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DPPH radical scavenging activity of extracts from *Urtica urens* (Urticaceae)

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In this study, hexane, chloroform, ethyl acetate and methanolic extracts from leaves, stem-bark and root of *Urtica urens* were evaluated for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The hexane, chloroform, ethyl acetate and methanolic extracts from leaves of *U. urens* showed scavenging activity ranging from 7.06±2.52 to 26.01±1.84, 10.86±1.81 to 41.48±1.91, 7.53±3.21 to 51.85±4.20 and 51.50±1.97 to 73.84±6.82%, respectively, at various concentrations. The hexane, chloroform, ethyl acetate and methanolic extracts from stem-bark of *U. urens* showed scavenging activity ranging from 3.26±1.84 to 38.54±2.78, 2.93±1.02 to 56.56±3.16, 19.19±1.77 to 53.99±2.18 and 30.10±0.07 to 62.80±1.90%, respectively, at various concentrations. The chloroform and methanolic extracts from root of *U. urens* showed scavenging activity ranging from 9.57±1.39 to 46.31±2.35 and 38.53±9.18 to 76.51±2.02%, respectively, at various concentrations. Additionally, the IC₅₀ values of these extracts were also determined and was found to be in the range of <200 to >3000 µg/mL. The positive control, ascorbic acid, exhibited an IC₅₀ value of <200 µg/mL. *U. urensis* reported to have many therapeutic applications, which include treating asthma, heart related problems, pulmonary tuberculosis and cleansing the bladder. Basotho tribes use *U. urens* during spring season to increase iron content in the blood. Further studies on *U. urens* are required to explore this plant for its commercial applications.

Key words: *Urtica urens*, DPPH radical scavenging assay, ascorbic acid, solvent extracts.

INTRODUCTION

Reactive oxygen species and free radicals are unstable and are highly reactive (Rodrigues et al., 2019). These species cause potentially harmful effects against biological system, which include damaging DNA, proteins and lipids (Rodrigues et al., 2019). The development of cardiovascular, neurodegenerative, metabolic and other chronic diseases are associated with the production of these reactive species (Rodrigues et al., 2019). Fortunately, these reactive species can be neutralized by some secondary metabolites called antioxidants (Mon et al., 2011; Rodrigues et al., 2019). Secondary metabolites such as polyphenols, phenolic acids and flavonoids from...
natural sources are reported to be very important antioxidants (Array et al., 2019; Rodrigues et al., 2019). Our dietary consumption is also a good source of production of these antioxidants (Rodrigues et al., 2019). The low ability of body to neutralize these reactive species promotes a phenomenon called oxidative imbalance/oxidative stress (Array et al., 2019; Rodrigues et al., 2019).

Known by other names such as bobatsi, common nettle, small nettle, dwarf nettle and burning nettle, *Urtica urens* belongs to the Urticaceae family of the genus *Urtica* (Lati et al., 2017; Nencu et al., 2015; Moteetee and Van Wyk, 2011; Schellman et al., 2008). *U. urens* is an herbaceous shrub, native to Mediterranean Europe and grows to 75 cm height (Coleman et al., 2018; Moteetee and Seleteng-Kose, 2017; Schellman et al., 2008). *U. urens* usually grows in large patches in moist soils composed of high organic matter (Coleman et al., 2018; Jimoh et al., 2010). The stems of *U. urens* are covered with stinging hairs but the leaves are smooth and more delicate (Schottner et al., 1997; Wagner et al., 1994). *U. urens* has widely been used by the Basotho tribes as wild vegetable (vernacular name: *morotho*) during spring season to increase iron content in the blood. *U. urens* has long been used in a folk medicine in the Kingdom of Lesotho due to its prophylactic ability. Additionally, *U. urens* also finds wide applications in folk medicine, which include treating asthma, anemia, rheumatism and arthritis, diabetes mellitus, heart problems, ulcers and pulmonary tuberculosis (Barkaoui et al., 2017; Moteetee and Van Wyk, 2011). The leaves of *U. urens* contain vitamins D, C and E (Mzid et al., 2017). *U. urens* is rich in phenolics, terpenoids, carotenoids, fatty acids and flavonoids. (El-Seadawy et al., 2018; Kregiel et al., 2018).

