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Chemical composition and antioxidant capacity of the leaf extract of *Justicia insularis*

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The leaves of *Justicia insularis* have been generating a lot of interest among the Yoruba people in the Southern Nigeria. They provide useful source of human nutrition and medicinal value. In this study, *J. insularis* leaves were harvested, processed and analysed to determine their chemical compositions and also to investigate their antioxidant properties. The proximate analysis showed that the sample contained 15.955% ash, 4.48% crude protein, 2.144% lipid and 18.367MJ/kg energy. The contents of calcium, potassium, phosphorous and magnesium were 3.30, 1.68, 0.11, and 0.76, respectively, while the amounts of sodium, manganese, iron, copper and zinc were 631.04, 334.02, 4490.08, 6.31 and 49.72 mg/g, respectively. The free radical scavenging activity of ethanol extract of *J. insularis* was determined by three methods namely: scavenging effect on 2,2-diphenyl-1-picryhydrazyl radical (DPPH), hydroxyl radical and peroxide oxidation. Comparison of the results obtained with the three antioxidant standards used in the assay revealed that the ethanol extract of the plant possessed antioxidant activity. The results obtained revealed high value of iron in the leaf and free radical scavenging properties indicating the presence of primary antioxidants in the plant.

Key words: Antioxidants, chemical composition, free radical, *Justicia insularis.*

INTRODUCTION

Most tropical African countries are blessed with diversity of food stuffs which play a basic role in nutrition and healthy body development. Unfortunately, millions of people in developing countries still suffer from malnutrition especially infants and children of rural areas (WHF, 2005). Malnutrition can be tremendously reduced by consuming the foods that are rich in energy, proteins, iron and vitamins, most especially those from the rural environment (Richard et al., 2007). In order to have a healthy population the relation between food, nutrition and health should be reinforced (Atasie et al., 2009). One way of achieving this is through the exploitation of available local resources such as local indigenous vegetable, since human population in Africa largely depend on edible indigenous vegetables to supplement their minerals and vitamins (Achu et al., 2005).

Numerous vegetables, crops, spices and medicinal herbs have been studied in an effort to identify new and potentially useful antioxidants (Vinson et al., 1998; Ganthavorn and Hughes, 1997; Jitoe et al., 1992; Zheng and Wang 2001). More recently, it has become evident that phenolic natural products may reduce oxidative stress by indirect antioxidant action. For example, various flavonoids, which are found naturally in fruits, vegetables

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and some beverages, have been demonstrated to exert antioxidant effects through a number of different mechanisms (Nijveldt et al., 2001). Knowledge of nutritional and medicinal status of local soup ingredients and food stuffs is important in order to encourage their cultivation and consumption. *Justicia insularis* is an annual or perennial herb up to 2 m tall. Stems are angular, basal part often swollen with aerial root. Leaves are decussately opposite. This herbaceous plant occurs in a wide range of habitats from moist forest to dry savannah regions (Enoch et al., 2009). It is used to make vegetable soup or eaten cooked as spinach. In Western Cameroon, it is added to groundnut soup, while extracts of the boiled leaves are given to babies to loosen their bowels and the leaves are applied to wounds to promote healing in Togoland and North East Ghana (Hepper, 1965).

The neglect of some local vegetables coupled with the growing reduction in their consumption prompted this research.

Therefore, the aim of this work is to assess the chemical composition and antioxidant properties of *J. insularis* in order to ascertain its nutritional suitability as well as health benefits.

MATERIALS AND METHODS

Sampling and sample preparation

Fresh samples of *J. insularis* were collected from different locations at Federal College of Education (Special), Oyo and referenced at the Forest Research Institute of Nigeria Ibadan, Oyo State. The samples were washed with clean water and air-dried for 2 weeks. The dried samples were ground into fine powder with electric grinder and stored in a well labelled air tight polythene bag for further analysis.

Proximate analysis

Crude fat was extracted by the Soxhlet method with petroleum ether (40 to 60°C) for 8 h. Crude nitrogen was determined based on the Kjeldhal procedure and crude protein value was obtained by multiplying the nitrogen value by a factor of 6.25. These, as well as energy and total ash content were estimated as described by the Association of Official Analytical Chemistry (AOAC, 1990).

