

POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF LEAVES OF *URTICA SIMENSIS* GROWN IN ETHIOPIA

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Abstract— This study aimed to investigate the antioxidant activity and polyphenolic content of a wild vegetable, *Urtica simensis*, grown in Ethiopia. Total phenolics, tannin and flavonoid content of leaves extract were determined by the Folin Ciocalteu, Folin Ciocalteu/protein precipitation and aluminum chloride methods, respectively. The antioxidant activity was tested by the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method. Results of the determination revealed that total phenols ranged from 15.75 to 22.67 mg gallic acid equivalent/g of dried leaves. Total tannin content ranged between 0.496 to 1.54 mg gallic acid equivalent/g of dried leaves. Similarly, total flavonoids concentration as catechin equivalent varied between 6.89 to 9.03 mg catechin equivalent/g of dried leaves. Leaves of *U. simensis* were found to have a substantial antioxidant activity ranging between 2.28-2.42 mg ascorbic acid equivalent/g of dried leaves. This study reveals that *U. simensis* leaves accumulated a substantial amount of polyphenols, particularly flavonoids, and pose a substantial antioxidant activity.

Keywords— *Urtica simensis*; phenolics; tannins; flavonoids; antioxidant activity.

I. INTRODUCTION

Cultivated and wild grown leafy vegetables are rich sources of vitamins, proteins, minerals and a variety of bioactive compounds such as phenolic compounds, which provide health benefits beyond basic nutrition (Aletor *et al.*, 2002; Yadav *et al.*, 2013). Consumption of green leafy vegetables plays important role in the prevention of human diseases, such as cancer, blood pressure, cardiovascular diseases and aging, in which free radicals are involved (Yadav *et al.*, 2013).

Nettle is one of the wild plants found all over the temperate region of the world. It is the common name for 30-45 species of flowering plants that belong to the genus *Urtica* of the family *Urticaceae* (Assefa *et al.*, 2013; Mamta and Preeti, 2014). Different species of the plant occur as a perennial plant in temperate zones of Asia, America and Europe. It is commonly found growing in rich soils in forest clearings, old fields and wasted places (Golalipour *et al.*, 2011; Mamta and Preeti, 2014).

Urtica simensis (known as Samma in Ethiopia) is one of the Nettle species that is endemic in Ethiopia. It is a dark green perennial wild plant and the leaves are

used as popular vegetable in some areas of Ethiopia (Friis, 1989; Assefa *et al.*, 2013). The plant grows all around the year and, therefore, can be harvested whenever there is a need. Leaves and young shoots are also eaten in times of famine in some areas of Ethiopia.

Furthermore, *U. simensis* has been traditionally used as a medicinal plant. To mention a few of its medicinal properties, the plant is effective in the treatment of blood pressure, diabetes, and prostate hyperplasia, rheumatoid arthritis, allergic rhinitis, diarrhea, cough and other problems (Dar *et al.*, 2012; Lahigi *et al.*, 2001). Despite of its medicinal and nutritional importance, the plant is not yet fully exploited by most of the ethnic groups of Ethiopia. Furthermore, its potential contribution to food security, nutrition, health, and income generation for the well-being of mankind is still largely underexploited (Assefa *et al.*, 2013).

Phenolic compounds are widely distributed in all plants, the most common polyphenols classes being phenolic acids, flavonoids, and tannins. Polyphenols are an integral part of the human diet. Antioxidant, anti-inflammatory, anti-carcinogenic, and other bioactivities demonstrated for various polyphenols suggest that they have beneficial effects on human health and provide protection against such chronic diseases as cardiovascular diseases, neuro-degenerative disorders, and cancers (Ovaskainen *et al.*, 2008).

Contrary to their medicinal importance, tannins are defined as anti-nutrients that can precipitate proteins, reduce mineral ions and vitamins utilization and prevent the activities of digestive enzymes (Sulaiman *et al.*, 2013).

