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Full Length Research Paper

Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*

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In this present study, a comparative evaluation of the antioxidant capacities, phenol and polyphenol composition, membrane stabilization, and cytotoxicity to brine shrimps (*Arthemia salina*) of the leaf and stem extracts of *Cissus multistriata* were carried out. 2,2- Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging effect was observed in the stem extract with IC_{50} of 29.25 µg/ml. The potency of radical scavenging effect of the stem extract was close to the synthetic antioxidant quercetin with IC_{50} of 21.05 µg/ml. The antioxidant activities of each extract increased with increasing concentration of extracts. The phenol and polyphenol content of the stem varied between 0.564 ± 0.000 to 6.200 ± 0.557% and that of the leaf content is lower (0.523 ± 0.000 to 3.400 ± 0.355%). The higher amount of phenolic compound in the stem could be contributory to the greater radical scavenging effects observed. The methanolic extracts exhibited minimum and maximum percentage membrane stability of 51.364 ± 11.241 and 56.098 ± 14.654% on human erythrocyte. The highest membrane stabilizing activity was observed in the stem extract with percentage stability of 56.098 ± 14.654%. The activity of the extracts was comparable to the standard anti-inflammatory drug (Indomethacin) used. The extracts are less toxic to the cell (*Arthenia salina*) with their LC/EC_{50} higher than that of standard potassium dichromate used. The results obtained in this present study indicate that *C. multistriata* can be a potential source of natural antioxidant and is relatively safe.

Key words: *Cissus Multistriata*, antioxidant, radical scavenger, polyphenols, cytotoxicity, *Arthemia salina* membrance stabilization.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies (Kumar et al., 2005). The World Health Organization (WHO, 1980) has recommended that this should be encouraged especially in places where access to conventional treatment is not adequate. Studies have shown that many plants have chemical components and biological activities that produce definite physiological actions in the body and, therefore, could be used to treat various ailments. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic com-pounds (Hill, 1952; Edeoga et al., 2005).

Flavonoids, a group of polyphenolic compounds with known properties, such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and anti-inflammatory action (Frankel 1995; Pourmorad et al., 2006) have been isolated from plants.

Several investigations have shown that many of these plants have antioxidant activities that could be therapeutically beneficial and it has been mentioned that the

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anti-oxidant potential of plants might be due to their phenolic components (Cook and Samman, 1996).

It is well known that oxidative stress induced by oxygen-free radicals and resultant tissue damage is the hallmarks of several chronic disorders and cell death (Mates et al., 2002). The therapeutic potentials of medicinal plants as natural antioxidants in reducing such free radical induced tissue damage (Pourmorad et al., 2006) and in the maintenance of health and protection from some age-related degenerative disorders such as cancer and coronary heart diseases (Hill, 1952) have been reported. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plant species and the search for novel antioxidants (Oke and Hamburger, 2002). Again, the effect of anti-inflammatory drugs including herbal preparation on the stabilization of erythrocyte membrane exposed to hypotonic and heat has been studied extensively (Oyedapo et al., 1997). The erythrocyte membrane resemble lysosomal membrane as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Kumar and Sadique, 1987; Oyedapo et al., 2004; Oyedapo, 1996; Oyedapo et al., 1997).

Cissus multistriata is a well known plant to the traditional medicine practitioners in Nigeria. It is used in the treatment of infertility, stomach ailments in children, malnutrition diseases such as Kwashiokor and marasmus. It is also used as cough remedy, for fracture healing, arthritis, etc. Since reactive oxygen species (ROS) have been implicated in some of these disorders and since polyphenolic compounds are able to booster biological resistance against ROS, the antioxidant capacity of *C. multistriata* need to be investigated. This forms the basis of this study which is designed to evaluate the antioxidant capacity and membrane stabilizing activity of the methanolic extract of *C. multistriata* on human erythrocytes exposed to heat and hypotonic induced lyses to ascertain whether the extract is suitable for stabilizing and maintaining the integrity of membrane under stressful conditions. In addition, the biosafety of this extract will be investigated to ascertain the level of its cytotoxicity using *Arthemia salina*

MATERIALS AND METHODS

Chemicals

DPPH (2,2-diphenly-1- picrylhydrazyl) and quercetin were purchased from Sigma Chemical Company (Sigma Germany). Vitamin C used was a product of Glaxo Smithklein. Methanol, Folin ciocalteu reagent, potassium dichromate, ferric chloride and amylalcohol were products of BDH. The Tannic acid used was M&B product.

Plant materials

The leaves and stems of *Cissus multistriata* were collected from Kogi State University Staff quarters, Anyigba. The plant material was washed with water to remove dirt and was air-dried in the laboratory for two weeks. The dried plant material was pulverized using mortar and pestle. Weighed portion of each plant part powder was then extracted.

Table 1. Percentage yield of crude extracts.

