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Antioxidant and Antibacterial Activity of *Ludwigia octovalvis* on *Escherichia coli* O157:H7 and Some Pathogenic Bacteria

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Abstract: *Ludwigia octovalvis* (Jacq.) P. H. Raven (Family: Onagraceae) is traditionally used to treat skin diseases, diarrhea and flatulence. This study assayed twelve extracts of *L. octovalvis* for their total phenolic content (TPC), antioxidant and antibacterial activity. The highest TPC at 264.76 ± 0.23 GAE mg/g dry weight and antioxidant activity (evaluated by 2, 2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power assays) at 1080.84 ± 6.07 µM TE/mg dry weight and 1256.88 ± 5.38 µM TE/mg dry weight, respectively, were detected in 80% methanol extract of the leaf. A strong correlation between TPC and antioxidant activities of both assays was observed ($r > 0.98$). Eighty percent methanol extract of the leaf gave the lowest minimum inhibitory concentration (MIC, 62.5 µg/mL) and minimum bactericidal concentration (MBC, 125.0 µg/mL) against *Escherichia coli* O157:H7, *Escherichia coli* ATCC 25922 and *Bacillus spizizenii* (ATCC 6633), while 80% methanol extract of the root gave similar MIC and MBC values against *Pseudomonas aeruginosa* (ATCC 27853). Our findings suggest that leaf of *L. octovalvis* as a possible new source of natural mixture of antioxidant and anti-*E. coli* O157:H7.

Key words: *E. coli* O157:H7 · *Ludwigia octovalvis* · Total phenolic · DPPH · FRAP

INTRODUCTION

E. coli O157:H7 is a foodborne pathogen that causes diarrhea, hemorrhagic colitis and, in a subset of patients, hemolytic uremic syndrome, which is a life-threatening complication [1]. It was first isolated in the USA in 1975 from sporadic cases of hemorrhagic colitis, but is now implicated with disease outbreaks around the globe [2]. Initial outbreak was associated with undercooked hamburgers and unpasteurized milk, but currently a variety of foods, cow-manure contaminated water and vegetables, swimming in contaminated waters and indirect or direct animal contact have been implicated as sources of the pathogen [3-6]. Plant extracts have been developed and used in foods as natural antioxidants and antimicrobials [7]. There have been studies on antibacterial activity against *E. coli* O157:H7 and other pathogens by plant extracts or metabolites for their potential use in controlling foodborne pathogens [8-10].

Ludwigia octovalvis (Jacq.) P. H. Raven (Family: Onagraceae) is a medicinal plant that is widely distributed in Malaysia [11]. A poultice of an entire plant is externally applied to heal dermatitis, boil, ulcer, impetigo and pimple. Its decoction is drunk as a health drink and to treat gastrointestinal complaints such as diarrhea and flatulence [12, 13]. A review performed on major published series such as PUB MED (http:www.pub.med, accessed on 3rd October 2011) revealed that reported antibacterial activities of this plant are limited only to against Streptococcus *mutans* [14], *Helicobacter pylori* [15] and dermatological bacteria [16].

Similarly, antioxidant activity of *L. octovalvis* is only limited to one report [17] and there was no mentioned of chemical compounds associated with the activity. Subsequent studies [18-20] reported on the isolation of new compounds from *L. octovalvis* with anticancer activity and some of these compounds like luteolin, quercetin, apigenin and gallic acid are known to have antibacterial and antioxidant activity. Emerging evidence is pointing to the beneficial dual role of phenolic phytochemicals having antioxidant activity as well as anti-*E. coli* O157:H7 agent [21]. The synergistic contribution of phenolics and antioxidant activity may have implications for extending the shelf life of foods as well as reduction of foodborne diseases due to *E. coli* O157:H7.

In the present study we reported on the antioxidant and antibacterial activity of methanol extract of *L. octovalvis* against *E. coli* O157:H7 and other bacteria. Antioxidant activity correlates strongly with total phenolic content.

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MATERIALS AND METHODS

Plant Material: The whole plant of *L. octovalvis* was harvested at its mature stage from the wet area of the Universiti Sains Malaysia main campus, Pulau Pinang. A voucher specimen number (11090) was deposited at the herbarium of the School of Biological Sciences, Universiti Sains Malaysia.

