ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 5:1 (2011) 29-42

records of natural products

Comparison of the Nutritive Value, Antioxidant and Antibacterial Activities of *Sonchus asper* and *Sonchus oleraceus*

Florence O. Jimoh[1], Adeolu A. Adedapo[2*] and Anthony J. Afolayan [1]

[1]*Department of Botany, University of Fort Hare, Alice 5700, South Africa*
[2]*Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria*

(Received February 3, 2010; Revised September 24, 2010; Accepted October 7, 2010)

Abstract: Many local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. For this reason, the nutritional, phytochemical, antioxidant and antibacterial activities of the acetone, methanol and water extracts of the leaves of *Sonchus asper* and *Sonchus oleraceus* were investigated. The proximate analysis showed that the plants contained appreciable percentage of moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate. The plants are also rich in minerals, flavonoids, flavonols, proanthocyanidins, total phenols and low levels of saponins, phytate and alkaloids. The extracts of the 2 plants also showed strong antioxidant antibacterial properties.

Keywords: Antibacterial properties; Antioxidant activities; *Sonchus asper*; *S. oleraceus*; nutritional value.

1. Introduction

Most developing countries depend on starch-based foods as the main staple food for the supply of both energy and protein. This accounts in part for protein deficiency which prevails among the populace as recognized by Food and Agricultural Organization [1-2].

Many of the local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. Though several works reporting compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries abound in literature, much still need to be done. Many workers [3-7] have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries.

It is surprising that few 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, thoug; h known, are considered as weeds and were not

* Corresponding author: adedapo3a@yahoo.co.uk.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 12 /15/2010 EISSN: 1307-6167

eaten by the people. This is in spite of the fact that these vegetables grow spontaneously and in abundance around the rural homesteads.

The consumption of vegetables has however been linked to reduction in the incidence of oxidative-stress related diseases due to beneficial health functionality of phenolic antioxidants present in them. Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals ($O_2^{\cdot-}$) and hydroxyl radicals ($OH^{\cdot}$), as well as non-free radical species (H_2O_2) and the singled oxygen ($^1O_2$) [8-11]. Also, excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer [12-14]. Some plant phenolics have also shown antimicrobial effects [15].

Sonchus asper (L.) Hill (Asteraceae) is an herbaceous annual or winter annual; entire plant contains a sticky, white latex; taproot short, bushy with many lateral roots; stems erect, hollow, stout, unbranched or slightly branched, 30 to 150 cm tall, often reddish; may have gland-tipped hairs on upper stems; leaves alternate, 4 to 18 cm long, 0.5 to 5 cm wide, crisped, many-lobed (5 to 11 lobes on each side) with fewer lobes on upper leaves [16]. A native of Europe but is now a cosmopolitan weed [17]. The leaf-gall fly (*Cystiphora sonchi*) of Europe and the former Soviet Union offer hope of suppressing *S. asper* and *S. arvensis* in Canada. Females lay eggs on the undersides of leaves, forming up to 270 galls/plant [16].

Sonchus oleraceus L was named by Carolus Linnaeus in 1753 in his "Species Plantarum." "Sonchus" is the Greek name for sow thistle and means "hollow." This is a reference to the hollow stems. The epithet oleraceus means "kitchen vegetable." Sonchus has the questionable distinction of being considered one of the world's worst weeds, a pest in more than 55 countries [18]. It has various functions such as abortifacient [Houma]; anticancer (sap); antidiarrheal [Houma]; anti-inflammatory [China]; "blood purifier"[Hoama]; calms the nerves (leaves); cathartic [Pima (stem juice), Turkey]; clears infections [China]; cure for opium addiction [China, Pima]; digestive purgative [Pima]; diuretic [Turkey]; emmenagogue; emollient [Haiti]; febrifuge (leaves and roots infused); gynecological aid [Potawatomi (*S. arvensis*)]; heart medicine [Navaho (*S. asper*)]; hepatic; hydrogogue (stem juice) [Turkey]; insecticide; lactogogue [Turkey]; mild laxative [New Zealand]; narcotic [China]; pectoral; pediatric aid [Houma, Iroquois (*S. asper*)]; poison [Navaho (*S. asper*)]; poultice; refrigerant [Spain, Turkey]; sedative [China, Iroquois (*S. asper*)]; stop bleeding [China]; to prevent infection (topical) [New Zealand]; tonic [Sudan, Turkey]; toothache remedy [Houma]; vermicide [Tanzania] [17-21].

Since the plants are regarded as wild vegetables, the study was aimed at assessing their nutritional qualities and possible biological activities.

2. Materials and Methods

2.1 Plant collection and extract preparation

Fresh plant materials of *Sonchus asper* and *Sonchus oleraceus* were collected in November 2006 from the wild around the University of Fort Hare campus (Alice, South Africa). The area falls within the latitudes 30°00-34° 15´S and longitudes 22° 45´ -30° 15´E. It is bounded by the sea in the east and the drier Karoo (semi-desert vegetation) in the west [22]. 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. 2006/7). The plant material was allowed to air-dry at ambient temperature (±24◦C) and then milled. Twenty grams each of the sample were extracted with 200 mL each of acetone, methanol, and water, respectively, at ambient temperature, with agitation for 18–24 h. Each extract was filtered using Whatman no. 1 filter paper and concentrated under reduced pressure to dryness below 40◦C. The water extract was freeze-dried. The extract yields

(w/w) were acetone (1.86%), methanol (6.6%), and water (9.5%), respectively for *S. asper* but 2.1% (acetone), 7.1% (methanol) and 8.9% (water) respectively for *S. oleraceus*. The dried extracts thus obtained were used directly for the determination of the antioxidant and antibacterial activities [23]. Determinations of chemical and nutritive values of this plant were carried out using the dried sample was that was ground into powder form.

