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Research Article

STUDY OF NUTRITIONAL QUALITY, PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES BY DIFFERENT SOLVENTS OF NETTLE (*Urtica urens*) FROM MADIKERI-KARNATAKA STATE

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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. The aim of this study was to determine the nutritional and medicinal potential of leaves of *Urtica urens*. To asses this we analysed the phytochemical and antioxidant activities of leaves in acetone, ethanol, methanol, water and ethyl acetate solvents used for extraction, using standard analytical methods. The proximate analysis showed that the leaves contained appreciable percentage of nutrition and was good in ash and protein content. The extracts contain range 6.76±0.02-21.46±0.04 mg/g levels of polyphenols by showing DPPH and ABTS radical scavenging activity by the solvents. Among them methanol showed 78% and 97% activity at 0.5mg/ml concentration respectively. FRAP activity in acetone was found to be higher than standard BHT compared to other solvents. Macro and microelements showed higher value in decreasing order: iron>manganese>zinc> copper>calcium>potassium> magnesium> phosphorus>sodium. When plant was compared with recommended dietary allowance (RDA) values, the results revealed that the leaves contain an appreciable amount of nutrients, minerals, and phytochemicals. Obtained results provide support for the use of this plant in traditional medicine and suggest its further advanced investigation.
Key**words:** Nutritional, Antioxidant, Polyphenols, Minerals, *Urtica urens*

INTRODUCTION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [19]. Indian subcontinent is rich/ bestowed with a wide variety of flora and fauna. This variation is due to the varied climatic condition, vegetation, topography etc. resulting in enriched heterogeneity. As a result, many herbs with potential medicinal value are left unnoticed. These herbs may possess medicinal values, domestic values and therapeutic values. It has been proved since ages the benefits of using these natural agents for curing various diseases. This property may be due to the presence of some active compounds that are different for each plant.

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 [2]. Epidemiological studies have demonstrated that people eating vegetarian diets have a reduced risk of heart diseases and obesity [6].

Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet [9]. Interest in polyphenols has greatly increased recently because these phytochemicals are known to suppress rates of degenerative processes such as cardiovascular disorders and cancer [9, 13, 22]. 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 [28]. 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 [27, 11]. *Urtica urens* (dwarf nettle) is a member of the family Urticaceae and it 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 [39,20, 34]. 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 [39, 20, 21, 8]. The aim of this study is to evaluate chemical constituents and antioxidant activities of aqueous, acetone, ethanol, methanol and ethyl acetate extract of leaves of nettle. And preliminary phytochemical screening of the extract was also done along

the determination of total phenolic content to compare the solvent efficiency in extraction and potential media.

MATERIALS AND METHODS

Plant material

Fresh leaves of Nettle (*Urtica urens*) were collected from Madikeri, Karnataka state, India August 2012. Identification of the plant was carried out by Dr. Nagendra .N, Department of Botany, College of Bharathi Education Trust, Affiliated to the University of Mysore, Karnataka,
India.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Potassium persulphate, Gallic acid, Catechin, ButylatedHydroxytoluene (BHT), Quercetin and FeCl3 were purchased from Sigma Chemical, Folin-Ciocalteu's Phenol Reagent and Sodium Carbonate from Merck Chemical Supplies. All the other chemicals used including the solvents, were of analytical grade.

Sample preparation

The plants were cleaned and leaves cut into small pieces, and then air dried at ambient temperature (±24°C). The dried samples were then pulverized into fine powder in a grinder, which was then stored at 4°C until use.

Preparation of extract

Twenty grams of dried plant samples were each extracted with 200mL of acetone, methanol, ethanol, water and ethyl acetate 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 2.1% (acetone), 7.6% (methanol), 6.8% (ethanol), 7.8% (water) and 5.9% (ethyl acetate) obtained. The dried extracts obtained were used directly for the determination of the antioxidant analysis [38].