Chemicals and Reagents: The following chemicals and reagents were used in this study: Fisher Scientific, Springfield, USA: *n*-Hexane, chloroform, ethyl acetate and methanol. Sigma-Aldrich Chemical, St. Louis, USA: Folin-Ciocalteau's (FC) reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (98% purity), 2,4,6-tris (2-pyridyl)-1,3,5-triazine (TPTZ), ferric chloride hexahydrate, sodium acetate, acetic acid glacial, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and *p*-iodonitrotetrazolium chloride (INT). QRec, Germany: Dimethylsulphoxide (DMSO). Oxoid, England:nutrient broth and nutrient agar. Bendosen Laboratory Chemicals, England: Anhydrous sodium carbonate.

Plant Extraction: The leaves, stems and roots of the plant were separated and washed. The samples were then dried at 40°C for 24 h and ground into powder. Each powdered plant part (30 g) was sequentially extracted (cold extraction) in a flask with 200 ml of *n*-hexane by continuous shaking at 40°C for 8 h. The extract was filtered using 150 mm diameter filter paper (Whatman, U.K.). The residue was then dried, similarly and successively extracted using chloroform, ethyl acetate and finally 80% (v/v) methanol all at 40°C. Each extract was concentrated using a rotary evaporator and stored at -4°C until further use. Overall, a total of 12 extracts was obtained.

Determination of Total Phenolic Content: The total phenolic content (TPC) in all the extracts was estimated by a colorimetric assay [22, 23], with slight modifications. The TPC was calculated from the calibration curve using gallic acid as a standard. The results are expressed as milligram of gallic acid equivalents per gram dry weight of extract (mg GAE/g d.w.). DMSO (10%v/v) was used to dilute the extract to obtain an initial concentration of 1 mg/ml. Briefly, 0.5 ml of each extract was pipetted in a test tube followed by 1.0 ml of 10% dilution of FC reagent. The contents of the test tube were thoroughly mixed. After 3 min, 3 ml of sodium carbonate (1% w/v) was added. The mixture was kept in the dark for 2 h at 25°C. The absorbance was measured at 760 nm using a spectrophotometer (Hitachi Model U-1900) with 10 % (v/v) DMSO as blank. The procedure was repeated using different concentrations of standard gallic acid solutions (0.05–0.2 mg/ml).

Experiments were carried out in triplicate and the mean value was recorded. The TPC was calculated as gallic acid equivalent (GAE) using the following equation:

Y (absorbance) = 8.982 X (µg gallic acid) – 0.01412, $r2$ = 0.9925 that was obtained from the standard gallic acid graph. The absorbance value was inserted in the abovementioned equation and the total amount of phenolic compound was calculated.

Determination of Antioxidant Activity

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay: The free radical scavenging activity of all the extracts was measured using DPPH scavenging activity [24, 25], with slight modifications. Briefly, 150 µl of 300 µM ethanolic DPPH solution was added to 50 µl of 1 mg/ml extracts (diluted in DMSO) in 96-microwell plates. DMSO was used as negative control. The reaction mixture was incubated in the dark at 37°C for 30 min. The decrease in absorbance value was then measured at 515 nm using a microplate reader (Thermo, Multiskan Ex, Finland). All measurements were carried out in triplicate.

Ferric Reducing Antioxidant Power (FRAP) Assay: Reducing power was determined using a FRAP assay [26], with slight modifications. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 µM TPTZ solution in 40 mM HCl and 20 mM ferric chloride hexahydrate at a proportion of 10:1:1 (v:v). The FRAP reagent was freshly prepared before analysis and warmed to 37°C prior to use. The extracts were dissolved in DMSO at a concentration of 1 mg/ml. Extracts (20 µl) were allowed to react with 180 µl of FRAP solution for 5 min in the dark. The absorbance of the reaction mixture was then measured at 593 nm using a microplate reader (Thermo, Multiskan Ex, Finland). All measurements were carried out in triplicate.