2.2 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 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 (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

2.3 Determination of total phenolics

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method [24]. 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 sec and allowed to stand for 30 min at 40^{o}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 was the absorbance and y was the tannic acid equivalent (mg/g).

2.4 Determination of total Flavonoids

Total flavonoids were estimated using the method of Ordon Ez et al. [25]. 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 contents were calculated as quercetin (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

2.5 Determination of total Flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran [26]. 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) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where x was the absorbance and was the quercetin equivalent (mg/g).

2.6 Determination of total proanthocyanidins

Determination of proanthocyanidin was based on the procedure reported by Sun et al. [27]. 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 contents 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 was the absorbance and y is the catechin equivalent (mg/g).

2.7 Determination of antioxidant activity

2.7.1 ABTS radical scavenging assay

For ABTS assay, the method of Re et al. [28] was adopted. The stock solutions included 7 mM $ABTS^{\cdot+}$ 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 $ABTS^{\cdot+}$ solution with 60 mL methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\cdot+}$ solution was prepared for each assay. Plant extracts (1 mL) were allowed to react with 1 mL of the $ABTS^{\cdot+}$ solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The $ABTS^{\cdot+}$ scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as ABTS radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$ where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract /standard.

2.7.2 DPPH radical scavenging assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana & Shahidi [29]. 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 0.02–0.1 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.

2.7.3 Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain [30] 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 using. Plant extracts (150 µL) were allowed to react with 2850 µL 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 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT, ascorbic acid and catechin.

2.8 Proximate Analysis

The recommended methods of the Association of Official Analytical chemists [AOAC, 1999] were used for the determination of moisture, ash, crude lipid, crude fibre and nitrogen content. 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 [31].

2.9 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 420nm. The elements comprising sodium, calcium, potassium, magnesium, iron, zinc, copper, manganese, potassium, nitrogen and phosphorus were determined in this way [32]. Macro and micronutrients were determined using Perkin Elmer; Analyst 700, single beam atomic absorption spectrometer and the data was obtained in parts per million (ppm), (1ppm=1mg/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. [33] for plant samples.

2.10 Analysis of secondary metabolites

Determination of alkaloid and saponins were as described by Obadoni and Ochuko [34]. The alkaloid content of the leaves of the plant was determined gravimetrically. 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 is 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 60ºC 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 55ºC. 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 90ºC. 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 [34-35]. Phytate was estimated by the method of Haug and Lentzsch [36] and Wheeler and Ferrel [37]. The finely ground plant sample was extracted with 0.2N HCl by shaking at room temperature. Phytic acid in the extract was determined colorimetrically.

2.11 Antibacterial assay

The bacterial cultures used in this study were obtained from the Department of Biochemistry and Microbiology, Rhodes University, South Africa. They consisted of five Gram-positive and five Gram-negative strains (Tables 6 and 7). Each organism was maintained on nutrient agar plates and was recovered for testing by growth in nutrient broth for 24 hrs. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth [38].

Test organisms were streaked in a radial pattern on sterile nutrient agar plates containing filtered extracts at final concentrations of 0.1, 0.5, 1.0, 2.5 and 5.0 mg/ml [15, 39]. Plates containing only nutrient agar and another set containing nutrient agar and the respective solvents served as controls. After inoculation, the plates were incubated at 37ºC for 24 to 48 hours. Each treatment was performed

in triplicate and complete inhibition of bacterial growth was required for an extract to be declared bioactive.

2.12 Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the Statistical Analysis System (SAS, 1999) program. P Values < 0.05 were regarded as significant and P values < 0.01 as very significant.

3. Results and Discussion

With respect to the total polyphenol and flavonoids, the acetone and methanol extracts of S. asper had higher content of this constituent than that of water extract. The result was similar to that of *S. oleraceus*. The methanol and water extracts of *S. asper* had higher content of proanthocyanidins relative to acetone but for *S. oleraceus*, the acetone and methanol extracts had less of proanthocyanidins than those of water extract i.e. 2.8, 2.6 and 4.1 respectively. In the case of total flavonol, the water extract did not show any trace of this constituent for the 2 plants but the acetone and methanol extracts had similar level for the 2 plants (Table 1). Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties [40], which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The results strongly suggest that phenolics are important components of these plants, and some of their pharmacological effects could be attributed to the presence of these valuable constituents. The results from this study showed that the polyphenolic contents of these 2 plants were very similar.

