

Antimicrobial activities of extracts from *Urtica urens*<sup>1\*</sup>Pillai, M.K., <sup>1</sup>Matamane, R.P. and <sup>2</sup>Mekbib, S.B.<sup>1</sup>Department of Chemistry and Chemical Technology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, P. O. Roma 180, Kingdom of Lesotho, Southern Africa<sup>2</sup>Department of Biology, Faculty of Science and Technology, National University of Lesotho, Roma Campus, P. O. Roma 180, Kingdom of Lesotho, Southern Africa**Article history:**

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**DOI:**[https://doi.org/10.26656/fr.2017.4\(5\).088](https://doi.org/10.26656/fr.2017.4(5).088)**Abstract**

The antibacterial and antifungal activities of various extracts from *Urtica urens* were evaluated against selected microorganisms using agar hole-plate diffusion method. A total of ten extracts namely *U. urens* hexane leaf extract (HLE), chloroform leaf extract (CLE), ethyl acetate leaf extract (ELE), methanolic leaf extract (MLE), hexane stem-bark extract (HSB), chloroform stem-bark extract (CSB), ethyl acetate stem-bark extract (ESB), methanolic stem-bark extract (MSB), chloroform root extract (CRT) and methanolic root extract (MRT) were prepared. These extracts were evaluated against two Gram-positive bacteria viz. *Listeria monocytogenes* and *Staphylococcus aureus*, four Gram-negative bacteria viz. *Serratia marcescens*, *Pseudomonas aeruginosa*, *Escherichia coli* (wild strain) and *Escherichia coli* (O157:H7) and two fungal isolates viz. *Candida albicans* and *Penicillium digitatum*. The inhibition zones of these extracts were found to be in the range of 9.0±0.0 to 40.5±0.7 mm against bacterial isolates and 16.0±4.2 to 17.5±4.9 mm against *C. albicans*. However, all these extracts exhibited no visible zone of inhibition against *P. digitatum*. Additionally, the minimum inhibitory concentrations (MICs) of these extracts were also determined and were found to be in the range of <31.25 to >1000 µg/mL against both bacterial and fungal isolates. From this study, we concluded that ELS, MLE, CRT and MRT showed promising antimicrobial activities.

**1. Introduction**

*Urtica urens* belongs to the Urticaceae family of the *Urtica* genus (Schellman *et al.*, 2008; Nencu *et al.*, 2015; Lati *et al.*, 2017). It is also known by other names such as small nettle, dwarf nettle and burning nettle. *U. urens* is native to Mediterranean Europe. It is herbaceous shrub and grows to 75cm height (Moteetee and Kose, 2017; Coleman *et al.*, 2018). *U. urens* commonly found in moist soils composed of high organic matter (Coleman *et al.*, 2018). In Southern Africa, *U. urens* has widely been consumed by Basotho tribes during spring season. *U. urens* has also widely been used as a traditional medicine around the world to treat anemia, asthma, rheumatism and arthritis, diabetic mellitus, heart problems, ulcers and pulmonary tuberculosis (Moteetee and Van Wyk, 2011; Barkaoui *et al.*, 2017). *U. urens* is rich in phenolics, terpenoids, carotenoids and fatty acids. (El-Seadawy *et al.*, 2018; Kregiel *et al.* 2018). The leaves of *U. urens* contains vitamins D, C and E (Mzid *et al.*, 2017). The antimicrobial activity of chloroform, ethanolic and

aqueous extracts from leaves of *U. urens* collected in Tunisia and Eritrea have previously been reported (Kaushik *et al.*, 2015; Barkaoui *et al.*, 2017; Mzid *et al.*, 2017). However, our literature search showed that this plant has not been explored well for its biological and pharmacological studies, particularly, the species gathered from the Kingdom of Lesotho. The objective of the current study was to evaluate the antibacterial and antifungal activities of hexane, chloroform, ethyl acetate and methanolic extracts from leaves, stem-bark and root of *U. urens* collected from the Kingdom of Lesotho, Southern Africa.

**2. Materials and methods****2.1 Plant materials**

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 leaves, stem-bark and root were separated from the whole plants using a pair of scissors. A voucher specimen for leaves

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(Matamane/UULE/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, Maseru, Kingdom of Lesotho, Southern Africa.

## 2.2 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). Approximately, 622.01, 450.17 and 128.36 g of powdered leaves, stem-bark and root, respectively, were obtained.

## 2.3 Preparation of plant extracts

A total 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 hr. Approximately, 5.12 g of combined hexane extract was obtained after removal of the solvent. The same procedure was repeated separately with chloroform, ethyl acetate and methanol. Approximately, 1.93, 1.49 and 3.29 g of chloroform, ethyl acetate and methanol leaves 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 of materials.