Determination of FRAP and DPPH Inhibition Values: Trolox was used as reference in both assays. Two different standard curves were obtained using Trolox standard solution in 10 % (v/v) DMSO at various concentrations. The absorbance of the reaction sample was compared to that of the Trolox standard. The results are expressed in terms of microMolar of Trolox equivalents per milligram dry weight of extract (µM TE/mg d.w.).

Antibacterial Activity

Bacterial Strains and Growth Media: Antibacterial activity was evaluated on following strains of bacteria, Gram positive bacteria: *Bacillus cereus* (ATCC 10876), *Bacillus licheniformis* (ATCC 12759), *Bacillus spizizenii* (ATCC 6633), *Staphylococcus aureus* (ATCC 12600), *Staphylococcus epidermidis* (ATCC 12228) and *Streptococcus mutans* (ATCC 25175), Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Escherichia coli* 0157:H7, *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Pseudomonas stutzeri* (ATCC 17588) and *Shigella boydii* (ATCC 9207). *E. coli* O157:H7 was obtained from National Laboratory for Food Safety and Quality of Malaysia. All bacteria strains were cultured on nutrient agar at 37°C for 18 h. and stored on nutrient agar slant at 4°C until use.

Disc Diffusion Method: A disc diffusion method [27] was used to determine the antibacterial activities of all the extracts. Stock solutions of each extracts were prepared by dissolving 20 mg of the extract in 1 ml 10% DMSO and filtered (0.2 µm, Whatmann, UK). Stock solution of antibiotics (chloramphenicol or doxycycline) used as positive controls were prepared at 1mg/ml.

For a particular bacterial species tested, cells from a 18h culture at 37°C on nutrient agar, were suspended in saline solution (0.85% w/v) and turbidity was adjusted to a scale of 0.5 on the MacFarland standard (Biomerieux, France). Briefly, 100 µl of the bacterial suspension was inoculated on nutrient agar and swabbed three times, rotating the plates 120 degrees between swabbing to distribute the cells uniformly. Seven discs were gently pressed on the agar with sterile forceps *viz*. two as negative controls (each impregnated with 20 µl water or 10%v/v DMSO) and one impregnated with 20 µl of chloramphenicol (20 ug/disc) and the remaining 4, each impregnated with 20 µl of n-hexane, chloroform, ethyl acetate and 80% methanol extract of leaf (400µg extract/disc). This was done in triplicate and repeated but using doxycycline as a positive in place of chloramphenicol. The whole test was then repeated but using extracts of stem and roots. These plates were incubated aerobically overnight at 37°C and the diameters of the inhibition zones were measured.

Broth Micro-Dilution Method: Stock solution of 80% methanol extract of leaf was prepared in 10% DMSO and filtered (0.2 µm, Whatmann UK). A broth micro-dilution bioassay in 96-well plates was used to determine MIC and MBC of the extract or antibiotic at two-fold dilution against *E. coli* O157:H7 in a total volume of 200 µl [28]. Cells (5×10^5) were cultured in nutrient broth containing final concentration of the extract ranging from 1000 µg/ml to 7.8 µg/ml. In two positive controls, cells (5×10^5) were cultured in nutrient broth containing final concentration of chloramphenicol or doxycycline ranging from 250 µg/ml to 2.0 µg/ml. A negative control with cells (5×10^5) cultured in nutrient broth only and another control containing extract and nutrient broth but without cells were also included in the 96-well plate. All tests were in triplicates.

The plates were covered and incubated aerobically at 37°C. After 18h, 40 µl of 0.2 mg/ml INT was added to each well and the plates were further incubated for 30 min. Bacterial growth in the wells was indicated by development of red-pink color, while growth inhibition was indicated by no change in the colour of cell suspensions. The MIC of each extract is defined as the lowest concentration inhibiting growth. To determine the MBC, samples from all wells showing no growth as well as sample from the lowest concentration showing growth in the MIC assay, were subcultured on freshly prepared nutrient agar. Plates were incubated for 24h at 37°C to check for any macrocolonies developing. The MBC is the lowest concentration of extract that killed viable cells, hence no macrocolonies were formed on the recovery medium.