Ethanol and water extracts from leaves of *U. urens*, collected from Tunisia, have previously been reported for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity (Mzid et al., 2017). Additionally, acetone, methanolic and water extracts obtained from whole plants of *U. urens* have also been evaluated previously for their ferric reducing antioxidant power (FRAP) (Jimoh et al., 2010). Our literature search showed that *U. urens* has not been explored well for their biological and pharmacological activities. The objective of this current study was to evaluate the DPPH radical scavenging activity of extracts from leaves, stem-bark and root of *U. urens* collected from the kingdom of Lesotho and to determine the IC_{50} values.

**MATERIALS AND METHODS**

**Plant**

Fresh whole plants of *U. urens* were collected in October 2018 at Lithabaneng Ha Keiso and Roma village of Maseru district, Lesotho, Southern Africa. The plant material was identified by a botanist from the Department of Biological Sciences, NUL. The leaves, stem-bark and root were separated from the whole plants using a scissors. Voucher specimen for leaves (Matamane/UULS/2018), stem-bark (Matamane/UUSB/2018) and root (Matamane/UURT/2018) were kept in the Organic Research Laboratory, Department of Chemistry and Chemical Technology, National University of Lesotho, Roma Campus, Masau, the Kingdom of Lesotho, Southern Africa.

**Processing of materials**

The air-dried plant materials were pulverized into powder using a laboratory blender (Waring Blender, Blender 80119, Model HGB2WT93, 240V AC, 3.5 AMPs, Laboratory and Analytical Supplies). 642.01, 450.17 and 128.36 g of powdered leaves, stem-bark and root, respectively, were obtained.

**Preparation of plant extracts**

A mass of 170.35 g powdered leaves were macerated with 800 mL of hexane for three days at room temperature with occasional agitation. The solution was filtered off using a vacuum filter (ATB, Model: 284065-H, Power: 230V 3.0A, 1320/min 50 Hz) and the solvent was removed by vacuo. The procedure was repeated twice. Finally, the sample was refluxed with 800 mL of hexane for 10 h. A mass of 5.12 g of combined hexane extract was obtained after removal of solvent. The same procedure was repeated separately with chloroform, ethyl acetate and methanol. A mass of 1.93, 1.49 and 3.29 g of chloroform, ethyl acetate and methanol leaf extracts were obtained, respectively from 150.37, 150.68 and 170.35 g of powdered leaves. Using similar extraction procedure, 1.12, 1.67, 1.49 and 3.29 g of hexane, chloroform, ethyl acetate and methanol stem-bark extracts were obtained, respectively from 99.89, 99.78, 100.18 and 150.22 g of powdered stem bark. Similarly, 0.71 and 3.21 g of chloroform and methanol root extracts were obtained, respectively from 64.92 and 63.44 g powdered roots. However, we did not obtain hexane and ethyl acetate extracts from this root powder due to shortage root materials.

**Chemicals and solvents used**

Hexane (AR Grade 99.5%), Chloroform (AR Grade 99.5%), ethyl acetate (AR Grade 99%), methanol (AR Grade 99.5%), Tris (hydroxymethyl) aminomethane and 1,1-diphenyl-2-picrylhydrazyl were all purchased from Sigma-Aldrich. L-Ascorbic acid was purchased from Associated Chemical Enterprises.