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

Phenolics	***S. asper***			***S. oleraceus***		
	Acetone	**Methanol**	**Water**	**Acetone**	**Methanol**	**Water**
Total polyphenol	10.14±0.44	10.53±1.29	5.00±0.24	10.71±0.32	9.72±1.06	6.07±0.07
Flavonoids	1.04±0.05	0.98±0.10	0.63±0.12	1.09±0.01	1.21±0.01	0.66±0
Proanthocyanidins	1.74±0.63	2.24±0.15	2.63±0.78	2.78±0.93	2.59±0.42	4.08±0.87
Total Flavonol	0.75±0.04	0.91±0.26	-	0.70±0.10	0.60±0.13	-

Total polyphenol is expressed as mg tannic acid/g of dry plant material. Flavonoid is expressed as mg quercetin/g of dry plant material.Proanthocyanidins is expressed as mg quercetin/g of dry plant material. Total flavonol is expressed as mg quercetin/g of dry plant material * indicates that this value is significantly different from the other at P<0.05

The ferrous reducing antioxidant power (FRAP) value for the 2 plants showed that methanol extract is much than that of acetone while acetone in turn is higher than those of water extracts. The FRAP values for the methanol and acetone extracts of the 2 plants were higher than those of BHT but lower than those of catechin, ascorbic acid (1632.1) and quercetin (3107.3). The FRAP value for water extracts of both plants was the lowest (Table 2). The antioxidant potentials of the extracts of the leaves of these plants were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [24]. The result showed that FRAP activity was highest in the methanol extract followed by acetone and least in water for the 2 plants. It thus meant that these plants exhibited similar activity.

Table 2. FRAP activity of the acetone, methanol and water extracts of *S. asper* and *S. oleraceus*

Extracts	***S. asper***	***S. oleraceus***
Acetone	158.67 ± 21.89	94.01 ± 3.41
Methanol	298.56 ± 32.52	201.3±28.72
Water	18.32 ± 5.79	29.86 ± 4.85
Ascorbic acid	1632.1 ± 16.95	1632.1 ± 16.95
BHT	63.46 ± 2.49	63.46 ± 2.49
Catechin	972.02 ± 0.61	972.02 ± 0.61
Quercetin	3107.29 ± 31.28	3107.29 ± 31.28

FRAP is expressed in units of µmol Fe (II)/g.

At 1 mg/mL, the acetone extract of *S. asper* caused 97.8% ABTS radical scavenging inhibition while the methanol, water, and BHT caused inhibition at 98.0, 99.1 and 99.3% respectively. For *S. oleraceus* at the same concentration, the results were 99.4, 95.7, 93.7 and 99.3% for acetone, methanol, water and BHT respectively. It must be stated however that at 0.05 mg/mL, the percentage inhibition for water extract of *S. oleraceus* was 99.2 while for the acetone extract it was 98.0% (Fig. 2). 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 [41]. Higher concentrations of the extracts were more effective in quenching free radicals in the system. At 1 mg/mL concentration, all the extracts for the 2 plants produced similar or equal ABTS radical scavenging activity.

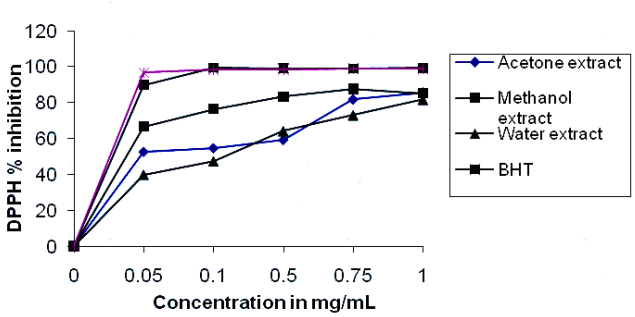

Figure 1. DPPH scavenging activity of *S. asper*

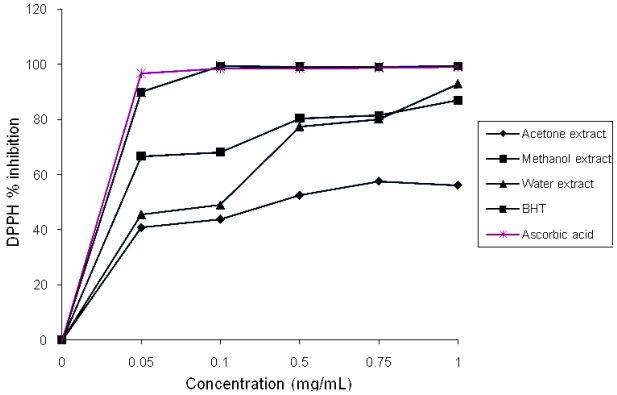

Figure 2. DPPH radical scavenging activity of *S. oleraceus*

At 1 mg/mL, the acetone, methanol, water and ascorbic acid caused DPPH radical scavenging activity at 85.6, 85.3, 81.8 and 99.8% respectively for *S. asper* while for *S. oleraceus* at 1mg/mL; the results were 56.1, 86.9, 92.9 and 100% for acetone, methanol, water and ascorbic acid respectively. For BHT, at 0.05 mg/mL, the DPPH radical scavenging activity was 100% (Fig. 1). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [42]. The DPPH radical scavenging abilities of the water extract of *S. oleraceus* at 1mg/mL was 92.9% and slightly less than those of ascorbic acid (100%) and BHT (100), showing that the extract has the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The results for other extracts of the 2 plants were not as high. The scavenging of the ABTS radical by the extracts at 1mg/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 [43]. Wang et al. [44] found that some compounds which have ABTS scavenging activity did not show DPPH scavenging activity. In an antioxidant study conducted by Alpinar et al. [45], it was reported that *Sonchus asper* and *Sonchus oleraceus* may be considered as a potential source of natural antioxidants, and can be especially recommended to be incorporated in diets to protect human health and produce general wellness.

