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Full Length Research Paper

Evaluation of bio-safety and antioxidant activity of the fruit and leaf of *Saba florida* (Benth.) from Ibaji forest

Omale James* and Omajali Jacob B

Department of Biochemistry, Kogi State University, Anyigba, Kogi State, Nigeria.

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This study aimed to determine the activity of *Saba florida* extracts against brine shrimps (*Artemia salina*) and to investigate *in vitro* antioxidant activity of the extracts as well. Extracts of the fruit pulp, fruit pericarp and leaf of the plant were subjected to bioscreening study to detect cytotoxic activity by the brine shrimp lethality bioassay. The results obtained showed that the crude alcohol extracts were relatively safe when compared with the reference potassium dichromate (LC_{50} = 296.825 µg/ml) but however, the toxicity of the extracts were increased by heating at different temperatures. The ability of the extracts in scavenging free radical was measured by DPPH reduction spectrophotometrically. In the DPPH scavenging assay, the order from strongest to the weakest was: fruit pericarp (IC_{50} = 38.115 µg/ml), leaf (IC_{50} = 46.185 µg/ml) and fruit pulp (IC_{50} = 90.782 µg/ml). On the whole, the results suggested that the plant parts investigated are relatively safe for the purposes utilized and are potent antioxidant source that could offer protection against oxidative stress.

Key words: *Saba florida*, DPPH, brine shrimps and antioxidants.

INTRODUCTION

Plants are among the most important and common sources of food and potentially valuable new drugs. There-fore, there is need to investigate the biological properties of food sources and of medicinal plants in order to develop new drugs (Alade and Irobi, 1993; Esimone et al., 2003). When screening for biologically active plant constituents, the selection of the plant species to be studied is obviously a crucial factor for the ultimate success of the investigation. Plants used as food and in traditional medicine are more likely to yield pharma-cologically active compounds (Hamburger and Hostettmann, 1991).

Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical ($^{\cdot}OH$) are often generated as by-products of biological reactions or from exogenous factors (Cerutti, 1991) . The involvement of these species in the pathogenesis of a large number of diseases including rheumatoid arthritis, atherosclerosis, skin-aging, nephritis, reperfusion injury, asthma, diabetes mellitus and carcinogenesis are well documented (Steinberg et al; 1989; Stadtman and Oliver, 1991; Stadtman, 1990; Cerutti, 1994; Freig et al., 1994; Florence; 1995; Dandona et al., 1996). A potent scavenger of these species may serve as a possible preventive intervention for free radical mediated diseases (Ames et al., 1995). In recent times therefore, the search for natural antioxidant and other preparations of plant origin to achieve this objective has been intensified.

S. florida belongs to the family *Apocynaceae.* The plant is very abundant in undisturbed forest, coastal areas and around Great Lake regions of Africa from sea level to 1250 m (Maundu et al., 1999) but rare in open areas. The plant is found in Ibaji and other parts of Kogi State, Nigeria. The fruit is edible and it makes a refreshing sour drink. The fruit does not abscise and must be harvested when it turns yellow. The stem yield latex that is an inferior rubber. Traditionally, bark decoctions are used to treat rheumatism. The leaves are used in Senegal to prepare sauces and condiments as salty appetizer. In Cote d'Ivoire, the latex is used as an adhesive for poison preparation for arrows as it hardens upon exposure. The inferior rubber produced from the latex is sometimes used to adulterate genuine rubber (FAO, 1983).

In Kogi State, the latex is used as trap for birds, rats and smaller rodents. The leaves are eaten as antidote against vomiting and the bark decoction are administered

*Corresponding author. E-mail: jamesomale123@yahoo.com. Tel: +234 -08068291727.

for diarrhea and food poison. The leaves are also used in the treatment of skin ulcer, children's headache and catarrhs .The fruit is a special delicacy for the monkeys in the forest and humans are beginning to compete with monkeys for the fruit. It appears in the local markets during the fruiting season. It is called hunters lunch fruit.