Proximate analysis

The recommended methods of the Association of Official Analytical Chemists [1] were used for the determination.

Mineral analysis

Inductively coupled plasma–optical emission spectrometer (ICP-OES Perkin Elmer. USA) was used in the analysis of minerals and metals. Sample was made to ash and dissolves in 10% nitric acid, filtered and makes up to 100 ml and fed to ICP-OES. Instrument is calibrated using multi standard elements (Perkin Elmer Life & Analytical Sciences USA) with 10 % nitric acid as sample blank.

Determination of total phenolic content

Total phenolic contents of all dry plants were determined with slight modifications using Folin- Ciocalteu assay as described by Atanassova [5]. An aliquot (1 ml) of extracts or a standard solution gallic acid was added. A reagent blank using (dd H2O) was also prepared. One ml of (1:1) Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min., 1 ml of 7% Na2CO3 solution was added to the mixture. The solution was diluted to 10 ml with dd H_2O and mixed. After incubation for 90 min. at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm using spectrophotometer. The data for the total phenolic content of the sample were expressed as milligram of gallic acid equivalents (GAE) per gram dry mass (mg GAE/g).

Determination of total flavonoids

Total flavonoids were estimated using the method of Ordoñez [31]. 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 evaluate at a final concentration of 0.1mg/mL. Total flavonoid content was calculated as quercetin equivalent (mg/g).

Determination of total flavonols

Total flavonols in plant extracts were estimated using the method of Kumaran & Karunakaran
[26]. 2 mL of 2% $AlCl_3$ ethanol and 3 mL (50g/L) sodium acetate solutions were added to 2 mL 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 equivalent (mg/g).

Determination of total proanthocyanidins

Determination of proanthocyanidin was based on the procedure reported by Sun [37]. 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).

Free radical scavenging activity

The scavenging activity of DPPH free radicals developed according to the method reported by Gyamfi [17]. 0.02-0.1mg of the extract in methanol was mixed with 1 ml of 0.135 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, read the absorbance at 517 nm BHT was used as standard. The percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}]}{[\text{Absorbance of control}]} \times 100$$

ABTS radical scavenging assay

The method of Re [32] 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.

$$\% \text{ Inhibition} = \frac{[(\text{Abs control} - \text{Abs sample})]}{[(\text{Abs control})]} \times 100$$

Where,
Abs control is the absorbance of ABTS radical methanol;
Abs sample is the absorbance of ABTS radical sample extract/standard**.**

Total antioxidant activity (FRAP assay)
A modified method of Benzie & Strain [7] was adopted for the FRAP assay. The stock solutions included 300mM Acetate buffer (3.1g C2H3NaO2·3H2O and 16ml C2H4O2), pH 3.6, 10mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40mM HCl, and 20mM FeCl3·6H2O solution. The fresh working solution was prepared by mixing 25ml acetate buffer, 2.5ml TPTZ, and 2.5ml FeCl3·6H2O. 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 FeSO4. Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT.

Statistical Analyses
Assays were performed in triplicate, and the results were expressed as mean values with standard deviations (SD).

RESULTS

Proximate analysis
The results (Table 1) obtained from proximate analysis of the *U. urens* leaves establishes that they can be ranked as mineral rich leaves due to their relatively high content when compared with the other components of the leaves except moisture content. The proximate analysis expressed in percentages, showed the moisture, ash, crude protein, crude lipid, crude fibre, carbohydrate contents and metabolic energy in leaves of *U. urens* to be 56.11, 25.76, 17.44, 6.94, 15.56, 7.55% and 163.46Kcal/100g respectively.

Table 1: Proximate analysis of the leaves of *Urtica urens*.

