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Full Length Research Paper

Experimental classification of the antioxidant capacity of the leaf, stem and root barks of *Magnifera indica* and *Azadirachta indica*

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The ferric reducing antioxidant power (FRAP) and total phenolic concentration of the leaf, stem and root barks of *Magnifera indica* and *Azadirachta indica* growing in Ogbomoso, Nigeria were evaluated *in vitro*. Only the leaf of *A. indica* belonged to good FRAP. Both the stem and root bark of *A. indica* and all the parts of *M. indica* investigated belonged to high FRAP. Experimental results revealed that the antioxidant capacity ranged from 6.80 - 9.20, 12.40 - 13.00 and 10.20 -13.203 mM of reduced Fe^{3+} for the leaf, stem and root bark, respectively in *A. indica*. In *M. indica*, the antioxidant capacity ranged from 12.20 - 15.20, 11.00 - 11.80 and 11.20 - 12.20 mM of reduced Fe^{3+} for the leaf, stem and root bark, respectively. The total phenolic concentration and antioxidant capacity of *M. indica* stem bark showed a high significant positive correlation (r = 0.9439; p = 0.05). The total phenolic concentration of the root bark of *A. indica* showed a high positive significant correlation with antioxidant capacity (r= 0.9850; p= 0.05). All the plant parts examined might be exploited in clinical medicine as protective factors because of their good and high antioxidant capacities.

Key words: Ethno-medicine, natural product, drug development, plant phenolics.

INTRODUCTION

Medicinal plants, as source of remedies, are widely used as alternative therapeutic tools for the prevention or treatment of many diseases (Harnafi and Amrani, 2008). Herbal remedies are popular remedies for diseases used by a vast majority of the world's population (Kuruvilla, 2002). Because dietary plants contain several hundred different antioxidants, it would be useful to know the total concentration of electron-donating antioxidants (that is, reductants) in individual items (Halvorsen et al., 2002). Such data might be useful in the identification of the most beneficial dietary plants (Halvorsen et al., 2002). Results demonstrate that there are more than 1,000 fold differences among antioxidant concentrations of various herbs (Dragland et al., 2003). Antioxidants are molecules that can delay or prevent an oxidative reaction (Velioglu et al., 1998). Antioxidants may offer resistance against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and many other mechanisms and thus prevent disease (Marx, 1987). Herbs are most often defined as any part of a plant that is used in the diet for its aromatic properties (Smith and Winder, 1996). The word 'herb' has usually been used to refer to any plant part used for its medicinal, savory or aromatic qualities (Earl, 2000).

There are approximately 380,000 species of plants on earth that have been identified and several thousands are yet to be discovered (Earl, 2000). Sources of natural antioxidants are primarily plant phenolics (Pratt and Hudson, 1990). They can be found in fruits, vegetables, nuts, seeds, leaves, flowers and bark (Wollenweber and Dietz, 1981). The intake of antioxidants present in foods is an important health protecting factor (Szollosi and Varga, 2002). Herbal compounds known by ancient medicine are of growing interest in the domain of diseases prevention (Szollosi and Varga, 2002). Most of the natural antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors and synergists (Larson,

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1988).

Medicinal plants have a lot of types of antioxidants mostly polyphenols and flavonoids which exhibit high antioxidant activity (Rice-Evans et al., 1995). Phenolic antioxidant exhibit anti- inflammatory, atherosclerotic and carcinogenic properties (Kahl, 1991). Phenolic antioxidants inhibit oxidation reactions by generally acting as a hydrogen donor and afford relatively stable free radicals and/or non-radical products (Shahidi and Wanasundara, 1992). Katalinic et al. (2006) classified medicinal plants into their antioxidant capacity as very low FRAP (< 1 mM/L), low FRAP (1 – 5 mM/L), good FRAP (5 – 10 mM/L), high FRAP (10 – 20 mM/L) and very high FRAP (>20 mM/L).

Mango (*Magnifera indica*) is commercially the most important fruit crop of India, accounting for > 54% of the total mango produced worldwide (Tharanathan et al., 2006). It is known for its strong aroma, intense peel coloration, delicious taste and high nutritive value due to its high content of vitamin C, B-carotene and minerals (Tharanathan et al., 2006). Vimang is the brand name of a formulation containing an extract of *M. indica,* ethnopharmacologically used in Cuba for the treatment of immunophathological disorders including bronchial asthma, atopic dermatitis and other allergic diseases (Garcia et al., 2006). Magniferin, the major compound of Vimang, contributes to the anti-allergic effects of the extract (Garcia et al., 2006). Vimang tablets prevent age-associated oxidative stress in elderly humans, which could retard the onset of age-associated disease thus improving the quality of life for elderly persons (Pardo-Andreu et al., 2006).