In continuation of our efforts to verify the bio-safety of Nigerian traditional foods and medicine we have collected *S. florida* from Ibaji, in Kogi State based on its usage as food and ethno pharmacological information. In order to study the toxicity of this plant we have performed brine shrimp lethality bioassay which is based on the ability to kill laboratory cultured brine shrimp (*Artemia salina)*. The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts (Sam, 1993). The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the micro-well scale. The aim of this study therefore, was to evaluate the fruit and leaf of *S. florida* commonly used as food and medicine in our local communities for toxicity and antioxidant potential.

MATERIALS AND METHODS

Collection and preparation of plant sample

The plant samples were collected from Igboigbo- Unale in Ibaji Local Government Area, Eastern part of Kogi State, Nigeria during the rainy season. Dirt was removed from the plant by rinsing in clean water. The leaves were air-dried for three weeks and pulverized using motorized blender. The fruit pulp and pericarp were dried in oven at 40°C and powdered.

Preparation of extracts

Cold extraction method was followed. Portions (30 g) of the powdered samples were weighed into conical flasks and 200 ml of pure methanol (99.9%) was added and left for 72 h. The mixtures were filtered under vacuum pressure and the filtrates were concentrated using rotary evaporator and subjected for activity studies.

Plant Identification

The plant was identified in the Botany unit of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria as *S. florida* (Benth).

Chemicals

Quercetin dehydrate, DPPH (2, 2-diphenyl-1-picrylhydrazyl) were purchased from Sigma chemical company (Sigma, Germany). Potassium dichromate, sodium chloride, sodium hydroxide and Tween 80 used were products of BDH.

Cytotoxicity bioassay

Modified method of Solis et al. (1992) was used to determine the inhibitory activity of the extracts on *A. salina* in vial bottles. Brine shrimps (*A. salina*) were hatched using brine shrimps eggs in a plastic vessel (500 ml), filled with sterile artificial sea water (prepared using NaCl salt (38 g/L) and adjusted to pH 8.5 using 40% NaOH) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were harvested from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 5 ml of brine solution. A portion (50 µl) of different concentrations of crude methanol extracts(1000, 500, 250, 125, 62.5 and 31.25 µg/ml) solution in 0.25% Tween 80-artificial sea water was added into each well (vial bottles) containing 10 newly hatched brine shrimps and then incubated at room temperature for 24 h. All samples were repeated in two wells to make the overall tested organisms of 20 for each. The living brine shrimps were counted under a hand magnifying lens. Same procedure was followed using potassium dichromate as the reference standard. Other portions of the plant samples were heated at 60 and 100°C respectively for one hour and cooled to determine the effect of temperature on their toxicity.

Plot of % lethality versus log concentration, substituted Y = 50 in the resulted linear equation to obtain the X value. The antilog of X was then the LC_{50} (concentration of 50% lethality) value (Ballantyne et al., 1995).

Free radical scavenging activity determination

The stable 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was used for the determination of free radical-scavenging activity of the extracts. The modified method of Blois (1985) was employed. A portion (1 ml) each of the different concentrations (1000, 500, 250, 125, 62.5, 31.25 µg/ml) of extracts or standard (Quercetin) in test tubes was added 1 ml of 0.3 mM DPPH in methanol. The mixtures were vortexed and then incubated in a dark chamber for 30 min after which the absorbencies were 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:

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

IC_{50} values denote the concentration of sample which is required to scavenge 50% DPPH free radicals.

RESULTS AND DISCUSSION

Table 1 shows the DPPH radical scavenging action of *S. florida* parts. The values of the 50% inhibition concentration (IC_{50}) of the *S. florida* extracts for the DPPH radicals were 38.115, 46.185 and 90.782 µg/ml for the fruit pericarp, leaf and fruit pulp respectively. From Table 1, it was evident that the antioxidant property of the fruit pericarp was more than other parts.

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of these diseases (Davasagayam et al., 2004) . DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts (Koleva et al., 2002). The radical scavenging activity in the plant extracts decreased in the following order: Fruit pericarp > leaf > fruit pulp. The fruit pericarp scavenges DPPH free

Table 1. DPPH radical scavenging activity of *S. florida* crude methanol extracts.

