Polyphenolic and biological activities of leaves extracts of *Argemone subfusiformis* (Papaveraceae) and *Urtica urens* (Urticaceae)

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Abstract: Nowadays there is a resurgence of interest in wild plants for their possible medicinal value in diets, since some epidemiological studies have demonstrated their effectiveness against important diseases. Generally, foods of plant origin contain many bioactive compounds, proteins, energy, vitamins and specific minerals; in addition, the popular wild plant species provide fibre, essential fatty acids and enhance the taste and colour in diets. We studied the nutritional and medicinal potential of leaves of *A. subfusiformis* and *U. urens*, collected in Alice, South Africa in November 2006. To asses this we analyzed the phytochemical, antioxidant and antibacterial activities of leaves in acetone, methanol and water extracts, using standard analytical methods. The proximate analysis showed that the leaves of both plant species contained appreciable percentages of moisture; ashes; carbohydrates; crude proteins, lipids and fibres. Elemental analysis of macro and microelements showed higher values for *U. urens* that contained in decreasing order: iron>manganese>zinc>copper>calcium>potassium>nitrogen>magnesium> phosphorus>sodium, for *A. subfusiformis* resulted in iron>zinc>manganese>copper>calcium>potassium>nitrogen> magnesium>phosphorus>sodium. Besides, the chemical composition showed higher concentration of alkaloids, saponins and phytates in *A. subfusiformis*. The extracts also caused 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2-azinobis-3 ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activities which were comparable to those of ascorbic acid. The extracts of both plants contained appreciable levels of polyphenols and also caused varied inhibition of some bacterial strains used in this study. When the nutrient and chemical constituents of these plants were compared with recommended dietary allowance (RDA) values, the results revealed that the leaves contain an appreciable amount of nutrients, minerals, and phytochemicals and low levels of toxicants. Since the plants also exhibited some level of antibacterial activities, their use for medicinal purposes is to some extent being justified. Rev. Biol. Trop. 58 (4): 1517-1531. Epub 2010 December 01.

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Foods of plant origin contain many bioactive compounds in addition to conventionally identified nutrients such as proteins, energy, vitamins and specific minerals. More than 900 different phytochemicals have been identified as components of food and there may be more than 100 in just one vegetable (Akindahunsi & Salawu 2005). Epidemiological studies have demonstrated that people eating vegetarian diets have a reduced risk of heart diseases and obesity (Bagchi & Puri 1998).

Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet (Bravo 1998). Interest in polyphenols has greatly increased recently because these phytochemicals are known to suppress rates of degenerative processes such as cardiovascular disorders and

cancer (Bravo 1998, Duthie 2000, Huang *et al*. 2007). Some of these potential health benefits of polyphenolic substances, have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation (Li-Chen *et al*. 2005). As a group, phenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases (Kyung-Hee *et al*. 2005, Chen & Yen 2007).

A resurgence of interest has developed in wild species for their possible medicinal values in diets. Wild plant species provide minerals, fibre, vitamins and essential fatty acids and enhance taste and colour in diets. In addition, they have antibacterial, hepatoprotective and anticarcinogenic properties and therefore have medicinal values (Green 1992, Bianco *et al*. 1998, Yildrim *et al*. 2001).

It is interesting to note that few vegetables species are consumed in South Africa by the general public. 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 (pers. comm.). This is in spite of the fact that these vegetables grow spontaneously and in abundance around the rural homesteads. Many workers (Lockeett *et al*. 2000, Akindahunsi & Salawu 2005, Edeoga *et al*. 2006, Hassan & Umar 2006, Ekop 2007) have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries. The consumption of vegetables has also been linked to reduction in the incidence of oxidative-stress related diseases such as cancer, diabetes amongst others (Akubugwo *et al*. 2007). Some plant phenolics have also shown antimicrobial effects (Koduru *et al*. 2006).

Argemone subfusiformis Ownbey subsp. *subfusiformis* (Papaveraceae) has butter-yellow petals; seeds approximately 2mm diameter and a capsule with fewer and larger spines. This plant also known as American poppy, is native to the Americas and Hawaii. Commonly found as a weed of roadsides, mining dumps, rabbit warrens, recently cultivated paddocks, waste places, and over grazed pastures. It often occurs as dense stands in sandy stream beds and alluvial flats associated with intermittent inland streams. *A. subfusiformis* contains isoquinoline alkaloids including sanguinarine, which causes mucosal irritation and also has interference with pyruvate oxidation in brain tissue. The sanguinarine is excreted in cows' milk and may be linked to the occurrence of endemic primary glaucoma in humans. The seeds of *A. mexicana* and *A. ochroleuca* are frequent contaminants of grain fed to chicken, causing low egg production, edema, ataxia, comb cyanosis and gastroenteritis. Dried plants in hay may cause chronic heart failure in cattle (Everist 1974, Auld & Meld 1992; Wilson *et al*. 1995, Parsons & Cuthbertson 2001, Harden 2002).

