.

found in understory forest, and Savanna woodland through-out the region from Senegal to West Cameroon and Fermando Po and across Africa to Egypt. *T. neriifolia* is a shrub that grows up to a height of 6 m, native of Brazil and occurs widely in West African region as garden cultivates. The uses of each plant is summarized below in Table 1 (Burkill, 1984; Odugbemi, 2006).

Traditionally, *S. hispidus* and *V. africana* and *T. neriifolia* are used to treat various inflammatory conditions and cardiovascular diseases. *T. neriifolia* also appears to have both antiviral and antifungal properties (Table 1).

The study aims to investigate whether the plants possess antioxidant properties that may be responsible for some of their traditional uses as oxidative damage is implicated in some of the diseases treated by the plants. The presence of phenolic compounds such as flavonoids, tannins and terpenoids in the plant materials were investtigated, followed by rapid screening for free radical scavenging activity. Correlation between the free radical scavenging activity, the total phenolic, total flavonoid and proanthocyanidin contents were also investigated in order to establish if there is a relationship between these group of phytochemicals and free radical scavenging activity.

MATERIALS AND METHOD

Collection of plants

S. hispidus (stem and root), *V. africana* (stem and leaf) and *T. neriifolia* (stem and leaf) were collected in the morning from uncultivated farmlands located in the Southwestern part of Nigeria. Plants were collected in the month of June. All the three plants were identified by Dr S. A. Adesegun of the Department of Pharmacognosy, University of Lagos.

Extraction and phytochemical screening of plants

The plant materials were oven dried at 38˚C and milled into uniform dry powder. Extraction was carried out by soaking 150 g of dried powdered samples in about 600 ml of methanol (analar grade) for 3 days. The extracts were filtered first through cotton wool, then through Whatman filter paper No 42 (125 mm). The collected extract was dried using a rotary evaporator.

Phytochemical screening was performed using standard procedures as described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973).

Determination of antioxidant activity

Rapid-TLC screening for antioxidant activity was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. The plates were developed in methanol:ethylacetate (2:1) after which it was air-dried and sprayed with 0.2%w/v DPPH spray in methanol. The plates were visualized for the presence of yellowish spots.

The radical scavenging activity of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) was determined by measuring UV absorbance at 517 nm. Radical scavenging activity was measured by a slightly modified method of Brand-Williams et al. (1995). The following concentrations of extract were prepared that is, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml. Vitamin C was used as standard and the same concentrations of it were prepared as the test solutions. All the solutions were prepared with methanol (Sigma-Aldrich, Analar grade). One ml of each prepared concentrations were placed into test tubes and 0.5 ml of 1 mM DPPH solution in methanol was added. The experiments were carried out in duplicates. The test tubes were incubated for 15 min and the absorbance was read at 517 nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

% inhibition = {(AB – AA)/AB} x 100.

Where AB is the absorption of blank sample and AA is the absorption of tested extract solution.

Determination of total phenolic content

Total phenolic content was determined according to the Folin and Ciocalteau's method (1927). Gallic acid was used as a standard. Concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentrations of 0.1 and 1 mg/ml of plant extracts were also prepared in methanol and 0.5 ml of each sample was mixed with 2.5 ml of a ten-fold diluted Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature before the absorbance was read at 760 nm spectrophotometrically. All determinations were performed in triplicates. The total phenolic content was expressed as gallic acid equivalent (GAE).

Table 2. Phytochemical constituents of *S. hispidus, V. africana* and *T. neriifolia.*

Phytochemical Tests	*S. hispidus* (stem)	*S. hispidus* (root)	*V. africana* (stem)	*V. africana* (leaves)	*T. neriifolia* (stem)	*T. neriifolia* (leaves)
Saponins	-	-	+	+	-	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Terpenes	+	-	+	+	+	+
Phlobatannins	-	-	-	+	-	+
Anthraquinone	-	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+