Observations showed that the aqueous extract of *M. indica* stem bark have some effects on the haematopoietic system manifested by a positive increase in the levels of PCV (haematocrit), erythrocyte, leukocyte, platelet counts and lymphocytes while the haemoglobin (Hb) and neutrophil levels were decreased (Nwinuka et al, 2008). *M. indica* is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pain and malaria (Agoha, 1981; Madunagu et al., 1990). *M. indica* contains alkaloids and glycosides which are of great importance pharmacologically (Madunagu et al., 1990). Hypoglycemic effect of *M. indica* young leaf methanolic extract in experimental diabetes mellitus was reported by Ugbenyen and Odetola (2009).

Azadirachta indica A. Juss (neem) is a source of several bioactive components; however only azadirachtins have been commercially exploited (Sadeghian and Mortazaienezhad, 2007). Neem leaf extracts have been used for pesticides, fertilizers, corrosion inhibition and biosorbent for dyes (Sadeghian and Mortazaienezhad, 2007). Nimbidin, a major crude bitter principle extracted from the oil of seed kernels of *A. indica* demonstrated several biological activities. From this crude principle, some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acids have been isolated (Siddiqui, 1942; Mitra et al., 1971). The spermicidal activity of nimbidin and nimbin was reported in rats and humans as early as 1959 (Sharma and Saksena, 1959; Murthy and Sirsi, 1958). The chloroform extract of the stem bark is effective against carrageenin-induced paw oedema in rat and mouse ear inflammation (Jacobson, 1986). The aqueous extract of the leaf possesses potent immunostimulant activity as evidenced by both humoral and cell-mediated responses (Sen et al., 1992; Ray et al., 1996).

Neem oil has been shown to possess immunostimulant activity by selectively activating the cell-mediated immune mechanisms to elicit an enhanced response to subsequent mitogenic or antigenic challenge (Upadhyay et al., 1992). Recently, hypoglycaemic effect was observed with the leaf extract and seed oil in normal as well as alloxan-induced diabetic rabbits (Khosla et al., 2000). The aqueous leaf extract offers antiviral activity against *Vaccinia* virus (Rao et al., 1998). Neem leaf aqueous extract also effectively suppresses oral squamous cell carcinoma induced by 7,12-dimethylbenz[a]anthraxcene (DMBA), as revealed by reduced incidence of neoplasm (Balasenthil et al., 1999). The aqueous extract of neem leaf was found to offer protection against paracetamol-induced liver necrosis in rats (Bhanwra et al., 2000).

This study was carried out to classify the different parts (leaf, stem and root bark) of the two plants, *A. indica* and *M. indica* into their scale of antioxidant capacity using modified antioxidant capacity scale reported elsewhere (Katalinic et al., 2006) and to investigate the relationship between total phenolics and antioxidant activity of the different plant parts.

MATERIALS AND METHODS

Chemical reagents

Hydrated sodium acetate (BDH), ferric chloride (BDH), concentrated HCl (BDH), sodium carbonate (Eagle Scientific Limited), glacial acetic acid (Eagle Scientific Limited) and tripyridyl triazine (BDH) were used in the study.

Collection of samples

The samples of leaf, stem and root barks of both *A. indica* and *M. indica* were collected from Ladoke Akintola University of Technology, Ogbomoso campus in Nigeria. Ogbomoso is the second largest city in Oyo State, Nigeria.

Preparation of plant extract

1 g of the shredded dry plant material was soaked in 20 ml of distilled water for four days in sterilized sample bottles. The solution was filtered and the filtrate obtained was used immediately for analyses of total phenolic concentration and antioxidant capacity.

Biochemical assays

Total phenolic concentration of each plant extract was determined according to the method described elsewhere (Sava et al., 2001).