Sample	Concentration (µg/ml)	Log concentration	% Scavenging activity	IC_{50} (µg/ml)
Crude methanol pulp extract	1000	3.0000	92.11	90.782[a]
	500	2.6990	81.94	
	250	2.3979	74.01	
	125	2.0969	45.64	
	62.5	1.7959	41.50	
	31.25	1.4949	35.77	
Leaf extract	1000	3.0000	94.95	46.185[b]
	500	2.6990	94.61	
	250	2.3979	82.47	
	125	2.0969	63.77	
	62.5	1.7959	51.59	
	31.25	1.4949	44.84	
Pericarp extract	1000	3.0000	98.71	38.115[c]
	500	2.6990	93.63	
	250	2.3979	82.51	
	125	2.0969	57.34	
	62.5	1.7959	56.68	
	31.25	1.4949	51.78	
Quercetin	1000	3.0000	97.27	38.859[d]
	500	2.6990	96.85	
	250	2.3979	96.39	
	125	2.0969	92.79	
	62.5	1.7959	44.39	
	31.25	1.4949	39.98	

a) Linear equation: y = 40.945x - 30.193. (b) y = 37.805x - 12.927. (c) y = 35.182x - 5.628. (d) y = 42.46x - 17.49.

radicals more than the reference standard- quercetin and by this investigation the most potent antioxidant part of plant.

The generation of the reactive oxygen species (ROS) beyond what the ability of the body can cope with leads to oxidative stress (Gutteridge and Haliwell, 1994; Maxwell, 1995) and can lead to tissue damages and necrosis in many instances (Prasad et al., 1999). Furthermore, oxidative stress plays an important role in malaria (Kremser et al., 2000; Kulkarni et al., 2003). The immune system of the body is stimulated by infection, including malaria, thereby causing the release of reactive oxygen species. In addition, the malaria parasite also stimulates certain cells to produce reactive oxygen species, resulting in hemoglobin degradation (Das and Nanada, 1999; Loria et al., 1999). The antioxidant property of some plants may be responsible for malaria therapy (Ayoola et al., 2008). The implication of this is that *S. florida* which has antioxidant property could play a key role in malaria therapy and management. From the results obtained in this work, this plant sample is a probable potent source of antioxidant that could be used to mop up free radicals in oxidative stress condition. The vitamins A and E composition of this plant (Omale and Omajali, 2010) could be responsible for the antioxidant activity observed in this screening. The model of scavenging DPPH free radicals used in the rapid screening method commonly employed for evaluating antioxidant activities is based on their ability to donate hydrogen ion (Kumazawa et al., 2002). The DPPH is a free radical stable at room temperature. The methanol solution gives a purple coloration which when reduced by antioxidant molecule gives rise to a yellow solution. The low vitamin E composition of the pulp extract (Omale and Omajali, 2010) may account for the weak antioxidant activity observed.

Bio-safety screening

Based on the 24 h exposure results obtained from *S. florida* extracts on brine shrimps, the LC_{50} values of crude methanol *S. florida* extract of fruit pulp, leaf and fruit pericarp were 10076.275, 121310.949 and 10078.595 µg/ml respectively. The LC_{50} of the reference standard was lower (296.825 µg/ml) as seen in Table 2. The leaf extract showed the lowest toxicity when compared with the reference standard (Table 2). The extracts studied in this work showed low lethality against brine shrimp, which has been successfully used as a simple biological test to

Table 2. Inhibitory effect on brine shrimps of *S. florida* crude methanol extracts.

