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Article

Comparative *in vitro* antioxidant potential of different parts of *Ipomoea asarifolia,* Roemer & Schultes, *Guiera senegalensis*, J. F. Gmel and *Anisopus mannii* N. E. Brown

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In this study, the radical scavenging capacity of *Guiera senegalensis, Ipomoea asarifolia* and *Anisopus mannii* were compared. The ascorbic acid equivalent of the stem bark (2.1 mM), leaves (2.05 mM) and root barks (1.89 mM) of *Guiera senegalesis* are much higher than any part of *Ipomoea asarifolia* or *Anisopus manii*. The stem bark, leaves and root bark of *Guiera Senegalensis* had IC_{50} of 15.4, 20.0 and 17.0 µL respectively, while corresponding parts of *Ipomoea asarifolia* showed IC_{50} of 50, 42 and 65 µL, respectively. These results suggest that *Guiera senegalensis* and *Ipomoea asarifolia* possess significant antioxidant capacities to warrant further detailed studies on the role of this property in their therapeutic effects.

Uniterms: *Ipomoea asarifolia*/antioxidant potential. *Guiera senegalensis*/antioxidant potential. *Anisopus mannii*/antioxidant potential.

Comparou-se a capacidade de sequestrar radicais livres de *Guiera senegalensis, Ipomoea asarifolia* e *Anisopus mannii.* O equivalente em ácido ascórbico da casca do caule (2,1 mM), folhas (2,05 mM) e da casca da raiz (1,89 mM) de *Guiera senegalesis* foi muito mais alto do que qualquer parte da *Ipomoea asarifolia* e da *Anisopus manii*. A casca do caule, as folhas e a casca da raiz de *Guiera senegalensis* apresentaram IC_{50} de 15,4, 20,0 and 17,0 µLl, respectivamente, enquanto as partes correspondentes de *Ipomoea asarifolia* apresentaram IC_{50} de 50, 42 e 65 µL respectivamente. Esses resultados sugerem que *Guiera senegalensis* e *Ipomoea asarifolia* possuem capacidade oxidante significativa que demanda estudos posteriores detalhados acerca do papel dessa propriedade em seus efeitos terapêuticos.

Uniterms: *Ipomoea asarifolia*/potencial antioxidante. *Guiera senegalensis*/potencial antioxidante. *Anisopus mannii*/potencial antioxidante.

INTRODUCTION

Plants continue to be a major source of medicine, as they have always been throughout human history (Ampofo, 1977; Farnsworth *et al.*, 1985; Eisenberg *et al.*, 1998). A number of these plants from other continents have been reported to exert therapeutic efficacies through their antioxidant activities (Aruoma, 2003; Park, Pezzuto, 2002; Scartezzini, Speroni, 2000; Zhu *et al.*, 2004), but only a few Nigerian and African medicinal plants have been the focus of research (Atawodi, 2005). *Ipomoea asarifolia,* Roemer & J.A. Schultes, *Guiera Senegalensis*, J.F. Gmel and *Anisopus mannii,* N.E. Brown are three Nigerian plants whose potential antioxidant potential have not yet been clearly established.

Ipomoea asarifolia, Roemer & J.A. Schultes (synonyms: *Ipomoea hederifolia)* or Morning glory, known in the Hausa language of Nigeria as "Duman Karda" (Kaduna), "Duman ra'afii" (Kano) and "Woba boje" or "Daadi mayo" (Adamawa), is a hairless, succulent perennial weed of the family *Convolvulaceae* that grows in hydromorphic soils in low lying and inland valleys, streams, and river banks of temperate and tropical countries (Okozie, Agyakwa, 1998). *Guiera Senegalensis*, J.F. Gmel (Family: *Combretaceae*), known in Hausa as "Sabera" is a small

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shrub with opposite or sub-opposite leaves that are characterized with scattered black glands, which is widespread in West Africa ((Hutchinson *et al.,* 1954), while *Anisopus mannii,* N.E. Brown *(*Synonyms: *Anisopus bicoronatus, Anisopus rostriferus*) is a glabrous twining shrub, known locally as "Kafi-suga" or "Nanukuna", which is a self-supporting or climbing epiphyte belonging to the family *Asclepiadacecae.* This plant may possess succulent or non-succulent anticlockwise twines.