Statistical Analysis: All data are reported as the mean ± S.D. of triplicate determinations. The statistical analysis of the data was carried out using one-way ANOVA, followed by post hoc least-significant difference (LSD) test using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL) at a confidence level higher than 95% ($p < 0.05$). Pearson's correlation test was conducted to determine the correlation between antioxidant and TPC. The test was carried out using the Prism 3.02 statistical software of GraphPadPrism (San Diego, CA).

RESULTS

Total Phenolic Content (TPC): Eighty percent of methanol extracts of the leaf and stem contained significantly ($p < 0.05$) highest amount of TPC (264.76 ± 0.23 GAE mg/g d.w. and 239.05 ± 0.29 GAE mg/g dry weight, respectively. Generally TPC of extract declined with decreasing polarity of the solvent used, i.e 80% methanol, ethyl acetate, chloroform and n-hexane. But for the root, TPC was significantly higher ($p<0.05$) in ethyl acetate extract compared to that of 80% methanol extract (Table 1).

Table 1: TPC and antioxidant activity of *Ludwigia octovalvis* extracts

Plant part	Extract	TPC (mg GAE/g d.w.)	Antioxidant activity (µM TE/mg d.w.) DPPH	Antioxidant activity (µM TE/mg d.w.) FRAP
Leaf	Methanol	264.76±0.23[a]	1080.84±6.07[a]	1256.88±5.38[a]
	Ethyl acetate	68.45±0.17[e]	301.48±8.92[d]	253.45±5.97[d]
	Chloroform	47.85±0.28[i]	113.04±1.58[j]	136.83±3.48[g]
	n-Hexane	34.64±0.11[j]	91.00±0.31[k]	142.04±3.53[g]
Stem	Methanol	239.05±0.29[b]	905.00±7.37[b]	912.17±5.06[b]
	Ethyl acetate	70.23±0.23[d]	206.68±2.71[f]	166.02±2.3[f]
	Chloroform	68.45±0.17[e]	182.30±5.02[g]	188.30±6.73[e]
	n-Hexane	57.65±0.17[g]	146.15±5.87[i]	113.40±6.65[h]
Root	Methanol	62.32±0.17[f]	225.32±1.84[e]	203.67±7.05[c]
	Ethyl acetate	98.88±0.19[c]	369.28±8.76[c]	303.36±4.76[c]
	Chloroform	70.08±0.28[d]	174.52±5.71[gh]	159.90±2.07[f]
	n-Hexane	50.00±0.22[h]	162.06±4.7[hi]	195.73±6.32[de]

Values are mean ± standard deviation of three replicates. Values in the same columns with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA and post hoc least-significant difference (LSD) test

Antioxidant Activity (AOA): The results of antioxidant activity from both assays are expressed as µM Trolox equivalent (µM TE)/mg dry weight which is a more meaningful and descriptive expression, compared with antioxidant activity expressed as percentage of activity at a specific concentration (Table 1). Results may provide a direct comparison of the antioxidant activity with that of Trolox equivalent [29].

The 80% methanol extract of leaf exhibited the highest antioxidant activity with DPPH and FRAP values at 1080.84 ± 6.07 µM TE/mg dry weight and 1256.88 ± 5.38 µM TE/mg dry weight, respectively (Table 1). These values were followed by the 80% methanol extract of the stem with DPPH and FRAP values at 905.00 ± 7.37 µM TE/mg d.w. and 912.17 ± 5.06 µM TE/mgd.w., respectively. Like the TPC, generally antioxidant activity of extract also decreased with decreasing polarity of the solvents used except for the root when antioxidant activity was significantly higher ($p < 0.05$) in ethyl acetate extract than in 80% methanol extract.

Correlation Between TPC and AOA: The correlation between TPC and antioxidant activities of the extracts was tested using Pearson's correlation test. The TPC values (mg GAE/g dry weight) of all the extracts were plotted separately against those of DPPH and FRAP. Figures 1 and 2 show high correlation coefficients were observed between TPC and DPPH values ($r = 0.9926$, $p < 0.0001$) and between TPC and FRAP values ($r = 0.9814$, $p < 0.0001$).