Urtica urens (dwarf nettle) is a member of the Urticaceae and native to Eurasia. *Urtica* prefers wet, rich soil and tends to grow in large patches. The stems are covered with stinging hairs but the leaves are smooth and more delicate (Wagner *et al*. 1994, Hirano *et al*. 1994, Schottner *et al*. 1997). The plant produces inconspicuous green-white flowers in late spring or summer. The leaf, flower, seed, and root of nettle are used differently and contain different chemical constituents. Like all green vegetables, nettle leaf is a micronutrient dense, nutritious food; however, it should be steamed or cooked before ingestion to destroy the stinging hairs, which contain histamine, formic acid, acetylcholine, acetic acid, butyric acid, leukotrienes, 5-hydroxytryptamine, and other irritants. Contact with the hairs leads to a mildly painful sting, development of an erythematous macule, and itching or numbness for a period lasting from minutes to days. Medicinal extracts of nettle do not cause this reaction as the hairs are destroyed in processing (Wagner *et al*. 1994, Hirano *et al*. 1994, Hryb *et al*. 1995, Berges *et al*. 1998).

These two plant species were identified as wild vegetables in South Africa and the study

was therefore aimed at assessing their nutritional quality and possible biological activities.

MATERIALS AND METHODS

Plant collection and extract preparation: Fresh plant materials of *Argemone subfusiformis* and *Urtica urens* were collected in November 2006 from the wild around the University of Fort Hare campus, Alice, South Africa. The area falls within 30°00 34°15' S-22°45' 30°15' E. It is bounded by the sea in the East and the drier Karoo (semi-desert vegetation) in the West (Masika & Afolayan 2003). 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, and 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 of dried plant samples were each extracted with 200mL of acetone, methanol, and water, respectively, at ambient temperature, with agitation for 18-24h. 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 (2.5%), methanol (7.9%), and water (9.3%), respectively for *A. subfusiformis* but 2.7% (acetone), 8.7% (methanol) and 8.8% (water) respectively for *U. urens*. The dried extracts obtained were used directly for the determination of the antioxidant and antibacterial activities (Taylor *et al*. 1996). Determinations of chemical and nutritive values of these plants were carried out using the dried samples that were ground into powder.

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, Tannic Acid, Quercetin and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); vanillin from BDH, Folin-Ciocalteu's Phenol Reagent and Sodium Carbonate from Merck Chemical Supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.

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.

Mineral analysis: The automated procedure for determining cations in 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.

Anti-nutrient analysis: Determination of alkaloid and saponins were as described by Obadoni & Ochuko (2001). Phytate was estimated by the method of Wheeler & Ferrel (1971).

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 5ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4ml (75g/l) of Sodium Carbonate. The tubes were mixed for 15sec and allowed to stand for 30min at 40°C for color development. Absorbance was measured at 765nm using the Hewlett Packard UV-VS spectrophotometer. Samples of extracts were evaluated at a final concentration of 0.1mg/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).

Determination of total flavonoids: Total flavonoids were estimated using the method

of Ordoñez *et al*. (2006). A volume of 0.5ml of 2% $AlCl_3$ ethanol solution was added to 0.5ml of samples. After one hour at room temperature, the absorbance was measured at 420nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1mg/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 *y* was the quercetin equivalent (mg/g).

Determination of total flavonols: Total flavonols in plant extracts were estimated using the method of Kumaran & Karunakaran (2006). 2.0mL of 2% $AlCl_3$ ethanol and 3.0mL (50g/L) sodium acetate solutions were added to 2.0mL of the sample. The absorption at 440nm was read after 2.5h at 20°C. Extract samples were evaluated at a final concentration of 0.1mg/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 *y* was the quercetin equivalent (mg/g).