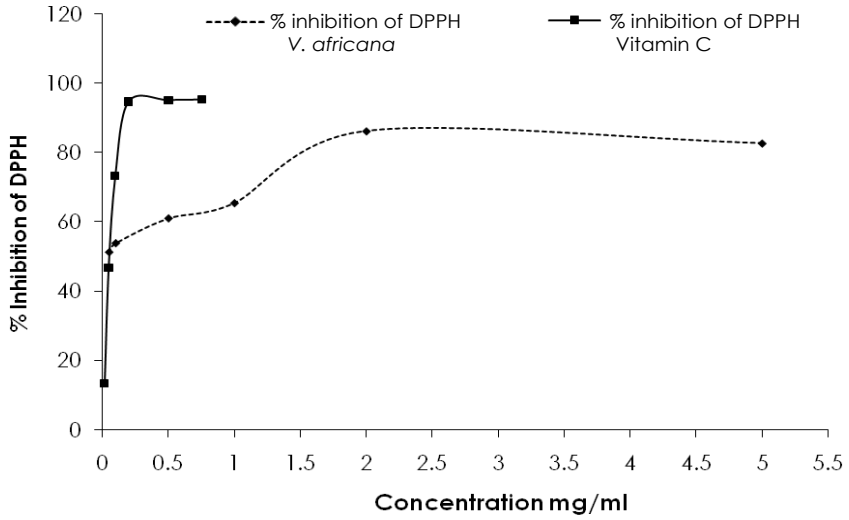

Figure 1. Graph of % Inhibition of DPPH against concentration of *V. africana* leaf extract and Vitamin C.

Determination of total flavonoid content

Total flavonoid content was determined using a method of Miliauskas et al. (2004). To 2 ml sample was added 2 ml of 2% $AlCl_3$ in ethanol. The UV absorption was measured at 420 nm after 1 h at room temperature. Concentrations of 0.1 mg/ml and 1 mg/ml of the extract in methanol, were used while rutin concentrations of 0.01, 0.02, 0.04, 0.08 and 0.10 mg/ml were used to obtain the calibration curve. Solutions were prepared in methanol. Total flavonoid content was calculated as rutin equivalent (RE) from the concentration of rutin equivalent obtained from the calibration curve.

Proanthocyanidin content

Proanthocyanidin content was determined as previously reported by Ayoola et al, 2006. Briefly, concentrations of 0.1 and 1 mg/ml of the sample extract were prepared and 0.025, 0.05, 0.1, 0.2 and 0.4 mg/ml of catechin were prepared as the standard solutions for the calibration curve. Solutions were prepared in methanol. 0.5 ml of HCl was added to each test tube and the solutions were allowed to stand for 15 min. The absorbance was measured at 500 nm. Proanthocyanidin content was measured as catechin equivalent (CE) from the concentration of catechin obtained from the calibration curve. All chemicals and reagents were obtained from Sigma-Aldrich, UK.

RESULTS

Phytochemical screening of all the plants tested revealed the presence of tannins, flavonoids and cardiac glycosides (Table 2). All except *S. hispidus* (stem) tested positive for the presence of anthraquinone and only *V. africana* (stem and leaves) and *Thevetia neriifolia* (leaves) tested positive for the presence of saponins. All except *S. hispidus* (root) tested positive for the presence of terpenoids. Only *V. africana* (leaves) and *T. neriifolia* (leaves) tested positive for the presence of phlobatannins.

Rapid TLC screening for antioxidant activity was negative for all the extracts except for the methanol extract of *V. africana* leaves where the colour of the DPPH spray changed from violet to yellowish spots. IC_{50} for DPPH inhibition was 0.048 and 0.054 mg/ml for *V. africana* and Vitamin C respectively (Figure 1).

Total phenolic content obtained for *V. africana* leaves was obtained from the regression equation of the calibration curve of gallic acid ($y = 3.5695x + 0.0062$, $R^2 = 1.0$), and expressed as gallic acid equivalent (GAE). Total phenolic content was recorded as 124 mg/g of plant

extract. A correlation of R^2 = 0.96 was obtained between the data for phenolic content and DPPH inhibition. Total flavonoid content was obtained from the regression equation of the calibration curve of rutin (y = 0.066x + 0.0324, R^2 = 0.94), and expressed as rutin equivalent (RE). Total flavonoid content was recorded as 90mg/g of plant extract. A correlation of R^2 = 0.96 was obtained between the data for total phenolic content and DPPH inhibition. Proanthocyanidin content was determined from the regression equation of the calibration curve of catechin (y = 0.066x + 0.0324, R^2 = 0.94) and expressed as catechin equi-valent (CE). Proanthocyanidin content was recorded as 90 mg/g of plant extract. A correlation of R^2 = 0.96 was obtained between the data for proanthocyanidin content and DPPH inhibition.