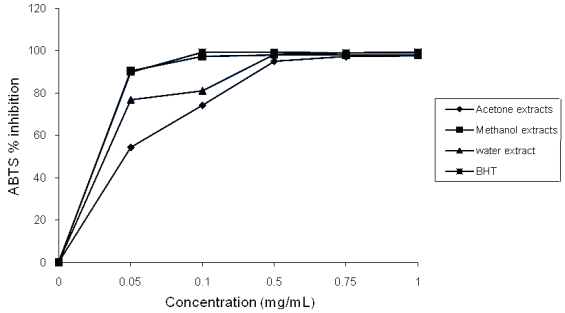

Figure 3. ABTS radical scavenging activity of *S. asper*

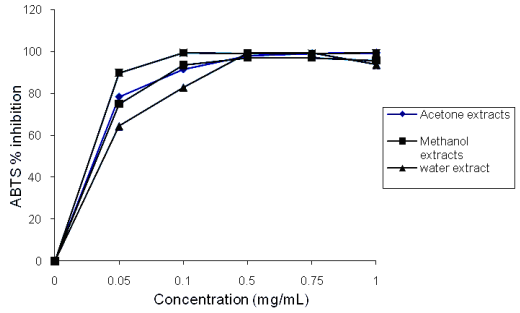

Figure 4. ABTS radical scavenging activity of *S. oleraceus*

The acetone extract of *S. asper* has activity against *Bacillus cereus* (5mg/mL), *Micrococcus kristinae* (5mg/mL), and *Staphylococcus aureus* (2mg/mL) and *Streptococcus pyrogens* (2mg/mL). The methanol extract only has activity against *Micrococcus kristinae* (5mg/mL). The water extract has activity against *Staphylococcus aureus* (2mg/mL), *Streptococcus pyrogens* (5mg/mL), *Micrococcus kristinae* (2mg/mL) and *Serratia marcescens* (5mg/mL), a Gram negative organism (Table 6). For *S. oleraceus*, the acetone extract has activity against all the organisms except *Streptococcus pyrogens*, *Salmonella pooni* and *Klebsiella pneumoniae*. The methanol extract was active against *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus kristinae* and *Pseudomonas aeruginosa*. The water extract did not have activity against any of the organism used in this study (Table 7). The acetone extract of *S. oleraceus* has activity against all the organisms except *Streptococcus pyrogens*,

Salmonella pooni and *Klebsiella pneumoniae*. It has also been shown that the plant extracts were active against most of the Gram-positive strains and less of the Gram-negative strains. This observation therefore supports the fact that, in general, the Gram-negative bacteria are less susceptible to antibacterial effect than the Gram positive ones [46-47]. Since these extracts show some activity against some of the organisms used in this study; the use of this plant for medicinal purpose may be justified.

The proximate analysis showed the percentage moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate of the leaves as of *S. asper* as 89.9, 18.8, 13.3, 7.8, 41.9 and 18.3%, respectively while its calorific value is 290.4 Kcal/100 g (Table 3). Elemental analysis in mg/100 g (DW) indicated that the leaves contained sodium (0.288), potassium (2.366), calcium (3.397), Magnesium (0.689), Iron (883), Zinc (50), phosphorus (0.297), Copper (16), Manganese (117), and Nitrogen (2.1) (Table 4). In the case of *S. oleraceus,* analysis showed the percentage moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate of the leaves as 85.4, 14.3, 17.5, 7.0, 46.0 and 15.3%, respectively while its calorific value is 317.3 Kcal/100 g (Table 3). Elemental analysis in mg/100 g (DW) indicated that the leaves of *S. oleraceus* contained sodium (0.05), potassium (4.558), calcium (2.992), Magnesium (0.61), Iron (139), Zinc (30), phosphorus (0.352), Copper (13), Manganese (191), and Nitrogen (2.8) (Table 4). The results of proximate composition of the leaves of *S. asper* (89.9) and S. oleraceus (85.4) show high moisture content (Table 3). This is within the reported range (81.4-90.3%) in some Nigerian green leafy vegetables [2]. Ash content, which is an index of mineral contents in biota, is 18.8% DW in *S. asper* and 14.3% DW in *S. oleraceus*. These compare favourably with the values reported for *Ipomea batatas* (11.10%), *Vernonia colorate* (15.86%) and *Moringa oleifera* (15.09% DW) [3, 48].

Table 3. Proximate analysis of the leaves of *S. asper* and *S. oleraceus*

Constituents	***S. asper***	***S. oleraceus***
Moisture	89.87± 1.87	85.37 ± 4.25
Ash	18.75± 0.25	14.25± 0.25[a]
Protein	13.25± 0.05	17.50 ± 0.08 [a]
Fat	7.75 ± 0.50	7.0 ± 0.23
Carbohydrate	41.92 ± 0.04	46.0 ± 0.07 [a]
Crude fibre	18.33 ± 0.72	15.25 ± 0.1 [a]
Energy (kcal)	290.43 ± 0. 15	317.25 ± 0.21 [a]

[a] The superscript showed that at P<0.05, a significant difference exists.

The value for *S. asper* is also, higher than that of some Nigerian leafy vegetable such as *Ocimum gratissium* (18.00% DW) *and Hibiscus esculentus* (8.00% DW) [4]. The crude protein content of *S. asper* (13.3% DW) and S. oleraceus (17.5%) are higher than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland [6, 49-50], but lower than those of *I. batatas* (24.85% DW), *Amaranthus candatus* (20.5% DW), *Piper guineeses* (29.78% DW) and *T. triangulare* (31.00% DW) [4, 48, 51]. According to Pearson [52], plant food that provides more than 12% of its calorific value from protein is considered good source of protein. Therefore, the protein content of the leaves of these 2 plants will go a long way in meeting the protein requirement of the local people.