Sensitivity of Bacteria Against Plant Extract: By disc diffusion assay, both positive control antibiotics inhibited all the bacteria tested (Table 2). For the extract, only methanol extract of stem and roots and all leaf extracts

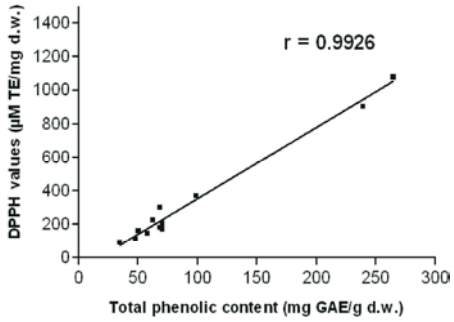

Fig. 1: Correlation between TPC and antioxidant activity using DPPH assay of 12 extracts of *Ludwigia octovalvis*

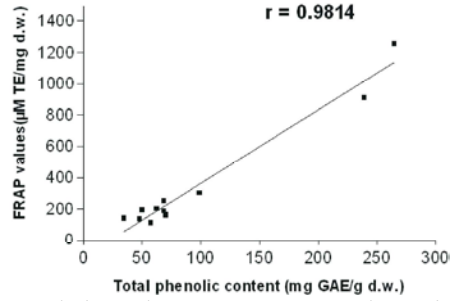

Fig. 2: Correlation between TPC and antioxidant activity using FRAP assay of 12 extracts of *Ludwigia octovalvis*.

possessed antibacterial activity, but they were unable to inhibit all the bacteria tested, unlike the control antibiotics. Generally methanol extracts were active against both gram-positive and gram-negative bacteria and methanol extract of leaf inhibited both the *E. coli* strains used. With additional factor that methanol extracts contained high TPC, they were preferred for determination of MIC and MBC against the tested bacteria.

Table 2: Antibacterial activities of extracts of *Ludwigia octovalvis* based on disc diffusion assay

Bacteria	Diameter of inhibition zone (mm)							
	Leaf				Stem	Root	Antibiotics	
Gram-positive	Methanol	Ethyl- acetate	Chloroform	n-Hexane	Methanol	Methanol	Chloramphenicol	Doxycycline
B. cereus	10.8±0.8^{b}	7.2±0.4^{a}	^{x}	^{x}	7.6±0.8^{a}	10.0±0.6^{b}	17.5±0.3^{fg}	16.5±0.5^{f}
B. licheniformis	12.2±1.2^{c}	8.4±0.5^{a}	^{x}	^{x}	9.7±0.6^{b}	^{x}	15.2±0.8^{e}	22.2±0.3^{i}
B. spizizenii	14.0±0.8^{de}	7.0±0.5^{a}	^{x}	^{x}	10.2±0.3^{b}	12.2±1.3^{c}	24.0±0.4^{i}	27.1±0.3^{j}
Staph. aureus	7.8±1.7^{a}	9.2±1.2^{b}	7.3±0.4^{a}	7.0±0.0^{a}	7.0±0.6^{a}	10.5±0.7^{b}	17.4±0.6^{f}	19.4±0.6^{h}
Staph.epidermidis	17.8±1.2^{fg}	9.5±0.5^{b}	8.3±0.5^{a}	7.0±0.0^{a}	11.3±1.0^{c}	10.0±0.5^{b}	20.0±0.5^{h}	13.5±0.5^{cd}
Strep. mutans	15.3±1.6^{e}	9.1±1.2^{b}	8.0±0.4^{a}	7.4±0.3^{a}	11.4±0.8^{c}	10.2±0.8^{b}	18.0±0.0^{fg}	22.0±0.4^{i}
Gram negative								
E. coli O157H7	12.0±0.4^{c}	10.6±0.5^{b}	9.5±1.5^{b}	7.0±0.0^{a}	^{x}	^{x}	15.8±1.2^{e}	14.7±0.8^{e}
E. coli ATCC	14.8±0.8^{e}	11.0±1.8^{c}	9.0±0.5^{b}	8.2±1.3^{a}	10.3±0.6^{b}	10.6±0.7^{b}	20.6±2.2^{h}	16.6±0.5^{f}
Kl. pneumoniae	^{x}	^{x}	^{x}	^{x}	^{x}	8.8±0.5^{a}	18.4±1.7^{g}	15.4±0.8^{f}
Ps.aeruginosa	9.8±1.4^{b}	8.0±1.0^{a}	7.6±0.1^{a}	7.0±0.0^{a}	8.2±0.2^{a}	14.2±1.4^{e}	16.4±1.5^{f}	18.4±0.5^{g}
Ps. Stutzeri	15.7±1.1^{e}	13.2±0.8^{cd}	10.7±1.1^{b}	9.3±0.8^{b}	11.3±0.8^{c}	7.7±1.0^{a}	12.9±1.5^{cd}	11.9±0.4^{c}
Sh. Boydii	8.0±0.8^{a}	9.4±0.3^{b}	9.0±0.0^{b}	8.7±1.0^{a}	7.0±0.0^{a}	10.2±0.8^{b}	17.0±0.2^{f}	18.0±0.2^{g}