Plant sample part	Extract yield (%)
C. *multistriata* stem	8.2
C. *multistriata* leaf	6.65

Preparation of plant extracts

Cold extraction method was used for the extraction. 20 g of the powdered sample were weighed into a conical flask. 150 ml of pure methanol was added and left for 72 h. The mixture was filtered and the filtrate was concentrated using a rotatory evaporator, and the percentage yield of the extract was calculated.

Total phenols and poly phenols determination

The total phenols composition was determined using the Folin-ciocalteu reagent as described by McDonald et al. (2001). The method of Harbone (1973) was employed in the determination of the total flavonoid content. The colorimetric method of van-Burden and Robinson (1981) was used in the determination of tannin composition.

DPPH free radical scavenging activity determination

The free radical scavenging activities of the plant extracts were determined using the modified method of Blois (1985). 1 ml of different concentrations (500, 250, 125, 62.5, 31.25 µg/ml) of extracts or standard (vitamin C and quercetin) in a test tube was added 1 ml of 0.3 mM DPPH in methanol making the final concentrations of 250, 125, 62.5, 31.25 and 15.62 µg/ml. The mixture was vortexed and then incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. Percentage scavenging activity was calculated using the expression:

% Scavenging activity = [(Absorbance of control – Absorbance of sample) / Absorbance of control] x 100

IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals, and this was computed using Jandel Scientific Sigma Plot for windows version 1.2

Rapid radical scavenging screening

The method of Mensor et al. (2001) as modified by Burits and Bucar (2000) and Adebajo et al. (2007) was followed in screening for the antioxidant property of the extracts. With the aid of capillary tube, stock solutions (1 mg/ml) of extracts were spotted on silica gel thin layer chromatographic (TLC) plate and developed with a solvent system of ethanol : methanol (90:10). After development, the chromatograms were dried and sprayed with a 0.3 mM solution of the stable radical DPPH. Purple spot formed against people background were taken as positive results. The duration for the development of yellow colour indicated whether the antioxidant activity was strong or not.

Table 2. Percentage phenol and polyphenol contents in the studied plant part.

Plant parts	Phenol (%)	Tannin (%)	Flavonoid (%)
C. *multistriata* stem	0.564 ± 0.000	0.256 ± 0.000	6.2000 ± 0.557
C. *multistriata* leaf	0.523 ± 0.000	0.384 ± 0.013	3.400 ± 0.355

Each value in the table was obtained by calculating the average of three experiments ± standard error of mean (SEM).

Table 3. DPPH radical scavenging activity of *C. multistriata* crude methanol extracts.

Sample	Concentration (µg/ml)	Log concentration	Scavenging (%)	IC_{50} (µg/ml)
Crude methanol stem extract	250	2.3979	91.2	29.25 [a]
	125	2.0969	76.98	
	62.50	1.7959	67.9	
	31.25	1.4949	59.9	
	15.62	1.1937	30.2	
Crude methanol leaf extract	250	2.3979	86.1	45.67 [b]
	125	2.0969	78.6	
	62.50	1.7959	62.1	
	31.25	1.4949	38.3	
	15.62	1.1937	22.7	
Quercetin	250	2.3979	93.8	21.05 [c]
	125	2.0969	75.9	
	62.50	1.7959	65.8	
	31.25	1.4949	54.3	
	15.62	1.1937	48.4	
Vitamin C	250	2.3979	68.6	17.53 [d]
	125	2.0969	62.8	
	62.50	1.7959	59.5	
	31.25	1.4949	54.3	
	15.62	1.1937	48.8	

[a] Linear equation: y = 46.198x – 17.729
[b] Linear equation: y = 55.505x – 42.118
[c] Linear equation: y = 37.335x + 0.5927
[d] Linear equation: y = 15.95x + 30.163

Membrane stabilizing activity assay

The method of Sadique et al. (1989) as modified by Oyedapo and Famurewa (1995) and Oyedapo et al. (2004) was employed in the membrane stabilizing activity assay. The assay mixture consisted hyposaline (2 ML), 1 ml of 0.15 M sodium phosphate butter at pH 7.4. Varying volumes of drugs (2 mg/ml) (0.0 - 1.0 ml) and 2% (v/v) erythrocyte suspension in isosaline (0.5 ml) were made up with isosaline to give a total assay volume of 4.5 ml. The control was prepared as above except the drug was omitted, while drug control (4.5 ml) lacked erythrocyte suspension. The reaction mixtures were incubated at 56ºC for 30 min. The tube was cooled under running water followed by centrifugation at 5,000rpm. The supernatant were collected followed by reading of the absorbance of the released hemoglobin at 560 nm. The percentage membrane stability was estimated using the expression:

Membrane stability = 100 – [100 x (Drug test value – Drug control value)/ Control value]

Cytotoxicity to brine-shrimps

Modified method of Solis et al. (1992) and Potduang et al. (2007) was used to determine the inhibitory activity on *A. salina*. 50 µl of different concentration of crude methanolic extracts (1000, 500, 250, 125 µg/ml) and control (methanol) was added into graduated vial bottles containing 10 newly hatched brine-shrimps in 5 ml of artificial sea water, and then incubated at room temperature for 24 h. All samples were repeated in 2 wells to make the overall tested organisms of 20 for each. The living brine shrimps were counted under a hand magnifying lense. Same procedure was followed using potassium dichromate as the reference standard and the data were analyzed based on U.S E.P.A probit analysis programme version 1.5 (Finney, 1971) to determine the LC50 at 95% confidence limit.