Mineral analysis

The sample was digested into solution by wet digestion using a mixture of conc. Nitric, perchloric and sulphuric acids in the ratio 9:2:1, respectively. Fe, Zn, Cr, Co, Mg, Ca, Cu, Mn and Pb were determined by Atomic absorption spectroscopy (AAS), Na and K were determined using Atomic Emission Spectrometer and colorimetric method was used to determine phosphorus.

Antioxidant activities

Free radical scavenging assay

The free radical scavenging activity of ethanol extract of *J. insularis* was measure by 2,2-diphenyl-1-picryhydrazyl radical (DPPH) according to Blois method (Blois, 1958). An aliquot of DPPH (1 ml, 0.1 mM) solution in methanol was added to 3 ml of ethanol extract of *J. insularis* (100 µg/ml) in water, shaken vigorously, allowed to stand for 10 min in the dark and the absorbance was measured at 517 nm in an ultraviolet (UV)-visible spectrophotometer. Gallic acid was used as positive control. The percentage DPPH scavenging effect was calculated as follows:

(%) $A_{cont} - A_{test}/A_{cont}$

Hydroxyl radical scavenging assay

The ability of *J. insularis* to scavenge OH was determined by the method described by Kunchandy and Rao (1990) 1 ml of the assay mixture (containing 100 µl of 2-deoxyl-D-ribose, 500 µl of the extract, 200 µl EDTA and 200 µl of $FeCl_3$, 100 µl of H_2O_2 and 100 µl of ascorbic acid), was incubated at 37°C for 1 h. 1 ml of thiobarbituric acid and 1 ml of trichloroacetic acid were added to the mixture after an hour of incubation and incubated again at 100°C for 20 min. After cooling, absorbance was taken at 532 nm against a blank sample. Gallic acid was used as positive control.

Scavenging of hydrogen peroxide

The ability of *J. insularis* to scavenge H_2O_2 was determined according to the method of Ruch (Ruch et al., 1989). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4) and the concentration of H_2O_2 was determined spectrophotometrically by measuring the absorbance at 230 nm. Ascorbic acid was used as positive control.

RESULTS AND DISCUSSION

Proximate composition

The results of proximate composition of *J. insularis* are presented in Table 1. The results revealed high ash content (15.955%) when compared to that reported in ten green leafy vegetables in South-Western Nigeria (0.2 to 3.9 g/100 g) (Olaiya and Adebisi, 2010). High ash content indicates the level of the mineral deposit in the plant materials. From the result, the value of fat was observed to be 2.144%. This value is similar to percentage fat reported in ten green leafy vegetables in South-Western Nigeria. The low value of fat recorded showed that *J. insularis* can be recommended as a weight reducing diet since low fat food reduces the level of cholesterol and obesity (Gordon and Kessel, 2002). The protein content of the sample (4.48%) was found to be similar to values observed in ten green leafy vegetables in South-Western Nigeria. Although *J. insularis* have low protein concentration it can still serve as a source of protein considering the level of protein deficiency in the society. The energy value (18.367 MJ/kg) obtained was lower than some common green vegetables in South-Western Nigeria (Gordon and Kessel, 2002). The results of the proximate analysis revealed that *J. insularis* is nutritious when compared to other vegetables consumed in Nigeria.

Table 1. Proximate composition of *Justicia insularis.*

Parameter	Concentration percent
Ash	15.955
Protein	4.48
Fat	2.144
Energy	18.367 MJ/kg

Table 2. Mineral composition of *Justicia insularis*.

Element	Concentration percent
Calcium	3.30
Magnesium	0.76
Potassium	1.68
Phosphorous	0.11
Sodium	631.04 mg/g
Manganese	334.02 mg/g
Iron	4.490.08 mg/g
Copper	6.31 mg/g
Zinc	49.72 mg/g

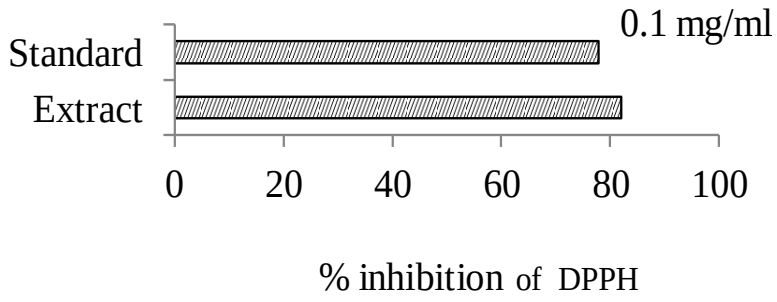

Figure 1. Scavenging activity of ethanol extract.