This is based on the principle that the phenolic group present in the plant extract reacts with Folin-Ciocalteau in an alkaline medium using sodium carbonate (Na_2CO_3) solution thus giving a blue colour. 0.1 ml of each plant extract was added to 0.5 ml of Folin-Ciocalteau reagent (10% dilution) followed by the addition of 0.4 ml (7.5%) of $Na_2CO_{3(aq)}$ solution. The solution was mixed and allowed to stand in the dark at room temperature for 30 min. The absorbance of the blue color developed was read at 765 nm on a UV visible spectrophotometer. The total phenolic concentration of each sample was calculated using the formula stated elsewhere (Sava et al., 2001).

$$\text{Total phenolic concentration (mg/dl)} = 95.1 \times A_{765} - 2.6$$

The antioxidant capacity (ferric reducing antioxidant power, FRAP) of each sample was estimated according to the traditional method of Benzie and Strain (1963) described elsewhere (Olabinri, 2006). In FRAP assay, Fe^{3+} reacts with tripyridyl triazine to form Fe^{3+} tripyridyl complex. The complex formed is reduced to Fe^{2+} tripydyl complex in the presence of an electron donating antioxidant from the sample. 2.90 ml of distilled water was added to 0.1 ml of sample, followed by the addition of 1.5 ml of the FRAP reagent (prewarmed to 57°C). The solution was mixed and allowed to stand for 15 min in the laboratory. The absorbance of the blue colour was read on a UV/visible spectrophotometer at 593 nm using FRAP reagent as blank. The antioxidant capacity (ferric reducing antioxidant power) was deduced from a standard FRAP curve which was prepared from iron (II) sulphate. Antioxidant capacity (FRAP value) was expressed in mM of reduced Fe^{3+}.

Statistical analysis

Student's t- test was used for statistical analysis. $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Table 1 shows the class of antioxidant capacity of the different plant parts of *A. indica* and *M. indica*. The order of antioxidant capacity for *A. indica* is as follows: Stem bark > root bark >leaf. The order of antioxidant capacity for *M. indica* is as follows: Leaf > root bark > stem bark. In this research, the modified method of scale of antioxidant capacity of Katalinic et al. (2006) was employed. A three fold increase in total phenolic concentration was observed in the root bark of *A. indica* when compared to the leaf (Table 2). Also, a three fold increase in the stem bark of *A. indica* was observed when compared to the leaf of *A. indica*. The order of total phenolic concentration for *A. indica* is as follows: Stem bark >root bark > leaf. The order of total phenolic concentration for *M. indica* was as follows: Leaf > root bark > stem bark.

In this work, the total phenolic concentration of *M. indica* stem bark showed a high significant positive correlation (r= 0.9439; p= 0.05) with antioxidant capacity of *M. indica* leaves as shown in Table 3. Also, in this work, the total phenolic concentration of the leaves of *M. indica* showed a high positive significant correlation with antioxidant capacity of *M. indica* leaves (r= 0.9095; p= 0.05) (Table 3). The total phenolic concentration of the root bark of *M. indica* showed a weak non-significant positive correlation with antioxidant capacity (r= 0.2161; p= 0.05) as indicated in Table 3. The root bark of *A. indica* total phenolic concentration showed a high positive significant correlation with antioxidant capacity (r= 0.9850; p= 0.05) while the stem bark and leaf total phenolic concentration also demonstrated positive correlation with their antioxidant capacity (r= 0.8454; p= 0.05 for stem bark, r= 0.8308; p= 0.05 for the leaf) as shown in Table 4. However, the correlation between both parameters in the plant parts was found to be insignificant.

The total phenolic concentration of *M. indica* stem bark was higher than that of the root bark of the same plant (Table 2), and the difference was significant ($P<0.05$) as shown in Table 5. Also, the total phenolic concentration of the leaf of *M. indica* was higher than that of the root bark of the same plant (Table 2), and the difference was significant ($P<0.05$) (Table 5). The total phenolic concentration of the stem bark of *A. indica* was greater than that of the leaves (Table 2) and the difference was significant ($P<0.05$) (Table 6). Also, the total phenolic concentration of *A. indica* root bark was significantly higher than that of the leaves (Table 2) and the difference

Table 1. Classification of *M. indica* and A. *indica* plant parts into their antioxidant capacity (ferric reducing antioxidant power, FRAP).