Constituents	*Urtica urens*
Moisture	**56.11±0.08**
Ash	**25.76±0.31**
Protein	**17.44±0.31**
Fat	**6.94±0.07**
Carbohydrates	**7.55±0.52**
Crude fiber	**15.56±0.48**
Metabolic Energy (Kcal)	**163.46±0.97**

Phytochemical analysis
Phytochemical analysis of plants extracts were carried out for dry plant samples. It showed presence of phytochemicals. However, sensitive phytochemicals were not detected. This could be because as time goes by few phytochemicals might get exhausted. The qualitative determination of these phytochemicals has been presented in table 2. The phytoconstituents detected in the plant materials could be responsible for their antimicrobial activity though their exact mode of action was not understood.

Table 2: Phytochemical analysis of dry leaves of *Urtica urens.*

S.No	Phytochemicals	Acetone	Ethanol	Methanol	Water	Ethyl acetate
1	Reducing sugar	+	+	+	+	-
2	Non-reducing sugar	*	+	+	+	*
3	Non-reducing Polysaccharides (Starch)	-	-	-	-	-
4	Proteins	-	-	-	-	-
5	Amino acids	*	+	+	+	+
6	Steroids	-	-	-	-	-
7	Cardiac glycosides	+	+	+	+	*
8	Anthraquinone glycosides	-	-	*	*	-
9	Saponins	-	-	-	+	-
10	Flavonoids	+	+	+	+	++
11	Alkaloids	+	+	+	+	++

(-) indicate absence, (+) indicates presence at good concentration, (*) indicates presence at low concentration, (++) indicates presence at high concentration.

Macro and microelements analysis
Minerals as inorganic elements function as co-factors in enzyme catalyzed reactions, regulation of acid-base balance, nerve conduction, muscle irritability and structural elements of the body. Elemental analysis in mg/100g (DW) indicated that leaves of *U. urens* (Table-3) contained the following order from higher to lower concentrations of essential minerals: iron (812.666±0.258), manganese(107.333±0.258),zinc(50.333±0.258),copper(12.106±0.049), calcium (11.352±0.022), potassium (3.360±0.000), magnesium (0.686±0.002), phosphorus (0.395±0.001) and sodium (0.067±0.000). 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 [15]. Therefore, consumption of *U. urens* would probably reduce

high blood pressure diseases because their Na/K is less than one.

Table 3: Macro and Micro elements of leaves of *Urtica urens*.

Elements in mg/100g	Urtica urens
Calcium	11.352±0.022
Copper	12.106±0.049
Iron	812.666±0.258
Magnesium	0.686±0.002
Manganese	107.333±0.258
Phosphorous	0.395±0.001
Potassium	3.360±0.000
Sodium	0.067±0.000
Zinc	50.333±0.258

Total polyphenol

The results for total polyphenols by different solvent extracts showed good phenolic content (Table-4). It is well known that plant polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations Solvent ethyl acetate had the highest levels of polyphenols (21.82±0.40) compared to methanol, ethanol, acetone and water in the order of 15.56±0.22, 12.60±0.10, 6.76±0.02 and 4.13±0.03 respectively. The antioxidant activity depends largely on their chemical composition, such as phenolics, flavonoids, enzymes, organic acids, amino acids, Maillard reaction products, ascorbic acid, carotenoids.

Flavonoids

Flavonoids are low molecular weight polyphenolic compounds present in all vascular plants. They are primarily recognized as the pigments responsible for autumnal burst of hues of yellow, orange and red shades in flowers and fruits. Flavonoids have been shown to possess a variety of biological activities at nontoxic concentrations in living organisms. Among the solvents ethyl acetate had highest amount (0.99±0.01, table.4).

Flavonols

Flavonols are phytochemical compounds found in high concentrations in a variety of plant-based foods and beverages. Based on their structure (3-hydroxyflavone backbone), flavonols are classified as flavonoids that include the following compounds: quercetin, kaempferol, and myricetin. The results obtained for flavonols showed no greater difference obtained. The ethyl acetate extracts of *U. urens* showed high amount of flavonols. The other solvents showed slightly similar activity present in decreasing order for methanol, ethanol, acetone and water (Table.4).