Determination of total proanthocyanidins: Determination of proanthocyanidin was based on the procedure reported by Sun *et al*. (1998). A volume of 0.5ml of 0.1mg/ml of extract solution was mixed with 3ml of 4% vanillin-methanol solution and 1.5ml hydrochloric acid; the mixture was allowed to stand for 15min. The absorbance was measured at 500nm. Extract samples were evaluated at a final concentration of 0.1mg/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* was the absorbance and *y* was 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 & Shahidi (2003). A solution of 0.135mM DPPH in methanol was prepared and 1.0ml of this solution was mixed with 1.0ml of extract in methanol containing 0.02-0.1mg of the extract. The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%)=$[(Abs_{control}-Abs_{sample})]/(Abs_{control})]x100$ 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 this assay. The stock solutions included 7mM ABTS solution and 2.4mM Potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12h at room temperature in the dark. The solution was then diluted by mixing 1ml ABTS solution with 60ml methanol to obtain an absorbance of 0.706±0.001 units at 734nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1ml) were allowed to react with 1ml of the ABTS solution and the absorbance was taken at 734nm after 7min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT, and the percentage inhibition calculated as ABTS radical scavenging activity (%)=$[(Abs_{control}-Abs_{sample})]/(Abs_{control})]x100$ 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 & Strain (1996) was adopted for the FRAP assay. The stock solutions included 300mM Acetate buffer (3.1g $C_2H_3NaO_2 \cdot 3H_2O$ and 16ml $C_2H_4O_2$), pH 3.6, 10mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40mM HCl, and 20mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was

prepared by mixing 25ml acetate buffer, 2.5ml TPTZ, and 2.5ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before using. Plant extracts (150µL) were allowed to react with 2850µL of the FRAP solution for 30min in a dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593nm. The standard curve was linear between 200 and 1000µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT, ascorbic acid and catechin.

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 24hr. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan & Meyer 1997).

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 5.0mg/ml (Koduru *et al*. 2006). Plates containing only nutrient agar and another set containing nutrient agar and the respective solvents served as controls. Streptomycin and chloramphenicol served as standard. After inoculation, the plates were incubated at 37ºC for 24 to 48h. Each treatment was performed in triplicate and complete inhibition of bacterial growth was required for an extract to be declared bioactive.

The experimental results were expressed as mean±standard deviation (SD) of three replicates. When 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.

TABLE 1
Proximate analysis of the leaves of Argemone subfusiformis *and* Urtica urens

Constituents	*Argemone subfusiformis*	*Urtica urens*
Moisture	72.80±8.96	57.16±2.18
Ash	14.5±0.00	27.75±0.25
Protein	19.19±0.24	18.36±0.10
Fat	5.5±0.01	7.25±0.25
Carbohydrate	36.69±0.30	30.29±0.4
Crude fibre	21.13±0.7	16.08±0.76
Energy (kcal)	285.02±0.20	260.93±0.1

RESULTS

Proximate analysis: The proximate analysis expressed in percentages, showed the moisture, ash, crude protein, crude lipid, crude fibre and carbohydrate contents in leaves of *A. subfusiformis* as 72.8, 14.5, 19.2, 5.5, 21.1 and 36.7%, respectively, while its calorific value is 285.0Kcal/100g. In the case of *U. urens*, analysis showed the moisture, ash, crude protein, crude lipid, crude fibre and carbohydrate content of the leaves as 57.2, 27.8, 18.4, 7.3, 16.1 and 30.3%, respectively, while its calorific value is 260.9Kcal/100g (Table 1).

Macro and microelements analysis: Elemental analysis in mg/100g (DW) indicated that leaves of *A. subfusiformis* contained the following ordered from higher to lower concentrations: iron (396), zinc (72), manganese (71), copper (17), calcium (4.8), potassium (3.2), nitrogen (3.1), magnesium (0.7), phosphorus (0.3), and sodium (0.09). Furthermore, leaves of *U. urens* contained iron (839), manganese (104), zinc (55), copper (13), calcium (12.3), potassium (3.3), nitrogen (2.3), magnesium (0.7), phosphorus (0.5) and sodium (0.09) (Table 2).

Antinutrient analysis: The chemical composition in mg/100g (DW) for alkaloid, saponins, and phytate were 2.4, 5.8 and 4.3 respectively for *A. subfusiformis*. In the case of *U. urens*, these values were alkaloids (0.6), saponins (3.3) and phytate (4.4) (Table 3).

Polyphenols: For *A. subfusiformis,* the acetone and methanol extracts showed higher

TABLE 2
Macro and micro elements constituents of leaves of Argemone subfusiformis *and* Urtica urens

Macro and micro elements (mg/100g dwb)	*Argemone subfusiformis*	*Urtica urens*
Calcium	4.787	12.262
Copper	17	13
Iron*	396	839
Magnesium	0.663	0.683
Manganese	71	104
Phosphorus	0.272	0.463
Potassium	3.218	3.251
Sodium	0.094	0.092
Total Khedjal Nitrogen	3.07	2.98
Zinc	72	55

*Iron= ppm.

