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ORIGINAL ARTICLE

Phytochemicals derived from *Leucaena leucocephala* (Lam.) de Wit (Fabaceae) biomass and their antimicrobial and antioxidant activities: HPLC analysis of extracts

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Abstract
The biomass production from *Leucaena leucocephala* (Lam.) de Wit (family Fabaceae) is a valuable source for chemical biorefinery. The bioactive molecules from the methanol extracts (MEs) from various parts of *L. leucocephala* grown in Egypt were evaluated. The antibacterial activity against the growth of *Erwinia amylovora*, *Agrobacterium tumefaciens*, and *Staphylococcus aureus* was determined by the inhibition zones (IZs) and the minimum inhibitory concentrations (MICs). The antifungal activity against the growth of *Rhizoctonia solani*, *Fusarium solani*, and *Alternaria solani* was recorded by measuring the fungal growth inhibition (FGI %) and MICs. The phytochemical compounds in the MEs were identified by HPLC–DAD, where the higher compounds identified (mg/kg ME) in seeds were benzoic acid (1520.44), myricetin (848.73), and rosmarinic acid (792.46); in roots, were benzoic acid (554.04), naringenin (419.99), and myricetin (205.51); in leaves were rosmarinic acid (4768.16), resveratrol (2983.99), quercetin (2052.43), myricetin (1432.63), and naringenin (1182.39); in branches, were rosmarinic acid (2230.26), resveratrol (1605.3), o-coumaric acid (691.16), and myricetin (681.93); in fruits were rosmarinic acid (431.43) and resveratrol (261.07); in stem-wood, were ellagic acid (1319.75), *p*-coumaric acid (1051.59), and ferulic acid (512.45); and in stem-bark, were resveratrol (1079.01), benzoic acid (1071.11), and catechol (305.51). The MEs at the concentration of 4000 mg/L from stem-wood, leaves, and stem-bark, the higher IZs against the growth of *E. amylovora*, *A. tumefaciens*, and *S. aureus* with values of 4.06 cm, 2.5 cm, and 2.63 cm, respectively, were found. The range of MICs values of MEs was 75–500 mg/L, 75–125 mg/L, and 75–125 mg/L, against the growth of *A. tumefaciens*, *E. amylovora*, and *S. aureus*, respectively. MEs prepared from seeds, fruits (pod), and stem-bark at 4000 mg/L showed the higher FGI (100%) against the growth of *A. solani*; MEs from seeds and branches observed the higher FGI values of 63.83% and 63.6%, respectively, against the growth of *F. solani*, and all MEs showed potent antifungal activity (FGI 100%) against *R. solani* except for leaf ME (88.06%). MICs were in the range of 250–500, 250–500, and 500–1000 mg/L against *A. solani*, *F. solani*, and *R. solani*, respectively. At 500 mg/L, the roots ME showed the highest total antioxidant activity (94.30%) compared to vitamin C (VC) (98.30%) at 100 mg/L. The EC50 values of the MEs from seeds, fruits, stem-bark, branches, stem-wood, leaves, and roots were 424.24 mg/L, 131.40 mg/L, 341.78 mg/L, 380.50 mg/L, 153.59 mg/L, 153.59 mg/L, and 129.89 mg/L compared with VC (6.88 mg/L). In conclusion, the botanical parts of *L. leucocephala* have several bioactive compounds, which can act as promising antimicrobial and antioxidant properties.

Keywords *Leucaena leucocephala* · Phytochemical compounds · Antimicrobial activity · Antioxidant activity · HPLC

1 Introduction

Leucaena leucocephala (Lam.) de Wit (family Fabaceae), the small-fast growing tropical mimosoid tree with multipurpose uses, is a native to southern Mexico and northern Central America [1]. Seeds of *Leucaena* are used as vegetables in cooking, since it contained more than 5.5% of fat [2] with the main fatty acids palmitic, behenic, stearic, oleic, lignoceric, and linoleic acids are used as coffee substitutes [3].

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Legumes of the tree provide high-protein cattle fodder [4]; however, the seeds contain mimosine, the anti-nutritional factor, and the non-protein amino acid, which is known to be toxic to ruminants [5–8]. Polysaccharides from *L. leucocephala* seed gum induced its cancer chemopreventive and anti-proliferative activities [9].

The plant extracts have been demonstrated to possess strong antibacterial and antifungal activities [10, 11]. It has been shown to be very effective at stopping the bacterial growth of *Dickeya solani* and *Agrobacterium tumefaciens* [12–14]. Botanical extracts have been observed to inhibit the mycelial growth and spore germination of some plant pathogenic fungi [15, 16]. Promising antifungal activity against *Rhizoctonia solani* was observed as wood samples from *Melia azedarach* treated with pomegranate peel extracts, where the HPLC analysis showed the presence of phenolic acid compounds, syringic, *p*-coumaric, benzoic, caffeic, gallic, ferulic, salicylic, cinnamic, and ellagic as well as catechol and pyrogallol [17]. Olive leaf extract showed potential antimicrobial activity and compounds oleuropein, caffeic acid, luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside verbascoside, and luteolin 4'-*O*-glucoside were identified by HPLC/DAD [18].