Table 4. Macro and micro elements constituents of the leaves of *S. asper* and *S. oleraceus*

Macro and Micro elements (mg/100g dwb)	***S. asper***	***S. oleraceus***
Magnesium	0.689	0.610
Calcium	3.397	2.992
Potassium	2.366	4. 558
Phosphorus	0.297	0.352
Sodium	0.288	0.050
Iron (ppm)	883	193
Zinc	50	30
Copper	16	13
Manganese	117	191
Total Khedjal nitrogen	2.12	2.8

The crude lipid content of *S. asper* (7.8 % DW) and *S. oleraceus* (7.0% DW) is slightly lower than the reported values (8.3 - 27.0% DW) in some vegetables consumed in West Africa [53-54]. However, it compares favorably with 4.2% reported for *Calchorus africanum* leaves and 1.85 - 8.71% DW in some edible green leafy vegetables of Southern India and Nigeria [55-56]. The carbohydrate content of *S. asper* (41.9% DW) and *S. oleraceus* (46.0% DW) is higher than 20, 23.7 and 39.05% reported for *Senna obtusfolia, Amaranthus incurvatus,* and *Momordica balsamina* leaves, respectively [6, 57]. This is however; lower than reported values for *Corchorus tridens* (75.0% DW) and sweet potatoes leaves (82.8%) [58]. The crude fibre content of *S. asper* (18.3% DW) and *S. oleraceus* (15.3% DW) is high when compared to *Ipomea batatas* (7.20%), *T. triangulare* (6.20%) *P. guineensis* (6.40%), *Corchorus olitorius* (7.0%), and *Vernonia amygdalina* (6.5%) [4, 48]. Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [59-60]. The RDA of fibre for children, adults, pregnant and lactating mothers are 19 – 25, 21-38, 28 and 29 g, respectively. The estimated calorific value for *S. asper* (290.4 kcal/100 g DW) and *S. oleraceus* (317.3% DW) leaves compare favourably to 248.8–307.1 Kcal/100 g DW reported in some Nigerian vegetables [2, 48, 61]. Asibey–Berko and Tayie [58] also reported comparable energy content in some Ghanaian green leafy vegetables. Thus, the calorific value agreement with general observation that vegetables have low energy values [62].

The mineral composition of *S. asper* and *S. oleraceus* is as shown in Table 4. The Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended [63]. Therefore, consumption of *S. asper* and *S. oleraceus* would probably reduce high blood pressure diseases because their Na/K is less than one. Iron content of the leaves of *S. asper* (883 mg/100 g) and *S. oleraceus* (193 mg/100 g) is very high when compare with the value reported in *I batatas* (16.00 mg/100 g) [48]. 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 [2, 64]. The Zinc content of *S. asper* (50 mg/100 g) and *S. oleraceus* (30 mg/100 g) compares favourably to most values reported for green leafy vegetables in literatures [6, 65]. Zinc is involved in normal function of immune system.

The chemical composition in mg/100 g (DW) for alkaloid, saponins, and phytate were 0.5, 4.5 and 5.2 respectively for *S. asper.* In the case of *S. oleraceus*, these values were alkaloids (0.43), saponins (4.5) and phytate (5.1) (Table 5). Analysis of the antinutrient contents of the plant showed that alkaloid level in *S. asper* (0.5) and *S. oleraceus* (0.43mg/100g) is lower than the values reported for the leafy vegetables like *Aspilia africana*, *Bryophyllum pinnatum*, *Cleome rutidosperma* and *Emilia coccinea* consumed in Nigeria [2, 5, 66]. The results also showed that in *S. asper* (4.5 mg/100g) and *S. oleraceus* (4.5mg/100g) levels of saponins are present. These levels of saponins in these plants are much less than the value reported for some medicinal plants used in Nigeria. Although the phytate level in *S. asper* (5.2 mg/100g) and *S. oleraceus* (5.1mg/100g) (9.3mg/100g) is slightly higher than the value reported for *I. batatas* and *G. africana* leaves [42, 67]; this value is still within the tolerable limits and can easily be detoxified by soaking, boiling or frying [2, 68-70]. It should be noted that saponins are one of the many secondary metabolites found in natural sources, with particular

abundance in various plant species [71]. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. There is evidence of the presence of saponins in traditional medicine preparations [72-73]

.

Table 5. Analysis of anti-nutrients contents of *S. asper* and *S. oleraceus*

Secondary metabolites	***S. asper***	***S. oleraceus***
Alkaloids	0.5 ± 0.15	0.43 ± 0.04
Saponins	4.5 ± 0	4.5±0
Phytate	5.16 ± 0	5.12 ± 0.3

The results of this study showed that the leaves of *S. asper* and *S. oleraceus* contain appreciable amount of proteins, fat, fibre, carbohydrate and calorific value, mineral elements, polyphenols, and generally low level of toxicants. Their antioxidant and antibacterial activities further lend credence to the biological value of this plant. Thus, it can be concluded that *S. asper* and *S. oleraceus* leaves can contribute significantly to the nutrient requirements of man and should be used as a source of nutrients to supplement other major sources. The plants also have high biological activities hence may be of great medicinal value.