TABLE 3
Analysis of anti-nutrients contents of Argemone subfusiformis *and* Urtica urens

Anti-nutrients	*Argemone subfusiformis*	*Urtica urens*
Alkaloids	2.35±0.35	0.58±0.03
Saponins	5.75±0.2	3.25±0.1
Phytate	4.30±0.61	4.39±1.10

concentrations of polyphenols than the water extract; whereas for *U. urens,* the methanol extract had greater content of total polyphenol than its acetone and water counterparts. In the case of flavonoids, acetone extract (1.22) resulted higher than water (0.63) and methanol (0.35) for *A. subfusiformis,* whereas for *U. urens* the flavonoids contents of acetone extract (0.89) was higher than methanol (0.46) and water (0.36) ones (Table 4).

Flavonols: The results for flavonols showed no difference for the acetone and methanol extracts of *A. subfusiformis,* though they were higher than those for the water extract. In the case of *U. urens,* the methanol extract had greater content of flavonols than the other two extracts. For proanthocyanidins, the acetone extracts of *A. subfusiformis* and *U. urens* were upper than those of methanol; besides, the acetone extract of *U. urens* was considerably high (8.12) (Table 4).

DPPH: At 1mg/ml, the acetone, methanol, water extracts and BHT caused DPPH radical scavenging activity at 84.9, 92.3, 69.5 and 99.3% respectively for *A. subfusiformis,* while for *U. urens* (at 1mg/ml) results were 60.8, 91.2, 63.5 and 100% for acetone, methanol, water and ascorbic acid respectively (Figs. 1 A & B).

ABTS: At 1mg/ml, the acetone extract of *A. subfusiformis* caused 98.2% ABTS radical scavenging inhibition while the methanol, water extracts and BHT caused inhibition at 99.4, 95.9 and 99.3%, respectively. For *U. urens* at the same concentration, the results were 97.5,

TABLE 4
Total polyphenol and flavonol contents of the acetone, methanol and water extracts of the leaves of Argemone subfusiformis *and* Urtica urens *(n=3, X±SEM)*

Phenolics	*Argemone subfusiformis*			*Urtica urens*		
	Acetone	Methanol	Water	Acetone	Methanol	Water
Total polyphenol	14.97±0.46	14.0±0.89	6.18±0.27*	7.28±0.23*	14.42±0.51	4.58±1.40*
Flavonoids	1.22±0.03	0.35±0.12*	0.63±0.01	0.89±0.08	0.46±0.02	0.36±0.03*
Proanthocyanidins	3.36±0.06*	2.36±0.40*	0.61±0.10*	8.12±0.36	4.57±1.12	2.92±0.38*
Total Flavonol	0.90±0.03	0.97±0.45	0.43±0.14*	0.81±0.01	1.11±0.35	0.71±0.02

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 catechin/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 $p<0.05$.