## 2.4 Microorganisms

Eight strains of microorganisms were used for this study. These are six bacterial isolates namely *Listeria monocytogenes*, *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Escherichia coli* (wild strain) and *Escherichia coli* O157:H7 and two fungal isolates namely *Penicillium digitatum* and *Candida albicans*. All these microorganisms were obtained from the culture collection at the Department of Biology, National University of Lesotho, Roma, Kingdom of Lesotho, Southern Africa.

## 2.5 Evaluation of antibacterial and antifungal activities of the *U. urens* extracts

The antibacterial and antifungal activities of various extracts of leaf, stem-bark and root of *U. urens* were screened by *in vitro* agar hole-plate diffusion method as described in literature (Manilal et al., 2009; Alghazeer et al., 2012). Various extract solutions were prepared separately at a concentration of 100 mg of extract in 1 mL of DMSO. The solutions were then separately filtered using 0.20 µm filter and then used for both antimicrobial and antifungal activities. A 100 µL of the broth culture of each bacterial strain was spread-plated on the nutrient agar (NA) plates separately. A sterile cork-borer was used to punch out 4 mm height and 6 mm diameter agar wells on the NA plates and each well was filled with 35 µL aliquots of the extract. The plates were then incubated at 37°C for one day. Tetracycline served as positive control for *E. coli* (wild type), *E. coli* (O157:H7), *S. aureus* and *L. monocytogenes*. Amoxicillin served as positive control for *S. marcescens* and *Pseudomonas aeruginosa*. DMSO served as negative control. For antifungal assay, the Petri dishes filled with 20 mL of potato dextrose agar (PDA) were used. A total of 100 µL of each fungal broth culture was spread-plated on the PDA medium. The cylindrical wells with the size of 4 mm height and 6 mm diameter were punched using an autoclaved cork-borer and then the wells were filled with 35 µL aliquots of the extract. The plates were then incubated at 24°C for two days. Miconazole nitrate served as positive control for *C. albicans* and DMSO served as negative control. However, positive control was not available for *P. digitatum*. All experiments were conducted in duplicates and the results were reported as the mean of two experiments. A clear zone > 10 mm are considered as positive results (Lima-Filho et al., 2002). Inhibition zones of <12, 12-20 and ≥20 mm diameter are expressed as weak, moderate and strong activities, respectively (Emmanuel et al., 2012; Matela et al., 2018).

## 2.6 Determination of minimum inhibitory concentrations (MICs)

The minimum inhibitory concentration (MIC) value refers to the minimum concentration needed to inhibit the visible growth of microorganism in a 24-hour period (Alghazeer et al., 2012; Alghazeer et al., 2017). The MIC of < 100 µg/mL, 100 to ≤ 625 µg/mL and > 625 µg/mL, were considered as significantly active, moderately active and weakly active, respectively (Emmanuel et al., 2012; Njimoh et al., 2015; Alghazeer et al., 2017). The MIC values of various *U. urens* extracts were determined as described in literature (Daud et al., 2005; Alghazeer et al., 2017). Briefly, stock solutions of various extracts of

*U. urens* was prepared separately at a concentration of 1000 µg/mL. From each stock solution, two-fold serial dilutions of 1000, 500, 250, 125, 62.5 and 31.25 µg/mL were prepared. A suspension of the microorganism is prepared at a concentration of one million to two million colony forming units CFU/mL by means of growing the bacterial isolate in nutrient broth in an incubator with continuous agitation (Matela et al., 2018). Agar wells with the size, 4 mm height and 6 mm diameter were punched using an autoclaved cork-borer and were then filled with 35 µL aliquots of the extract. The plates were incubated at 37°C for a period of 24-hours.

2.7 Statistical analysis

Data analysis was performed using the SPSS 17.0 statistics program by means of two-way analysis of variance. The differences were considered statistically significant when  $p \leq 0.05$ .