The phytochemical screening of the fresh aqueous leaf extracts from *Leucaena leucocephala* showed the presence of tannins, saponins, coumarins, flavonoids, cardiac glycosides, steroids, phenols, carbohydrates, and amino acids [19]. *L. leucocephala* leaves from Malaysia, extracted using different solvents, showed the presence of squalene, phytol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 3,7,11-tridecatrienenitrile, and 4,8,12-trimethyl as analyzed by GC–MS [20]. Squalene was identified in extracts of *L. leucocephala* whole plant from China using several solvents [21]. Bioactive compounds were identified in the genus *Leucaena* like hydrocynamic acid, leucaenine, apigenin, quercetin-3-*O*-arabinofuranoside, epicatechin-3-*O*-gallate, and quercetin-3-Orhamnoside [22]. Condensed tannins were found in different parts of the *Leucaena* tree [23], gallocatechin, epicatechin and epigallocatechin [24], and quercetin and myricetin glycosides [25].

Fruit extract at lower concentrations exhibited lipolytic activity, which could contribute to its "insulin-like" property [26]. The seed extract has been recognized to act as a hypoglycemic agent [27]. The extracts and isolated bioactive compounds from the plant could be a promising alternative to conventional anthelmintic to treat gastrointestinal parasites of small ruminants [28], and the protein extracts observed good anthelmintic activity on *Haemonchus contortus* [29]. Extracts of leaves were used for the biosynthesis of copper oxide [19], cadmium oxide [30], and silver nanoparticles [31], with potent antimicrobial activities against several pathogenic bacteria and fungi.

Several bioactive chemical groups were identified in leaf extracts from *L. leucocephala*, such as phenolic compound, aromatic amide, and carboxylic acid, while phenolic compound and carboxylic acid were observed in root extracts [32] with promising nematicidal effects against the root-knot nematode. Leaf extract showed the presence of principal constituent 2-(H)-benzofuranone-5,6,7,7a-tetrahydro-4,4,7a-trimethyl with other compounds pentadecanoic acid-14-methyl-methyl ester and 6,10,14-trimethyl-2-pentadecanone a ketone [33]. The whole plant extract showed the presence of 5α,8α-epidioxy-(24R)-ergosta-6,22-dien-3β-ol, β-sitosterol, β-sitostenone, stigmastenone, lupeol (5), 1,3-dipalmitoyl-2-oleoylglycerol, methylparabene, and isovanillic acid [34]. Mimosine, gallic acid, caffeic acid, β-sitosterol, luteolin-7-*O*-glucoside, chrysoenol, and kaempferol-3-*O*-rutinoside were identified in the extract [35]. Extracts from different parts of the tree showed the presence of several bioactive compounds such as quercetin from leaf extract [36]. The whole plant showed the presence of several compounds such as polyprenol, lupeol, squalene, and β-sitostenone [21]. Leaf and seed extracts showed antioxidant and antidiabetic activities [37]. In model systems, the antioxidant activity of seed extracts showed inhibitory effects against lipid oxidation [38].

The present work aims to maximize the utilization of *L. leucocephala* biomass by identifying the chemical compounds in the methanolic extract from the botanical parts of the tree and to pinpoint the effect of these extracts on the growth of some pathogenic bacteria and fungi, as well as antioxidants.

2 Materials and methods

2.1 Source of plant materials and the extraction procedure

Leucaena leucocephala plant collected from Alexandria, Egypt, was identified by Prof. Dr. Ahmed A.A. El-Settawy at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The plant materials were divided into the following parts: seeds, roots, leaves, stem-wood, fruits, branches, and stem bark (Fig. 1). All the botanical parts were air-dried under the laboratory conditions until each of them could be transferred to powder using a small laboratory mill which were used for the extraction. About 50 g from each powdered part was soaked in 150 mL methanol (80%) for 1 week under the laboratory conditions of 65 ± 5% relative humidity (RH) and 27 ± 2 °C. After the extraction, the materials obtained were filtrated using filter paper (Whatman no. 1) and the methanol extracts

Fig. 1 Botanical parts of *Leucaena leucocephala* tree: (**1**) seeds, (**2**) fruits, (**3**) stem wood, (**4**) roots, (**5**) leaves, (**6**) stem bark, (**7**) branches, and (**8**) the prepared powdered materials (photos were taken by coauthor Nourhan Elsayed Elbanoby)

(MEs) then poured into Petri dishes to complete the dryness and then concentrated [39]. MEs were prepared at the concentrations of 4000, 2000, 1000, 500, and 250 mg/L by dissolving them in 10% dimethyl sulfoxide (10% DMSO).

2.2 Pathogenic microorganisms

The antimicrobial evaluation of the MEs was tested against the growth of three plant pathogenic fungi (Seed Pathology Laboratory, Plant Pathology Institute, Agriculture Research Center (ARC), Alexandria, Egypt) and two plant pathogenic bacteria, in addition to *Staphylococcus aureus*, a pathogen that infects humans (Bacterial Plant Diseases Laboratory, Plant Pathology Department, Faculty of Agriculture, Alexandria University, Egypt). The fungal and bacterial isolates are presented in Table 1, and the symptoms of their infection are shown in Fig. 2.

2.3 Assessing of antibacterial and antifungal activities of the extracts

The potential of the plant MEs activity was tested against the growth of some fungal and bacterial pathogens. The plant MEs were prepared at the concentrations of 4000, 2000, 1000, 500, and 250 mg/L. For bacteria, the agar circle dissemination strategy was utilized for the assurance of antimicrobial activities of the MEs. Substantially, the tested bacteria were spread over the surfaces of the nutrient agar (NA) medium in Petri dishes (9 cm). Sterilized filter paper disks of 7-mm diameter were loaded with 50 µL of the ME and placed on the Petri dishes with the tested bacteria and the inhibition zones (IZs) diameters were registered in millimeters. Tetracycline was used as a positive control (10 µg/L), and 10% DMSO as a negative control for the tested bacteria was incubated at 28 °C for 24 h before comparisons were made. Minimum inhibitory concentrations (MICs) were performed using serial dilutions [40] of the ME ranging between 4 and 4000 mg/L.

