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*Full Length Research paper*

# Polyphenol compositions and antioxidant capacity: A comparison of ripe and unripe fruit extract of *Cissus multistriata* (Vitaceae) plant

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**The present study was carried out to evaluate the antioxidant activities of *Cissus multistriata* (Vitaceae) fruit in various system. Nitric oxide radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, and *in vivo* antioxidant enzymes were assayed to evaluate the biological activities of the extract. The percentage antioxidant activity of the methanol extracts increased in a concentration dependent fashion. In DPPH radical scavenging assays the $IC_{50}$ value of the unripe fruit extract was 0.684 µg/ml. The ripe fruit extract inhibited the nitric oxide radicals generated from sodium nitroprusside with an $IC_{50}$ of 0.184 µg/ml, comparable to 0.0161 µg/ml for standard vitamin C. The polyphenol contents of the extracts were also determined and the plant extracts contain tannins, flavonoids and anthocyanins. *In vivo* antioxidant and rapid radical scavenging activities were also screened which were both positive for the two fruit extracts. This study suggests that possible mechanism of the biological activities may be due to free radical-scavenging and antioxidant characteristics which may be due to the presence of polyphenols in the fruit extracts.**

**Key words:** Antioxidant, DPPH, polyphenols.

## INTRODUCTION

The use of plant as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied (Kirby, 1996).

In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies (Kirby, 1996; Hostettmann and Marston, 2002). Therefore, it is a new challenge to seek for antioxidant, membrane stabilizing activities of natural compounds such as polyphenols from ethnomedicinal plants. Polyphenols are a group of highly hydroxylated phenolic compounds present in the extractive fraction of several plant materials. Polyphenols in plants include hydroxy coumarins, flavonols, flavones, anthocyanins and tannins. The evaluation of the antioxidant activities of polyphenols from ethnomedicinal plants may also be necessary because they are among desired medicinal properties of plant due to their nutracuetical effects (Zhu et al., 2004). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides (Gulcin, 2006).

Antioxidant activities of polyphenols have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* observations (Moosmann and Behl, 1999; Parr and Bolwell, 2000). Polyphenolic compounds in plant, including the catechins, exert anticarcinogenic, antimutagenic and cardioprotective effects linked to their free radical scavenging (Parr and Bolwell, 2000; Santos-Buelga and Scalbert, 2000). They are reported to be chemo-preventive agents by lowering cholesterol (Ferreira and Slade, 2002). In addition to their individual

efforts, antioxidants interact in synergistic ways and have sparing effect against oxidative destruction. These justify the overwhelming interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Parr and Bolwell, 2000).

In Nigeria, *Cissus multistriata* is widely used in the management of diverse diseases such as kwashiorkor, marasmus, arthritis etc. It is used in the treatment of infertility, stomach ailment in children, as well as sure remedy for cough. Its application to fracture site have provided healing as claimed by traditional herbal medicine practitioners. The fruit is popular hook bait and a major attractant to big fishes in the riverine area of Ibaji, Kogi State. The medicinal properties of the plant have been claimed extensively. Since reactive oxygen species (ROS) have been implicated in some of the diseases claimed to be cured by this plant and since polyphenolic compounds are able to boost biological resistance against ROS, the antioxidant capacity of *C. multistriata* fruit need to be assessed. Various biological activities of the crude methanol fruit extract of *C. multistriata* was investigated to provide new fields for utilization study of the plant.

## MATERIALS AND METHODS

### Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Griess reagent were purchased from sigma chemical company (Sigma Germany), vitamin C used was a product of Glaxo Smithklein. Methanol, Folin-Ciocatteu reagent, ferric chloride, amyl alcohol and sodium nitroprusside were products of BDH. Tannic acid used was M and B product.

The Glutathione and superoxide dismutase kit were obtained from Randox laboratories, U.K.