Our survey revealed that *Guiera senegalensis*, J.F. Gmel, *Ipomoea asarifolia and Anisopus mannii,* N.E. Brown are used either individually or in combination with other herbs, for the treatment of gastrointestinal disorders and/or diabetes, respectively, in Northern Nigeria. These diseases are known to have biological oxidation as a major etiological factor (Dandona, 1996; Boynes,1991; Ames *et al.*, 1995; Collier *et al.*, 1990; Cerruti, 1994; Atawodi, 2005). However, there is little or no information in the literature on the mechanism(s) by which *Guiera senegalensis*, *Ipomoea asarifolia and Anisopus mannii* exert their therapeutic effects. Therefore, it was considered necessary to evaluate their comparative antioxidant potential to ascertain if this property is one of the underlying bases for their usefulness in folk medicine.

MATERIALS AND METHODS

Plant collection and identification

The leaves, stem and root barks of the plants were collected from Sakuru and Dumbi villages of Zaria Local Government Area in Kaduna State, Nigeria. The identities of the three plants were confirmed by the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, as *Ipomoea asarifolia* (Voucher No.6958), *Guiera senegelensis* (Voucher No.1823) and *Anisopus mannii* (Voucher No. 217).

Sample preparation and extraction

Parts of the plants (leaves, stem and roots) were collected, washed rapidly under running water and dried in open air in the laboratory. Dried materials were pounded in a laboratory mortar to a relatively fine texture with a particle size of about 0.2mm.

One gram (1.0 g) each of pulverized dry plant parts were weighed into 150 mL conical flasks and 50 mL of deionized water was added. The mixtures were then covered with aluminum foil and incubated in a water bath at 37 ^{o}C for two (2) hours, with occasional shaking. Thereafter, 10 mL of the extracts were centrifuged at 3,000 rpm for 15 minutes. The extraction was repeated in duplicate for all parts of the plants. The supernatants were transferred into clean labeled sample bottles and kept in the refrigerator at 4 ^{o}C until ready for use, usually within the same day.

Assay for antioxidant activity

The antioxidant assay utilized was based on the scavenging ability of antioxidant(s) in plant extracts towards the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which is deep purple in color, to form the corresponding hydrazine with accompanying color change to light purple or golden yellow. This color change is inversely proportional to increasing concentration of the antioxidant in the mixture.

As described by Gyamfi *et al.* (1999), the reaction tubes contained 1.0 mL of 0.1 mM DPPH-ethanol solution, 1.0 mL of ethanol (98% ethanol), 0.95 mL of 0.05 M Tris-HCl buffer (P^{H}7.4), and 50 µL of either the extract or standard (Vitamin C) solution wrapped in aluminium foil. The absorbance was measured at 517 nm exactly 30 seconds after adding each of the extracts, as loss of absorbance at this wavelength is a measure of the radical scavenging capacity of the extracts.

To appreciate the results from the DPPH method, the Ascorbic Acid Equivalent (AAEq) and "Inhibitory Concentration" or IC_{50} value (the concentration of the substrate that causes 50% inhibition of the DPPH activity) were established. The IC_{50} values were estimated by extrapolation from plots of the percent (%) antioxidant activity against the concentration/volume of the test solutions, while Ascorbic Acid Equivalent was obtained by extrapolation from the standard curve for the ascorbic acid antioxidant activity.