Moncut 25% WP (flutolanil), the referenced chemical fungicide, was prepared at the concentrations of 1500 mg/mL and assessed using the broth dilution method according to the Clinical and Laboratory Standards Institute [41]. Three fungal isolates were cultivated on a potato dextrose

Table 1 The species of the fungal and the bacterial isolates agents were used in this study

Fungal and bacterial species	Hosts	Diseases	Accession numbers
Alternaria solani	Tomato	Early blight	MT279570
Fusarium solani	Zucchini	Crown rot	MW947256
Rhizoctonia solani	Tomato	Root rot	MN398397
Erwinia amylovora	Pear	Fire blight	HG423347
Agrobacterium tumefaciens	Guava	Crown gall	MG706145
Staphylococcus aureus	Human bacterial	Skin and soft tissue	ATCC 6538

Fig. 2 The natural infection symptoms for **A** *Erwinia amylovora* (Guava), **B** *Agrobacterium tumefaciens* (pear), **C** *Rhizoctonia solani* (tomato), **D** *Alternaria solani* (tomato), and **E** *Fusarium solani* (zucchini) (photos were taken by Coauthor Abeer A. Mohamed)

agar (PDA) medium for 1 week. After that, a single 5-mm diameter culture disk of the fungus was placed in the middle of the Petri dishes that contain the prepared concentrations of plant MEs. The Petri dishes were incubated for one week at 28 °C, and three replications were used for each isolate. The assessment of antifungal activity was calculated with the formula of the fungal linear growth inhibition (%) = [DC − DT/DC] × 100, where DC and DT are the average fungal linear growth (mm) under the control and experimental treatments, respectively. Three replicates were carried out for all of the treatments. Minimum inhibitory concentrations (MICs) were measured by the serial dilution method for the studied MEs [42].

2.4 Antioxidant activity of the methanol extracts

Free radical scavenging activity of the obtained MEs from seeds, roots, leaves, stem wood, fruits, branches, and stem bark was assayed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (absorbance at 517 nm) [43]. Serial dilution was used from each ME (500, 400, 300, 200, 100, 50, 25, 12, and 6 mg/L) to measure the total antioxidant activity (TAA%) and the concentration of the reference compound ascorbic acid (AA) or vitamin C (from 2 to 100 mg/L) responsible for 50% of inhibition of DPPH radical (EC_{50}) was measured by the scatterplot analysis to find the regression equations [44].

2.5 Phytochemical compositions analysis by HPLC

The phytochemicals identified in the MEs from all the studied parts of *L. leucocephala* were analyzed using An Agilent 1260 Infinity HPLC Series (Agilent, Santa Clara, CA, USA), equipped with a Quaternary pump and a Zorbax Eclipse plus C18 column (100 mm × 4.6 mm i.d.) (Agilent Technologies, Santa Clara, CA, USA [45]. The instrument was operated at 30 °C and the injection volume was 20 µL with the following ternary linear elution gradient; (A) HPLC grade water 0.2% H_3PO_4 (v/v), (B) methanol, and (C) acetonitrile.