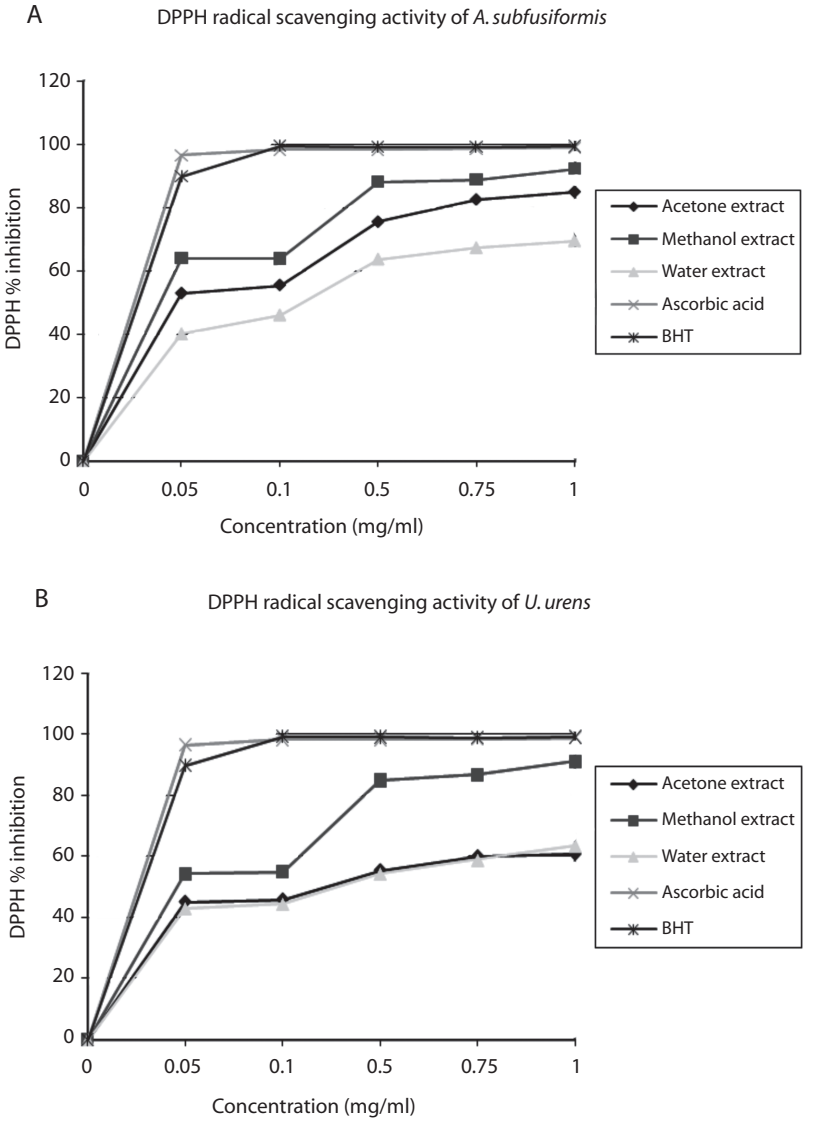

Fig. 1. (**A**) DPPH radical scavenging activities of *Argemone subfusiformis* and (**B**) DPPH radical scavenging activities of *Urtica urens*.

95.2, 98.5 and 99.3% for acetone, methanol, water and BHT respectively. At 0.5mg/ml, the percentage inhibition for methanol extract of *U. urens* was 99.5 (Fig. 2 A & B).

FRAP: The acetone and methanol extracts of both plants had higher ferrous reducing antioxidant power (FRAP), when compared to that of water extract and BHT, but less activity compared to catechin, ascorbic acid and quercetin (Table 5).

Antibacterial assay: The acetone extract of *A. subfusiformis* has activity against all the