### Plant materials

The fruit of *C. multistriata* was obtained from Ekwanali – Igboigbo, Kogi State, Nigeria. The plant material was washed with water to remove dirt and oven dried at 45°C. The dried plant material was pulverized using electric blender. Weighed portion of the sample was subjected to cold extraction.

### Preparation of plant extracts

Cold extraction method was employed for the extraction. 20 g of the powdered samples were weighed into a conical flask. 150 ml of pure methanol was added and left for 72 h. The mixtures were filtered and the filtrates were concentrated using rotary evaporator.

### Determination of polyphenol composition

The total phenol 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 and anthocyanin content. The colorimetric method of Van-Burden and Robinson (1981) was used in the determination of tannin composition.

### Nitric Oxide radical inhibition assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions which were measured by Griess reaction (Marocci et al., 1994; Green et al., 1982). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the varying concentration (1000, 500, 250, 125 and 62.5 µg/ml) of the extract and reference compound were incubated in a water bath at 25°C for 150 min. After incubation, 1.5 ml of the reaction mixture was removed and 1.5 ml of Griess reagent was then added. The absorbance of the chromophore formed was evaluated using spectrophotometer at 546 nm. Percentage inhibition of the nitric oxide generated was calculated using the formula:

$$\frac{I - E}{C} \times 100$$

Where C = absorbance of the fully oxidized control.
E = absorbance in the presence of extract.

### Rapid radical scavenging screening

The method of Mensor et al. (2001) 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. Yellow spot formed against purple background were taken as positive results. The duration for the development of yellow color indicated whether the antioxidant activity is strong or not.

### Determination of DPPH free radical scavenging activity

The free radical scavenging activities of the plant extracts were measured employing the modified method of Blois (1958). 1 ml each of the different concentrations (1000, 500, 250, 125 and 62.5 µg/ml) of extracts or standard (vitamin C) in a test tube was added 1 ml of 0.3 mM DPPH in methanol. 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 (Gulcin, 2009):

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### *In vivo* antioxidant enzyme activity assay

#### *Animals*

Wister albino rats (male) were used for the study of the crude extracts. The animals were kept at 27 ± 2O°C, relative humidity 44 - 56% and light and dark cycles of 10 and 14 h, respectively for one week before and during the experiment. Animals were provided with standard diet (Mouse cubes – Top feed, Anyigba) and the food was withdrawn 18 - 24 h before the start of the experiment and given water adlibitum.

**Table 1.** Percentage phenol and polyphenol composition *of C. multistriata* fruit extract.

| Plant parts | Tannins (%) | Flavonoid (%) | Phenol (%) | Anthocyanins (%) |
|---|---|---|---|---|
| *C. multistriata* ripe fruit | 0.24 ± 0.00 | 0.36 ± 0.08 | 0.39 ± 0.01 | 0.89 ± 0.02 |
| *C. multistriata* unripe fruit | 0.24 ± 0.01 | 0.35 ± 0.00 | 0.38 ± 0.01 | 0.05 ± 0.1 |

**Table 2.** Rapid radical scavenging screening of *C. multistriata* fruit extracts.

| Plant parts | Reaction speed | Intensity of spots |
|---|---|---|
| C. *multistriata* ripe fruit | Very fast | +++ |
| C. *multistriata* unripe fruit | Fast | ++ |

+++ = Strong intensity (immediate reaction)
++ =Intermediate intensity (1 - 15 min before color development).

**Animal grouping and crude extract administration**

Rats were divided into six groups of four rats each. Group 1 (control) animals were administered a single dose of water (1 ml/kg) orally, daily for 5 days and received liquid paraffin (1 ml/kg S.C). Group 2 ($CCl_4$) received water (1 ml/kg b.w orally) once daily for 5 days and received $CCl_4$: liquid paraffin (1:1, 2 ml/kg b.w, S.c).

Test group animals (Group 3 - 6) were administered orally a dose of 200 mg/kg b.w of methanol extracts in the form of aqueous suspension once daily. The groups (4 and 6) animals were administered simultaneously $CCl_4$: liquid paraffin (1:1, 2 ml/kg b.w, S.C) after 30 min. Animals were sacrificed after the last treatment. Blood samples were collected via cardiac puncture into EDTA bottles and refrigerated until used for the biochemical investigations.