Sample	Concentration (µg/ml)	Log concentration	% Lethality	LC_{50} (µg/ml)
Crude methanol pulp extract	1000	3.0000	40	10076.275[a]
	500	2.6990	30	
	250	2.3979	20	
	125	2.0969	20	
	62.5	1.7959	10	
	31.25	1.4949	20	
Leaf extract	1000	3.0000	20	121310.949[b]
	500	2.6990	20	
	250	2.3979	30	
	125	2.0969	10	
	62.5	1.7959	0	
	31.25	1.4949	10	
Pericarp extract	1000	3.0000	40	10078.595[c]
	500	2.6990	20	
	250	2.3979	20	
	125	2.0969	10	
	62.5	1.7959	10	
	31.25	1.4949	10	
Potassium dichromate	1000	3.0000	70	296.825[d]
	500	2.6990	70	
	250	2.3979	40	
	125	2.0969	40	
	62.5	1.7959	30	
	31.25	1.4949	10	

a) Linear equation: y = 15.187x- 10.798. b) Linear equation: y = 12.339x - 12.73. c) Linear equation: y = 18.034x - 22.197. d) Linear equation: y = 37.017x- 41.526.

guide the fractionation process of plant extracts in order to detect antitumor compounds (McLaughlin et al., 1993). These plant extracts may not be probable anti-tumor source due to low toxicity at room temperature. The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties (McLaughlin et al., 1993).

The potency or antitumor effect was well exhibited when the plant parts were heated at 60 and 100°C respectively and cooled before testing on the brine shrimps than the extract that was not heated (Tables 2 and 3). Heating the plant extracts at elevated temperatures (and cooled) could induce anti-tumor activity as more of the biological organisms (Brine shrimps) were killed when tested on them. The degree of lethality was found to be directly proportional to the concentration of the extracts. Maximum deaths took place at a concentration of 1000 µg/ml whereas least mortalities were at 31.25 µg/ml concentration. The LC_{50} values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii killed against the log concentration of the extracts and the best -fit line was obtained from the data by means of regression analysis. The significant lethality of the extracts to the brine shrimps when heated is an indication of the presence of potent cytotoxic components which are heat sensitive and that calls for further investigation. At room temperature the plant extracts are relatively safe as seen on Table 2.

Conclusion

This study provided evidence that the crude methanol extracts are potential source of antioxidant and may be favorably disposed for the treatment of diseases caused by ROS. Characterization of the active components of *S. florida* with radical scavenging action is needed which may be our next line of action. In this study also, the brine shrimp lethality assay really has proven to be a convenient system for monitoring biological activities of plant which are used as food and medicine in our community and has also provided some biochemical basis for the ethno medicinal uses of *S. florida*. However, further and more specific bioassays are necessary in order to confirm this conclusion.

Table 3. Effect of temperature on the toxicity of *S. florida* extracts.

Sample	Concentration(µg/ml)	Log concentration	% Lethality	LC_{50} (µg/ml)
Pulp extract Heated at 60°C	1000	3.0000	80	149.000[a]
	500	2.6990	60	
	250	2.3979	80	
	125	2.0969	40	
	62.5	1.7959	40	
	31.25	1.4949	20	
Pulp extract heated at 100°C	1000	3.0000	80	138.989[b]
	500	2.6990	80	
	250	2.3979	80	
	125	2.0969	60	
	62.5	1.7959	60	
	31.25	1.4949	40	
Leaf extract heated at 60°C	1000	3.0000	60	368.859[c]
	500	2.6990	60	
	250	2.3979	40	
	125	2.0969	40	
	62.5	1.7959	20	
	31.25	1.4949	40	
Leaf extract heated at 100°C	1000	3.0000	80	27.376[d]
	500	2.6990	80	
	250	2.3979	80	
	125	2.0969	80	
	62.5	1.7959	60	
	31.25	1.4949	40	
Pericarp extract heated at 60°C	1000	3.0000	60	106.902[e]
	500	2.6990	60	
	250	2.3979	60	
	125	2.0969	60	
	62.5	1.7959	40	
	31.25	1.4949	40	
Pericarp extract heated at 100°C	1000	3.0000	100	16.396[f]
	500	2.6990	100	
	250	2.3979	100	
	125	2.0969	80	
	62.5	1.7959	60	
	31.25	1.4949	60	

a) Linear equations: y = 37.69x - 31.99. b) y = 26.57x + 6.939. c) y = 20.88x - 3.596. d) y = 24.67x + 14.54. e) y = 15.18x + 19.20. f) y = 32.27x + 10.80.

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