RESULTS

In this study, the radical scavenging capacity of three plants, namely, *Guiera Senegalensis*, J.F. Gmel, *Ipomoea asarifolia and Anisopus mannii,* N.E. Brown, were evaluated and compared using the DPPH radical scavenging method while utilizing ascorbic acid as the standard antioxidant. Thus, during the screening stage, antioxidant activity was expressed as Vitamin C equivalent. Samples with reasonable antioxidant activity were further evaluated for their 50% inhibitory concentration (IC_{50}).

Table I compares the ascorbic acid equivalent of parts of the plant under study. The Ascorbic Acid Equivalent of the stem bark (2.1mM), leaves (2.05mM) and root barks (1.89mM) of *Guiera senegalesis* were much higher than any part of *Ipomoea asarifolia* or *Anisopus*

manii plants. Consequently, the average antioxidant activity expressed as Ascorbic Acid Equivalent was highest in *Guiera senegalensis* (2.05 mM), followed by *Ipomea asarifolia* (1.7mM) and *Anisopus manii* (0.17 mM), in this order (Figure 1).

TABLE I - Ascorbic Acid Equivalent of different parts of *Guiera senegalensis, Ipomoea asarifolia* and *Anisopus mannii*

Plant	Part	IC_{50} for Extracts (µL/3 mL)	Radical scavenging activity -Vitamin C equivalent (mM)
Guiera	stem	15.4	2.10 ± 0.09
senegalensis	leaves	20.0	2.05 ± 0.14
	root	17.0	1.89 ± 0.08
Ipomoea	leaves	42.0	1.33 ± 0.06
asarifolia	root	65.0	0.52 ± 0.07
	stem	50.0	0.50 ± 0.04
Anisopus	stem	ND	0.20 ± 0.03
mannii	root	ND	0.19 ± 0.05
	leaves	ND	0.15 ± 0.06

Vitamin C Equivalent was calculated based on the triplicate analysis of two extracts per sample; ND = Not determined

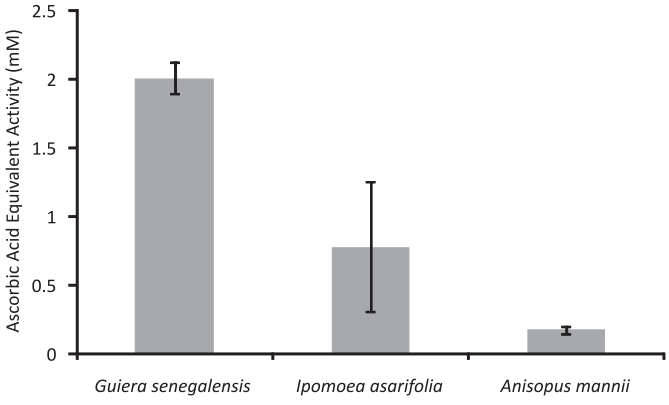

FIGURE 1 - Comparison of average antioxidant activity of all parts of *Guirea senegalensis*, *Ipomea asarifolia* and *Anisopus manii*

The IC_{50} of those plant parts that were amenable to the procedure adopted are presented in Table I. It should be noted that the lower the IC_{50} value, the stronger the antioxidant potential. Using extracts of 1 g samples boiled in 150 mL water, the stem bark, leaves and root barks of *Guiera senegalensis* had IC_{50} of 15.4, 20.0 and 17.0 µL respectively, while corresponding parts of *Ipomoea asarifolia* showed IC_{50} of 50, 42 and 65 µL, respectively (Table I). Thus, all parts of *Guiera senegalensis* possessed greater antioxidant potential than those of *Ipomoea asarifolia* and obviously, also more potential than *Anisopus mannii,* whose antioxidant activity was too low to be amenable to the IC_{50} assessment procedure adopted.

