

Short Communication

Antioxidant properties of *Ximenia americana*

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***Ximenia americana*, a medicinal plant, was evaluated for free radical scavenging and antioxidant activity. The extracts from the stem bark was obtained using methanol. The methanol portion was again fractionated into flavonoid and saponin portions. The antioxidant activity of the portions was assessed by the 2, 2 diphenyl-1-picryl-hydrazyl (DPPH) assay. The flavonoid portion revealed a significant ($P < 0.05$) antioxidant activity with the Rc_{50} value of 8. The study reveals that *X. americana* could be used as a potential antioxidant.**

Key words: *Ximenia americana*, flavonoid, antioxidant activity.

INTRODUCTION

There is renewed interest in the potentials of medicinal plants as antioxidants in reducing free radical-induced tissue injury (Atawodi, 2005; Pourmorad et al., 2006) and usage as antioxidant in foods and cosmetics. It has been reported that antioxidant activity of plants might be due to their phenolic compounds (Duh et al., 1999). Flavonoids are polyphenolic compounds that are ubiquitous in nature and can be categorized according to their chemical structure, into flavanols, flavones, flavanones, catechins, anthocyanidins and chalcones. They are widely distributed in plants fulfilling many functions. They have been referred to as "nature's biological response modifiers" due to strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens (Atawodi, 2005). They show anti allergic, anti-inflammatory, antimicrobial and anticancer activity (Cushine and Lamb, 2005).

They have been reported also to have free radical scavenging inhibition of hydrolytic oxidative enzyme properties (Frankel, 1999). *Ximenia americana* "Wild olive or Plum" in English or locally called "Tsada" in Hausa and "Chabbuli" in Fulani is a medicinal plant that is bushy and spiny shrub, 4 – 5 m high with an open crown. The fruits are green but turn golden yellow or red when ripe. The

fruit when eaten is refreshing and has an almond acid taste. The plant is used in traditional medicine for treatment of malaria, fever, leprotic ulcers and skin infections of mixed origin in Northern parts of Nigeria (Ogunleye and Ibitoye, 2003). Arbonnier (2004) reported the medicinal uses of *X. americana* to include treatment of fever, stiffness, onchocerciasis, sore throat, asthma and headaches. Maikai et al. (2007) reported that the stem bark has trypanocidal activity, while, Onyekwelu et al. (2000) also reported that the roots have transient antitrypanosomal activity. The roots are used for treating abdominal pains, dysentery, inflamed joints and mouth ulcers (Ake and Guinko, 1991). Phytochemical screening of the leaves revealed the presence of saponins, cyanogenic glycosides, flavonoids and tannins (Ogunleye and Ibitoye, 2003). Thus, this study examines the antioxidant activity of the extract of stem bark of *X. americana*.

MATERIALS AND METHODS

Collection of plant material

Fresh stem bark of *X. americana* was collected from Afaka village, 35 km to Kaduna and taken to Department of Biological Sciences, Ahmadu Bello University Zaria for identification the voucher No.1612. The bark was dried at room temperature before crushing into powder then stored in air tight container and kept at 4°C until needed.

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Table 1. Phytochemical screening of *Ximenia americana* methanol extracts.

Extract	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Pylobatannins	Saponnins	Tannins	Terpenoids
methanolic extract	++	+	++	++	++	+++	+++	+++
Flavonoid extract	-	-	-	+++	-	-	-	-
Saponnin extract	-	-	-	-	-	+++	-	-

+++ = highly present, ++ = moderately present, + = faintly present, - = absent.

Table 2. Antioxidant activity of *Ximenia americana* methanol extracts using DPPH assay.