Proanthocyandins

Proanthocyanidins are a class of biologically active flavonoids found throughout the plant kingdom, and are one of the most potent antioxidants in nature. Here acetone keeps upper solvent for extraction of proanthocyandins (9.07±0.07). Ethyl acetate obtained second position by showing 7.18±0.07 content. Remaining solvent recorded values in increasing order: water, ethanol, methanol with 2.27±0.02, 3.68±0.06 and 5.76±0.03 respectively (Table-4).

Table 4: Total polyphenol, flavonoid, flavonol and proanthocyanidins of acetone, methanol, ethanol, water and ethyl acetate extracts of the leaves of *Urtica urens*.

Phenolic	Leaves of Urtica urens				
	Acetone	Ethanol	Methanol	Water	Ethyl acetate
Total polyphenol mean ± SD ($mg_{gallicacid}$/g)	6.76±0.02	12.60±0.10	15.56±0.22	4.13±0.03	21.82±0.40
Flavonoids mean ± SD ($mg_{quercetin}$ /g)	0.76±0.02	0.31±0.00	0.59±0.00	0.58±0.01	0.99±0.01
Flavonol mean ± SD ($mg_{quercetin}$ /g)	0.78±0.01	0.94±0.01	1.37±0.03	0.68±0.01	2.07±0.05
Proanthocyandins mean ± SD ($mg_{catechin}$/g)	9.07±0.07	3.68±0.06	5.76±0.03	2.27±0.02	7.18±0.07

DPPH radical scavenging activity

From the methodological point of view the DPPH method is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts. The results of radical scavenging activities measured using DPPH assay and plotted in fig: 1. *U. urens* leaf extract by methanol showing higher activity (78% at 0.5mg/ml) among the other solvents and ethyl acetate showing lower activity compared to ethanol, water and acetone. Activity can be determined through reduction of DPPH radicals at 516 nm.

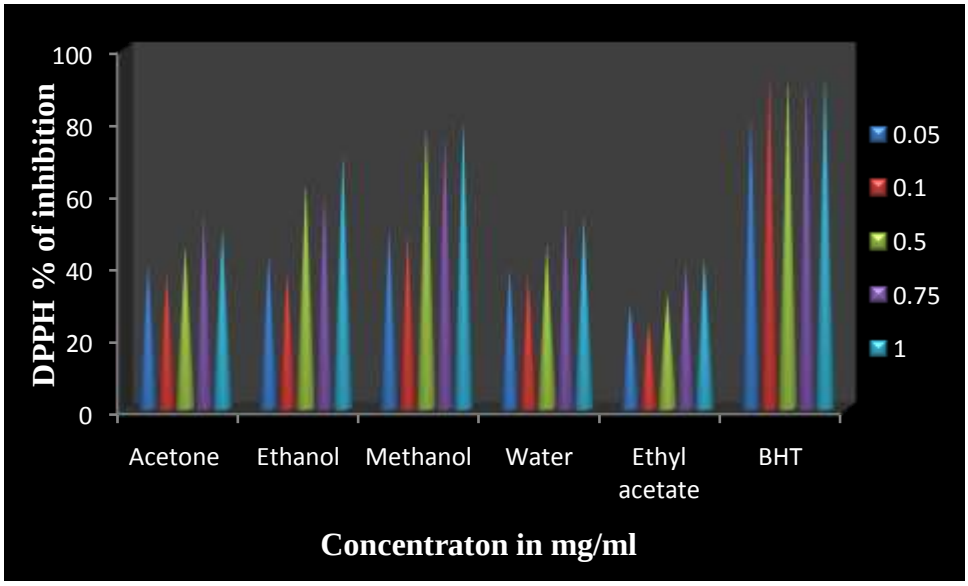

Fig 1: DPPH radical scavenging activity in different solvent extracts of *Utica urens*.