3. Results and discussion

The antimicrobial activity of various extracts from leaves, stem-bark and root of *U. urens* are summarized in Table 1. A total of ten extracts namely *U. urens* hexane leaf extract (HLE), chloroform leaf extract (CLE), ethyl acetate leaf extract (ELE), methanolic leaf extract (MLE), hexane stem-bark extract (HSB), chloroform

stem-bark extract (CSB), ethyl acetate stem-bark extract (ESB), methanolic stem-bark extract (MSB), chloroform root extract (CRT) and methanolic root extract (MRT) were prepared. Against *L. monocytogenes*, all extracts showed a moderate activity with inhibition zones in the range of 11.5±0.7 to 21.5±1.4 mm and ELE showed a significant strong activity with an inhibition zone of 21.5±1.4 mm. The positive control, tetracycline, showed an inhibition zone of 30.5±0.7 mm. Against *S. aureus*, all these extracts showed a weak to moderate activities with inhibition zones in the range of 11.0±1.4 to 17.0±0.7 mm. The positive control, tetracycline, showed an inhibition zone of 23.5±0.7 mm. Against *S. marcescens*, all these extracts also showed a weak to moderate activities with inhibition zones in the range of 10.5±2.1 to 16.5±6.3 mm. The positive control, amoxicillin, exhibited an inhibition zone of 21.5±2.1 mm. Against *P. aeruginosa*, all these extracts showed weak to strong inhibition zones in the range of 9.5±1.4 to 40.5±0.7 mm. MLE, CRT and MRT exhibited significantly higher activity with inhibition zones of 22.0±2.8, 21.5±0.7 and 40.5±0.7 mm. respectively and the remaining extracts showed a weak to moderate activities. Against *E. coli* (wild strain), all these extracts showed a weak to moderate activities with inhibition zones in the range of 9.0±0.0 to 18.5±0.7 mm. The positive control, tetracycline, showed an inhibition zone of 18.5±3.5 mm.

Table 1. Inhibitory effect of various extracts from leaves, stem-bark and root of *U. urens* against selected microorganisms.

Extracts	Zones of inhibition (mm)							
	Gram-positive bacteria		Gram-negative bacteria			Fungal isolates		
	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>S. marcescens</i>	<i>P. aeruginosa</i>	<i>E. coli</i> (wild strain)	<i>E. coli</i> (O157:H7)	<i>C. albicans</i>	<i>P. digitatum</i>
HLE	15.0±4.2 <sup>b</sup>	16.5±2.1 <sup>a</sup>	12.0±4.2 <sup>b</sup>	9.5±1.4 <sup>a</sup>	15.0±2.8 <sup>a</sup>	10.0±0.0 <sup>a</sup>	16.0±2.8 <sup>a</sup>	-
CLE	13.5±3.3 <sup>a</sup>	10.5±2.1 <sup>a</sup>	10.5±2.1 <sup>a</sup>	18.0±2.8 <sup>a</sup>	12.0±1.4 <sup>a</sup>	13.5±3.5 <sup>a</sup>	17.0±4.2 <sup>b</sup>	-
ELE	21.5±1.4 <sup>a</sup>	12.5±4.9 <sup>b</sup>	11.5±3.5 <sup>a</sup>	17.0±4.2 <sup>b</sup>	13.0±1.4 <sup>a</sup>	10.5±0.7 <sup>a</sup>	17.0±7.0 <sup>b</sup>	-
MLE	12.5±0.7 <sup>a</sup>	9.5±0.7 <sup>a</sup>	12.0±0.0 <sup>a</sup>	22.0±2.8 <sup>a</sup>	15.5±0.7 <sup>a</sup>	16.0±4.2 <sup>b</sup>	17.0±1.4 <sup>a</sup>	-
HSB	17.0±2.8 <sup>a</sup>	13.5±2.1 <sup>a</sup>	16.5±6.3 <sup>b</sup>	9.5±1.4 <sup>a</sup>	16.5±6.3 <sup>b</sup>	17.5±3.9 <sup>a</sup>	16.0±4.2 <sup>b</sup>	-
CSB	16.5±2.1 <sup>a</sup>	11.5±2.1 <sup>a</sup>	16.0±1.4 <sup>a</sup>	12.0±1.4 <sup>a</sup>	13.0±1.4 <sup>a</sup>	12.5±2.1 <sup>a</sup>	17.0±2.8 <sup>a</sup>	-
ESB	11.5±0.7 <sup>a</sup>	16.5±0.7 <sup>a</sup>	13.5±3.3 <sup>a</sup>	13.5±4.9 <sup>b</sup>	18.5±0.7 <sup>a</sup>	12.5±2.1 <sup>a</sup>	17.5±4.9 <sup>b</sup>	-
MSB	16.0±2.8 <sup>a</sup>	11.0±1.4 <sup>a</sup>	12.5±2.1 <sup>a</sup>	14.5±0.7 <sup>a</sup>	9.0±0.0 <sup>a</sup>	10.5±1.4 <sup>a</sup>	16.5±2.1 <sup>a</sup>	-
CRT	11.5±0.7 <sup>a</sup>	15.5±0.7 <sup>a</sup>	12.5±0.7 <sup>a</sup>	21.5±0.7 <sup>a</sup>	10.0±1.4 <sup>a</sup>	10.5±0.7 <sup>a</sup>	16.5±0.7 <sup>a</sup>	-
MRT	11.5±0.7 <sup>a</sup>	17.0±0.7 <sup>a</sup>	13.5±2.2 <sup>a</sup>	40.5±0.7 <sup>a</sup>	16.5±2.1 <sup>a</sup>	12.5±2.1 <sup>a</sup>	17.5±3.3 <sup>a</sup>	-
Positive controls	30.5±0.7 <sup>a</sup>	23.5±0.7 <sup>a</sup>	21.5±2.1 <sup>a</sup>	09.5±0.0 <sup>a</sup>	18.5±3.5 <sup>a</sup>	24.5±2.1 <sup>a</sup>	23.5±1.8 <sup>a</sup>	#