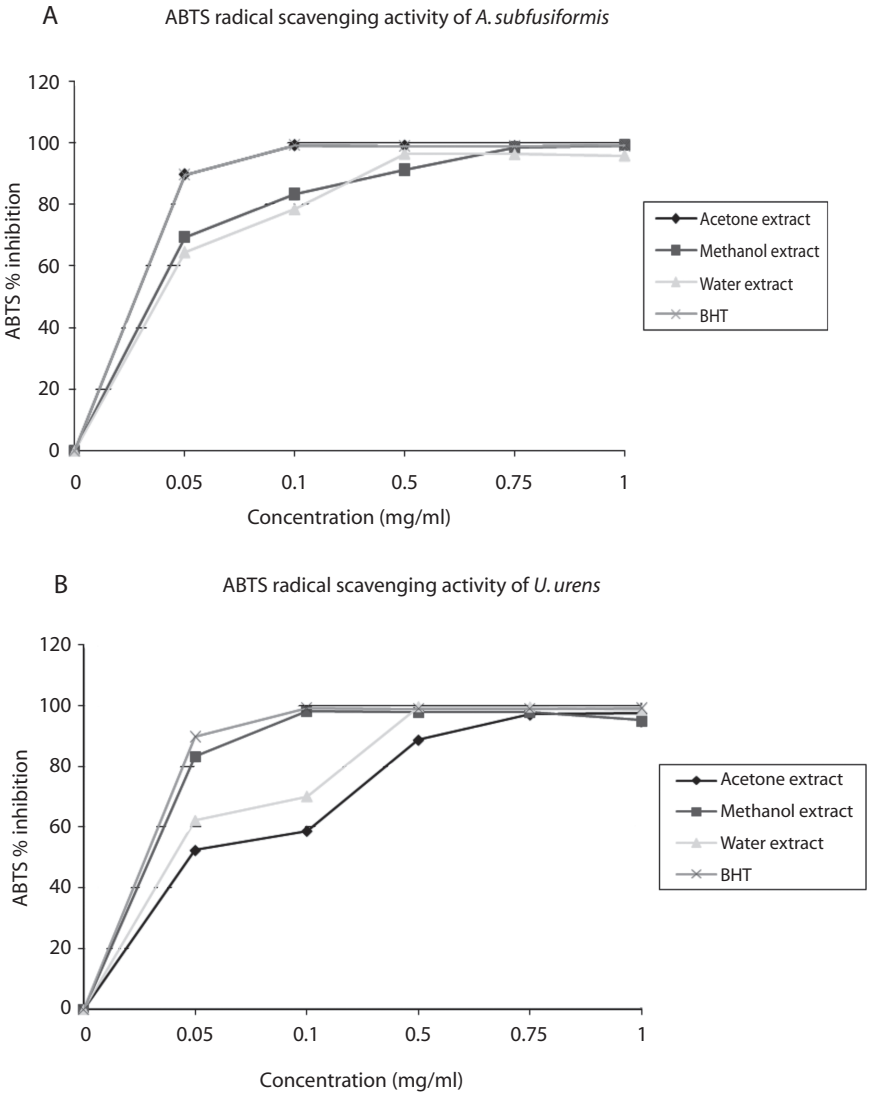

Fig. 2. (**A**) ABTS scavenging activities of *Argemone subfusiformis* and (**B**) ABTS scavenging activities of *Urtica urens*.

Gram positive organisms except *Staphylococcus aureus*. It was also active against two Gram negative organisms i.e. *Escherichia coli* and *Salmonella pooni*. The methanol extract was active against three gram positive organisms and one Gram negative organisms used in this study. The water extract of *A. subfusiformis* however showed activity against all the Gram positive organisms as well as two Gram negative strains used in this study. The acetone extract of *U. urens* showed activity against *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *E. coli*. The water extract showed activity against all Gram positive strains except *Streptococcus pyrogens*. The methanol extract of *U. urens* was active against four Gram positive and one Gram negative strains. The water extract was the

TABLE 5
FRAP activity of the acetone, methanol and water extracts of Argemone subfusiformis *and* Urtica urens

Extracts/Standards	*Argemone subfusiformis*	*Urtica urens*
Acetone	487.73±29.46	248.39±5.73
Methanol	175.44±13.2	90.57±13.65
Water	13.43±1.38	18.77±3.01
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.

most active since it had activity against all the organisms used in this study and even at the lowest concentration (0.1 and 1.0 mg/ml) (Tables 6 and 7).

DISCUSSION

Proximate analysis: The results of proximate composition of the leaves of *A. subfusiformis* (72.8%) and *U. urens* (57.2%) showed relatively low moisture content. This is below the reported range (81.4-90.3%) in some Nigerian green leafy vegetables (Akubugwo *et al*. 2007). Ash content, which is an index of mineral contents in biota, is 14.5 DW in *A. subfusiformis* and 27.8% DW in *U. urens*. These compare favourably with the values reported for *Ipomea batatas* (11.10%), *Vernonia colorata* (15.86%) and *Moringa oleifera* (15.09% DW) (Lockeett *et al*. 2000, Antia *et al*. 2006). The value for *U. urens* is also, higher than that of some Nigerian leafy vegetable such as *Ocimum gratissium* (18.00% DW) and *Hibiscus esculentus* (8.00% DW) (Akindahunsi & Salawu 2005). The crude protein content of *A. subfusiformis* (19.2% DW) and *U. urens* (18.4%) are greater than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland (Ogle & Grivetti 1985, Isong & Idiong 1997, Hassan & Umar 2006). According to Pearson (1976), plant food that provides more than 12% of its calorific value is considered good source of protein. Therefore, the protein content of the leaves of these two plants will go a long way in meeting the protein requirement of the local people.

The crude lipid content of *A. subfusiformis* (5.5 % DW) and *U. urens* (7.3% DW) resulted slightly lower than reported values (8.3-27.0% DW) in some vegetables consumed in West Africa (Ifon & Bassir 1980, Sena *et al*. 1998). 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 (Agbo 2004, Gupta *et al*. 2005). The carbohydrate content of *A. subfusiformis* (36.7% DW) and *U. urens* (30.3% DW) is higher than 20 and 23.7% reported for *Senna obtusfolia* and *Amaranthus incurvatus* leaves, respectively

TABLE 6
Antibacterial activity of leaves extracts of Argemone subfusiformis *(A.s.)*