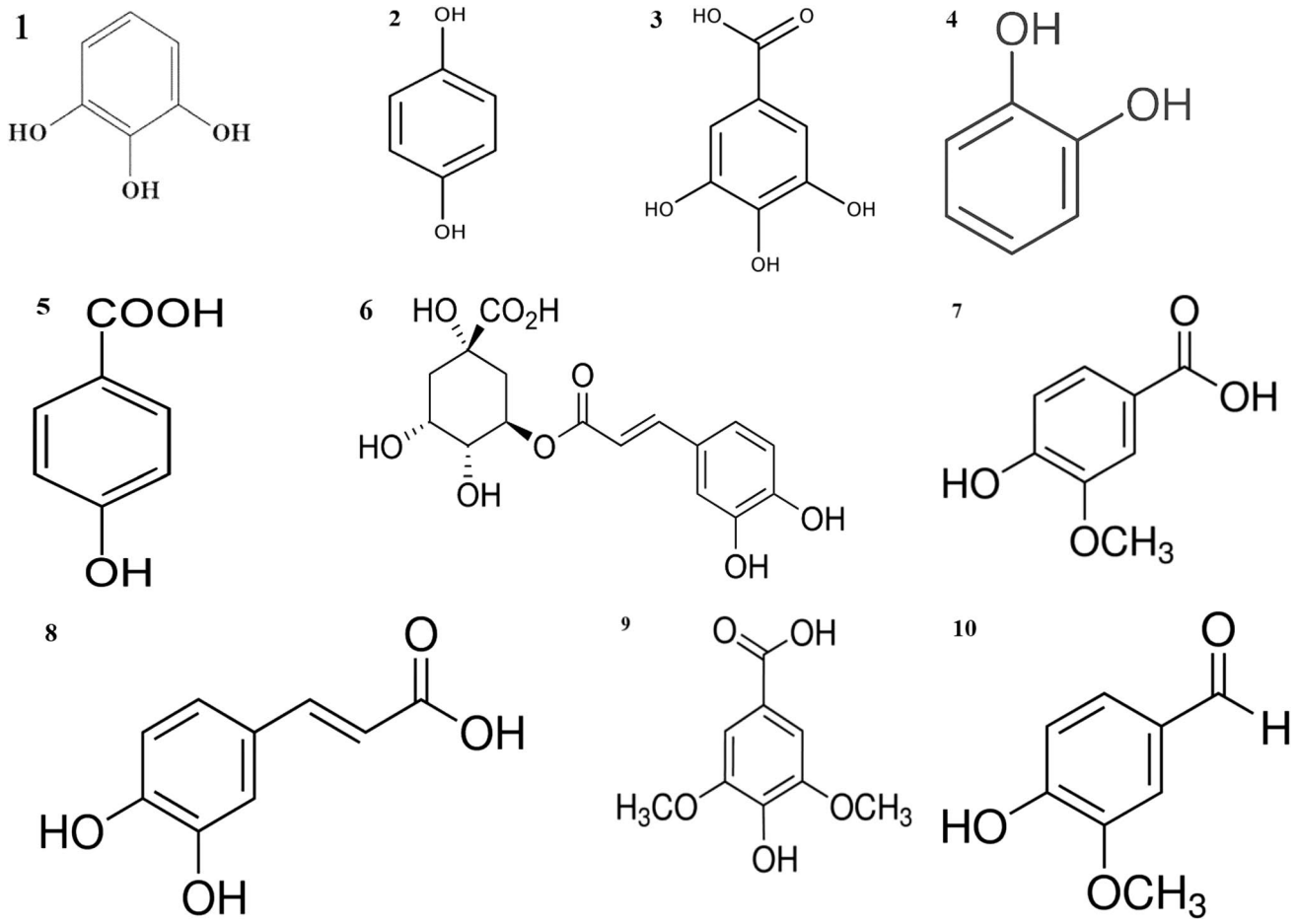

Fig. 3 Chemical structure of the phytochemical compounds

Standard HPLC grade phenolic and flavonoid compounds (Fig. 3) including pyrogallol (1), quinol (2), gallic acid (3), catechol (4), *p*-hydroxybenzoic acid (5), chlorogenic acid (6), vanillic acid (7), caffeic acid (8), syringic acid (9), vanillin (10), *p*-coumaric acid (11), ferulic acid (12), benzoic acid (13), rutin (14), ellagic acid (15), *o*-coumaric acid (16), salicylic acid (17), resveratrol (18), cinnamic acid (19), myricetin (20), quercetin (21), rosmarinic acid (22), naringenin (23), and kaempferol (24) as well as caffeine (25), were used for the HPLC analysis. The detection was set at 284 nm to identify the existed compounds.

2.6 Statistical analysis

The results of the inhibition zones observed against the growth of the bacterial strains (*E. amylovora*, *A. tumefaciens*, and *S. aureus*) as well as the percentages and the fungal linear inhibition of *A. solani*, *F. solani*, and *R. solani* as affected by five concentrations (4000, 2000, 1000, 500, and 250 mg/L) of the MEs of several parts of *L. leucocephala* were statistically analyzed with two-way analysis of variance (ANOVA) using SAS software (SAS Institute, Release 8.02, Cary, North Carolina State University, Raleigh, NC, USA) [46]. The means of the treatments were compared against the control treatments according to the least significant difference (LSD) test at a 0.05 level of probability.

3 Results

3.1 Antibacterial activity

According to the statistical analysis presented in Table 2, the plant parts, the concentrations of methanol extracts (MEs) from *Leucaena leucocephala*, and their interaction were observed significant effects on the growth of *Erwinia amylovora*, *Agrobacterium tumefaciens*, and *Staphylococcus aureus*.

To find out the best plant part with the potent ME concentration, Table 3 shows the effect of interaction between *L. leucocephala* botanical parts and their ME concentrations.

