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Full Length Research Paper

Assessing the polyphenolic, nutritive and biological activities of acetone, methanol and aqueous extracts of *Rumex sagittatus* Thunb.

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The nutritional, phytochemical and antioxidant activities of acetone, methanol and aqueous extracts of the leaves of *Rumex sagittatus* Thunb. were investigated using standard analytical methods in order to assess the therapeutical potential of the leaves of this plant. The proximate analysis showed that the leaves contained appreciable amount of ash, crude protein, lipids, fibre and carbohydrates. Elemental analysis in mg/100 g (DW) indicated that the leaves contained appreciable sodium, potassium, calcium, magnesium, iron, zinc, phosphorus, copper, manganese and nitrogen. The chemical composition in mg/100 g (DW) showed the presence of alkaloid, saponins and phytate. The extracts also exhibited DPPH and ABTS radical scavenging activities, which were comparable to that of ascorbic acid and BHT. Extracts also contained appreciable levels of polyphenolic compounds. In comparing the nutrient and chemical constituents with recommended dietary allowance (RDA) values, the results reveal that the leaves contain an appreciable amount of nutrients, minerals, and phytochemicals and low levels of toxicants.

Key words: Antioxidant activities, *Rumex sagittatus,* nutritional value.

INTRODUCTION

Rumex sagittatus Thunb. (Polygonaceae) belong to a genus of about 200 species. The plant is native to southern Africa, where it occurs from Malawi and Zambia (south) to South Africa. It has become naturalized in many parts of Australia near urban areas, from Queensland to eastern Tasmania and warmer locales in New Zealand (Arnold and De Wets, 1993; Wolf, 1999).

The docks and sorrels, genus *Rumex* L., are a genus of about 200 species of annual, biennial and perennial herbs in the buckwheat family Polygonaceae. Members of this family are very common perennial herbs growing in acidic, sour soils mainly in the northern hemisphere, but have been introduced almost everywhere. Many are nuisance weeds (and are sometimes called dockweed or dock weed), but some (notably the Common Sorrel, *Rumex acetosa*) have edible leaves, used in soups and salads. The leaves of most species contain oxalic acid and tannin and many have astringent and slightly purgetive qualities. In Western Europe, dock leaves are a traditional remedy for the sting of nettles, and suitable larger docks such as *Rumex obtusifolia* often grown conveniently in similar habitats as compared to *Urtica dioica*. It has been shown that dock leaf has high levels of chlorphenamine, a powerful antihistamine, finally, providing hard evidence for the medicative power of *R. patientia* (Bicker t al., 2009).

Many workers have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries (Lockeett et al., 2000; Akindahunsi and Salawu, 2005; Edeoga et al., 2006). The consumption of vegetables particularly plants containing functional phytochemicals has also been linked to reduction in the incidence of oxidative-stress-related diseases (Akubugwo et al., 2007).

New developments in biopharmaceuticals point to the involvement of reactive oxygen species (ROS) in many diseases (Behera et al., 2006; Adedapo et al., 2008). Free radicals attack the unsaturated fatty acids in the biomembranes resulting in membrane fluidity (Dean and

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David, 1993). Free radicals have been implicated in the causation of several degenerative diseases and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Di Matteo and Esposito, 2003; Behera et al., 2006). Plants containing flavonoids have been reported to possess strong antioxidant properties (Raj and Shalini, 1999; Badami et al., 2003; Behera et al., 2006; Tripathi et al., 2007).

It is surprising that few indigenous vegetables species were consumed in South Africa by the general populace. The interview conducted during the course of our research in Alice and its surrounding villages indicated that many of these species, though known, are considered as weeds and were not eaten by the people. This is in spite of the fact that these vegetables grow spontaneously and in abundance around the rural homesteads. *R. sagittatus* was one of the wild vegetables identified and the study was therefore, aimed at assessing its nutritional quality as well as antioxidant activities of the some extracts of the plant.