**Estimation of glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity**

These two biochemical parameters, glutathione peroxidase (GPX) (Plagia and Valentine, 1967) and superoxide dismutase (SOD) (Woodlliams et al., 1983) were assayed using assay kits (Randox laboratories limited, Ireland).

## RESULTS AND DISCUSSION

The phytochemical composition of the extracts were determined and showed that the plant extracts contained appreciable quantity of tannins, flavonoids, and anthocyanincs (Table 1).

Each value in the table was obtained by calculating the average of three determinations ± S.E.M.

The analysis of the phytochemical composition of the fruit extracts has shown the presence of flavonoids, and other polyphenolic compounds which have been known for antioxidant and protection against free radical damages (Dicarlo et al., 1999). The percentage in the plant extracts are shown in Table 1. It appeared that the ripe fruit extract of *C. multistriata* had the highest content of polyphenolic compounds. Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities. The results of the DPPH radical scavenging activities of the extracts are as presented on Tables 2 and 3 and suggest that the fruit extracts contain free radical scavenging activity which could exert a beneficial action against pathological alterations caused by the generation of free radicals. Lipid peroxidation is accelerated when free radicals are formed as a result of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major anti-oxidation mechanisms to inhibit the chain reaction of lipid peroxidation. The free radical scavenging activity of the crude drug extracts were evaluated by DPPH assay. DPPH is known to obstruct labile hydrogen (Constantin et al., 1990; Matsubara et al., 1991). Scavenging of DPPH radical is related to the inhibition of lipid peroxidation (Ratty et al., 1988; Rekka and Kourounaskis, 1991).

*In vivo* antioxidant activity was assayed by estimation of Glutathione peroxidase (GPX) and superoxide dismutase (SOD) levels. The results of *C. multistriata* fruit extracts on the activities of antioxidant enzymes are as presented on Table 5. GPX and SOD content increased in extract treated groups, whereas, $CCl_4$–intoxicated groups have shown decrease in levels of enzyme activity compared to control group.

To prevent damage to cellular components, there are numerous enzymatic antioxidant defenses designed to scavenge reactive oxygen species in the cell. These natural antioxidants are manufactured in the body, provide an important defense against free radicals. These enzymes include Glutathione peroxidase, catalase, and superoxide dismutase. Some of these enzymes are also present in plants. In plants, SOD isoensymzes are present in the cytosol and mitochondria. As presented on Table 5, *C. multistriata* unripe fruit extract showed highest activity of GPX while activity of SOD is highest with ripe fruit extract.

$CCl_4$ is being used extensively to investigate hepatoprotective and antioxidant activity on various experimental animals (Bhathal et al., 1983). A major

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

| Plant part/ standard | Concentration (µg/ml) | Log concentration | % Scavenging activity | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| Unripe fruit | 1000 | 3.00000 | 77.86 | 0.684[a] |
| | 500 | 2.69897 | 76.86 | |
| | 250 | 2.39794 | 75.91 | |
| | 125 | 2.09691 | 69.66 | |
| | 62.5 | 1.79588 | 67.29 | |
| Ripe fruit | 1000 | 3.00000 | 78.50 | 18.13[b] |
| | 500 | 2.69897 | 77.90 | |
| | 250 | 2.39794 | 72.70 | |
| | 125 | 2.09691 | 63.50 | |
| | 62.5 | 1.79588 | 58.90 | |
| Vitamin C | 1000 | 3.00000 | 96.95 | 0.016[c] |
| | 500 | 2.69897 | 90.73 | |
| | 250 | 2.39794 | 89.57 | |
| | 125 | 2.09691 | 85.93 | |
| | 62.5 | 1.79588 | 85.11 | |

a. Linear equation: y = 9.141 X + 51.51
b. Linear equation: y = 17.80 X + 27.60
c. Linear equation: y = 9.4 X + 66.97.

