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Phytochemical analysis and antioxidant properties of *Urtica urens*

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Abstract
Urtica urens finds therapeutic applications in the traditional medicine. Chloroform extract from leaves, ethyl acetate extract from leaves, methanolic extract from leaves, chloroform extract from stem-bark, ethyl acetate extract from stem-bark, methanolic extract from stem-bark were prepared separately from *U. urens* by maceration techniques. The presence of flavonoids, phenolics, alkaloids, tannins, phlobatannins, terpenoids, saponins, steroids, anthocyanins and coumarins classes of compounds were identified in these extracts by phytochemical analysis. These extracts showed 7.00±0.00 to 96.55±2.43% of hydrogen peroxide scavenging activity. These extracts also showed varying IC_{50} values in the hydrogen peroxide scavenging activity. The methanolic extract from stem-bark has lowest IC_{50} value of <25µg/mL and the IC_{50} value of positive control ascorbic acid was found to be 36.92µg/mL. The ferric reducing power of these extracts was in the range of 0.138±0.020 to 1.21±0.0 9 at 700nm. Additionally, the total flavonoid contents and total phenolic contents of these extracts were determined to be 6.02±1.21 to 31.57±0.67mg QE/g and 18.72±056 to 131.67±2.04mg GAE/g, respectively. From this study, we conclude that *U. urens* have various classes of phytochemicals with antioxidant potential.

Keywords: *Urtica urens*, Urticaceae, phytochemical analysis, antioxidant potential

1. Introduction
Urtica urens belongs to the Urticaceae family of *Urtica* genus [1]. *U. urens* is also known by vernacular names such as bobatsi, burning nettle, dwarf nettle, common nettle and small nettle [1-3]. *U. urens* are commonly found in wasteland, roadsides and along riverbanks and grow up to 75cm height [4, 5]. *U. urens* has oval to elliptical-shaped leaves and the presence of stinging hairs cover the stems and leaves [6, 7]. *U. urens* has a wide variety of therapeutic applications which include in the treatment of diabetes, anemia, asthma, ulcers, heart related ailments, pulmonary tuberculosis, arthritis, bronchitis and rheumatism of the muscles and joints [8, 9]. In the Kingdom of Lesotho, the Basotho tribes consume the leaves of *U. urens* during spring season for health benefits. The ethanolic and aqueous extracts obtained from the aerial parts of Tunisian species of *U. urens* have previously been subjected to phytochemical analysis [2, 10-13]. The antibacterial, antifungal and free radical scavenging activities of various extracts from *U. urens* have also been reported previously [14, 15]. The objective of the current study was i) to analyze the phytochemical profiles, ii) to evaluate the hydrogen peroxide scavenging activity and ferric reducing power and iii) to determine the total flavonoid contents (TFCs) and total phenolic contents (TPCs) of chloroform, ethyl acetate and methanolic extracts obtained from the leaves and stem-bark of *U. urens*. This is the first report of this kind particularly the species gathered from the Kingdom of Lesotho.

2. Materials and Methods
2.1 Chemicals and reagents
AR grade of the following solvents and chemicals were used in this study. Chloroform (99.5%), methanol (99.5%), ethanol (96.5%), gallic acid, quercetin, trichloroacetic acid and hydrochloric acid (32-33%) (Sigma Aldrich). Sodium hydroxide, ferric chloride, potassium ferricyanide, magnesium ribbons, ammonia solution (35%) and aluminum chloride (Associated Chemicals). Folin-Ciocalteau reagent, ethyl acetate (AR grade, 99.5%), ascorbic acid and hydrogen peroxide (30%) were purchased from Promark Chemicals. Sodium monohydrogen phosphate, sodium dihydrogen phosphate, benzene, mercuric chloride and potassium iodide (Prestige Laboratory Supplies).

2.2 Plant materials
Fresh leaves and stem-bark of *U. urens* were collected in June 2021 at Thoteng Ha Skauti village of the Maseru district, Lesotho. Voucher specimen *viz*. Matamane/UULS/2021 for leaves and Matamane/UUSB/2021 for stem-bark were deposited at Organic Chemistry Research Laboratory at Department of Chemistry NUL.