**DPPH radical scavenging assay and determination of IC_{50} values**

The antioxidant activity of the various extracts was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described in literature with slight modifications (Blois, 1958; Sasidharan et al., 2007). Briefly, stock solutions of each extract were prepared at a concentration of 3.0 mg of extract in 1 mL of 50% methanol (v/v). Serial further dilutions, such as 3000, 2000, 1500, 1000, 800, 500 and 200 µg/mL, were made from these stock solutions. Solutions without extract concentration served as negative controls. 100 µL of each aliquot was mixed with 100 µL of 0.1 mM DPPH solution in absolute methanol and 100 µL of 50 mM Tris-HCl buffer solution maintained at pH 7.4. The reaction mixture was vortexed and then incubated in a dark room at room temperature for 30 min. The optical density (absorbance) of the mixture was measured at 517 nm using an MRC spectrophotometer (Model Spectro UV – 11 S/N: UEB 1704020). The following equation was used to calculate the percentage DPPH radical scavenging activity of extracts.
Table 1. The percentage inhibition of DPPH radical scavenging activity of leaves, stem-barks and root extracts of *U. urens* at various concentrations.

| Extract                  | Concentration (µg/mL)/(% Inhibition) |
|--------------------------|--------------------------------------|
|                          | 200        | 500        | 800        | 1000       | 1500       | 2000       | 3000       |
| UUHXLS                   | 7.06±2.52  | 10.84±0.44 | 12.54±1.63 | 14.83±3.02 | 16.43±2.18 | 19.85±1.14 | 26.01±1.84 |
| UUCHLS                   | 10.86±1.81 | 13.91±0.38 | 17.13±2.39 | 22.53±1.16 | 24.85±4.07 | 33.94±0.24 | 41.48±1.91 |
| UUEALS                   | 7.53±3.21  | 10.04±1.81 | 15.30±0.07 | 17.36±0.54 | 26.84±3.68 | 32.16±1.27 | 51.85±4.20 |
| UUMELS                   | 51.50±1.97 | 55.40±1.17 | 57.77±3.01 | 59.00±5.32 | 61.02±0.78 | 65.69±4.11 | 73.84±6.82 |
| UUHXSB                   | 3.26±1.84  | 5.05±2.96  | 11.18±6.60 | 13.85±0.08 | 20.01±1.36 | 25.71±4.71 | 38.54±2.78 |
| UUCHSB                   | 2.93±1.02  | 9.64±1.39  | 14.45±0.77 | 26.19±0.17 | 29.38±2.13 | 49.80±4.30 | 56.56±3.16 |
| UUEASB                   | 19.19±1.77 | 36.99±1.43 | 38.42±2.08 | 43.44±3.83 | 44.96±2.09 | 53.28±3.66 | 53.99±2.18 |
| UUMESB                   | 30.10±0.07 | 40.61±1.16 | 43.89±1.75 | 47.97±4.22 | 51.88±1.35 | 58.01±7.83 | 62.80±1.90 |
| UUCHRT                   | 9.57±1.39  | 20.46±0.67 | 30.52±4.02 | 32.69±2.16 | 34.30±1.94 | 38.57±1.71 | 46.31±2.35 |
| UUMERT                   | 38.53±9.18 | 57.30±2.31 | 60.41±0.79 | 65.58±0.08 | 66.88±0.38 | 74.18±3.65 | 76.51±2.02 |
| Asc. acid                | 59.44±0.14 | 63.78±0.04 | 64.61±0.33 | 66.82±1.12 | 69.18±2.13 | 72.30±2.73 | 83.96±6.08 |

UUHXLS = *U. urens* hexane leaves extract; UUCHLS = *U. urens* chloroform leaves extract; UUEALS = *U. urens* ethyl acetate leaves extract; UUMELS = *U. urens* methanolic leaves extract; UUHXLS = *U. urens* hexane stem-bark extract; UUCHLS = *U. urens* chloroform stem-bark extract; UUEASB = *U. urens* ethyl acetate stem-bark extract; UUMESB = *U. urens* methanolic stem-bark extract; UUCHRT = *U. urens* chloroform root extract; UUHMERT = *U. urens* chloroform root extract. Asc. acid = Ascorbic acid, which served as positive control. The experiments were carried out in triplicate. Each value is expressed as mean±standard deviation, (n=3).

DPPH Scavenged (%) = \( \frac{(A_{\text{cont}} - A_{\text{test}})}{A_{\text{cont}}} \times 100 \)

where \( A_{\text{test}} \) = Absorbance in the presence of extract or positive control and \( A_{\text{cont}} \) = Absorbance of negative control (that is, without extract).