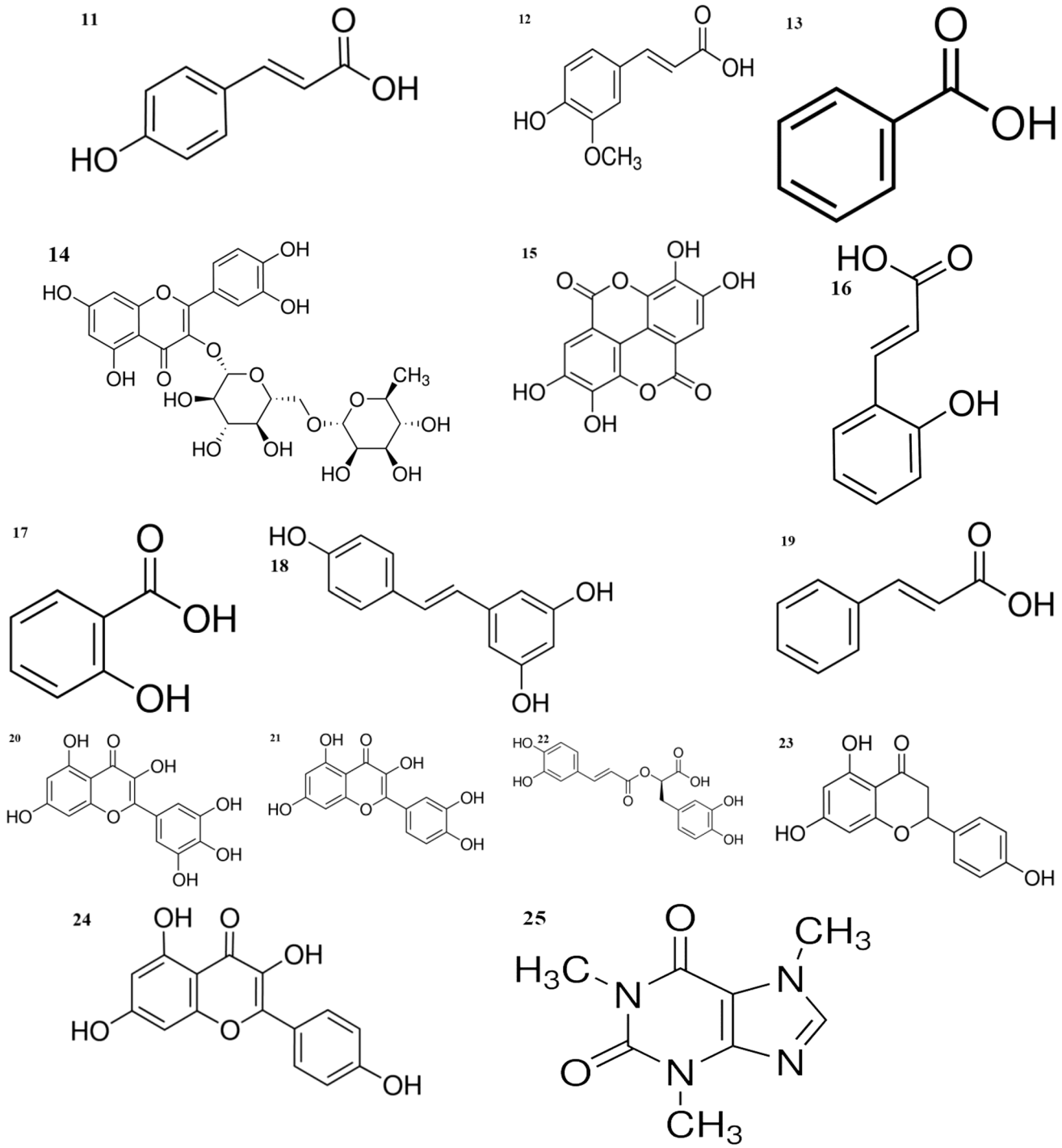

Fig. 3 (continued)

At 4000 mg/L, the MEs from stem-wood, roots, and branches displayed the highest inhibition zones (IZs) against the growth of *E. amylovora* with values of 4.06 cm, 3.53 cm, 3.33 cm, and 3.3 cm, respectively, compared to the positive control used (4 cm). Additionally, at 2000 mg/L, the MEs from seeds, stem-wood, and branches showed IZ values of 3.2, 3.06, and 3.06 cm, respectively, and fruits at 4000 mg/L with IZ values of 3.06 cm. On the other hand, using 250 mg/L, all MEs have induced the lowest IZs. Leaf MEs at the concentration of 4000, 2000, 1000, and 500 mg/L showed the highest IZ values against the growth of *A. tumefaciens* with 2.5, 2.16, 2.06, and 1.86 cm, respectively,

Table 2 Mean squares of the antibacterial activity of the methanol extracts from *L. leucocephala* and their concentrations against the growth of *E. amylovora*, *A. tumefaciens*, and *S. aureus*

S.O.V	d.f	MS		
		E. amylovora	*A. tumefaciens*	*S. aureus*
Plant parts (A)	6	1.24**	1.952**	0.412**
Concentrations (B)	6	35.088**	7.927**	12.899**
A*B	36	0.201*	0.16*	0.139*
Error	98	0.034	0.011	0.055

*and ** indicates significance at 0.05 and 0.01 levels; *S.O.V.*, source of variance; *d.f.*, degrees of freedom; *MS*, mean squares

compared to the positive control used (2 cm). Using the concentration of MEs with 4000, 2000, and 1000 mg/L of stem-bark, the highest IZs of 2.63, 2.36, and 2.3 cm, respectively, were found against the growth of *S. aureus*, compared to the positive control (2.5 cm). Using 250 mg/L of all plant sources has induced the lowest IZ.

Table 4 sets out the MICs values observed by the application of MEs from several parts of *L. leucocephala* against the tested bacteria. The MICs values were ranged between 75 and 500 mg/L against the growth of *A. tumefaciens*, where the lowest MICs were found in the ME of seeds and roots. The range of MIC values was from 75 to 125 mg/L against the growth of *E. amylovora* and the lowest MICs (75 mg/L) were from the MEs in leaves, fruits, and branches. All of the MEs observed MIC values of 125 mg/L against *S. aureus*, while the lowest value was in stem-bark ME (75 mg/L).

3.2 Antifungal activities

There are highly significant effects of plant MEs from different parts and the concentration of *L. leucocephala* ME. Additionally, there is a significant impact of the interaction between the extracted source of *L. leucocephala* and its concentration, in terms of fungal growth inhibition (FGI) (%) of *Rhizoctonia solani*, *Fusarium solani*, and *Alternaria solani* as the statistical analysis of the variance has revealed in Table 5.

