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Antioxidant Power of *Macaranga barteri* Leaf

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Abstract: Methanolic extract of *Macaranga barteri* Mull-Arg (Euphorbiaceae) was investigated for antioxidant properties using different *in vitro* tests, including DPPH free radical scavenging, reducing, inhibition of linoleic acid lipid peroxidation, iron chelating power. The total phenolic content was also determined and expressed in gallic acid equivalent. The antioxidant activities increased with increasing concentration of the extract to certain extent then level off with further increase. The radical scavenging activity of the extract was comparable to that of BHT and ascorbic acid and no significant difference between them ($p>0.05$). The extract of *M. barteri* showed strong inhibition of lipid peroxidation in linoleic acid system and moderate reducing properties. It demonstrated poor iron chelating capacity compared to EDTA used as positive control, suggesting metal chelation plays very little role in antioxidant properties of the extract. This study showed that the leaf extract of *M. barteri* is a potential source of antioxidants.

Key words: Phenolic content, radical scavenging effect, reducing power, iron chelating power, inhibition of lipid peroxidation, *M. barteri*

INTRODUCTION

Some reactive molecules such nitric oxide, superoxide, hydrogen peroxide etc are physiologically useful but can also be harmful if present in excess or in-appropriate situations (oxidative stress). These oxidants can react with various components of living cell, such as proteins, DNA or lipids/fats thus causing damage by changing the chemical structure of these components. Such damage had been linked to a number of pathological conditions-aging, carcinogenesis, atherosclerosis etc. (Aruoma, 1998; Wasser, 2002; Klaunig and Kamendulis, 2004). The human body however has evolved a large array of endogenous antioxidant defense system against oxidative stress, including antioxidant enzymes such as superoxide dismutase, catalase and various peroxidases, the hormone melatonin and uric acid. These endogenous antioxidants do not completely protect against the sum total of oxidative stresses challenging the body and thus there is net oxidative damage that in the long term contributes to aging and various diseases (Watkins and Li, 2001; Willcoox *et al*., 2004; Balaban *et al*., 2005). Foods or antioxidant supplements may however be used to help minimize oxidative damage in the body.

Macaranga barteri Mull-Arg (Euphorbiaceae) is shrub or tree common in Guinea, Southern Nigeria and Equatorial Guinea. The plant is used as vermifuge and febrifuge in Congo Brazzaville and Nigeria. It is also used to relieve cough and bronchitis. The leaves are used in Sierra Leone for gonorrhea and the plant is also used as an aperient and anti-anaemic tonic in Ivory Coast. Previous studies on the plant have reported that no alkaloid in the bark and trace amount was detected in the leaves (Burkill, 1994). A search in the literature revealed very little information about the plant. The present study is designed to investigate the antioxidant power including radical scavenging ability, reducing effect, ferrous chelating and ability to inhibit peroxidation in the linoleic acid system. The total phenolic content was also determined.

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MATERIALS AND METHODS

Materials

The studies were conducted in Lagos, Nigeria in 2006/2007. The leaves of *M. barteri* were collected at Ibadan, in Oyo, Nigeria and were authenticated by Mr. K.I. Odewo of Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria after comparing with voucher specimen with number FHI 107230. The leaves were dried at 30°C and hand crushed to fine powder.

Chemicals

Folin-Ciocalteu phenol reagent, gallic acid, anhydrous sodium carbonate, ascorbic acid, linoleic acid, ammonium thiocyanate, 2,6-Di-tert-buytl-4-methylphenol (BHT), 90% 1,1-diphenyl-2-picrylhydrazyl (DPPH), $FeCl_2$ tetrahydrate, anhydrous ferric chloride, potassium ferricyanide, [4,4'- [3-(2-pyridinyl-1,2,4-triazine-5,6-diyl]bisbenzenesulfonic acid] (ferrozine) and trichloroacetic acid were purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals were of analytical grade BDH Chemical Laboratory (England, UK).

Preparation of Extract

About 500 g of powdered leaf was extracted with methanol (3 L) using Soxhlet apparatus for 48 h. The extract was concentrated under reduced pressure until a semi-solid sticky mass was obtained. The yield was 17.55% w/w.

Phytochemical Screening

The leaves of *M. barteri* were tested for the presence of alkaloid, anthraquinone, cardiac glycoside, steroidal nucleus, saponins, cyanogenetic glycoside and phenolic compounds using the method of Sofowora (1982).

Determination of Total Phenolic Content

The total phenolic content of the extract was determined with the Folin-Ciocalteu reagent (Mcdonald *et al*., 2001). The extract (100 mg mL^{-1}, 1.0 mL) was mixed with 5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 4 mL (0.7 M) sodium carbonate. The mixture was allowed to stand for 1 h and the absorbance was measured at 765 nm. The analysis was carried out in triplicate and gallic acid was used as the standard for calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of methanolic extract.