Plant part	FRAP value (mM of Fe^{2+})	Scale of antioxidant capacity
A. indica		
Stem bark	12.40 ± 0.37	High FRAP
root bark	12.24 ± 1.42	High FRAP
Leaf	8.04 ± 1.08	Good FRAP
M. indica		
Stem bark	11.36 ± 0.47	High FRAP
Root bark	11.64 ± 0.55	High FRAP
Leaf	14.00 ± 1.11	High FRAP

The values are mean ± SD of five analyses.

Table 2. Changes in the levels of total phenolic concentration in the leaf, stem and root bark of *A. indica* and *M. indica.*

Plant part	Total phenolic concentration (mg/dL)
A. indica	
Stem bark	5768.37 ± 191.39
root bark	5683.27 ± 507.46
Leaf	1727.7 ± 350.98
M. indica	
Stem bark	4270.16 ± 287.95
Root bark	3955.79 ± 439.43
Leaf	6180.89 ± 486.40

The values are mean ± SD for five analyses.

Table 3. Pearson correlation between total phenolic concentration and antioxidant capacity of *M. indica* plant parts.

Plant part	Pearson correlation coefficient (r)	Significant level	Remark
Stem bark	0.9439	0.05	SG
Root bark	0.2161	0.05	NS
Leaves	0.9095	0.05	SG

SG indicates significant; NS indicates not significant.

Table 4. Pearson correlation between total phenolic concentration and antioxidant capacity of *A. indica* plant parts.

Plant part	Pearson correlation coefficient (r)	Significant level	Remark
Stem bark	0.8454	0.05	NS
Root bark	0.9925	0.05	SG
Leaves	0.8308	0.05	NS

SG indicates significant; NS indicates not significant.

Table 5. Significant testing between total phenolic concentration in different plant parts of *M. indica.*

Plant parts	Experimental t value	Remark
Stem and root bark	2.08	SG
Stem bark and leaves	7.58	SG
Root bark and leaves	5.86	SG

Table 6. Significant testing between total phenolic concentration in different plant parts of *A. indica*.

Plant parts	Experimental t value	Remark
Stem and root bark	1.24	NS
Stem bark and leaves	20.92	SG
Root bark and leaves	21.10	SG

Table 7. Significant testing between FRAP value in different plant parts of *M. indica*.

Plant parts	Experimental t value	Remark
Stem and root bark	0.99	NS
Stem bark and leaves	4.97	SG
Root bark and leaves	4.41	SG

Table 8. Significant testing between FRAP value in different plant parts of *A. indica*.

Plant parts	Experimental t value	Remark
Stem bark and root bark	0.62	NS
Stem bark and leaves	9.12	SG
Root bark and leaves	5.61	SG

was significant (P<0.05).

The antioxidant capacity of *M. indica* leaf was slightly greater than that of the stem bark (Table 1), and the difference was found to be significant (p<0.05) as shown in Table 7. The antioxidant capacity of the stem bark of *A. indica* was higher than the antioxidant capacity of the leaves (Table 1) and the difference was significant (p< 0.05) as shown in Table 8. Also, the antioxidant capacity of *A. indica* stem bark was slightly higher than the antioxidant capacity of the root bark and the difference was not significant (p> 0.05) as shown in Table 8.

Plant based natural antioxidants can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, e.t.c. (Gordon and David, 2001); that is any part of the plant may contain active components. FRAP is a sensible and practicable indicator of total antioxidant capacity (Vassalle et al., 2004). Results show that FRAP method is sensitive in the measurement of total antioxidant power of fresh biological fluids, such as plant homogenates and pharmacological plant products (Szollosi and Varga, 2002). FRAP assays is based on ferric reducing ability (Vassalle et al., 2004). Ferric to ferrous reduction at low pH causes a blue ferrous tripyridyl triazine complex to form (Benzie and Strain, 1996; Olabinri, 2006). The FRAP assay offers a putative index of antioxidant or reducing potential of biological fluid within the reach of every laboratory and researcher interested in oxidative stress and its effects (Benzie and Strain, 1996). The FRAP assay uses antioxidants as reductants in a redox – linked colorimetric reaction (Benzie and Strain, 1996).

In conclusion, the leaf, stem and root bark of *M. indica* belong to high FRAP. Both the root and stem bark of *A. indica* belong to high FRAP. The leaf of *A. indica* belongs to good FRAP and antioxidant activity depends on total phenolic concentration for stem bark and leaf of *M. indica*

and for the root bark of *A. indica*.

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