2.3 Processing of plant materials
The leaves and stem-bark of *U. urens* were separated from the plant materials using a scissors. The separated plant materials were air-dried at room temperature for two weeks. They were ground into powder separately using a hand-screw mill. A mass of 310.49 and 245.22g of powdered leaves and stem-bark of *U. urens*, respectively were obtained.

2.4 Preparation of plant extracts
A mass of 100g of powdered leaves was extracted with 500mL of chloroform in 1L Erlenmeyer flask using a mechanical shaker for 24 hours. The solution was filtered and concentrated using a Buchi-Rotavapor. The crude extract thus obtained was kept in a pre-weighed beaker. The extraction procedure was repeated twice and a mass of 9.61g combined chloroform leaf crude extract was obtained. Following similar extraction procedure, 10.04 and 18.39g of combined ethyl acetate and methanolic extracts were obtained respectively from 100.44 and 100.20g powdered leaves, respectively. Similarly, 7.12, 8.48 and 14.29g of combined chloroform, ethyl acetate and methanolic stem-bark crude extracts were obtained respectively from 80.19, 80.52 and 80.35g of powdered stem-bark.

2.5 Phytochemical analysis
The phytochemical analysis of various extracts obtained from the leaves and stem-bark of *U. urens* was were carried out using methods reported in literature [16, 17].

2.6 Evaluation of hydrogen peroxide scavenging activity
The hydrogen peroxide scavenging activity of these extracts from *U. urens* was evaluated using a method given in the literature[16]. Briefly, a stock solution of each extract (1000µg in 1.0mL of methanol) and further dilutions such as 25, 50, 100, 200, 300, 400 and 500 µg/mL were prepared separately and followed the rest of the procedure as per literature[16]. The absorbance of the resulting mixture was measured at 230nm using MRS Spectro UV-11 spectrophotometer. The hydrogen peroxide scavenging capacity of the extract was calculated using the equation given below.

H_2O_2 scavenging activity (%) = (1 – absorbance of sample/absorbance of blank) x 100

The IC_{50} values of all these extracts were also determined using MS Excel. A lower IC_{50} value represents a higher antioxidant activity of extract and *vice versa*.

2.7 Evaluation of ferric reducing power
The ferric reducing power of these extracts from *U. urens* was evaluated using a method given in the literature[18]. Briefly, a stock solution of each extract (0.2mg in 1.0mL of methanol) and further dilutions such as 12.5, 25, 50, 100 and 200µg/ml were prepared separately and followed the rest of the procedure as per literature [18]. The absorbance of the resulting mixture was measured at 700nm using the same MRS Spectro UV-11 spectrophotometer. The higher the absorbance of the reaction mixture, the higher the reducing ability of the extract and *vice versa* [16].

2.8 Determination of total flavonoid contents (TFCs)
The total flavonoid contents (TFCs) of these extracts from *U. urens* was determined using the aluminum chloride calorimetric method as described in literature literature[19]. Briefly, a stock solution of quercetin (25mg in1.0 mL of methanol) and further dilutions such as 25, 20, 15, 10 and 5µg/mL were prepared and followed the rest of the procedure as per literature [19]. The absorbance of the resulting mixture was measured at 420nm using the MRS Spectro UV-11 spectrophotometer. Each experiment was conducted in triplicates. The TFCs are expressed as milligrams of quercetin equivalents per gram of extract dry weight (mg QE/g DW).

2.9 Determination of total phenolic contents (TPCs)
The total phenolic contents (TPCs) of these extracts from *U. urens* was determined using Folin-Ciocalteau assay[20-22]. Briefly, a stock solution of gallic acid (10.0mg in 10.0mL of methanol) and further dilutions such as 25, 50, 100, 200 and 400µg/mL were prepared and followed the rest of the procedure as per literature[20-22]. The absorbance of the resulting mixture was measured at 765nm using MRS Spectro UV-11 spectrophotometer. The experiment was conducted in triplicates. The TPCs are expressed as milligrams of gallic acid equivalents per gram of extract dry weight (mg GAE/g DW).

2.10 Statistical analysis
The statistical analysis was carried out using one way analysis of variance (ANOVA) coupled with Turkey's test. When $p \leq 0.05$, the differences were statistically significant.