There are some studies done in other countries on the different species of *Urtica* (*U. dioica*, *U. pilulifera*, *U. urens*, etc.) to investigate their usefulness for medicinal activities. Studies from Iraq, Turkey and India showed that *Urtica* leaves contain flavonoids, alkaloids, fatty acid, phenols, saponins and tannins (Ghaima *et al.*, 2013; Kumar *et al.*, 2013; Kan *et al.*, 2009; Das *et al.*, 2011).

In Ethiopia, a few studies have also been undertaken, mainly emphasized on the nutrient content and antinutrient content of *U. simensis* leaves (Assefa *et al.*, 2013). Therefore, this study is aimed to determine the total phenolics, flavonoids, tannins contents and antioxidant activity of leaf extract of *U. simensis* of Ethiopian origin.

II. METHODS

A. Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade with the highest purity. These chemicals and reagents were: sodium carbonate (anhydrous, Research Lab Fine Chem Industries, Mumbai, India), sodium molybdate dehydrate (98%, BDH Laboratories Supplies, Poole, England, UK), sodium sulfate (anhydrous, Research-Lab Fine Chem Industries, Mumbai, India), anhydrous AlCl_3 and NaNO_2 (Fluka, Lausanne, Switzerland); ovalbumin, sodium carbonate ($\times 10\text{H}_2\text{O}$), and ethanol (Research Lab Fine Chem, Mumbai, India); sodium acetate, NaOH, and L-ascorbic acid (98%, BDH Chemicals Ltd., Poole, England, UK); D-catechin, HCl, 1,1-diphenyl-2-picryldrazyl (DPPH) (Sigma-Aldrich, Poole, Dorset, England, UK); methanol (Merck, Darmstadt, Germany); sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) and phosphomolybdic acid (Scharlau Chemie s.a., Sentmenat, Spain).

B. Equipments

A UV-VIS Spectrophotometer (Perkin Elmer UV Win Lab Lambda 900, USA) equipped with 1 cm path length quartz cells was used for absorbance measurements.

C. Collection of plant materials

Leaves of *U. simensis* were collected from three different areas of the capital city, Addis Ababa (namely: Ayertena, Akaki and Kotebe) and one from the Oromia region, specifically from Holeta town in November 2013. The samples were well rinsed with distilled water to remove dust particles and then dried in the shade at room temperature. Once dried, the samples were ground, sieved and stored in clean plastic bottles until analysis.

D. Extract preparation

A known amount (4 g) of each sample was macerated with agitation at room temperature in 100 mL of 80% methanol in water for 24 h. The extract was filtered using Whatman No. 1 filter paper and the aliquots were analyzed for their antioxidant capacity, total phenol content, total flavonoids content and total tannin content using the methods subsequently described. Each sample was extracted and analyzed for each assay in triplicate.

E. Preparation of Folin-Ciocalteu phenol reagent

Ten grams of sodium tungstate and 2.5 g of sodium molybdate were dissolved in 70 mL of distilled water; then, 5 mL of 85% phosphoric acid and 10 mL of concentrated hydrochloric acid were added and refluxed for 10 hours. Subsequently, 15 g of lithium sulfate, 5 mL of distilled water and one drop of bromine were added. The mixture was refluxed for 15 min. Then, it was cooled at room temperature and diluted to 100 mL with distilled water. During the reaction process, the hexavalent phosphomolybdic/phosphotungstic complex was formed.

F. Determination of total phenolic content

Total phenolic concentration in the plant extract was spectrophotometrically determined by the Folin-

Ciocalteu assay (Singleton and Rossi, 1965; Singh and Maurya, 2010), using gallic acid as a standard. An aliquot of 200 μL of each plant extract was mixed with 2 mL of 10% Na_2CO_3 solution. After 5 min, two mL of Folin-Ciocalteu phenol reagent were added and shaken. A reagent blank using distilled water was also prepared. After incubation for 90 min, at room temperature, the absorbance against the prepared reagent blank were measured at 760 nm using a double beam spectrophotometer. The concentration of total phenolic compounds in the extract was expressed as milligram of gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g dw) of sample. All the samples were analyzed in triplicate.