The \( IC_{50} \) value is defined as the concentration (in µg/mL) of extract that inhibits the formation of DPPH radical by 50% (Moyo et al., 2013; Ndhlala et al., 2013). A lower value of \( IC_{50} \) represents higher antioxidant activity. The \( IC_{50} \) values were calculated from graphs by plotting extract concentrations vs. percentage inhibition of DPPH radical using Microsoft Excel. Each experiment was carried out in triplicate and the averages of the three values were used to calculate \( IC_{50} \) values. Standard deviation was calculated for each concentration from the three values of the experiment.

Statistical analysis

All determinations were performed in triplicate and the results were expressed as mean ± standard deviation. Statistical significance was achieved using one way analysis of variance (ANOVA) at p < 0.05.

RESULTS AND DISCUSSION

Table 1 summarizes the percentage inhibition of DPPH radical scavenging activity of hexane leaves extract (UUHXLS), chloroform leaves extract (UUCHLS), ethyl acetate leaves extract (UUEALS) and methanolic leaves extract (UUMELS) of *U. urens*; hexane stem-bark extract (UUHXSB), chloroform stem-bark extract (UUCHSB), ethyl acetate stem-bark extract (UUEASB) and methanolic stem-bark extract (UUMESB) of *U. urens* and chloroform root extract (UUCHRT) and methanolic root extract (UUMERT) of *U. urens*. In all cases, ascorbic acid in 50% methanol served as positive controls. UUHXLS exhibited 7.06±2.52, 10.84±0.44, 12.54±1.63, 14.83±3.02, 16.43±2.18, 19.85±1.14 and 26.01±1.84% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. On the other hand, the positive control, ascorbic acid, showed 59.44±0.14, 63.78±0.04, 64.61±0.33, 66.82±1.12, 69.18±2.13, 72.30±2.73 and 83.96±6.08% of radical scavenging activity at the same concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result revealed that UUHXLS showed relatively very weak radical scavenging activity compared to positive control at all concentrations. UUCHLS exhibited 10.86±1.81, 13.91±0.38, 17.13±2.39, 22.53±1.16, 24.85±4.07, 33.94±0.24 and 41.48±1.91% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Therefore, UUCHLS showed a weak radical scavenging activity compared to the positive control at low concentrations, but showed significant scavenging activity at a concentration of 3000 µg/mL. UUEALS showed 7.53±3.21, 10.04±1.81, 15.30±0.07, 17.36±0.54, 26.84±3.68, 32.16±1.27 and 51.85±4.20% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result showed that UUEALS also exhibited a weak radical scavenging activity at lower concentrations but has a significant antioxidant activity at a concentration of 3000 µg/mL. UUMELS exhibited 51.50±1.97, 55.40±1.17, 57.77±3.01, 59.00±5.32, 61.02±0.78, 65.69±4.11 and 73.84±6.82% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result showed that UUMELS exhibited remarkably strong radical scavenging activity at all
Table 2. The IC$_{50}$ values of various leaves, stem-barks and root extracts of *U. urens* based on their % inhibition of DPPH radical.

| S/N | Extract          | IC$_{50}$ (µg/mL) |
|-----|------------------|-------------------|
| 1   | UUHXLS           | >3000             |
| 2   | UUCHLS           | >3000             |
| 3   | UUEALS           | 2976.48           |
| 4   | UUMELLS          | <200              |
| 5   | UUHXSB           | >3000             |
| 6   | UUCHSB           | 2427.83           |
| 7   | UUEASB           | 2086.87           |
| 8   | UUMESB           | 1481.20           |
| 9   | UUHCR            | >3000             |
| 10  | UUMERT           | 472.67            |
| 11  | Asc. acid        | <200              |

UUHXLS = *U. urens* hexane leaves extract; UUCHLS = *U. urens* chloroform leaves extract; UUEALS = *U. urens* ethyl acetate leaves extract; UUMELLS = *U. urens* methanolic leaves extract; UUHXLS = *U. urens* hexane stem-bark extract; UUCHLS = *U. urens* chloroform stem-bark extract; UUEALS = *U. urens* ethyl acetate stem-bark extract; UUMELLS = *U. urens* methanolic stem-bark extract; UUCHRT = *U. urens* chloroform root extract; UUMELLS = *U. urens* methanolic root extract. Asc. acid = Ascorbic acid, which served as positive control. The experiments were carried out in triplicate. Each value is expressed as mean±standard deviation (n=3).