3. Results
Chloroform extract from leaves (E1), ethyl acetate extract from leaves (E2), methanolic extract from leaves (E3), chloroform extract from stem-bark (E4), ethyl acetate extract from stem-bark (E5) and methanolic extract from stem-bark (E6) were prepared separately from *U. urens*. Phytochemical analysis of these extracts showed the presence of ten classes of phytochemicals *viz.* flavonoids, phenolics, tannins, phlobatannins, alkaloids, sterols, terpenoids, saponins, coumarins and anthocyanins classes of compounds were identified from these extracts and the results are summarized in Table 1.

Table 1: Phytochemical analysis of various extracts from *U. urens*.

Phytoconstituents	Extracts					
	E1	E2	E3	E4	E5	E6
Phenolics	+	+	+	+	+	+
Flavonoids	-	+	+	-	+	+
Tannins	+	+	+	-	+	+
Phlobatannins	-	-	-	-	-	+
Alkaloids	-	+	+	-	-	-
Sterols	+	-	+	+	-	+
Terpenoids	-	+	+	+	-	+
Saponins	-	-	+	-	-	+
Coumarins	-	+	-	-	-	+
Anthocyanins	-	-	+	-	-	+
Quinones	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

Chloroform extract from leaves (E1), ethyl acetate extract from leaves (E2), methanolic extract from leaves (E3), chloroform extract from stem-bark (E4), ethyl acetate extract

from stem-bark (E5) and methanolic extract from stem-bark (E6). (+) and (-) signs denote the presence and absence of the phytoconstituent, respectively.

The hydrogen peroxide scavenging activity of E1-E6 is summarized in Table 2. The leaf extracts, E1-E3 exhibited hydrogen peroxide scavenging activity in the range of 8.34±0.76 to 56.25±0.47%, 11.93±2.10 to 64.04±1.68% and 37.49±0.07 to 89.23±1.58% of at a concentration range of 25 to 500µg/mL, respectively (Table 2). This result showed that E3 has higher hydrogen peroxide scavenging activity followed by E2 and E1. The stem-bark extracts, E4-E6 showed hydrogen peroxide scavenging activity in the range of 7.00±0.00 to 57.28±0.87%, 33.90±1.06 to 78.23±2.23% and 51.18±1.33 to 96.55±2.43% at a concentration range of 100, 200, 300, 400 and 500 µg/mL, respectively (Table 2). This result showed that E6 has higher hydrogen peroxide scavenging activity followed by E5 and E4. The positive control, ascorbic acid showed hydrogen peroxide scavenging activity in the range of 44.01±2.60 to 94.04±0.95% at a concentration range of 25 to 500µg/mL, respectively. Additionally, the IC_{50} values of E1-E6 were also determined and are listed in Table 2. Extracts, E1-E6 showed IC_{50} values of 406.53, 319.69, 52.14, 413.09, 178.58 and <25µg/mL, respectively. Extract E6 was found to be the most potent with IC_{50} value of <25µg/mL. Ascorbic acid showed an IC_{50} value of 36.92µg/mL Table 2.

Table 2: Hydrogen peroxide scavenging potential of various extracts from *U. urens* and determination of their IC_{50} values.

Extracts	Concentration of extracts (µg/mL)							IC_{50} values (µg/mL)
	25	50	100	200	300	400	500	
E1	8.34±0.76^{a}	17.47±1.61^{c}	29.67±0.94^{a}	39.11±2.72^{c}	42.16±2.83^{b}	49.16±1.40^{d}	56.25±0.47^{a}	406.53
E2	11.93±2.10^{c}	21.09±0.88^{a}	36.17±1.58^{c}	37.86±2.16^{c}	48.29±0.76^{a}	56.29±3.66^{c}	64.04±1.68^{b}	319.69
E3	37.49±0.07^{a}	48.81±1.12^{d}	54.79±1.00^{b}	66.28±2.01^{d}	75.03±1.43^{b}	81.68±0.29^{e}	89.23±1.5^{b}	52.14
E4	7.00±0.00^{e}	13.06±0.67^{b}	24.70±3.01^{f}	30.67±1.66^{b}	38.94±1.11^{d}	50.42±2.15^{c}	57.28±0.87^{a}	413.09
E5	33.90±1.06^{b}	41.52±0.04^{a}	45.35±2.17^{d}	52.83±0.74^{e}	55.97±4.51^{d}	64.73±0.56^{b}	78.23±2.23^{b}	178.58
E6	51.18±1.33^{b}	53.32±0.84^{e}	60.39±1.09^{b}	67.28±3.46^{d}	79.16±0.44^{e}	89.32±1.88^{c}	96.55±2.43^{c}	<25
Asc. acid	44.01±2.60^{c}	58.59±0.45^{a}	73.12±1.06^{b}	80.31±0.78^{a}	86.53±2.42^{d}	89.88±1.19^{b}	94.04±0.95^{a}	36.92