MATERIALS AND METHODS

Plant collection and extract preparation

Fresh leaves of *R. sagittatus* were collected in November 2006 around the University of Fort Hare campus Alice, South Africa (30°00-34° 15´S; 22° 45´ -30° 15´E). These areas consist of villages, which are generally classified as rural and poor. Professor D. Grierson of the Department of Botany, University of Fort Hare, authenticated the species. A voucher specimen was prepared and deposited in the herbarium of the Department of Botany (Jimoh Med. 2006/710B). The plant material was allowed to air-dry at ambient temperature (±24˚C) and then milled. Twenty grams per sample was extracted from 200 ml of acetone, methanol, and water, respectively, with stirring at ambient temperature for 18 to 24 h. Each extract was filtered using Whatman no. 1 filter paper and concentrated under reduced pressure to dryness below 40˚C. The aqueous extract was freeze-dried. The extract yields (w/w) were acetone (3.2%), methanol (8.9%), and aqueous (9.3%). The dried extracts thus obtained were used directly for the determination of the antioxidant activities (Taylor et al., 1996).

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2 -azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3-(2-pyridyl)-5,6-diphenyl- 1,2,4-triazine-4 ,4 -disulfonic acid, potassium ferricyanide; catechin, butylated hydroxytoluene (BHT), ascorbic acid, catechin, tannic acid, quercetin, TPTZ and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA)., vanillin from BDH; Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.

Determination of total phenolics

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40˚C for color development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-VS spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content were expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where x is the absorbance and y is the tannic acid equivalent (mg/g).

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordoñez et al. (2006). To 0.5 ml of sample, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) equivalents using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x is the absorbance and y is the quercetin equivalent (mg/g).

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran (2006). To 2.0 ml of sample (standard), 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h at 20˚C. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin (mg/g) equivalents using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x is the absorbance and y is the quercetin equivalent (mg/g).

The quantitation of total proanthocyanidins

The quantitation of proanthocyanidin was based on the procedure reported by Sun et al. (1998) . A volume of 0.5 ml of 0.1 mg/ml of extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total proanthocyanidin content were expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and y is the catechin equivalent (mg/g).

Determination of antioxidant activity

DPPH radical scavenging assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2003). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02 to 0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$ where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample extract /standard.

ABTS $^{+}$ radical scavenging assay

The method of Re et al. (1999) was adopted for ABTS $^{+}$ radical scavenging assay. The stock solutions included 7 mM ABTS $^{+}$ solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS $^{+}$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS $^{+}$ solution was prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS $^{+}$ solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS $^{+}$ scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as ABTS $^{+}$ radical scavenging activity (%) = [($Abs_{control}$ - Abs_{sample})]/($Abs_{control}$)] × 100, where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract /standard.

Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before use. Plant extracts (150 l) were allowed to react with 2850 l of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 M $FeSO_4$. Results are expressed in M Fe (II)/g dry mass and compared with that of BHT, ascorbic acid and catechin.

Proximate analysis

The proximate analysis (carbohydrates, fats, proteins, moisture and ash) of the plant sample was determined by using AOAC methods. Carbohydrate was determined by difference method [100 - (Protein +Fats +moisture +ash)]. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method. The nitrogen value was converted to protein by multiplying to a factor of 6.25. The moisture and ash were determined using weight difference method, while determination of crude lipid content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 - 60°C). All the proximate values were reported in percentage (AOAC, 1999; Okwu and Morah, 2004).

Mineral analysis

The automated procedure for determining cations in the plant materials utilizes the reaction between a particular cation and molybdovanate to form a complex. The complex is then measured colorimetrically at 420 nm. The elements comprising sodium, calcium, potassium, magnesium, iron, zinc, copper, manganese, nitrogen and phosphorus were determined in this way (Coomes, 1997). Macro and micronutrients were determined using Perkin Elmer; Analyst 700, single beam atomic absorption spectrometer and the data were obtained in parts per million (ppm), (1 ppm = 1 mg/kg). Calibration curve was established using working standards for each element. Laboratory procedures for the preparation and determination of macro and micronutrients were used as outlined by Shah et al. (2009) for plant samples.