Bacterial species	Gram +/-	Minimum inhibitory concentration (mg/ml)				
		Acetone	Methanol	Water	Chloramphenicol	Streptomycin
Bacillus cereus	+	1.0	2.0	1.0	<2	<2
Staphylococcus epidermidis	+	1.0	na	1.0	<2	<2
Staphylococcus aureus	+	na	na	1.0	<2	<2
Micrococcus kristinae	+	2.0	2.0	1.0	<2	<2
Streptococcus pyrogens	+	5.0	5.0	1.0	<2	<2
Escherischia coli	-	5.0	5.0	na	<2	<2
Salmonella pooni	-	5.0	na	1.0	<2	<2
Serratia marcescens	-	na	na	1.0	<2	<2
Pseudomonas aeruginosa	-	na	na	na	<20	<5
Klebsiella pneumonae	-	na	na	na	<2	<2

TABLE 7
Antibacterial activity of leaves extracts of Urtica urens

Bacterial species	Gram +/-	Minimum inhibitory concentration (mg/ml)				
		Acetone	Methanol	Water	Chloramphenicol	Streptomycin
Bacillus cereus	+	2.0	5.0	0.1	<2	<2
Staphylococcus epidermidis	+	2.0	5.0	0.1	<2	<2
Staphylococcus aureus	+	2.0	5.0	0.1	<2	<2
Micrococcus kristinae	+	na	5.0	0.1	<2	<2
Streptococcus pyrogens	+	na	na	0.1	<2	<2
Escherischia coli	-	2.0	5.0	0.1	<2	<2
Salmonella pooni	-	na	na	0.1	<2	<2
Serratia marcescens	-	na	na	0.1	<2	<2
Pseudomonas aeruginosa	-	na	na	1.0	<20	<5
Klebsiella pneumonae	-	na	na	1.0	<2	<2

(Faruq *et al*. 2002, Hassan & Umar 2006). This is however lower than reported values for *Corchorus tridens* (75.0% DW) and sweet potatoes leaves (82.8%) (Asibey-Berko & Tayie 1999). The crude fibre content of *A. subfusiformis* (21.1% DW) and *U. urens* (16.1% 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%) (Akindahunsi & 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 & Newmark 1998, Ishida *et al*. 2000). The estimated calorific value for *A. subfusiformis* (285.0kcal/100g DW) and *U. urens* (260.9% DW) leaves compare favourably to 248.8-307.1Kcal/100g DW reported in some Nigerian vegetables (Isong *et al*. 1999, Antia *et al*. 2006, Akubugwo *et al*. 2007). Asibey-Berko & Tayie (1999) also reported comparable energy content in some Ghanaian green leafy vegetables. Thus, confirming that vegetables have low energy values (Lintas 1992).

Macro and micro element analysis: 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 (FND 2002). Therefore, consumption of *A. subfusiformis* and *U. urens* would probably reduce high blood pressure diseases because their Na/K is less than one. Iron content of leaves of *A. subfusiformis* (396mg/100g) and *U. urens* (839mg/100 g) is very high when compared with the value reported in *I. batatas* (16.00mg/100g) (Antia *et al*. 2006). Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and for the oxidation of carbohydrates, protein and fats (Adeyeye & Otokiti 1999, Akubugwo *et al*. 2007). The zinc content of *A. subfusiformis* (72mg/100g) and *U. urens* (55mg/100g) compares favourably to most values reported for green leafy vegetables in literatures (Ibrahim *et al*. 2001, Hassan & Umar 2006). Zinc is involved in normal function of immune system.

Anti-nutrient analysis: Analysis of the antinutrient contents of the plant showed that alkaloid level in *A. subfusiformis* (2.4mg/100g) and *U. urens* (0.6mg/100g) is lower than the values reported for the leafy vegetables like *Aspilia africana*, *Bryophyllum pinnatum*, *Cleome rutidosperma* and *Emilia coccinea* consumed in Nigeria (Edeoga *et al*. 2006, Okwu & Josiah 2006, Akubugwo *et al*. 2007). The levels of saponins in these plants are much less than the value reported for some medicinal plants used in Nigeria. The phytate level in these vegetables is still within the tolerable limits and can easily be detoxified by soaking, boiling or frying (Akubugwo *et al*. 2007).

Polyphenols: Polyphenols are the major plant compounds with antioxidant activity. Some of the potential health benefits of polyphenolic substances have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation (Wichi 1988, Li-Chen *et al*. 2005). As a group, phenolic compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species, the major cause of many chronic human diseases (Kyung-Hee *et al*. 2005, Chen & Yen 2007). Phenolic compounds are plant substances which posses in common an aromatic ring bearing hydroxyl substituent. They may occur combined with sugar, as glycosides and they are usually located in the vacuole of the plant cells. The results strongly suggest that phenols 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 *A. subfusiformis* in general were higher than those of *U. urens*. The acetone extract of *U. urens* however showed considerably high level of proanthocyanidins. The antioxidant activity of *A. subfusiformis* is relatively greater than that of *U. urens*. This may be due to relatively higher polyphenolic contents of the extracts of *A. subfusiformis*.