Measurement of DPPH Radical Scavenging Activity

The method of Koleva *et al*. (2002) with some modifications was used for the determination of free radical scavenging activity of the extract. The extract (0.02-0.75 mg mL^{-1}) of 1.0 mL was diluted with 4.0 mL of methanol and mixed with 1.0 mL of methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (1 mM). The mixture was shaken immediately and allowed to stand at 20°C in dark for 30 min. The decrease in absorbance at 517 nm was then measured using spectrophotometer. BHT and ascorbic acid were tested and used as reference standards. All analysis was performed in triplicate and the ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging Effect (\%)} = [(Ao - A_1)/Ao] \times 100$$

Where,

Ao : The absorbance of the control

A_1 : The absorbance in the presence of the sample of extract or standard.

Evaluation of Reducing Ability

The reducing power of the extract was determined according to the method of Yen and Chen (1995) with some modifications. The extract (1.0 mL, 0.1-1.5 mg mL^{-1}) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) was added then the mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture and centrifuged at 1000 g for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm against a blank in the spectrophotometer. Ascorbic acid and BHT were used as positive controls. Higher absorbance of the reaction mixture indicated increased reducing power.

Measurement of Ferrous Ion Chelating Capability

The Fe^{2+}-chelating power was measured using the method of Decker and Welch (1990) with some modifications. The extract (1 mL, 0.1-10.0 mg mL^{-1}) was mixed with 4 mL of deionised water then treated with $FeCl_2$ (0.1 mL, 2.0 mM) and ferrozine (0.2 mL, 5 mM) The mixture was shaken and left to stand at 20°C for 20 min. The absorbance of the final solution was determined at 562 nm. EDTA was used as positive control and ability to chelate ferrous ion was determined using the following equation:

$$\text{Chelating effect (\%)} = [(Ao - A_1)/Ao] \times 100$$

Where,

Ao : The absorbance of the control

A_1 : The absorbance in the presence of the sample of extract or standard.

Antioxidant Activity

The antioxidant activity was measured using the method of Chang *et al*. (2002) with modifications. The extract (0.5 mL, 0.5-7.5 mg mL^{-1}) was mixed with linoleic acid emulsion (2.5 mL, 0.56% w/v, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0) and incubated at 60°C in the dark for 12 h to accelerate oxidation. Ferrous chloride solution and ammonium thiocyanate were added to 0.1 mL solution of the sample in sequence and the absorbance was read at 500 nm to determine the peroxide value. Ascorbic acid and BHT were used as positive controls. The solution without the extract or the controls was used as blank. The inhibitory effect of the extract in the linoleic acid system was determined as follows:

$$\text{Inhibition (\%)} = [(Ao - A_1)/Ao] \times 100$$

Where,

Ao : The absorbance of the blank

A_1 : The absorbance in the presence of the sample of extract or standard.

Statistical Analysis

All data were expressed as mean±standard deviation. Analysis of variance was performed by ANOVA procedures and $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The results of phytochemical investigation of the leaves of *M. barteri* showed presence of saponins and phenolic compounds. However, alkaloids, anthraquinones, cardiac and cyanogenetic glycosides were not detected. The total phenolic content of leaf extract of *M. barteri* was found to be 48.88 mg g^{-1}, as gallic acid. Phenolic compounds in plants have been reported to play a significant role

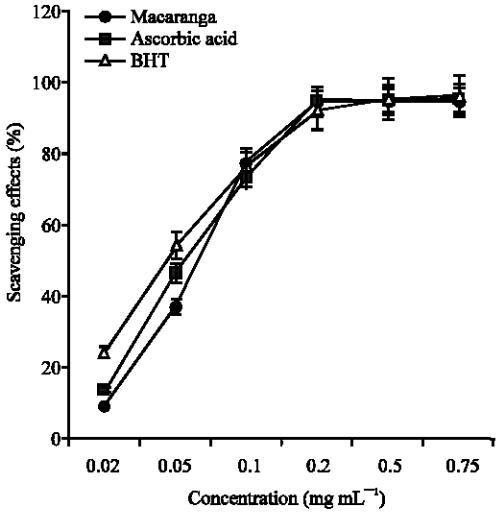

Fig. 1: Scavenging effects of *M. barteri*, Ascorbic acid and BHT on DPPH radical. Each value represents mean±standard deviation (n = 3)

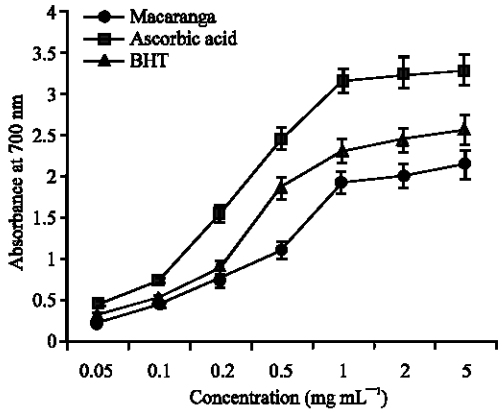

Fig. 2: Reducing power of *M. barteri*, Ascorbic acid and BHT. Each value represents mean±standard deviation (n = 3)

in scavenging effect and prevention of lipid peroxidation due to the presence of hydroxyl moiety (Kahkonen *et al*., 1999). They may be responsible for the antioxidant activities observed in the tested extract.