Values are means of three replicates ± standard deviation. Values with different superscript letters are significantly different ($p < 0.05$) based on one-way ANOVA and post hoc least-significant difference (LSD) test. ^{x}no inhibition

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 80% methanol extracts of *Ludwigia octovalvis*

Bacteria	MIC and MBC values (µg/mL)									
	Leaf		Stem		Root		Chloramphenicol		Doxycycline	
Gram-positive	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. cereus	125.0	250.0	500.0	1000.0	125.0	250.0	15.60	31.25	15.60	31.25
B. licheniformis	500.0	1000.0	250.0	500.0	>1000	>1000	15.60	31.25	15.60	31.25
B. spizizenii	62.5	125.0	500.0	1000.0	125.0	500.0	7.80	15.60	7.80	15.60
S. aureus	500.0	1000.0	1000.0	>1000	250.0	1000.0	15.60	31.25	15.60	31.25
S. epidermidis	250.0	500.0	125.0	250.0	250.0	500.0	7.80	15.60	31.25	62.50
S. mutans	125.0	500.0	125.0	250.0	125.0	250.0	15.60	31.25	7.80	15.60
Gram- negative										
E. coli O157H7	62.5	125.0	>1000	>1000	>1000	>1000	15.60	31.25	15.60	31.25
E. coli ATCC	62.5	125.0	125.0	250.0	125.0	250.0	15.60	31.25	7.80	15.60
K. pneumoniae	125.0	250.0	>1000	>1000	125.0	500.0	15.60	31.25	15.60	31.25
P. aeruginosa	125.0	1000.0	500.0	1000.0	62.5	125.0	15.60	31.25	7.80	15.60
P. stutzeri	125.0	250.0	125.0	250.0	125.0	250.0	31.25	62.50	31.25	62.50
S. boydii	125.0	500.0	500.0	1000.0	125.0	250.0	15.60	31.25	15.60	31.25

In view of the limitation of disc diffusion assay to objectively indicate actual activity of the plant extracts, broth microdilution technique was applied to determine the MIC and MBC of methanol extracts. The lowest MIC and MBC were 62.50 µg/mL and 125.00 µg/mL, respectively and this was demonstrated by leaf methanol extract against both *E. coli* strains and *B. spizizenii* and root methanol extract against *P. aeruginosa*. Other extracts gave higher MIC and MBC values and were considered ineffective (Table 3).

None of the extracts demonstrated comparable MIC and MBC values with the positive controls. The antibacterial activity of leaf methanol extract against E. coli O157:H7 is of great importance considering that it is an emerging human pathogen.

DISCUSSION

In this study, three different parts of *L. octovalvis* were separately and successively extracted by increasing the polarity of solvents and each extract was quantified for its TPC and evaluated for its antioxidant and antibacterial activities. Our finding that 80% methanol extract of stem and leaf contained high amount of TPC explains its broad spectrum antibacterial activity as shown by disc diffusion assay. It is known that plant phenolics exert their bactericidal activity by accumulating at the cell membrane [30] and disrupting the membrane fluidity [31] leading to efflux of K + ions, a major cytoplasmic action and involved in several key functions of growing bacterial cells.