Table 6. Antibacterial activity of the leaves extracts of *Sonchus asper*

Bacterial species	Gram +/-	Minimum inhibitory concentration (mg/mL)				
		Acetone	Methanol	Water	Chloramphenicol	Streptomycin
Bacillus cereus	+	5.0	na	na	<2	<2
Staphylococcus epidermidis	+	na	na	na	<2	<2
Staphylococcus aureus	+	2.0	na	2.0	<2	<2
Micrococcus kristinae	+	5.0	5.0	2.0	<2	<2
Streptococcus pyrogens	+	na	na	5.0	<2	<2
Escherichia coli	-	na	na	na	<2	<2
Salmonella pooni	-	na	na	na	<2	<2
Serratia marcescens	-	na	na	5.0	<2	<2
Pseudomonas aeruginosa	-	na	na	na	<20	<5
Klebsiella pneumonae	-	na	na	na	<2	<2

na: not active

Table 7. Antibacterial activity of the leaves extracts of *Sonchus oleraceus*

Bacterial species	Gram +/-	Minimum inhibitory concentration (mg/mL)				
		Acetone	Methanol	Water	Chloramphenicol	Streptomycin
Bacillus cereus	+	2.5	2.5	na	<2	<2
Staphylococcus epidermidis	+	5.0	na	na	<2	<2
Staphylococcus aureus	+	2.5	5.0	na	<2	<2
Micrococcus kristinae	+	2.5	5.0	na	<2	<2
Streptococcus pyrogens	+	na	na	na	<2	<2
Escherichia coli	-	1.0	na	na	<2	<2
Salmonella pooni	-	na	na	na	<2	<2
Serratia marcescens	-	2.5	na	na	<2	<2
Pseudomonas aeruginosa	-	2.5	2.5	na	<20	<5
Klebsiella pneumonae	-	na	na	na	<2	<2

na: not active

Acknowledgments

The authors are grateful to the National Research Foundation (NRF) of South Africa for funding this research.

References

[1] O. Ladeji, Z.S. Okoye and T. Ojobe (1995). Chemical evaluation of the nutritive value of leaf of fluted pumpkin (*Telferia occidentalis*), *Food. Chem.* **53,** 353-355.

[2] I.E. Akubugwo, N.A. Obasi, G.C. Chinyere and A.E. Ugbogu (2007). Nutritional and chemical value of *Amaranthus hybridus* L. leaves from Nigeria, *Afr. J. Biotech.* **6** (24), 2833-2839.

[3] C.T. Lockeett,C.C. Calvert and L.E. Grivetti (2000). Energy and micronutrient Composition of dietary and Medicinal wild plants Consumed during drought: Study of Rural Fulani, Northeastern Nigeria, *Int. J. Food. Sci. Nutr.* **51**, 195-208.

[4] A.A. Akindahunsi and S.O. Salawu (2005). Photochemical screening and nutrient-anti-nutrient composition of selected tropical green vegetables, *Afr. J. Biotech.* **4,** 497-501.

[5] H.O. Edeoga, G. Omosun and L.C. Uche (2006). Chemical composition of *Hyptis sauveolens* and *Ocimum gratissium* hybrids from Nigeria, *Afr. J. Biotech.* **5**(10), 892-895.

[6] L.G. Hassan and K.J. Umar (2006). Nutritional value of Balsam Apple (*Momordica balsamina* L.) leaves. *Pak J Nutr* **5**(6): 522-529.

[7] A.S. Ekop (2007). Determination of chemical composition of *Gnetum africana* (AFANG) seeds, *Pak. J. Nutr.* **6**(1), 40-43.

[8] B. Halliwell (1995). How to characterize an antioxidant: an update, *Bioch. Soc. Sym.* **61,** 85-91.

[9] G.L. Squadriato and, W.A. Peyor (1998). Oxidative chemistry of nitric oxide: the role of superoxide, peroxynitrite, and carbon dioxide. *Free Radical Biol Med* **25**: 392-403.

[10] A. Yildrim, M. Oktay and V. Bilaloglu (2001). The antioxidant activity of the leaves of *Cydonia vulgaris, Turkish J. Med. Sc.* **31,** 23-27.

[11] I. Gulcin, M. Oktay, I.O. Kufrevioglu and A. Aslan A (2002). Determination of antioxidant activity of Lichen *Cetraria islandica* (L) Ach, *J. Ethnopharmacol.* **79,** 325-329.

[12] A.P. Kourounakis, D. Galanakis and K., Tsiakitzis (1999). Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities, *Drug Dev. Res.* **47,** 9-16.

[13] I. Gulcin, M.E. Buyukokuroglu, M. Oktay and I.O. Kufrevioglu (2002). On the *in vitro* antioxidant properties of melatonin, *J. Pineal. Res.* **33,** 167-171.

[14] I. Gulcin, M.E. Buyukokuroglu, M. Oktay and I.O. Kufrevioglu (2003). Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. *Subsp. pallsiana* (Lamb.) Holmboe, *J. Ethnopharmacol,* **86,** 51-58.

[15] S. Koduru, D.S. Grierson and A.J. Afolayan (2006). Antimicrobial activity of *Solanum aculeastrum, Pharm. Biol.* **44** (4), 283-286.

[16] Holm LD and Eric P, Jaun HJ (1997) World weeds: natural histories and distribution. John Wiley & Sons.

[17] W.L. Wagner, D.R. Herbst and S.H. Sohmer (1999). Manual of the flowering plants of Hawaii. Revised edition. Bernice P. Bishop Museum special publication. University of Hawaii's Press/Bishop Museum Press, Honolulu.

[18] L.D. Holm, V.H. Juan, P.P. James and L. Donald (1979). A geographical atlas of world weeds. John Wiley & Sons.