Analysis of secondary metabolites

The alkaloid content of the leaves of the plant was determined gravimetrically (Haborne, 1973) . Briefly, 5 g of plant sample was weighed and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 h before it was filtered. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60°C for 30 min, transferred into desiccators to cool and then reweighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed. For the determination of saponins, 20 g of plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven into a constant weight. The saponin content was calculated in percentage (Nahapetian and Bassiri, 1975; Obadoni and Ochuko, 2001). Phytate was estimated using the method of Haug and Lentzsch (1983). The finely ground plant sample was extracted with 0.2N HCl by shaking at room temperature. Phytic acid in the extract was determined colometrically.

RESULTS

The methanol extract caused the highest DPPH radical scavenging activity (Figure 1), while the acetone extract caused the highest ABTS $^{+}$ radical scavenging inhibition (Figure 2). Table 1 shows that the methanol and acetone extracts of the plant had higher content of total polyphenol, flavonoids, total flavonols and proanthocyanidins than those of aqueous extract, while Table 2 indicates that the ferrous reducing antioxidant power (FRAP) values of the methanol extract is higher than those of other extracts.

The proximate analysis showed the percentage moisture content, ash content, crude protein, crude lipid, crude fiber, calorie values as well as carbohydrate content of the leaves of *R. sagittatus* (Table 3). Elemental analysis also indicated that the leaves of *R. sagittatus* contained sodium, potassium, calcium, magnesium, iron, zinc, phosphorus, copper, manganese and nitrogen (Table 4). Table 5 shows that alkaloid, saponins and phytate were some of the secondary metabolites present in the leaves of the plant.

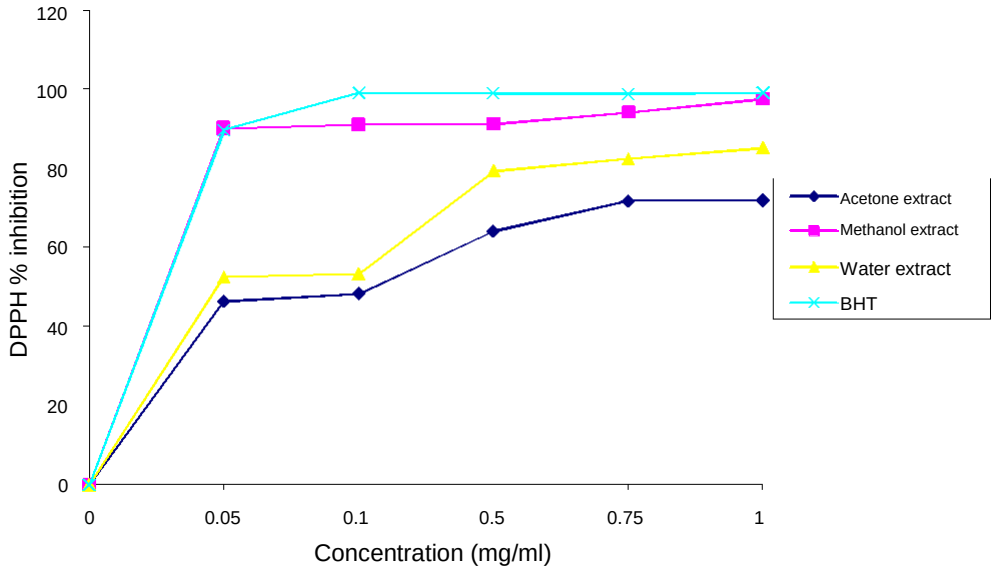

Figure 1. DPPH radical scavenging activity of the extracts of *R. sagittatus*.