Result from the study also showed some bacteria were less sensitive towards 80% methanol extract. This is not unexpected as earlier report [32] showed similar result, albeit using a different *Ludwigia* species that is *L. adscendens*. Our result that polar extract were active against wide range of bacteria also concur with earlier report on polar solvent extract of *L. octovalvis* which exhibited significantly higher antibacterial activity against dermatological bacteria than did the non-polar extracts such as petroleum ether, hexane and chloroform [16].

For the root, TPC and AOA of 80%methanol extract was lower than that of ethyl acetate, which may be due to the differences in morphological and anatomical characteristics of the different parts of a plant. This necessitates a different extraction solvent system to ensure optimum recovery of TPC [33]. TPC in ethyl acetate extract is three times lower than in leaves and stem, so its contribution to the observed antibacterial activity was possibly minor.

To the best of our knowledge, activity of *L. octovalvis* against *E. col*i O157:H7, a newly emerging pathogen appears lacking. By microdilution assay, only 80% methanol extract of leaf inhibited both *E. coli* strains at the lowest MIC (62.5µg/ml) and MBC (125 µg/ml) values. This finding suggests the potential of leaf extract as a natural source of both, phenolics which are antibacterials and antioxidants. Only one publication reported the MIC of *L. octovalvis* and that was against *S.mutans* [14]. Our study showed that MIC of all 80% methanol extracts against *S. mutans* was 125.0 µg/ml, much lower than that of the aqueous extract of the entire plant at 2000 µg/ml. This showed 80% methanol as the preferred solvent for extracting antibacterial phenolic compounds from *L. octovalvis*. Overall, antioxidant and antibacterial activities of the extracts vary with parts of *L. octovalvis* and types of solvent used [34]. Bioactive components of different species and parts of plants have different solubility levels in different solvents [35].

The extracts were also examined for their antioxidant activities using DPPH and FRAP assays. Despite representing different mechanism of actions, these assays generated a similar conclusion that TPC correlates strongly ($r > 0.98$) with AOA. These results supported earlier studies [36, 37], who found a significant correlation between TPC and antioxidant activities, evaluated by three different analytical methods. Although none of the extract demonstrated a comparable MIC and MBC values with positive controls, our finding is important because *E. coli* O157:H7 was sensitive to the phenolics-containing extract. This indicates the potential beneficial synergistic role of phenolic phytochemicals having an antioxidant activity as well as antibacterial activity especially *E. coli* O157:H7. Emerging epidemiological evidence is increasingly pointing to the beneficial effects of phenolic phytochemicals having antioxidant activity in managing infectious diseases [38]. Synergistic phenolics and antioxidant activity inhibits bacterial growth and this has implications for diet-based management of infectious disease which is an inexpensive blocker against infectious diseases [39]. A mixture of phenolics phytochemicals in whole foods is effective in protectively supporting human health compared to isolated individual phenolic phytochemicals. *L. octovalvis* is potentially useful as sources of phenolics and therefore future studies should identify the profile of phenolic phytochemicals which contributes to the functionality of the plant from synergistic interaction of constituent phenolic phytochemicals.

CONCLUSIONS

Extracting solvents and plant parts influenced the TPC, antioxidant and antibacterial activities of the extracts. The most polar solvent used (80% methanol) extracted the most total phenolics from leaf and stem, which correlates strongly with their high antioxidant and explains why the extract has antibacterial activity. Eighty percent methanol extract of leaf inhibited both *E. coli* 0157:H7 and *E. coli* ATCC 25922 and *Bacillus spizizenii* (ATCC 6633), while that of the root inhibited *Pseudomonas aeruginosa* (ATCC 27853) at MIC and MBC values of 62.5 ug/ml and 125 ug/ml, respectively. Other extracts were considered ineffective as the MIC and MBC were higher than these values. We conclude that the 80% methanol extract of the leaf of *L. octovalvis* could possibly be a new source of natural mixture of antioxidant and anti *E. coli* O157:H7.

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