E1-E6 = refer to footnote of Table 1. Asc. Acid = ascorbic acid. Values in the same column with different superscript letters are statistically significant.

The ferric reducing power of leaf extracts (E1-E3), stem-bark extracts (E4-E6) and ascorbic acid is summarized in Table 3 and shown in Figure 1 and was found to be in the range of 0.193±0.013-1.109±0.103, 0.138±0.020-1.216±0.093 and 0.488±0.007-1.691±0.020 at 760nm, respectively (Table 3). Among leaf extracts, E3 exhibited highest ferric reducing power followed by E2 and E1. Among stem-bark extracts, E6 exhibited highest ferric reducing power followed by E5 and E4 (Table 3).

Table 3: Ferric reducing power of various extracts from *U. urens*.

Extracts	Ferric reducing power/Concentration of extracts (µg/mL)				
	12.5	25	50	100	200
E1	0.193±0.013^{a}	0.211±0.110^{d}	0.263±0.031^{b}	0.342±0.025^{c}	0.489±0.008^{e}
E2	0.312±0.009^{a}	0.347±0.028^{b}	0.418±0.005^{a}	0.619±0.071^{d}	0.841±0.033^{c}
E3	0.402±0.036^{c}	0.517±0.003^{a}	0.733±0.071^{c}	0.897±0.006^{a}	1.109±0.103^{d}
E4	0.138±0.020^{b}	0.198±0.008^{e}	0.221±0.037^{b}	0.260±0.016^{b}	0.337±0.065^{c}
E5	0.203±0.086^{d}	0.219±0.034^{b}	0.333±0.014^{a}	0.421±0.000^{e}	0.595±0.065^{c}
E6	0.361±0.023^{b}	0.538±0.060^{f}	0.808±0.130^{d}	1.009±0.104^{f}	1.210±0.093^{f}
Asc. acid	0.488±0.007^{e}	0.763±0.011^{a}	0.974±0.096^{f}	1.320±0.053^{c}	1.691±0.020^{b}

E1-E6 = refer to footnote of Table 1. Asc. Acid = ascorbic acid. Values in the same column with different superscript letters are statistically significant.