Table 2 summarizes the IC$_{50}$ values of UUHXLS, UUCHLS, UUEALS, UUMELLS, UUHXSB, UUCHSB, UUEASB, UUMESB, UUCHRT and UUMERT. The positive control, ascorbic acid, showed an IC$_{50}$ value of <200 µg/mL. The leaves extracts viz. UUHXLS, UUCHLS, concentrations and the scavenging activity was very much comparable to positive control at all concentrations (Table 1). UUHXSB showed 3.26±1.84, 5.05±2.96, 11.18±4.60, 18.35±0.08, 24.01±1.36, 25.71±4.71 and 38.54±2.78% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. Compared to the positive control, UUHXSB exhibited a weak scavenging activity at all concentrations. UUCHLS exhibited 2.93±1.02, 9.64±1.39, 14.45±0.77, 26.19±0.17, 29.38±2.13, 49.80±4.30 and 56.56±3.16% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result indicated that UUCHLS showed a weak radical scavenging activity at lower concentrations but showed significant radical scavenging activity at higher concentrations of 2000 to 3000 µg/mL. UUEASB showed 19.19±1.77, 36.99±1.43, 38.42±0.28, 43.44±3.83, 44.96±2.09, 53.28±3.66 and 53.99±2.18% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result revealed that UUEASB has a weak radical scavenging activity at lower concentrations but at concentrations 500 to 3000 µg/mL, it showed significant radical scavenging activity (Table 1).

UUCHRT exhibited 9.57±1.39, 20.46±0.67, 30.52±4.02, 32.69±2.16, 34.30±1.94, 38.57±1.17 and 46.31±2.35% of radical scavenging activity at concentrations of 200, 500, 800, 1000, 1500, 2000 and 3000 µg/mL, respectively. This result showed that UUCHRT has a weak radical scavenging activity at lower concentrations relative to the positive control. However, at concentration of 3000 µg/mL, it showed significant radical scavenging activity. UUMELLS, UUMESB and UUMERT showed higher radical scavenging activity compared to other extracts (Table 1). Particularly, UUMERT exhibited highest radical scavenging activity and its scavenging activity was very much comparable to positive control, ascorbic acid (Table 1).
UUEALS and UUMELS showed IC$_{50}$ values of >3000, >3000, 2976.48 and <200 µg/mL, respectively. Therefore, among the leaves extracts, UUMELS was the most potent with IC$_{50}$ value of <200 µg/mL, which was comparable to positive control. The stem-bark extracts viz. UUHXSB, UUCHSB, UUEASB and UUMESB exhibited IC$_{50}$ values of >3000, 2427.83, 2086.87 and 1481.20 µg/mL, respectively. Therefore, among the stem-bark extracts, UUMESB was the most potent with IC$_{50}$ value of 1481.20 µg/mL. Nevertheless, its scavenging activity remained weaker than positive control. The IC$_{50}$ value of root extracts viz. UUCHRT and UUMERT were found to be >3000 and 472.67 µg/mL, respectively. UUMERT was found to be the most potent with IC$_{50}$ value of 472.67 µg/mL. Nevertheless, its scavenging activity remained weaker than positive control (Table 2). For comparison and clarity, we have included bar diagrams for each extract together with positive control (Figures 1, 2 and 3). Figures 1, 2 and 3 show the percentage of radical scavenging activity of UUHXLS, UUCHLS, UUEALS, UUMELS and the positive control, UUHXLS, UUCHLS, UUEALS, UUMELS and the positive control and UUCHRT, UUMERT and the positive control, respectively. In all cases, the percentage radical scavenging activity is increased with increasing concentration of the extracts. This means that the free radical scavenging activity of the extracts is dose-dependent. Additionally, the solvent also played an important role in extracting active constituents from the plant materials and subsequently in determining the percentage of radical scavenging activity. In the present cases, methanolic extracts showed higher scavenging activities than other extracts. The results were statistically significant, when p < 0.05.