<sup>a</sup> = the difference is statistically significant; <sup>b</sup> = the difference is not statistically significant.

HLE = *U. urens* hexane leaf extract; CLE = *U. urens* chloroform leaf extract; ELE = *U. urens* ethyl acetate leaf extracts; MLE = *U. urens* methanol leaf extract; HSB = *U. urens* hexane stem-bark extract; CSB = *U. urens* chloroform stem-bark extract; ESB = *U. urens* ethyl acetate stem-bark extract; MSB = *U. urens* methanol stem-bark extract; CRT = *U. urens* chloroform root extract; MRT = *U. urens* methanol root extract. Tetracycline served as a positive control against *L. monocytogenes*, *S. aureus*, *E. coli* (wild strain) and *E. coli* (O157:H7). Amoxicillin served as a positive control against *S. marcescens* and *P. aeruginosa*. Miconazole nitrate served as a positive control against *C. albicans*. DMSO served as a negative control. # : No positive control used. - : No activity observed.

Against *E. coli* (O157:H7), all these extracts also showed a weak to moderate activities with inhibition zones in the range of 10.0±0.0 to 17.5±3.9 mm. The positive control, tetracycline, showed an inhibition zone of 24.5±2.1 mm. Against *C. albicans*, all these extracts showed a moderate activity with inhibition zones in the range of 16.0±2.8 to 17.5±3.3 mm. The positive control, miconazole nitrate, exhibited an inhibition zone of 23.5±1.8mm. We also evaluated the antifungal activity of these extracts against *P. digitatum*. However, none of the extracts showed any visible zone of inhibition against these fungal isolates.

Additionally, we also evaluated the minimum inhibitory concentration (MIC) of these extracts and the results are summarized in Table 2. Against *L. monocytogenes*, HSB, CSB, MSB and MRT were significantly active with MIC values of 62.5, 62.5, 62.5 and <31.25 µg/mL, respectively. Other extracts showed weak to moderate activities. Against *S. aureus*, HLE was significantly active with MIC value of 62.5 µg/mL and all other extracts showed a weak to moderate activities. Against *S. marcescens*, CLE, CRT and MRT exhibited a significant activity with MIC values of 62.5, <31.25 and <31.25 µg/mL, respectively and other extracts showed moderate activity. Against *P. aeruginosa*, HLE, ELE, HSB, CSB, ESB, CRT and MRT exhibited a significant activity with MIC values of 62.5, <31.25, 62.5, <31.25, <31.25, <31.25 and 62.5 µg/mL, respectively. However, CLE, MLE and MSB exhibited moderate activity. Against *E. coli* (wild strain), HLE, CLE, ELE, MLE and HSB showed significant activity with MIC values of 62.5, <31.25, <31.25, <31.25 and 62.5 µg/mL, respectively. MRT showed weak activity and CSB, ESB,

MSB and CRT showed moderate activity (refer to Table 2). Against *E. coli* (O157:H7), CLE and MLE exhibited a significant activity with MIC values of 62.5 and <31.25 µg/mL, respectively and all other extracts showed a moderate activity (refer to Table 2). Against *C. albicans*, HLE, ELE and HSB exhibited a strong activity with MIC values of <31.25 µg/mL, for each extract and all other extracts showed a weak to moderate activity (refer to Table 2). We did not conduct any experiment for the antifungal activity of these extracts against *P. digitatum* since these extracts did not show any visible inhibition zone in the preliminary study.