G. Determination of tannin contents

Tannin content in each sample was determined using egg albumen, which binds tannins as described by Sultana *et al.* (2012). Exactly 2 mL of properly diluted extract were mixed with 2 mL of 5 mg/mL egg albumen solution in acetate buffer of pH (4.6), vortexed, kept for 15 min at room temperature and then centrifuged for 10 min at 3,000 rpm. The supernatant possesses non-tannins phenols because the tannins were precipitated with the egg albumen powder. The phenolic content of the supernatant was measured following the same procedure as described above, using the same standard calibration curve. Tannin content was calculated as the difference between total and non-tannin phenolic content, and the concentration of non-tannin and tannin phenolic compounds in the extract was expressed as milligram of gallic acid equivalent (GAE) per gram of dry mass (mg GAE/g dw).

H. Determination of flavonoid content

Flavonoid content was determined using aluminum chloride (AlCl_3) according to Eghdami and Sadeghi (2010) method. The plant extract (1 mL) or a standard solution of catechin was added to 4 mL of distilled water followed by 5% NaNO_2 (0.3 mL) in a test tube. After 5 min, 10% AlCl_3 (0.3 mL) was added. After 6 min, the reaction mixture was treated with 2 mL of 1 M NaOH. Finally, the reaction mixture was diluted to 2.4 mL with distilled water and the absorbance was measured at 510 nm against a reagent blank. The results were expressed as milligram of catechin equivalent (CE) per gram of dry weight (mg CE/g dw) of sample.

I. DPPH radical-scavenging assay

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to the yellow-colored diphenylpicryl-hydrazine (Malik *et al.*, 2011). In this study, antioxidant activity was measured by a radical scavenging assay using the stable radical DPPH (Ayoola *et al.*, 2008). The scavenging activity of the samples was measured by monitoring the reduction of DPPH in the presence of the leaves extract. A solution of 16 $\mu\text{g/mL}$ DPPH in methanol was prepared by adding 40 mg of DPPH to a 250 mL volumetric flask and diluting

to the volume with methanol. In addition, 0.75 mg/mL of ascorbic acid standard was prepared in distilled water. A standard curve was constructed using five different concentrations of standard solutions of ascorbic acid (0.025, 0.0125, 0.00625, 0.00313 and 0.00156 mg/mL). A volume of 1 mL of the sample solution or standard was placed into a test tube containing 3 mL of distilled water. Then 2 mL of 16 µg/mL methanolic solution of DPPH were added to the mixture and incubated at room temperature in the dark for 1 h. Absorbance of the resulting mixture was measured at 514 nm. The scavenging effect was calculated using the following equation: DPPH radical scavenging activity (%) = $[(A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}}] \times 100$. Where, A_{Control} is the absorbance of the control (containing all the reagents except the sample) and A_{Sample} is the absorbance of the sample (Kataki *et al.*, 2012). A calibration curve was constructed from the standard solutions of ascorbic acid, as % scavenging activity versus concentration of standard ascorbic acid.

J. Statistical analysis

All the analysis was conducted in triplicate. The results were expressed as mean values. The presence of significant differences among the means were tested by one-way ANOVA, using SPSS software (Version 20.0 for Windows, SPSS Inc., Chicago, IL). Sample means were compared by least significant difference (LSD) multiple Duncan's range test. Differences at ($p < 0.05$) were considered to be significant.

III. RESULT AND DISCUSSION

A. Total phenolic content and total tannins

The total phenolic and total tannin contents of *U. simensis* leaves extract were determined. Total phenolic and total tannin contents are expressed as milligram of gallic acid equivalents (mg GAE/g) of dry weight of sample, and shown in Table 1. The total phenolic contents in the four samples (collected from Ayertena, Holeta, Akaki and Kotebe) were found to be 22.7 ± 2.23 , 21.0 ± 2.42 , 15.8 ± 0.21 and 20.2 ± 1.74 mg GAE/g, respectively. The ranges of these values are comparable with the data reported by Kumar *et al.* (2013) from India (15.5–21.8 mg/g dry weight basis). Statistically, a significant difference ($p < 0.05$) was noticed for total phenolic compounds content between samples from Akaki and other localities, while no significant differences ($p > 0.05$) between samples from Ayertena, Holeta and Kotebe were observed when one way ANOVA was carried out. Though the sampling sites have similar climatic conditions, this significant difference might arise due to the variability of the soil composition of the sampling sites.