[19] C.J. Webb, W.R. Sykes and P.J. Garnock-Jones (1988). Flora of New Zealand, Volume IV: Naturalized pteridophytes, gymnosperms, dicotyledons. Botany Division, DSIR, Christchurch.

[20] A.E. Orchard (1994). Flora of Australia. Vol. 49, Oceanic islands 1. Australian Government Publishing Service, Canberra.

[21] F.R. Fosberg (1997). Preliminary checklist of the flowering plants and ferns of the Society Islands. U. California, Berkeley.

[22] P.J. Masika and A.J. Afolayan (2003). An ethnobotanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa, *Pharm. Biol.* **41,** 16-21.

[23] R.S.L. Taylor, F. Edel and N.P. Manandhar and G.H.N. Towers (1996). Antimicrobial activity of southern Nepalese medicinal plants.,*J Ethnopharmacol.* **45,** 67-70.

[24] K. Wolfe, X. Wu and R.H. Liu (2003). Antioxidant activity of apple peels, *J. Agric. Food Chem.* **1,** 609-614.

[25] A.A.L. Ordoñez, J.G. Gomez, M.A. Vattuone and M.I. Isla (2006). Antioxidant activities of *Sechium edule (*Jacq.) Swart extracts, *Food Chem.* **97,** 452-458.

[26] A. Kumaran and R.J. Karunakaran (2006). Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus, Food Chem.* **97,** 109-114.
[27] J.S. Sun, Y.W. Tsuang, I.J. Chen, W.C. Huang, Y.S. Hang and F.J. Lu (1998). An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury, *Burns* **24,** 225-231.
[28] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Bio. Med.* **26,** 1231–1237.
[29] C.M. Liyana-Pathiranan and F. Shahidi (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum L*) as affected by gastric pH conditions, *J. Agric. Food Chem.* **53,** 2433-2440.
[30] I.F.F. Benzie and J.J. Strain (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Anal. Biochem.* **239,** 70-76.
[31] D.E. Okwu and F.N. Morah (2004). Mineral and nutritive value of *Dennettia tripetala* fruits, Fruits **59**(6), 437-442.
[32] D.A. Coomes (1997). Nutrient status of Amazonian caatinga forests in a seasonally dry areas: nutrient fluxes in litter fall and analysis of soils, *Can. J. For. Res.* **27**(6), 831-839.
[33] M.T. Shah, S. Begum and S. Khan (2009). Pedo and biogeochemical studies of mafic and ultramafic rocks in the Mingora and Kabal areas, Swat, Pakistan", *Environ. Earth Sci.*, DOI: 10.1007/s12665-009-0253-8.
[34] B.O. Obadoni and P.O. Ochuko (2001). Phytochemical studies and comparative efficacy of the Crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, *Global. J. Pure. Appl. Sci.* **8,** 203-208.
[35] A. Nahapetian and A. Bassiri (1975) Changes in concentration and interrelationship of phytate, P, mg, Cu, Zn, in wheat during maturation, *J. Agric. Food Chem.* **32**, 1179-1182.
[36] W. Haug and H.J. Lentzsch (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products, *J. Sci. Food Agric*. **34,** 1423-1426.
[37] V.E. Wheeler and F.E. Ferrel (1971). A method of phytic acid determination in wheat fraction, *Cereal Chem.* **48,** 312-316.
[38] A.J. Afolayan and J.J.M. Meyer (1997). The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoot of *Helichrysum aureonitens, J. Ethnopharmacol.* **57,** 177–181.
[39] J.J.M. Meyer and A.J. Afolayan (1995). Antibacterial activity of *Helichrysum aureonitens* (Asteraceae), *J. Ethnopharmacol.* **47,** 109-111.
[40] H.P. Wichi (1988). Enhanced tum from the perspective effect on forestomach and oesophageal squamous epithelium, *Food Chem. Toxicol.* **26,** 717-723.
[41] H. Yoshida and S. Takagi (1999). Antioxidative effects of sesamol and tocopherols at various concentrations in oils during microwave heating, *J. Sci. Food Agric.* **79,** 220-226.
[42] L.Yu, S. Haley, J. Perret, M. Harris, J. Wilson and M. Qian (2002). Free radical scavenging properties of wheat extracts, *J. Agric. Food. Chem.* **50,** 1619–1624.
[43] W. Zheng and Y.S. Wang (2001). Antioxidant activity and phenolic compounds in selected herbs, *J. Agric. Food Chem.* **49,** 5165–5170.
[44] M. Wang, J. Li, M. Rangarajan, Y. Shao, E.J. La Voie, T. Huang and C. Ho (1998). Antioxidative phenolic compounds from Sage (*Salvia officinalis*), *J. Agr. Food Chem.* **46**: 4869–4873.
[45] K. Alpinar, M. Ozyurek, U. Kolak, K. Guclu, C. Aras, M. Altun, S.E. Celik, K.I. Berker, B. Bektasoglu, and R. Apak (2009). Antioxidant capacities of some food plants widly grown in Ayvalik of Turkey, *Food Sci. Technol. Res.* **15**(1), 59-64.
[46] D.S. Grierson and A.J. Afolayan (1999). Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa, *J. Ethnopharmacol.* **66,** 103–106.
[47] A.J. Afolayan (2003). Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi, *Pharm. Biol.* **41,** 22–25.
[48] B.S. Antia, E.J. Akpan, P.A. Okon and I.U. Umoren (2006). Nutritive and Anti-Nutritive Evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pak J Nutr* **5**(2): 166-168.
[49] B.M. Ogle and L.E. Grivetti (1985). Legacy of the chameleon: Edible wild plants in the Kingdom of Swaziland, Southern Africa. A cultural, ecological nutritional study. Part IV: Nutritional analysis and conclusion, *Ecol. Food. Nutr.* **17**, 41-64.
[50] E.U. Isong and U.I. Idiong (1997). Comparative studies on the nutritional and toxic composition of three varieties of *Leianthera Africana, Plants. Food. Hum. Nutr.* 51, 79-84.
[51] E.U. Etuk, M.N. Bassey, U.O. Umoh and E.G. Inyang (1998). Comparative nutritional studies on three local varieties of *Heinsia crinita, Plant Varieties Seeds* **11,** 151-158.