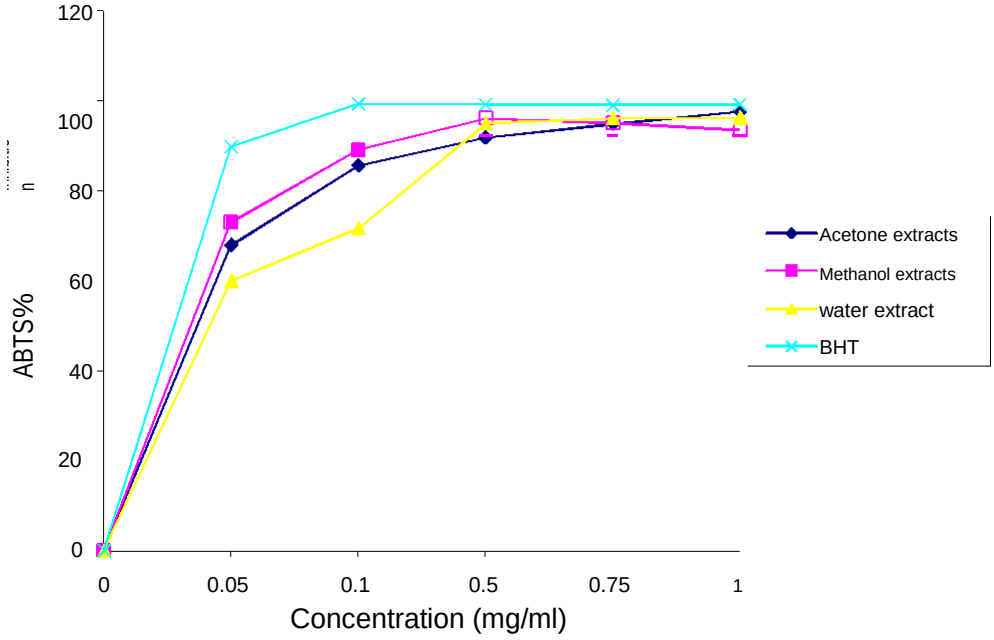

Figure 2. ABTS radical scavenging activity of the extracts of *R. sagittatus*.

DISCUSSION

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Yu et al., 2002). The DPPH radical scavenging abilities of the methanol extract of *R. sagittatus* was slightly less than that of ascorbic acid and BHT, showing that the extract has the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary anti-oxidants.

Proton radical scavenging is an important attribute of antioxidants. $ABTS^{+}$, a protonated radical, has characteristic absorbance maxima at 734 nm, which decreases with the scavenging of the proton radicals (Yoshida and Takagi, 1999). Higher concentrations of the extracts were more effective in quenching free radicals in the system. Since the extracts of the plant produced similar or equal ABTS radical scavenging activity, this

Table 1. Polyphenol contents of the acetone, methanol and water extracts of the leaves of *R. sagittatus* (n= 3, X ± SEM).

Phenolics	Acetone	Methanol	Water
Total polyphenol [a]	12.3 ± 0.7	26.8 ± 1.1	3.5± 0.3
Flavonoids [b]	1.3 ±0.1	1.7 ± 0.1	0.5± 0.01
Proanthocyanidins [d]	3.1 ± 6.1	5.6 ± 0.4	1.8 ± 0.3
Total Flavonol [c]	0.8 ± 0.1	0.9 ± 0.01	-

[a]Expressed as mg tannic acid/g of dry plant material.
[b,c,d]Expressed as mg quercetin/g of dry plant material.
*indicates that this value is significantly different from the other at P<0.05.

Table 2. FRAP activity of the acetone, methanol and water extracts of *R. sagittatus.*

Extracts	FRAP [e]
Acetone	384.64± 48.28
Methanol	707.26± 54.36
Water	47.88± 1.21
Ascorbic acid	1632.1± 16.95
BHT	63.46± 2.49
Catechin	972.02 ± 0.61
Quercetin	3107.29 ± 31.28

[e]Expressed in units of µmol Fe (II)/g.

Table 3. Proximate analysis of the leaves of *R. sagittatus.*

Constituents	Value
Moisture	89.85 ± 0.89
Ash	16.75 ± 0.25
Protein	18.0 ± 0.16
Fat	4.25 ± 0.25
Carbohydrate	43.94 ± 0.23
Crude fiber	17.06 ± 2.64
Energy (kcal)	286.01 ± 0.32

Table 4. Macro and micro elements constituents of the leaves of *R. sagittatus.*

Macro and Micro elements (mg/100g dwb)	Value
Magnésium	0.703
Calcium	2.309
Potassium	3.0
Phosphorus	0.381
Sodium	0.177
Iron (ppm)	441
Zinc	39
Copper	14
Manganese	80
Total Khedjal nitrogen	2.88

Table 5. Analysis of the secondary metabolites of *R. sagittatus*

Anti-nutrients	Value
Alkaloids	0.53± 0.03
Saponins	5.5± 0.2
Phytate	5.84± 0.24

plant could be said to possess anti-oxidant activity. The scavenging of the ABTS radical by the extracts at 1 mg/ml was found to be slightly higher than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (Zheng and Wang, 2001). Wang et al. (1998) found that some compounds, which have ABTS scavenging activity, did not show DPPH scavenging activity.