Microorganisms such as *P. aeruginosa*, *S. aureus*, *E. coli* and *C. albicans* have previously been used to screen the antimicrobial activity of chloroform, ethanol and aqueous extracts obtained from leaves of *U. urens* (Kaushik et al., 2015; Barkaoui et al., 2017; Mzid et al., 2017). The leaves have been collected at various geographic locations in Tunisia and Ethiopia. Some of these extracts exhibited remarkable antimicrobial against these microorganisms, while the others are not. However, the results obtained among these studies were not consistent, even among the same extracts (Kaushik et al., 2015; Barkaoui et al., 2017; Mzid et al., 2017). Additionally, we noticed that the chloroform extracts were common to our study also. Therefore, we have chosen the same microorganisms to compare our chloroform extracts results with previously reported results. Additionally, we also screened our other extracts namely hexane, ethyl acetate and methanol extracts against the same microorganisms. Furthermore, we have also chosen three more microorganisms namely *L. monocytogenes*, *S. marcescens* and *P. digitatum* for this

Table 2. The minimum inhibitory concentrations (MICs) of various extracts from *U. urens* on selected microorganisms.

Extracts	Zones of inhibition (mm)							
	Gram-positive bacteria			Gram-negative bacteria			Fungal isolates	
	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>S. marcescens</i>	<i>P. aeruginosa</i>	<i>E. coli</i> (wild strain)	<i>E. coli</i> (O157:H7)	<i>C. albicans</i>	<i>P. digitatum</i>
HLE	125	62.5	250	62.5	62.5	500	<31.25	N/T
CLE	>1000	250	62.5	125	<31.25	62.5	250	N/T
ELE	250	>1000	500	<31.5	<31.25	250	<31.25	N/T
MLE	500	>1000	250	250	<31.25	<31.25	250	N/T
HSB	62.5	500	125	62.5	62.5	250	<31.25	N/T
CSB	62.5	250	250	<31.25	500	500	125	N/T
ESB	>1000	125	125	<31.25	500	250	250	N/T
MSB	62.5	500	125	500	250	250	500	N/T
CRT	250	500	<31.25	<31.25	125	125	500	N/T
MRT	<31.25	125	<31.25	62.5	>1000	500	250	N/T

Refer to the footnote of Table 1.

study, since these three microorganisms have never been used previously to screen the antimicrobial activity of any of the extracts from *U. urens*.

In a previous report, the ethanolic extract from leaves of *U. urens* collected from Tunisia showed remarkable antibacterial activity against *S. aureus* and *P. aeruginosa* with minimum bactericidal concentration/minimum inhibition concentration (MBC/MIC) value of 2.0 µg/mL at a concentration of 150 µg/mL (Mzid et al., 2017). However, this ethanolic leaf extract did not exhibit any visible zone of inhibition against *E. coli* in the same study (Mzid et al., 2017). Again, in the same study, the water extract did not exhibit any visible zone of inhibition against *P. aeruginosa* and *S. aureus*. In another report, the chloroform and aqueous extracts from leaves of *U. urens* collected in Eritrea showed no inhibitory effect against *E. coli*, *S. aureus* and *C. albicans* (Kaushik et al., 2015). However, in our study, the chloroform leaf extract exhibited weak activity against *E. coli* and *S. aureus* with inhibition zones of 12.0±1.4 and 10.5±2.1 mm, respectively and a moderate activity against *C. albicans* with an inhibition zone of 17.0±4.2 mm. In another report, the chloroform and aqueous leaf extracts of *U. urens* collected from Tunisia exhibited no zone of inhibition against *S. aureus*, *E. coli* and *P. aeruginosa* (Barkaoui et al., 2017). The chloroform extract and these three microorganisms were common to our study also. However, in our study, the chloroform leaves extract showed a moderate activity with inhibition zones of 12.0±1.4, 10.5±2.1 and 18.0±2.8 mm, respectively, against *E. coli*, *S. aureus* and *P. aeruginosa*. This discrepancy may be due to change in chemical constituents and/or their relative proportions of plant materials since they were collected at different locations. Additionally, the seasonal variations will have a prominent role in determining the chemical constituents and/or their relative proportions and subsequently the antimicrobial activities.

#### 4. Conclusion

The antibacterial and antifungal activities of extracts from various parts of *U. urens* collected from the Kingdom of Lesotho, Southern Africa were evaluated. These extracts exhibited inhibition zones in the range of 9.0±0.0 to 40.5±0.7 mm against bacterial isolates and 16.0±4.2 to 17.5±4.9 mm against *C. albicans*. However, all these extracts exhibited no zone of inhibition against *P. digitatum*. Furthermore, the minimum inhibitory concentrations (MICs) of various extracts of *U. urens* were evaluated. The MIC values of these extracts were found to be in the range of <31.25 to >1000 µg/mL, against both the bacterial and fungal isolates. From this study, we concluded that ELS, MLE, CRT and MRT

showed promising antimicrobial activities.

#### Conflict of interest

The authors declare no conflict of interest.

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