[52] D. Pearson (1976). Chemical analysis of foods. (7th ed) churchchill, Livingstone, London.
[53] E.T. Ifon and O. Bassir (1980). The nutritive value of some Nigerian leafy vegetables- parts 2: The distribution of proteins, Carbohydrates (including ethanol-soluble simple sugars), Crude fat, Fibre and Ash. *Food Chem.* **5,** 231-235.
[54] L.P. Sena, D.J. VanderJagt, C. Rivera, A.T.C. Tsin, I. Muhammadu, O. Mahammadu, M. Milson, A. Pastosyn and R.H. Glew (1998). Analysis of Nutritional Components of eight famine foods of the Republic of Niger. *Plant Foods Hum. Nutr.* **52,** 17-30.
[55] J.T. Agbo (2004). Proximate nutrient composition of sickle pod. (*Cassia obtusfolia)* leaves and seeds, *Plants Prod. Res. J.* **8,** 13-17.
[56] S. Gupta, A.J. Lakshmi, M.N. Majunath and J. PrakashJ (2005). Analysis of nutrient and antinutrient content of under utilized green leafy vegetables. *LWT-Food Sci. Tech.* **38,** 339-345.
[57] U.Z. Faruq, A. Sani and L.G. Hassan (2002). Proximate composition of sickle pod. (*Senna obtusfolia*) leaves, *Niger. J. Basic. Appl. Sci.* **11,** 157-164.
[58] E. Asibey-Berko and F.A.K. Tayie (1999). Proximate analysis of some underutilized Ghanaian vegetables, *Ghana J, Sci,* **39,** 91-92.
[59] C.V. Rao and H.L. Newmark (1998). Chemo-preventive effect of Squalene on colon cancer, *Carcinogenesis* **19,** 287-290.
[60] H. Ishida, H. Suzuno, N. Sugiyama, S. Innami, T. Todokoro and A. Maekawa (2000). Nutritional evaluation of chemical component of leaves, stalks and stems of sweet potatoes (*Ipomea batatas* poir), *Food Chem.* **68,** 359-367.
[61]. E.U. Isong, S.A.R. Adewusi, E.U. Nkanga, E.E. Umoh and E.E. Offiong (1999). Nutritional and phytogeriatological studies of three varieties of *Gnetum africanum* (afang), *Food Chem.* **64,** 489-493.
[62] C. Lintas (1992). Nutritional aspects of fruits and vegetable consumption, *Options Mediterraennes* **19,** 79-87.
[63] F.N.D. (2002). Food and nutrition board, Institute of medicine. National Academy of Sciences. Dietary reference Intake for Energy, Carbohydrate, Fibre, Fat, Fatty Acids, Cholesterol, protein and Amino acid (micro-nutrients). www.nap.edu
[64]. E. Adeyeye and M.K.O. Otokili (1999). Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum* (Bell and cherry peppers), *Discov Innov* **11,** 75-81.
[65] N.D.G. Ibrahim, E.M. Abdurahhman and G. Ibrahim (2001). Elemental analysis of the leaves of *Vernonia amygdalina* and its biological evaluation in rats, *Niger. J. Nat. Prod. Med.* **5,**13-16.
[66] D.E. Okwu and C. Josiah (2006). Evaluation of the chemical composition of two Nigerian Medicinal plants, *Afr. J. Biotech* **5**(4), 357-361.
[67]. E.T. Ifon and O. Bassir (1979). The nutritive value of some Nigerian leafy green Vegetables-Part 1: Vitamin and mineral contents. *Food. Chem* **4**, 263-267.
[68] O.U. Eka and A.U. Osagie (1998). Nutritional quality of plant food. Post harvest publishers, University of Benin, Nigeria.
[69] A.S. Ekop, N.D. Eddy and P.G. Udofia (2004). Effect of Processing on the elemental composition of beans. Proceedings of 28th annual Conf. of Nig. Inst. of Food Sci Tec. (NIFEST), Ibadan
[70] A.S. Ekop and N.O. Eddy (2005). Comparative Studies of the level of toxicants in the seed of Indian Almond (*Terminalia catappa)* and African walnut (*Coula edulis*), *Chem. Class. J*. **2,**74-76.
[71] K. Hostettan, and A Marston (1995). Saponins: chemistry and pharmacology of natural products. Cambridge University Press, Cambridge, UK.
[72] M.N. Asl, and H. Hosseinzadeh (2008). Review of pharmacological effects of *Glycyrrhiza* spp and its bioactive compounds. *Phytother. Res.* **22**(6), 709-724.
[73] J. Hussain, Z. Muhammad, R. Ullah, F.U. Khan, I.U. Khan, N. Khan, J. Ali, and S. Jan (2010). Evaluation of the chemical composition of *Sonchus eruca* and *Sonchus asper, J. Am. Sci.* **6**(9), 231-235.