The effects of interactions between plant part ME and its concentration on the FGI are shown in Table 6. Upon the significant interaction between plant part and its concentration, the concentration level of 4000 mg/L of MEs from seeds, fruits (pods), and stem bark has displayed the highest FGI (100%) against the growth of *A. solani*, while using 4000 mg/L of each root, leaves, stem wood, and branches non-significant differences among them 74.43%, 78.76%, 79.46%, and 79.4%, respectively. Using 250 mg/L of all plant parts, MEs has the lowest FGI impact, seeds (13.43%), roots (7.06%), leaves (11.3%), stem wood (6.36%), fruits (15.53%), and stem bark (11.3%). However, using 250 mg/L of branch ME has no fungal activity was obtained (Table 5).

All ME concentrations at 4000 mg/L have induced the highest FGI against *F. solani*, but the MEs from seeds and branches showed the highest values (63.83% and 63.6%, respectively), followed by root and bark MEs at 4000 mg/L, which displayed the same value (56.9%). The concentration of 250 mg/L of root ME has no fungal activity (0%), moreover, the level of concentration 250 mg/L of all other MEs has induced the lowest FGI value. Additionally, there was a non-significant difference between the impact of 250 and 500 mg/L on the fungal activity (Table 5).

Based on the significant interaction between the plant part ME and its concentration on the growth of *R. solani*, the use of 4000 mg/L level of concentration of all plant-MEs has displayed the highest FGI (100%), except for leaf ME (88.06%). It was also detected that 250 mg/L of all plant parts-MEs has no fungal activity. However, the MEs at 500 mg/L level of concentration from seeds, leaves, and stem-wood have a low FGI %, compared with those of the other plant parts MEs (0.0%) (Table 6).

Table 7 presents the MICs values of MEs against the growth of *A. solani, F. solani*, and *R. solani*. MICs were in the range of 250–500, 250–500 and 500–1000 mg/L against the growth of *A. solani*, *F. solani*, and *R. solani*, respectively. MEs from roots, leaves, pods and stem-bark showed the lowest MIC value (250 mg/L) against *A. solani*, MEs from seeds, stem-wood, pods, branches and stem-bark with MIC value (250 mg/L against *F. solani*, and MEs from seeds and stem-wood with MIC value (500 mg/L) against *R. solani*.

3.3 Antioxidant activity

The total antioxidant activity percentage (TAA%) of the MEs from the botanical parts of *L. leucocephala* (Fig. 4) was increased with the increase of the extract concentration. Additionally, at 500 mg/L, the highest TAA% was as in the following order: roots ME > fruits ME > leaves ME > seeds ME > bark ME > branches ME > wood ME with the following percentages 94.3, 77.32, 72.12, 71.26, 60.78, 56.98, 52.81, and 43.01%, respectively, compared to 98.3% as it observed in case of the VC at 100 mg/L.

To measure the concentration of VC and MEs responsible for 50% of inhibition of DPPH radical (EC50), the correlation scatterplots of variables were made, whereas they were VC: ($Y = 46.51 + 0.507*X$; $r = 0.99$, $p = 0.0000$; $r^2 = 0.98$; EC50 = 6.88 mg/L); seeds ME: $Y = 8.55 + 0.097*X$; $r = 0.98$, $p = 0.00000$; $r^2 = 0.96$; EC50 = 424.24 mg/L); fruits ME: $Y = 40.0004 + 0.07*X$; $r = 0.91$, $p = 0.0005$; $r^2 = 0.84$; EC50 = 131.40 mg/L); bark: $Y = 23.92 + 0.07*X$; $r = 0.86$, $p = 0.0026$; $r^2 = 0.74$; EC50 = 341.78 mg/L); branches ME: $Y = 20.05 + 0.07*X$; $r = 0.90$, $p = 0.0009$; $r^2 = 0.81$; EC50 = 380.50 mg/L); wood ME: $Y = 12.67 + 0.06*X$; $r = 0.94$, $p = 0.0002$; $r^2 = 0.88$; EC50 = 550.46 mg/L); leaves ME: $Y = 37.29 + 0.08*X$; $r = 0.85$, $p = 0.0035$; $r^2 = 0.72$;

Table 3 Antibacterial activity of the interaction between the methanolic extracts and their concentrations from several parts of *L. leucocephala*