In a previous report, acetone, methanolic and water extracts obtained from whole plants of $U$. *urens* have been investigated for their DPPH scavenging activity. At a concentration of 1 mg/mL, these extracts showed 60.8, 91.2 and 63.5% of scavenging activity, respectively (Jimoh et al., 2010). This result revealed that methanolic extract has been the most potent with very high scavenging activity of 91.2% (Jimoh et al., 2010). Additionally, ATBS radical scavenging potential of these three extracts has also been reported previously (Jimoh et al., 2010). Again, at this concentration of 1 mg/mL acetone, methanolic and water extracts exhibited 97.5, 95.2 and 98.5% inhibition of ATBS, respectively (Jimoh et al., 2010). This result, therefore, revealed that all these three extracts showed very high activity in this ATBS radical assay. Mzid et al. (2017) collected leaves of $U$. *urens* from Tunisia and obtained ethanolic and water extracts. These ethanolic and water extracts exhibited DPPH radical scavenging activity of 65.33±10.72 and 45.67±10.21 mg TE/g extract, respectively (Mzid et al., 2017). These two extracts also exhibited ATBS radical scavenging activity of 560.33±29.45 and 350.33±18.73 mg TE/g extract, respectively (Mzid et al., 2017). Additionally, the IC$_{50}$ values of these two extracts have been found to be 245.65±10.2 and 142.94±10.54 µg/mL, respectively in the ATBS assay and 30.88±3.03 and 14.65±1.09 µg/mL, respectively in the DPPH assay (Mzid et al., 2017). In another study, DPPH radical scavenging activity of methanolic extract from whole plant of $U$. *urens*, collected from Palestine, exhibited an IC$_{50}$ value of
Figure 2. Percentage inhibition of DPPH radical by various stem bark extracts of *U. urens* at different concentrations. UUHXL = *U. urens* hexane stem-bark extract, UUCHLS = *U. urens* chloroform stem-bark extract, UUEALS = *U. urens* ethyl acetate stem-bark extract, UUMELS = *U. urens* methanolic stem-bark extract. Ascorbic acid served as positive control. The experiments were carried out in triplicate. Each value is expressed as mean±standard deviation (n=3).

Figure 3. Percentage inhibition of DPPH radical by chloroform and methanolic root extract at different concentrations. UUCHLS = *U. urens* chloroform root extract, UUMELS = *U. urens* methanolic root extract. Ascorbic acid served as positive control. The experiments were carried out in triplicate. Each value is expressed as mean±standard deviation (n=3).

29.70±0.60 µg/mL (Jaradat et al., 2016). The methanolic extract is common to the present study also. However, in our study, UUMELS showed IC$_{50}$ value≤200 µg/mL. However, UUMESB and UUMERT showed IC$_{50}$ values of 1481.20 and 472.67 µg/mL, respectively. This variation may be due to the presence of active constituents at various proportions in different parts of the plant and the collection of plants from different geographic locations.

Conclusion

The radical scavenging activity of hexane, chloroform, ethyl acetate and methanolic extracts from leaves, stem-
bark and root of *U. urens* collected from the Kingdom of Lesotho was found to be in the range of 7.06±2.52 to 73.84±6.82%. Additionally, the IC₅₀ values of all these extracts were also determined and was found to be in the range of <200 to >3000 µg/mL. The positive control, ascorbic acid, exhibited an IC₅₀ value of <200 µg/mL. Therefore, it was concluded that some extracts from *U. urens* exhibited significant radical scavenging activity, while others exhibited weak radical scavenging activity. *U. urens* is reported to have many therapeutic applications in folk medicine, which include treating asthma, heart related problems, pulmonary tuberculosis and cleanse the bladder. Therefore, further studies on *U. urens* will be useful to explore this plant for its commercial applications.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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