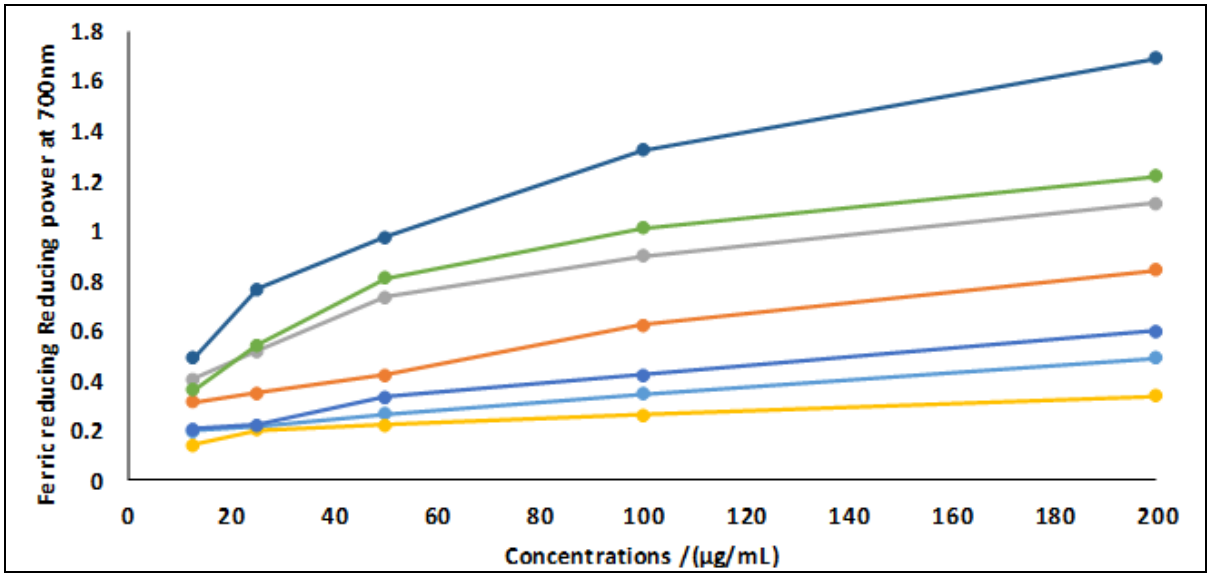

Fig 1: Ferric reducing power of various extracts from *U. urens*.

The TFCs of E1-E6 are summarized in Table 4. The TFCs of E1-E6 was determined from the calibration curve obtained from quercetin standard. The regression equation (y = 0.0614x – 0.028, R^2 = 0.9902) for quercetin standard is shown in Figure 2. The TFCs of E1-E6 were determined to be 6.02±1.21, 10.18±1.85, 31.57±0.67, 4.73±1.09, 17.40±3.06 and 14.07±1.33mg QE/g, respectively. Among the leaf extracts, E3 has the highest TFCs followed by E2 and E1. Among the stem-bark extracts, E5 has the highest TFCs followed by E6 and E4.

Table 4: The TFCs and TPCs of various extracts from *U. urens*.

Extracts	TFC (mg QE/g)	TPC (mg GAE/g)
E1	6.02±1.21^{c}	18.72±0.56^{a}
E2	10.18±1.85^{c}	44.28±2.69^{d}
E3	31.57±0.67^{b}	131.67±2.04^{b}
E4	4.73±1.09^{d}	37.41±1.35^{c}
E5	17.40±3.06^{d}	35.08±2.16^{b}
E6	14.07±1.33^{b}	68.51±0.87^{e}

E1-E6 = refer to footnote of Table 1. Values in the same column with different superscript letters are statistically significant.

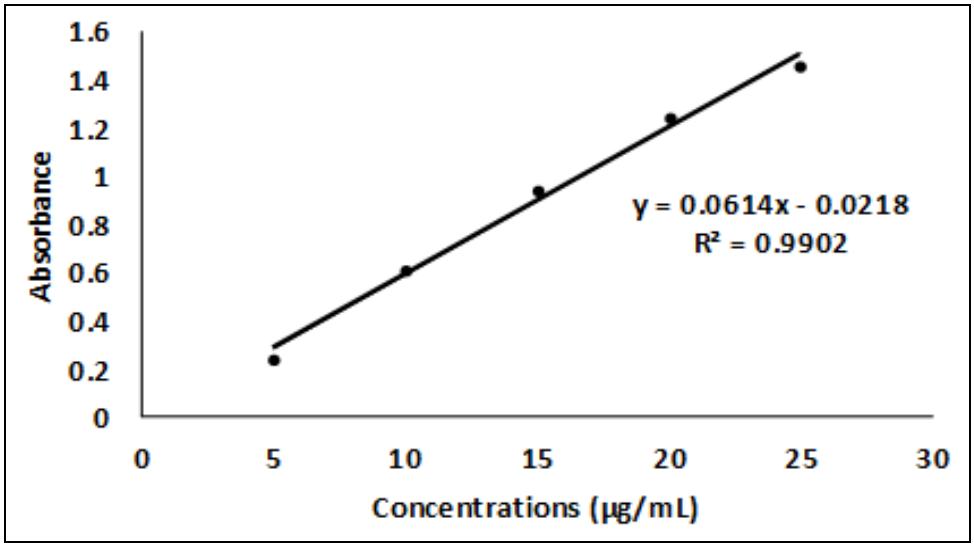

Fig 2: Calibration curve for quercetin (for estimating TFCs of various extracts).