The radical scavenging effect of the extract and positive controls were concentration dependent (Fig. 1). The scavenging effects increased with increasing concentration then level off with further increase in concentration. The radical scavenging activity of the extract and positive controls decreased in the following order: ascorbic acid > BHT > *M. barteri* with IC_{50} of 0.10, 0.15 and 0.18 mg mL^{-1}, respectively. DPPH radical scavenging activities have reported to be due to proton donating ability of the antioxidant (Yamagushi *et al*., 1998; Mokbel and Hashinaga, 2006) thus the antioxidant activities of the extract may be attributed to its hydrogen donating power. The radical scavenging activity of the extract is comparable to that of BHT and ascorbic acid and no significant difference between them $p>0.05$.

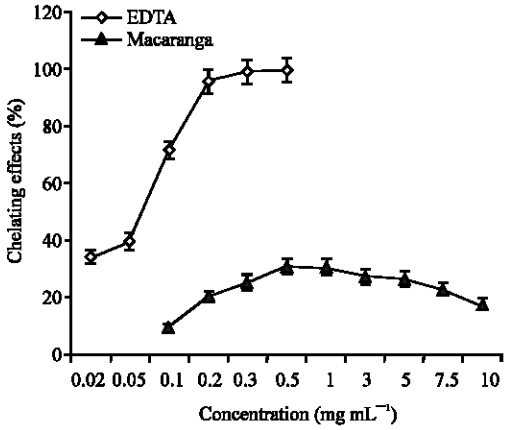

Fig. 3: Chelating effect of *M. barteri* leaf extract on Fe^{2+} ion. Each value represents mean±standard deviation (n = 3)

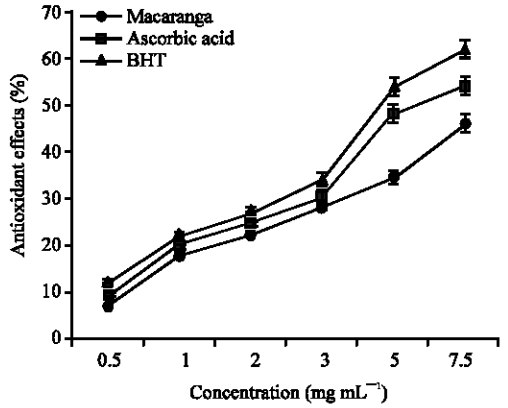

Fig. 4: Effects of *M. barteri* leaf extract on linoleic acid peroxidation. Each value represents mean±standard deviation (n = 3)

The reducing activities increase with increasing concentration of the antioxidant (Fig. 2). Reduction of Fe^{3+}/ferricyanide complex to the ferrous form is commonly used for the assessment of antioxidant properties of compounds. The reducing power of these compounds is due to their ability to donate electrons (Yen and Chen, 1995; Kahkonen *et al*., 1999). The results obtained showed *M. barteri* leaf extract as a reducing agent that could react with free radicals and convert them to more stable compounds. The reducing power of the extract of *M. barteri* and standard compounds follow the order of ascorbic acid > BHT > *M. barteri.*

The chelating ability also increased with increase in leaf extract concentration and reached 30.5% at concentration of 0.5 mg mL^{-1} then decreased as the concentration increased (Fig. 3). EDTA showed better chelating power (IC_{50} = 0.075 mg mL^{-1}) than the extract at all tested concentration. Iron chelating power is essential in antioxidant properties because it reduces the production of oxyradicals and lipid peroxidation thus protecting against oxidative damage (Willet, 1995; Nice, 1997; Gulcin *et al*., 2003). The results obtained showed that metal chelation plays very little role in antioxidant properties of the extract.

Lipid peroxidation involves oxidation of polyunsaturated fatty acid in the cell membrane (Fig. 4) and it was reported to be responsible for cell membrane destruction and cell damage (Yoshikawa *et al*.,

1997). The leaf extract of *M. barteri* inhibited lipid peroxidation in a dose dependent manner but its activity was significantly less ($p<0.05$) than that of BHT and ascorbic acid. The result suggested that the extract could be used in treatment of free radicals and oxidative damage induced diseases. The antioxidant capacity increased in the order macaranga < ascorbic acid < BHT with IC_{50} 10.21, 6.41 and 4.12 mg mL^{-1}, respectively.

We may conclude that the *M. barteri* leaf extract has a potent antioxidant property. Although the activity is relatively lower than that of BHT and ascorbic acid, the extract may be a veritable source of bioactive compounds with better activities after fractionation.

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