**Table 4.** Nitric oxide radical scavenging activity of *C. multistriata* fruit extracts.

| Plant part/ standard | Concentration (µg/ml) | Log concentration | % Inhibition | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| Ripe fruit | 1000 | 3.00000 | 91.32 | 0.184[a] |
| | 500 | 2.69897 | 91.32 | |
| | 250 | 2.39794 | 88.98 | |
| | 125 | 2.09691 | 84.06 | |
| | 62.5 | 1.79588 | 99.02 | |
| Unripe fruit | 1000 | 3.00000 | 92.97 | 1.33[b] |
| | 500 | 2.69897 | 90.04 | |
| | 250 | 2.39794 | 84.17 | |
| | 125 | 2.09691 | 76.91 | |
| | 62.5 | 1.79588 | 75.91 | |
| Vitamin C (Standard) | 1000 | 3.00000 | 96.95 | 0.0161[c] |
| | 500 | 2.69897 | 90.73 | |
| | 250 | 2.39794 | 89.57 | |
| | 125 | 2.09691 | 85.93 | |
| | 62.5 | 1.79588 | 85.11 | |

a. Linear equation: y = 11.91 X + 58.77.
b. Linear equation: y = 15.03 X + 48.15.
c. Linear equation: y = 9.460 X + 66.97.

defense mechanism involves the antioxidant enzymes, including SOD, catalase and glutathione peroxidase which convert active oxygen molecules into non-toxic compounds. The toxic metabolite $CCl_4$ radical is produced, which further reacts with oxygen to give trichloromethyl peroxyl radical. Cytochrome P4502 EI is the enzyme responsible for this conversion. This radicals bind covalently to the macromolecules and cause per

**Table 5.** Effects of *C. multistriata* fruit extracts on the activities of glutathione peroxidase (GPX) and superoxide dismutase (SOD).

| Group | Treatment | GPX (U/L) | SOD (U/ml) |
|---|---|---|---|
| 1. | Control | 375.42 ± 1.69 | 41 ± 15.56 |
| 2. | $CCl_4$ | 368.95 ± 1.89 | 18 ± 16.97 |
| 3. | Ripe fruit | 420.60 ± 1.89 | 88 ± 16.97 |
| 4. | Ripe fruit + $CCl_4$ | 369.51 ± 1.84 | 52 ± 0.01 |
| 5. | Unripe fruit | 588.84 ± 4.59 | 64 ± 16.97 |
| 6. | Unripe fruit + $CCl_4$ | 386.95 ± 1.89 | 53 ± 32.55 |

Each value in the table was obtained by calculating the average of four replication ± S.E.M.

oxidative degradation of lipid membrane of the adipose tissue.

In this study, increase in the activity of this enzyme in the extract treated groups could suggest stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $CCl_4$. The significant increase in GPX and SOD content of the whole blood used, suggested antioxidant activity of *C. multistriata* parts used. Thus, it can be inferred that the possible mechanism of antioxidant activity of the *C. multistriata* extracts may be due to their free-radical scavenging activity, which may be due to the presence of polyphenolic compounds in the extracts.

It is well known that nitric oxide has an important role in various types of inflammatory processes. In the present study, the crude fruit extracts were checked for their inhibitory effect on nitric oxide production. Table 4 illustrates the percentage inhibition of nitric oxide generation by *C. multistriata* fruit extracts. Vitamin C was used as a reference compound. The concentration of *C. multistriata* ripe fruit extract needed for 50% inhibition was 0.184 (µg/ml) which is comparable to the reference standard $IC_{50}$ = 0.0161 (µg/ml).

These extracts have shown maximum protection most especially unripe fruit extract. It appears that the plant part studied contain the antioxidant enzymes or probably induce their synthesis *in vivo.* The plant may be a good source of natural antioxidant and could be well integrated into the Nigerian folk medicine and food drugs.

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