RESULTS AND DISCUSSION

The crude methanol extract of the stem and leaf of *C. multistriata* yielded 8.2 and 6.65%, respectively (Table 1). The quantitative estimation of the phytochemical constituents of the leaves and stems of *C. multistriata* shows that the plant is rich in flavonoids, tannins, and phenols to some extent (Table 2). Phenolic compounds have been

Table 4. Radical Scavenging activities of the methonolic extracts from the stem and leaf of *C. multistriata* using rapid DPPH TLC screening.

Plant part	Reaction speed	Intensity of spots
C. *multistriata* stem extract	Fast	+ + +
C. *multistriata* extract leaf	Fast	+ + +

+ + + = Strong intensity (immediate reaction).

Table 5. Membrane-stabilizing activity of crude methanolic extracts of plant on human RBC subjected to heat and hypotonic stress.

Plant part/standard	Sabilization (%; mean ± S.E)	Max. Inhibition
C. *multistriata* stem extract	56.098 ± 14.654	90.76
C. *multistriata* extract leaf	51.364 ± 11.241	85.23
Indomethacin	59.700 ± 5.127	69.60

Table 6. Inhibitory effect on brine-shrimp of *C. multistriata* crude methanol extracts.

Sample	Concentration (µg/ml)	Log concentration	Lethality (%)	LC_{50} (µg/ml)
Crude methanol stem extract	1,000	3.0000	65.9	666.98 [a]
	500	2.6990	35	
	250	2.3979	25	
	125	2.0969	20	
Crude methanol leaf extract	1,000	3.0000	70	512.53 [b]
	500	2.6990	40	
	250	2.3979	35	
	125	2.0969	25	
Potassium dichromate	1,000	3.0000	100	44.20 [c]
	500	2.6990	80	
	250	2.3979	70	
	125	2.0969	70	

[a] Linear equation: y = 49.065 x – 88.565
[b] Linear equation: y = 46.507 x – 76.021
[c] Linear equation: y = 33.219 x – 4.6578

recognized as antioxidant agents, which act as free radical terminators (Shahidi and Wanasundara, 1992) and have been known to show medicinal activity as well as exhibiting physiological functions (Sofowora, 1993). It has been reported that compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants (Das and Pereira, 1990; Younes, 1981). The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003; Cook and Samman, 1996). The presence of these phytochemicals in *C. multistriata* stem and leaf is a significant finding in this present study.

The scavenging activity of *C. multistriata* leaf and stem and standards as determined in this study are presented in Table 3. *C. multistriata* stem extract gave the highest radical scavenging activity with $1C_{50}$ value of 29.25 µg/ml and is comparable with standard quercetin (IC_{50} = 21.05 µg/ml), while the radical scavenging activity of the leaf extract gave 45.67 µg/ml. The scavenging activity of this plant extracts may be related to the presence of phenol and polyphenolic compounds (Table 2). The result of the rapid radical scavenging screening of the plant parts confirmed their high radical scavenging activity (Table 4). DPPH stable free radical method is an easy, rapid and sensitive way to evaluate the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002).

The membrane stabilizing activities of the extracts are shown on Table 5. The results showed that the extracts are highly potent on human erythrocyte adequately protecting it against heat and hypotonic induced lyses. The activity was comparable to that of standard anti-inflammatory drug (Indomethacin). It has been reported that flavonoids exert profound stabilizing effects on lysosomes both *in vitro* and *in vivo* in experimental ani-

mals (Van-Cangeghem, 1972; Sadique et al., 1989; Middleton, 1996) while tannin and saponins have the ability to bind cations and other biomolecules, and are able to stabilize the erythrocyte membrane (Oyedapo, 2001; El-Shanbrany et al., 1997). The high membrane stabilizing activity of the stem extract of C. multistriata observed in this investigation may be due to its high flavonoids and tannin content. Earlier investigations have revealed that various herbal preparations are capable of stabilizing the red blood cell membrane and exert anti-inflammatory activity (Sadique et al., 1989; Olugbenga et al., 2005).

The inhibitory effects on brime-shrimp of *C. multistrata* extracts are shown on Table 6. The extract possessed very low cytotoxicity to brine shrimp with LC50 of 666.98 µg/ml for the stem extract and 512.53 µg/ml for the leaf extract. The mild brine-shrimp inhibition indicating low cytotoxicity of the plant could be correlated with its tradition uses of this for the management of diverse ailments.

The antioxidant capacity, phenol and polyphenol composition, membrane stabilization and cytotoxicity to brine shrimps of leaf and stem extracts of *C. multistriata* have been demonstrated in this study. The results obtained indicate that *C. multistriata* is a potential source of natural antioxidant and is relatively safe for the medicinal purposes it is being used for by the Nigerians.

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