Antioxidant activities: 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 acetone extract of *A. subfusiformis* and *U. urens* at 1mg/ml were 92.3% and 91.2% respectively, hence slightly less than those of ascorbic acid (99%) and BHT (99.3%). This showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals (Yoshida & Takagi 1999). At 1mg/ml concentration, all the extracts for the two plants produced similar or equal ABTS radical scavenging activity showing that higher concentrations of the extracts were more effective in quenching free radicals in the system. The scavenging of the ABTS radical by the extracts at 1mg/ml was found to be 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 & Wang 2001). Wang *et al*. (1998) found that some compounds which have ABTS scavenging activity did not show DPPH scavenging activity.

The antioxidant potentials of the leaves extracts of these plants were estimated from their ability to reduce TPTZ-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 (Wolfe *et al*. 2003). The acetone and methanol extracts of the two plants had higher ferrous reducing antioxidant power (FRAP) relative to water and BHT, but less activity compared to catechin, ascorbic acid and quercetin. It thus meant that these plants exhibited similar activity.

Antibacterial assay: The high activity shown by the extracts of these two plants may have justified their use for medicinal purposes. The water extract of *U. urens* particularly showed high activity against all the organisms used in this study. The German Commission E approves the use of nettle leaf as supportive therapy in patients with lower urinary tract infections (combined with immune and antimicrobial therapy) and to prevent and treat formation of urinary gravel (Blumenthal *et al*. 1998). 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 may have supported the fact that, in general, the Gram-negative bacteria are less susceptible to antibacterial effect than

the Gram positive ones (Grierson & Afolayan 1999, Afolayan 2003).

The results of this study showed that the leaves of *A. subfusiformis* and *U. urens* 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 *A. subfusiformis* and *U. urens* leaves can contribute significantly to the nutrient requirements of man and should be used as supplement nutrients to other major sources. 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.

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RESUMEN

En la actualidad se ha dado un resurgimiento del interés en las plantas silvestres por su posible valor en la dieta, como consecuencia de algunos estudios epidemiológicos que han demostrado efectividad contra importantes enfermedades. En general, los alimentos de origen vegetal contienen muchos compuestos bioactivos, proteínas, energía, vitaminas y minerales específicos; además, las especies silvestres populares proporcionan fibras, ácidos grasos esenciales y aditivos para mejorar el sabor y color de los alimentos. Estudiamos el potencial nutricional y medicinal de las hojas de *A. subfusiformis* y *U. urens*, recolectados en Alice, Sudáfrica, en noviembre de 2006. Analizamos los compuestos químicos y antioxidantes de estas plantas, la actividad antibacteriana de las hojas en extractos acuosos, con acetona y metanol, utilizando los métodos estándares de análisis. Encontramos que las hojas de ambas especies contienen apreciables porcentajes de humedad, cenizas, carbohidratos, proteínas, lípidos y fibras. El análisis de los macro y microelementos mostró valores altos para *U. urens* en el siguiente orden decreciente: >hierro>manganeso>zinc>cobre>calci o> potasio>nitrógeno>magnesio>fósforo>sodio y para *A. subfusiformis* fue: hierro>zinc>manganeso>cobre>calci o>potasio>nitrógeno>magnesio>fósforo>sodio. Además, la composición química mostró una alta concentración de alcaloides, saponinas y fitatos en *A. subfusiformis*. Estos extractos 1, 1 - difenil-2-picrilhidrazil (DPPH) y 2, 2-azinobis-3 ácido etilbenzotiazolina-6-ácido sulfúrico (ABTS) también causaron actividad en el barrido de radicales comparables con los del ácido ascórbico. Los extractos de ambas especies contienen niveles apreciables de polifenoles y también causaron una variada inhibición de algunas cepas bacterianas utilizadas en el estudio. Cuando los nutrientes y compuestos químicos de estas plantas se compararon con los valores dietéticos recomendados (RDA), los resultados revelaron que las hojas contenían una apreciable cantidad de nutrientes, minerales, compuestos químicos y bajos niveles de sustancias tóxicas. Dado que las plantas también mostraron un cierto nivel de actividad antibacteriana, su utilización para fines medicinales en cierta medida está justificada.

Palabras clave: antibacterial, antioxidante, valor nutritional, *Argemone subfusiformis, Urtica urens*.

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