The contents of total tannin of the *U. simensis* were also evaluated. The total tannin content is expressed in terms of milligrams of gallic acid equivalent per gram of dry mass of sample (mg GAE/g) and the data are given in Table 1. The total tannin contents in the studied samples ranged from 0.496 ± 0.28 to 1.54 ± 0.950 mg GAE/g of dried sample. No significant difference ($p <$

0.05) was observed among the studied samples from Akaki and Kotebe; while the other samples from Ayertena and Holeta showed significant differences between each other when pair wise one-way ANOVA analysis was carried out. The measured tannin contents are significantly different from the data reported by Assefa *et al.* (2013) in the range of 0.253 to 0.270 mg/g of dried leaves of *U. simensis* collected from other localities of Ethiopia. These significant differences might be due to differences in the climatic condition of the regions, physicochemical property of the soil and variation of sampling seasons.

Though relatively higher concentrations of total tannins were recorded in the present study, these values are still low compared to other indigenous wild vegetables reported by Addis *et al.* (2005).

B. Flavonoids

Flavonoids were also observed to be present in the *U. simensis*, which at the same time could contribute extensively to some biological properties that promote human health and reduce the risk of various diseases. In this study, the concentration of total flavonoids in plant extract was determined using a spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of milligrams of catechin equivalent per gram of dry sample (mg CE/g). Results are shown in Table 2. The contents of total flavonoids in the studied samples collected from the four sampling sites (Ayertena, Holeta, Akaki and Kotebe) were found to be 9.03 ± 0.42 , 8.13 ± 0.15 , 6.89 ± 0.13 and 7.13 ± 0.15 mg CE/g of dry weight, respectively. These values are significantly different ($p < 0.05$), except between samples from Akaki and Kotebe. The reason for the difference might be due to variation of the age of the harvested leaves and variation of the soil chemistry of the sampling area

It can be concluded that *U. simensis* could be a very good source of flavonoids. A World Health Organization survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, for their primary healthcare. These medicinal plants are rich sources for naturally occurring antioxidants, especially phenolic, tannins and flavonoids contents (Sultana *et al.*, 2012). Therefore, the presence of these compounds in *U. simensis* leaves might be responsible for the usefulness of the plant for traditional medicine. Furthermore, the various reports on the medical importance of this species might be related to one or more phenolic compounds present in this plant.

Table 1. Total phenolics, total tannins and total flavonoids contents (mean $\pm$ SD, $n = 3$) in *U. simensis* extract expressed per dry mass basis.

Sampling site	Total phenolics (mg GAE/g)	Total tannin (mg GAE/g)	Total flavonoid (mg CE/g)
Ayertena	22.67 ± 2.23^a	1.54 ± 0.95^a	9.03 ± 0.42^a
Holeta	21.02 ± 2.42^a	0.496 ± 0.28^b	8.13 ± 0.15^b
Akaki	15.75 ± 0.21^b	1.023 ± 0.25^c	6.89 ± 0.13^c
Kotebe	20.23 ± 0.74^a	0.98 ± 0.44^c	7.13 ± 0.15^c

Values in the same column that are followed by a different letter (a–c) are significantly different $p < 0.05$ by Duncan's multiple range tests.

Table 2. The antioxidant activity of *U. simensis* leaves extract in mg of AAE/g DW and % scavenging effect, $n = 3$, triplicate analyses.

Sample	Scavenging effect (%) mean $\pm$ SD	mg AAE/g of dry sample mean $\pm$ SD
Ayertena	91.1 $\pm$ 0.2	2.42 $\pm$ 0.1
Holeta	89.2 $\pm$ 1.4	2.35 $\pm$ 0.2
Akaki	86.9 $\pm$ 1.9	2.28 $\pm$ 0.2
Kotebe	87.3 $\pm$ 0.6	2.29 $\pm$ 0.2

C. Antioxidant activities

Antioxidant activities of plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. In this study, the antioxidant activities of *U. simensis* leaves extract were compared with ascorbic acid as a reference. Antioxidant activity was determined by the method of DPPH radical scavenging assay. The results are expressed as milligrams of ascorbic acid equivalents per gram (mg AAE/g) of dry weight of sample. The results of the determination are demonstrated in Table 2.