Sample	RC ₅₀ value µg/ml
Ascorbic acid (Standard)	10.60 ±2.20
Methanol extract	82.50 ±3.60
Flavonoid portion	8.20 ±1.10
Saponnin portion	148.70 ±4.35

Extraction and fractionation of flavonoids and saponin from stem bark of *X. americana*

The extraction and fractionation of flavonoids from stem bark of *X. americana* was carried out as described by Won et al. (1980). The air-dried stem bark powdered material (500 g) was successively extracted at room temperature using soxhlet extraction with petroleum ether 60-80°C to exhaustion. The petroleum ether portion was concentrated at room temperature to give a semi-oily mass of (5.20 g) which was coded "PXa". The defatted marc was exhaustively extracted at room temperature with methanol (4 X 300 ml) the combine methanolic extract was then successively partitioned with ether: water 1:1 (3 X 250 ml). The water portion was again partitioned with n butanol (4 X 350ml). The butanolic portion was collected and partitioned with 1% potassium hydroxide (3 X 350 ml), the butanolic portion was collected and concentrated at room temperature to give the saponin fraction coded "SpXa". The potassium hydroxide portion was again partitioned with 2% HCl : butanol 1:1 (3 X 300 ml). The butanol portion was collected in a porcelain dish and concentrated at room temperature to give the flavonoid fraction coded "FpXa".

Phytochemical screening of the extracts

The methanolic, flavonoid and saponnin extracts were screened as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Chemicals

DPPH was obtained from sigma Aldrich co. (St. Louis, USA). The others were from the BDH London, which were of analytical grade.

Preparation of the stock solution of extract

20 mg/ml was prepared using DMSO. The stock solutions were serially diluted to obtain 20, 40, 60, 80, 100, 200 and 500 µg/ml.

Antioxidant activity (DPPH free radical scavenging activity) of the extract

The antioxidant activity of the extract and the standards was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Braca et al., 2002). Briefly, the diluted solutions were all prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 100 µg/ml. 0.002% DPPH was prepared also in methanol. 1 ml of the solution was mixed with 1 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a spectronic against methanol. The RC₅₀ value, which is the concentration at which the test sample reduces 50% of the free radical concentration, was calculated. The blank contained 1 ml of 0.002% DPPH solution. The optical density was calculated using the formula:

$$\% \text{ of inhibition of DPPH activity} = A - B/A \times 100$$

Where A = optical density of blank and B = optical density of sample.

Statistical analysis

The results were expressed as means ± SEM. Level of significance was assessed by student's t test and ANOVA.

RESULTS AND DISCUSSION

Phytochemical screening of the extracts revealed the presence of alkaloids, anthraquinones, cardiac glycoside, flavonoids, saponnins, tannins and terpenoids in the methanolic extract (Table 1). The extract exhibited some significant activity in the DPPH assay. Table 2 shows the activities of the different portions of the extract. The flavonoid portion of the extract showed antioxidant activity with RC₅₀ value of 8.20 ± 1.00 µg/ml, while the saponnin showed 186.70 ± 2.00 µg/ml and ascorbic acid 10.60 ± 1.20 µg/ml.

The result indicated that the antioxidant activity of the flavonoid portion was higher than the ascorbic acid standard. Antioxidants are important in living systems as well as in food because they prevent the formation of free radical by donating atoms or scavenging them (Ruiz-Teran et al., 2008). Oxidative stress has also been

reported in the pathology of cancer, atherosclerosis and malaria (Ruiz-Teran et al., 2008). The results showed that the stem bark of methanolic extract of *X. americana*, a medicinal plant had antioxidant activity. The result agrees with that of several workers (Maduka and Okoye, 2002; Atawodi, 2005; Suresh Kumar et al., 2008; Ruiz-Teran et al., 2008) who reported similar antioxidant activities of some medicinal plants. Ruiz-Teran et al. (2008) reported that methanolic extracts of *L. arborea*, *S. macrocarpum* contained antioxidant activity. Similarly, the results also revealed that the methanolic portion of the plant exhibited antioxidant activity. It has been reported that the antioxidant activity of plants might be due to their phenolic compounds (Duh et al., 1998; Ruiz-Teran et al., 2008) which agrees with our results; the flavonoid portion which showed the highest activity is also a phenolic compound. The study reveals that *X. americana* could be a potential source of antioxidants which could be used medicinally or in foods to prevent oxidation.

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