DISCUSSION

This investigation compared the *in vitro* antioxidant potential of different parts of three medicinal plants which are used locally for treatment of several ailments in the northern part of Nigeria. Of these plants, *Guiera senegalensis* showed the highest antioxidant activity followed by *Ipomoea asarifolia* and *Anisopus mannii* in this order, as evidenced from their Ascorbic Acid Equivalent values (Table I and Figure 1) as well as their IC_{50} values (Table I)

Today, medicinal plants are showing tremendous promise for preventive intervention in the pathogenesis of many diseases, as well as in their treatment (Atawodi, 2005), especially the ROS-mediated diseases such as cancer (Mantle *et al.*, 2000; Tsao *et al.*, 2004; Surh, Ferguson, 2003; Mehta, Pezzuto, 2002; Park, Pezzuto, 2002; Bravo, 1998), ulcer (Reppeto, Llesuy, 2002; Borelli, Izzo, 2000), diabetes (Sabu, Kuttan, 2002) and others (Youdim, Joseph, 2001; Perry *et al.*, 1998; Lampe, 2003; Miller, 1998). The relationship between the antioxidant compounds in plants and their effectiveness in the treatment of these diseases have been previously described (Reppeto, Llesuy, 2002; Sabu, Kuttan, 2002; Yang *et al.*, 2001; Bravo, 1998; Manach *et al.*, 2004).

Guiera senegalensis is reportedly useful in the treatment of malaria (Ancolio *et al.*,2002), fungal diseases (Silva, Gomes, 2003) and snake bite (Abubakar *et al.*, 2000), while in Northern Nigeria, the plant is also reported to be useful in the treatment of gastrointestinal disorders and skin diseases. Although oxidative stress may have little or no role to play in the etiology of these diseases, except in gastrointestinal disorders such as ulcer, the strong antioxidant potential of this plant suggests that it is plausible it may exert therapeutic efficacy in oxidative-stress related diseases for which it is not currently recognized. This strong antioxidant activity is consistent with its phytochemical constituents which include alkaloids (Ancolio *et al.*, 2002), guirenone and other polyphenols (Silva, Gomes, 2003; Le Grand, 1989). In fact, Bucar *et al.* (1989) isolated 5-methylflavesperone and rhamnetin from *Guiera senegalensis* and demonstrated that while rhamnetin strongly inhibited peroxidation of phospholipids liposomes, the former possessed little or no such activity. Similarly, using a combination of techniques such as TLC, HPLC, UV spectroscopy and NMR, the presence of catechin, myrecitrin, rutin and quercetin in *Guiera senegalensis* have been described by other researchers (Ficarra *et al.*, 1997). That these phytochemicals are potent antioxidant compounds has been well demonstrated (Bravo, 1998; Manach *et al.*, 2004).

The moderate antioxidant potential observed for *Ipomoea asarifolia* is consistent with its reported content of the antioxidant compounds, triacylated and tetraglucosylated anthocyanins (Pale *et al.,* 2003) and its use as an anti-diabetic in Northern Nigerian traditional medicine. These assertions are further supported by information in the literature which suggest that other members of the *Convolvulaceae* family, which also contain the phytochemical, anthocyanin (Philpott *et al.,* 2004) are effective agents in the management of biological radical-induced diseases and pathological processes such as diabetes (Kusano *et al.*, 2001; Ludvik *et al.*, 2004), mutagenesis (Yoshimoto *et al.*, 1999) and memory loss as well as other age-related degenerative syndromes (Cho *et al.*, 2003).

*Anisopus manni*i is an anti-diabetic herb in Northern Nigeria, but a search of the literature appears to suggest no record of its phytochemistry or medicinal importance in any other part of the world. However, this investigation indicated that all parts of the plant have poor antioxidant activity. Therefore, its medicinal efficacy, if any, must be mediated by other mechanisms other than scavenging of biological free radicals.

Based on the results obtained in the present study, it may be concluded that *Guiera senegalensis* and *Ipomoea asarifolia* possess sufficient antioxidant activity to warrant further detailed study of their pharmacology and phytochemistry.

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