Generally, free radical scavenging and antioxidant activity of phenolics (e.g. flavonoids and tannins) mainly depend on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules. It is also affected by other factors, such as glycosylation of aglycones, other H-donating groups (-NH, -SH), etc.

As indicated in Table 2, the *U. simensis* samples have good antioxidant activity, in the range of 2.28–2.42 mg AAE/g of dry matter. These results demonstrated that *U. simensis* have potentially good antioxidant activity. Accordingly, the percent inhibition or percent radical scavenging power of the *U. simensis* samples ranged from 87.0 to 91.1%.

Generally, no significant difference ($p \geq 0.05$) was observed among the antioxidant activities of the studied samples. This might be due to the presence of comparable concentration of the bioactive constituents.

D. Correlation between the antioxidant capacity and phytochemicals content

Correlation between total phenolic, tannins and flavonoid contents in *U. simensis* and radical scavenging activity of the four samples, were analyzed by calculating the correlation coefficients (r) between them. The level of total polyphenols, flavonoids and tannins contents have been found to positively correlate with the radical scavenging activity in the *U. simensis* extracts. The results showed a positive linear correlation between the radical scavenging activity and total polyphenols ($r = 0.600$), flavonoids ($r = 0.819$) and tannins ($r = 0.204$) content, respectively. However total flavonoids content was strongly correlated ($r = 0.819$) with radical scavenging activity as compared to the other group of compounds.

A direct correlation between radical scavenging activity and phenolic and tannin contents of the samples failed to be demonstrated by linear regression analysis.

Table 3. Comparison of the total polyphenol, flavonoid, tannin and non-tannin contents of *U. simensis* with different species of the plant and other plants from other regions of the world.

Plant name	Polyp- henols (mg/g)	Flavo- noid (mg/g)	Tannin (mg/g)	Non- tannin (mg/g)	References
<i>U. urens</i> (India)	16-21	0.59	-	-	Kumar <i>et al.</i> , 2013
<i>U. dioic</i> (Iraq)	48.3	-	-	-	Ghaima <i>et al.</i> , 2013
<i>U. dioic</i> (India)	6.35	-	-	-	Kataki <i>et al.</i> , 2012
Spinach (India)	2.6	-	-	-	Gacche <i>et al.</i> , 2010
<i>A. hybridus</i> (Ivory Coast)	2.39	0.27	1.51	0.88	Patricia <i>et al.</i> , 2014
<i>U. urens</i> (S. Africa)	6.68	0.73	-	-	Afolayan and Jimoh, 2009
<i>A. hybridus</i> (Kenya)	-	-	0.0245	-	Alwala <i>et al.</i> , 2014
<i>U. simensis</i> (Ethiopia)	15.75 - 22.67	6.89- 9.03	0.496- 1.54	14.66- 21.1	This study

This lack of relationship is in agreement with other literature (Lizcano *et al.*, 2010). It is known that only phenolic compounds with a certain structure, particularly hydroxyl position in the molecule, can act as proton donors and show radical scavenging activity (Rice-Evans *et al.*, 1996).

E. Comparison of total polyphenols, flavonoid, tannin and non-tannin contents of *U. simensis* with literature values

The results obtained in this study were compared with the results of similar studies on the different species of the plant and other plants reported in the literature (Table 3). Wide range of variations in the concentration of the studied phytochemicals have been noticed between the Ethiopian *U. simensis* and other species of *Urtica* and other similar leafy vegetables from other countries. This variation is not only due to species variability but also variations in physicochemical properties of the soil and climatic conditions of the regions.

IV. CONCLUSIONS

From this study, it can be concluded that the leaves of *U. simensis* contain appreciable amount of phytochemical constituents (phenolics, flavonoids and tannins) with considerable antioxidant activity. Thus, this wild vegetable can be considered as an easily accessible and valuable natural source of antioxidants and dietary supplement.

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