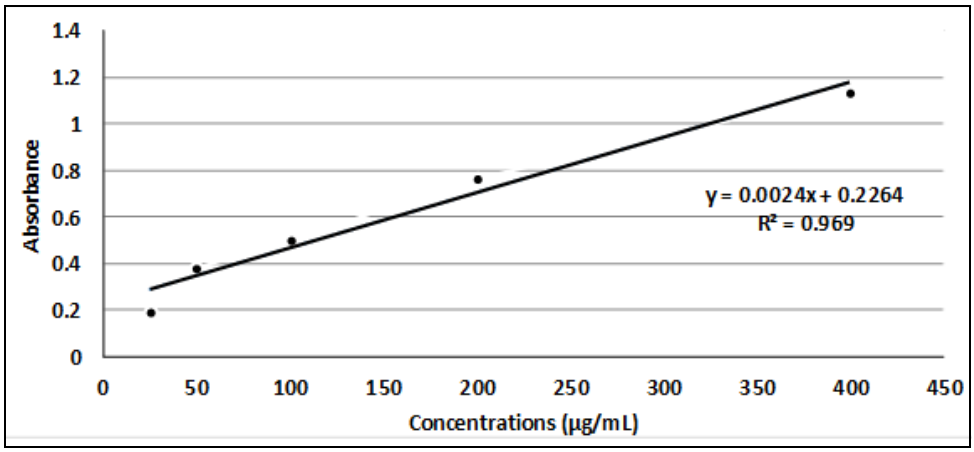

Fig 3: The calibration curve of gallic acid (for estimating TPCs of various extracts).

The TPCs of E1-E6 are also summarized in Table 4. The TPCs of E1-E6 was determined from the calibration curve of gallic acid standard. The regression equation, y = 0.0024x + 0.2244, R^2 = 0.969, for gallic acid standard is shown in Figure 3. The TPCs of E1-E6 were determined to be 18.72±0.56, 44.28±2.69, 131.67±2.04, 37.41±1.35, 35.08±2.16 and 68.51±0.87mg GAE/g, respectively. Among the leaf extracts, E3 has the highest TPCs followed by E2 and E1. Among the stem-bark extracts, E6 has the highest TFCs followed by E4 and E5.

4. Discussion

The generation of minimal amount of hydrogen peroxide in our body (Saeed *et al*., 2012) could produce hydroxyl free radicals [16]. These hydroxyl free radicals are responsible for lipid peroxidation and this process causes detrimental effects on the DNA [23]. Fortunately, the phytochemicals present in our diet have the potential to scavenge these harmful species. The present study showed that E1-E6 are a rich source of various classes of phytochemicals and these phytochemicals have the ability to scavenge hydrogen peroxide potentially. In other words, the phytochemicals present in E1-E6 could prevent the formation of harmful hydroxyl radical and therefore prevent the lipid peroxidation. Ferric (Fe^{3+}) ions have been one of the potent endogenous prooxidants[24]. Tannins, phenolics, tocopherols, carotenoids and flavonoids classes of phytochemicals significantly inhibit the oxidation processes induced by Fe^{3+} ions [25, 26]. In the current study, the presence of various classes of phytochemicals in E1-E6 might have caused the ferric reducing power. The TFCs and TPCs of the ethanolic and aqueous extracts from aerial parts of *U. urens* collected in Tunisia have been previously reported[11]. The TFCs of ethanolic and aqueous extracts have been reported as 6.81±1.72 and 5.34±0.21mg QE/g DW, respectively [11]. Similarly, the TPCs of ethanolic and aqueous extracts have been reported as 31.41±0.31 and 29.56±1.56

mgGAE/g DW, respectively [11]. In the present study, various solvents extracts from the leaves and stem-bark of *U. urens* have relatively higher TFCs and TPCs (Table 4).

5. Conclusion

The extracts from leaves and stem-bark of *U. urens* have various classes of phytochemicals. The hydrogen peroxide scavenging activity of these extracts was evaluated. In general, the methanolic extracts from both leaves and stem-bark showed highest hydrogen peroxide scavenging activity and showed highest ferric reducing power. Similarly, these two extracts also showed highest TFCs and TPCs. From this study, we conclude that *U. urens* have various classes of phytochemicals with antioxidant potential Therefore, further studies can be carried out to promote its therapeutic applications.

6. Conflict of interest statement

The authors declared that there is no conflict of interest.

7. Acknowledgements

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