Plant parts	Concentration (mg/L)	*E. amylovora*	*A. tumefaciens*	*S. aureus*
	Positive control	$4^{a} \pm 0.11$*	$2^{c-e} \pm 0.06$	$2.5^{a} \pm 0.18$
	Negative control	0^{s}	0^{t}	0^{m}
Seeds	4000	$4.06^{a} \pm 0.11$	$2.333^{ab} \pm 0.06$	$1.83^{d-g} \pm 0.18$
	2000	$3.2^{cd} \pm 0.11$	$1.7^{fg} \pm 0.06$	$1.53^{f-j} \pm 0.18$
	1000	$2.36^{hi} \pm 0.11$	$1.633^{gh} \pm 0.06$	$1.43^{h-j} \pm 0.18$
	500	$1.8^{lm} \pm 0.11$	$1.467^{h-j} \pm 0.06$	$1.3^{i-k} \pm 0.18$
	250	$1.43^{o-q} \pm 0.11$	$1.267^{k-m} \pm 0.06$	$1.16^{jk} \pm 0.18$
Roots	4000	$3.33^{bc} \pm 0.11$	$1.6^{g-i} \pm 0.06$	$1.76^{d-h} \pm 0.18$
	2000	$2.93^{d-f} \pm 0.11$	$1.2^{l-n} \pm 0.06$	$1.63^{d-i} \pm 0.18$
	1000	$2.7^{fg} \pm 0.11$	$1.13^{m-o} \pm 0.06$	$1.53^{f-j} \pm 0.18$
	500	$2.46^{gh} \pm 0.11$	$1.06^{n-p} \pm 0.06$	$1.53^{f-j} \pm 0.18$
	250	$1.73^{mn} \pm 0.11$	$0.96^{o-r} \pm 0.06$	$1.43^{h-j} \pm 0.18$
Leaves	4000	$2.86^{ef} \pm 0.11$	$2.5^{a} \pm 0.06$	$1.86^{d-f} \pm 0.18$
	2000	$2.36^{hi} \pm 0.11$	$2.16^{bc} \pm 0.06$	$1.73^{d-h} \pm 0.18$
	1000	$2.03^{j-l} \pm 0.11$	$2.06^{cd} \pm 0.06$	$1.66^{d-i} \pm 0.18$
	500	$1.6^{m-o} \pm 0.11$	$1.96^{de} \pm 0.06$	$1.53^{f-j} \pm 0.18$
	250	$1.2^{p-r} \pm 0.11$	$1.86^{ef} \pm 0.06$	$1.5^{f-j} \pm 0.18$
Stem-wood	4000	$3.53^{b} \pm 0.11$	$1.43^{i-k} \pm 0.06$	$1.93^{c-e} \pm 0.18$
	2000	$3.06^{c-e} \pm 0.11$	$1.3^{j-m} \pm 0.06$	$1.83^{d-g} \pm 0.18$
	1000	$2.36^{hi} \pm 0.11$	$1.06^{n-p} \pm 0.06$	$1.76^{d-h} \pm 0.18$
	500	$2.26^{h-j} \pm 0.11$	$0.93^{p-r} \pm 0.06$	$1.66^{d-i} \pm 0.18$
	250	$2.1^{i-k} \pm 0.11$	$0.86^{q-s} \pm 0.06$	$1.46^{g-j} \pm 0.18$
Fruits	4000	$3.06^{c-e} \pm 0.11$	$1.2^{l-n} \pm 0.06$	$2^{b-d} \pm 0.18$
	2000	$2.53^{gh} \pm 0.11$	$1.03^{n-q} \pm 0.06$	$1.86^{d-f} \pm 0.18$
	1000	$2.33^{hi} \pm 0.11$	$0.93^{p-r} \pm 0.06$	$1.73^{d-h} \pm 0.18$
	500	$2.1^{i-k} \pm 0.11$	$0.86^{q-s} \pm 0.06$	$1.66^{d-i} \pm 0.18$
	250	$1.46^{n-p} \pm 0.11$	$0.8^{rs} \pm 0.06$	$1.56^{e-i} \pm 0.18$
Branches	4000	$3.3^{bc} \pm 0.11$	$1.33^{j-l} \pm 0.06$	$1.86^{d-f} \pm 0.18$
	2000	$3.06^{c-l} \pm 0.11$	$1.2^{l-n} \pm 0.06$	$1.76^{d-h} \pm 0.18$
	1000	$2.73^{fg} \pm 0.11$	$1^{o-q} \pm 0.06$	$1.66^{d-i} \pm 0.18$
	500	$2.4^{h} \pm 0.11$	$0.93^{p-r} \pm 0.06$	$1.6^{e-i} \pm 0.18$
	250	$1.3^{p-r} \pm 0.11$	$0.9^{p-r} \pm 0.06$	$1^{kl} \pm 0.18$
Stem-bark	4000	$2.93^{d-f} \pm 0.11$	$1.26^{k-m} \pm 0.06$	$2.63^{a} \pm 0.18$
	2000	$2.03^{j-l} \pm 0.11$	$1.06^{n-p} \pm 0.06$	$2.36^{ab} \pm 0.18$
	1000	$1.83^{k-m} \pm 0.11$	$1.03^{n-q} \pm 0.06$	$2.3^{a-c} \pm 0.18$
	500	$1.16^{qr} \pm 0.11$	$0.86^{q-s} \pm 0.06$	$2.26^{a-c} \pm 0.18$
	250	$1.1^{r} \pm 0.11$	$0.7^{s} \pm 0.06$	$0.76^{l} \pm 0.18$

*Means with the same letter/s within the same column are not significantly different according to LSD at a 0.05 level of probability

Positive control: tetracycline (10 µg/L)

Negative control: 10% dimethyl sulfoxide

EC50 = 153.59 mg/L); and roots ME: Y = 37.78 + 0.09*X; $r = 0.89$, $p = 0.001$; $r^2 = 0.80$; EC50 = 129.89 mg/L).

3.4 Phytochemical compounds in *L. leucocephala* methanolic extracts by HPLC

The phytochemical compounds identified in various parts-MEs from *L. leucocephala* are shown in Table 8 with the HPLC chromatographic charts (Fig. 5a–g). The most abundant compounds in seed ME were benzoic acid (1520.44 mg/kg ME), myricetin (848.73 mg/kg ME), rosmarinic acid (792.46 mg/kg ME), ellagic acid (265.57 mg/kg ME), o-coumaric acid (247.98 mg/kg ME), and rutin (223.21 mg/kg ME). The main phytochemical compounds from root ME were benzoic acid (554.04 mg/kg ME), naringenin (419.99 mg/kg ME), and myricetin 205.51 mg/

Table 4 Minimum inhibition concentrations (MICs) of extracts against the growth of *A. tumefaciens*, *E. amylovora*, and *S. aureus*