ABTS radical scavenging activity

The free radical scavenging ability of *U.urens* phenolics was determined using ABTS radical cation, too. ABTS radical cation has been often used in the evaluation of antioxidant activity of single compounds and complex mixtures of various origins (body fluids, foods, beverages, plant extracts). In this assay ABTS radical cation was generated directly in stable form using potassium persulfate. Generation of radical before the antioxidants are added prevents interference of compounds, which affect radical formation. This modification makes the assay less susceptible to artifacts and prevents overestimation of antioxidant capacity. Reactions of phenols with ABTS radical cation are usually rapid, but the reactions with DPPH radical differ from compound to compound. We have observed rapid and strong inhibition of both, DPPH radical or ABTS radical cation, after the addition of *U.urens* phenolics. Methanolic extract had greater ABTS inhibition activity (97% at 0.5mg/ml) and ethyl acetate had very less activity compare to ethanol, water and acetone (Fig: 2).

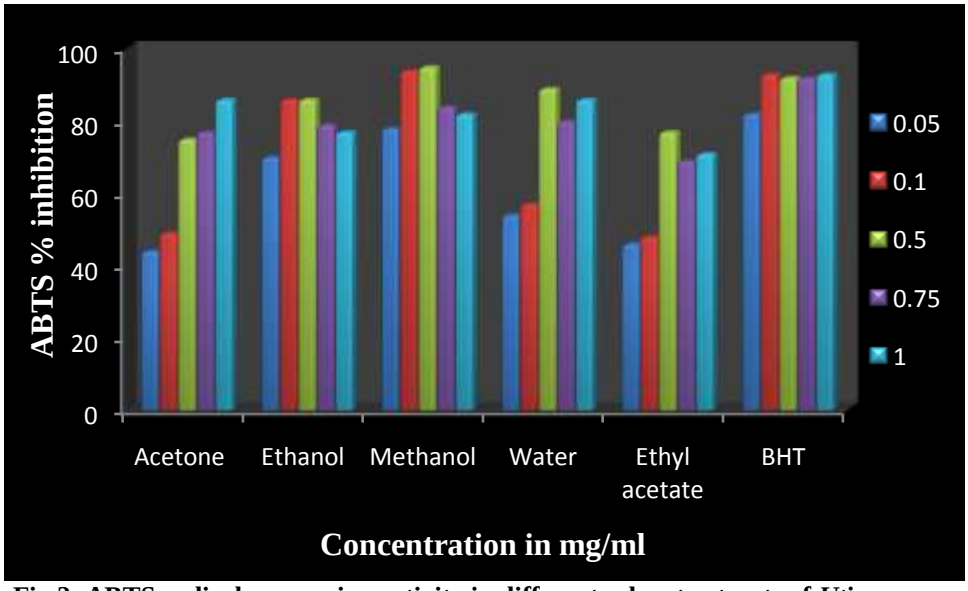

Fig 2: ABTS radical scavenging activity in different solvent extracts of *Utica urens*.

FRAP activity

In this study we used FRAP assay because it is quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present. This method was initially developed to assay plasma antioxidant capacity, but can be used to measure the antioxidant capacity from a wide range of biological samples and pure compounds to fruits, wines, and animal tissues. The solvent acetone extract had highest ferrous reducing antioxidant power compared to standard BHT which had 63.66 and other solvent extracts had antioxidant activity in increasing order: water<ethanol<methanol<ethyl acetate (Fig: 3).