DISCUSSION

Phytochemical screening of the plant materials revealed some differences in the phytochemical constituents of the plants tested. *V. africana* leaves and *T. neriifolia* leaves tested positive for all the phytochemicals tested in this study (Table 2). All the plant materials tested positive for tannins and flavonoids while only *S. hispidus* root tested negative for terpenoids.

Suprisingly, only *V. africana* leaves tested positive to DPPH inhibition despite the fact that all the plant extracts tested positive for the presence of tannins and flavonoids. The DPPH test shows the ability of the test compound to act as a free radical scavenger. DPPH is a free radical and it gives a strong absorption band at 517nm in the visible region of the electromagnetic radiation. It has a deep violet colour. This absorption diminishes as the electron is paired off resulting in decolourization with respect to the number of electrons taken up and the colour changes to a pale yellow. The result suggests that the flavonoid and tannin contents in the other plant materials as well as in *V. africana* stem were very low, such that their radical scavenging activities were not significant. The leaf extract of *V. africana* exhibited a good potential to act as a free radical scavenger. Moreover the IC_{50} for DPPH inhibition for *V. africana* (0.048 mg/ml) was comparable to that of Vitamin C (0.054 mg/ml) which is a known free radical scavenger. In fact, it appeared to be slightly better than Vitamin C at 50% inhibition. However a maximum inhibition was achieved at a higher concentration of 2 mg/ml (86.3%) for *V. africana* leaves compared to 0.75 mg/ml (95.6%) for Vitamin C. Hence a higher concentration of *V. africana* leaves will be required to achieve maximal inhibition of DPPH compared to Vitamin C.

Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers (Kahkonen et al., 1999). Therefore, it was reasonable to determine the total phenolic content in the plant extract. The total phenolic content in *V. africana* leaves was recorded as 124 mg/g of plant extract. A correlation of 0.96 was obtained between the data for phenolic content and the DPPH assay. This shows that plant phenolics are likely to play a part in the free radical scavenging activity of *V. africana* leaf extract.

Flavonoids are a ubiquitous group of polyphenolic substances which are present in most plants. Therefore it was also reasonable to determine the total flavonoids content in *V. africana* leaf extract. The total flavonoids content was obtained as 30 mg/g of plant extract. There was also a good correlation between the total flavonoids content and the DPPH assay (R^2 = 0.96), indicating that the flavonoids were contributory to the free scavenger activity of the plant extract. Proanthocyanidins are a type of bioflavonoid that have been shown to have very potent antioxidant activity. Proanthocyanidin content of the methanolic extract of *V. africana* leaf was obtained as 90 mg/g of extract. There was also a good correlation between the proanthocyanidin content and the DPPH assay (R^2 = 0.96). This indicates that proanthocyanidins present in the extract were likely to be involved in the free radical scavenging activity of the *V. africana* leaf extract.

Flavonoids have been shown to have antibacterial, anti-inflamatory, antiallergic, antineoplastic, antiviral, antithrombotic and vasodilatory activities (Miller, 1996). 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 *V. africana* leaf extract can be exploited in the treatment of the various disease conditions mentioned above. Traditional uses for fever and toothache suggest possible anti-inflammatory properties, use as cardiotonic, hypertension and improved mental alertness suggests possible antithrombotic and vasodilatory properties. These are in accordance with some of the properties of flavonoids.

Conclusion

The methanolic extracts of *S. hispidus* (stem and root), *Thevetia neriifolia* (stem and leaves) and *V. africana* (stem) did not exhibit free radical scavenging activity when screened with DPPH. Hence, it can be concluded that their traditional uses are not likely to be due to intrinsic free radical scavenging activities. Only the methanolic extract of *V. africana* leaves showed free radical scavenging activity. The antioxidant potential of *V. africana* leaves was comparable to that of Vitamin C (IC_{50} values of 0.048 and 0.054 mg/ml respectively). There was a good correlation between % inhibition of DPPH by the plant extract and the total phenolic content, total flavonoid and proanthocyanidin contents. The results show that there are flavonoids present in the leaf extract of *V. africana* with potent antioxidant activities. These bio-active constituents can be exploited as possible therapeutic agents in the treatment and prevention of various diseases such as cardiovascular, cancer, asthma, inflam-

matory conditions and macular degeneration.

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