Mineral composition

The mineral constituents of *J. insularis* are shown in Table 2. The contents of calcium, magnesium, potassium and phosphorus were 3.30, 0.76, 1.68 and 0.11%, respectively. These values, except that of calcium, are low compared to those reported in the ten green leafy vegetables in South-Western Nigeria. The iron, sodium, manganese, and zinc contents of the sample were observed to be very high compared with those reported in the ten green leafy vegetables in South-Western Nigeria. Consequently, the need for supplementary diet rich in K^+ (potassium) and Mg^{2+} (magnesium) to avoid metal deficiency syndrome. Distorted enzymatic activity and poor electrolyte balance of the blood fluid are related to inadequate K^+ and Mg^{2+} and as they among the most required elements of living cells (National research council, 1993).

2,2-Diphenyl-1-picryhydrazyl radical (DPPH) radical scavenging activity

The free radical scavenging activity of ethanol extract of *J. insularis* against the standard gallic acid is provided in Figure 1. The *J. insularis* extract gave DPPH scavenging activity (82.1%), which was higher than that obtained for the standard (77.9%). Thus *J. insularis* seem to be a good source of natural antioxidants.

Hydroxyl radical scavenging activity

The results are shown as an inhibition rate in Figure 2. *J.*

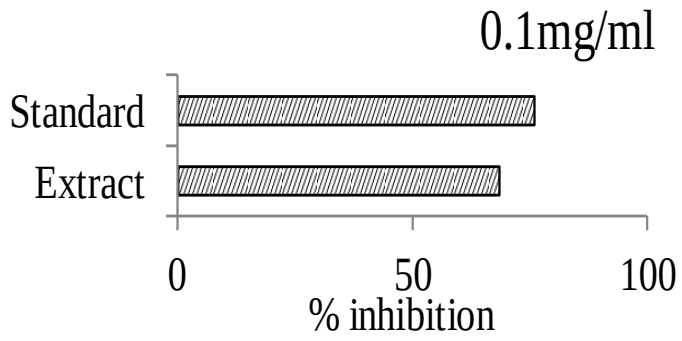

Figure 2. Inhibition rate.

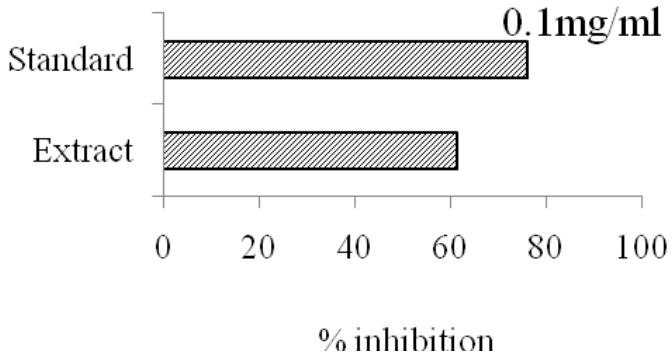

Figure 3. Lower H_2O_2 scavenging activity.

insularis exhibited the lower inhibition of 68.4% but standard gallic acid showed higher inhibition of 75.9%.

Scavenging of hydrogen peroxide

The ability of *J. insularis* to scavenge H_2O_2 was determined according to the method of Ruch et al. (1989). Figure 3 indicates that *J. insularis* exhibits a lower H_2O_2 scavenging activity of 61.5% compare to the standard of ascorbic acid whose scavenging effect is 76.3%.

Conclusion

This study has revealed that *J. insularis* consumed in Southern part of Nigeria can contribute useful amount of mineral elements to human diet. However, the protein and the fat contents of this vegetable are not enough to satisfy the recommended dietary allowances (RDAs) for these macronutrients. Extract from *J. insularis* showed antioxidant properties were similar to antioxidant properties of the standards used. There are few reports on the antioxidant capacity of *J. insularis* and the mechanism of *J. insularis* as antioxidative agents is still not fully understood. Hence, further research is recommended to analyze and isolate the active compounds responsible for the antioxidant activity from *J. insularis*

Conflict of Interest

The authors have not declared any conflict of interest.

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