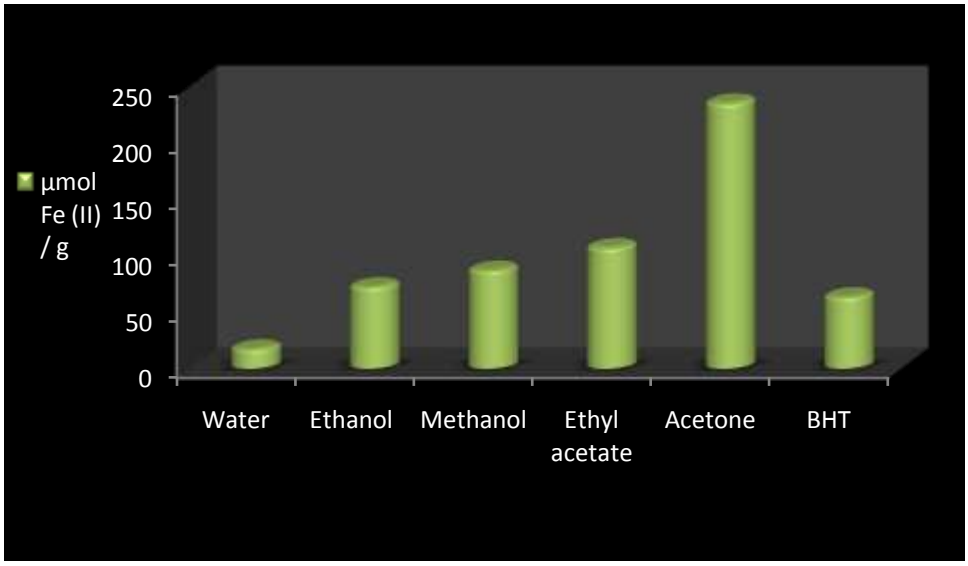

Fig 3: FRAP activities in acetone, ethanol, methanol, water and ethyl acetate extracts of *Urtica urens*.

DISCUSSION

The results of proximate composition of the leaves of *U. urens* showed relatively low moisture and fat compare to reports [3, 23, 35] for vegetables. Crude protein little greater than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland [30, 24, 18] and the estimated calorific value for *A. subfusiformis* (285.0kcal/100g DW) and *U. urens* (260.9% DW) leaves compare favourably to248.8-307.1Kcal/100g DW reported in some Nigerian vegetables [25, 4, 3]. Analysis of the phytochemical contents of the plant showed as like reported for the leafy vegetables like *Aspilia africana*, *Bryophyllum pinnatum*, *Cleome rutidosperma* and *Emilia coccinea* consumed in Nigeria [14, 29, 3]. Even mineral contents present in favorable level. Polyphenols are the major plant compounds which posses in common an aromatic ring bearing hydroxyl substituent 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 [40, 28].According to the Singleton and Rossi [36] various phenolic compounds have different responses in this assay. Flavonoids are the largest group of polyphenolic compounds found in higher plants and synthesized from the shikimic acid and malonic acid pathways [33]. This plant leaves contain high flavonoid and flavonol in ethyl acetate fraction than remaining solvents but proanthocyanidins higher in acetone fraction than other solvents in our obtained results. The DPPH assay results are highly reproducible and comparable to other free radical scavenging methods such as ABTS [16]. Methanol fraction showed high activity up to 78% and ABTS 97% activity at 0.5mg/ml concentration. There are many methods to determine antioxidant capacity. These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods particular antioxidants have varying contributions to total antioxidant potential [10]. And in FRAP assay showing acetone fraction had highest activity (230 µmol Fe(II)/g) than standard drug BHT. With this background the present study conclude that *U. urens* as a potential source of natural antioxidants. The presence of general phytochemicals and specific active compounds might be responsible for their therapeutic effects.

CONCLUSION

The results of this study concluded that the leaves of *U. urens* contain appreciable amount of proteins, fat, fibre, carbohydrate and calorific value, mineral elements and polyphenols. Their antioxidant activities further lend credence to the biological value of this plant. Thus, it can be concluded that *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 can show activity against bacteria's, this may be due to high phenolic content and presence of active compounds such as alkaloids and tannins. Therefore, the use of this plant for medicinal purpose may be justified.

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Conflict of Interest

The authors declare no conflict of interest.

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