Polyphenols are major plant compounds with antioxidant activity, which play an important role in quenching reactive oxygen species (Wichi, 1988). Polyphenolics are important components of *R. sagittatus* and some of their pharmacological effects could be attributed to the presence of these valuable constituents. The results from this study showed that the methanol extract had higher polyphenolic contents than the other extracts and could thus be more effective as an anti-oxidant.

The antioxidant potential of the extracts of *R. sagittatus* leaves was evaluated further by their ability to reduce TPTZ-Fe (III) to TPTZ-Fe (II). According to Wolfe et al. (2003), a highly positive relationship between total phenols and antioxidant activity tends to appear in many plant species. FRAP activity was highest in the methanol extract followed by acetone and least in water extract in this plant.

Leaves of *R. sagittatus* contained a high amount of water. This is within the reported range in some Nigerian green leafy vegetables (Akubugwo et al., 2007) . Ash content, which is an index of mineral contents in biota, compared favorably with *Ipomea batatas* Poir, *Vernonia colorata (*Willd.) Drake and *Moringa oleifera* Lam (Lockeett et al., 2000; Antia et al., 2006). The crude protein content of *R. sagittatus* is higher than protein

content of *Momordica foetida* Schumach leaves consumed in Nigeria and Swaziland (Ogle and Grivetti, 1985; Isong and Idiong, 1997; Hassan and Umar, 2006). According to Pearson (1976), plant foods that provide more than 12% of its caloric value from protein is considered a good source of protein. Therefore, the protein content of the leaves of *R. sagittatus* might contribute in meeting the protein requirements of the local people.

The crude fiber content of *R. sagittatus* is high when compared to *I. batatas* Poir, *T. triangulare* (Jacq.) Willd, *Corchorus olitorius* L, and *Vernonia amygdalina* Del (Akindahunsi and Salawu, 2005; Antia et al., 2006). Adequate intake of dietary fiber can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Rao and Newmark, 1998; Ishida et al., 2000). The relatively high level of ash, lipid, carbohydrate and others in this plant is attributed to its nutrient values.

The Na^+/K^+ ratio in the body is of great concern for the prevention of high blood pressure. Na/K ratio less than one is recommended (FND, 2002). Therefore, consumption of *R. sagittatus* would probably reduce high blood pressure and related diseases because their Na^+/K^+ are less than one. The iron content of the leaves of *R. sagittatus* is very high when compared with *I. batatas* Poir (Antia et al., 2006). Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats (Adeyeye and Otokiti, 1999; Akubugwo et al., 2007). The Zinc content of *R. sagittatus* was favorably compared to most values reported for green leafy vegetables (Ibrahim et al., 2001; Hassan and Umar, 2006).

Analysis of the secondary metabolites present in the plant showed that its alkaloid level is lower than the values reported for the leafy vegetables like *Aspilia africana* (Pers.) C.D. Adams, *Bryophyllum pinnatum* Lam, *Cleome rutidosperma* D.C. and *Emilia coccinea* (Sims) G. Don consumed in Nigeria (Edeoga et al., 2006; Okwu and Josiah, 2006; Akubugwo et al., 2007). The levels of saponins and phytate in this plant are much less than the value reported for some medicinal plants used in Nigeria and since these values are still within the tolerable limits; these metabolites can easily be detoxified by soaking, boiling or frying (Akubugwo et al., 2007).

The results of this study showed that the leaves of *R. sagittatus* contain appreciable amount of proteins, fat, fibre, carbohydrate and caloric value, mineral elements, polyphenols and generally low level of toxicants. Their antioxidant activities further lend credence to the biological value of this plant. Thus, it can be concluded that *R. sagittatus* leaves can contribute significantly to the nutrient requirements of man and should be used as a source of nutrients to supplement other major sources. Since these extracts show good antioxidant activities in this study; the use of this plant for medicinal purpose may be justified.

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