Plant material	MICs (mg/L)		
	A. tumefaciens	*E. amylovora*	*S. aureus*
Seeds	75	125	125
Roots	75	125	125
Leaves	125	75	125
Stem-wood	250	125	125
Pods (fruits)	250	75	125
Branches	250	75	125
Stem-bark	500	125	75

Table 5 Mean squares of *R. solani*, *F. solani*, and *A. solani* as affected by plant part extract, concentrations of the extract, and their interaction

S.O.V	d.f	M.S		
		R. solani	*F. solani*	*A. solani*
Plant part (A)	6	76.483**	95.367**	374.182**
Concentrations (B)	6	43,142.271**	11,561.834**	19,270.983**
A*B	36	67.307*	52.609*	87.509*
Error	98	0.673	3.165	6.112

*and ** indicates significance at 0.05 and 0.01 levels; *S.O.V.*, source of variance; *d.f.*, degrees of freedom; *MS*, mean squares

kg ME). The man abundant compounds from the leaves ME were rosmarinic acid (4768.16 mg/kg ME), resveratrol (2983.99 mg/kg ME), quercetin (2052.43 mg/kg ME), myricetin (1432.63 mg/kg ME), naringenin (1182.39 mg/kg extract), catechol (1134.56 mg/kg ME), kaempferol (931.85 mg/kg ME), and benzoic acid (789.01 mg/kg ME). The phytochemical analysis of branch ME showed the presence of rosmarinic acid (2230.26 mg/kg ME), resveratrol (1605.3 mg/kg ME), o-coumaric acid (691.16 mg/kg ME), myricetin (681.93 mg/kg ME), and *p*-hydroxybenzoic acid (589.53 mg/kg ME) as the most abundant compounds. In fruits ME, the most identified abundant compounds were rosmarinic acid (431.43 mg/kg ME), resveratrol (261.07 mg/kg ME), myricetin (174.38 mg/kg ME), and p-hydroxybenzoic acid (207.24 mg/kg ME).

Stem wood ME showed the presence of ellagic acid (1319.75 mg/kg ME), *p*-coumaric acid (1051.59 mg/kg ME), ferulic acid (512.45 mg/kg ME), quercetin (446.94), rosmarinic acid (405.23 mg/kg ME), chlorogenic acid (376.23 mg/kg ME), pyrogallol (319.85 mg/kg ME), and cinnamic acid (229.84 mg/kg ME) as the most abundant compounds. In the stem bark ME extract, the highest abundant compounds were resveratrol (1079.01 mg/kg ME), benzoic acid (1071.11 mg/kg ME), catechol (305.51 mg/kg ME), rosmarinic acid (234.75 mg/kg ME), and *p*-hydroxybenzoic acid (222.90 mg/kg ME).

4 Discussion

This study was conducted to confirm the antimicrobial activities of seeds, roots, leaves, branches, fruits (pods), stem wood, and stem bark extracts from *L. leucocephala* (Lam) de Wit. The evaluation of the antimicrobial activities has been poorly discussed and documented. Therefore, the present work aimed to maximize the benefit of its extracts against the growth of several pathogenic bacteria and fungi.

The phytochemical analysis of the MEs from the botanical parts of *L. leucocephala* by HPLC showed the presence of several bioactive phenolic and flavonoid compounds like catechol, *p*-hydroxybenzoic acid, catechin, chlorogenic acid, caffeic acid, *p*-coumaric acid, benzoic acid, ferulic acid, rutin, ellagic acid, o-coumaric acid, resveratrol, quercetin, rosmarinic acid, naringenin, myricetin, and kaempferol.

Other works showed that the *Leucaena* leaves had valuable phenolic components (µg/mL ethanolic extraction) like gallic acid (331.58), chlorogenic acid (99.76), catechin (131.5), methyl gallate (26), caffeic acid (31.49), syringic acid (21.18), pyrocatechol (13.6), rutin (81.29), ellagic acid (391.15), coumaric acid (42.89), vanillin (36.54), ferulic acid (2.69), naringenin (529.24), querectin (6.15), cinnamic acid (1.87), and kaempferol (5.69) [47]. *Trans*-coumaric and *cis*-coumaric acids were isolated from *L. leucocephala* whole plant extract [21]. Phytochemicals found in fresh leaves are contained quercetin and caffeic acid inhibited 90.49% of egg hatching of *Cooperia* spp. [48]. Several phenolic and flavonoid constituents including caffeic acid, isorhamnetin-3-*O*-galactoside, isorhamnetin, kaempferol-3-*O*-rubinoside, chrysoeriol, quercetin-3-*O*-rhamnoside, and luteolin-7-glucoside were isolated and identified from solvent fractions of aerial parts aqueous-alcoholic extract of *L. leucocephala* [35]. Quercetin was isolated from the ethyl acetate fraction obtained from leaf crude ME [36]. By HPLC, quercetin, caffeic acid, and scopoletin in proportions of 82.21%, 13.42%, and 4.37%, respectively, were identified in *Leucaena leucocephala* leaves [48]. The acetone and butanol extracts at 4000 µg/mL from the flowers of *C. viminalis* showed potential antibacterial activity against the growth of *A. tumefaciens* with IZ values of 15.07 mm and 13.33 mm, and MIC values of 16 µg/mL and 250 µg/mL respectively [49].

The active principle of *L. leucocephala* seed pod biomass has revealed showed the presence of palmitic acid (nematicidal activity, antioxidant activity, and lubricant agent), pelargonic acid (anti-inflammatory and antimicrobial properties), pyridine (antioxidant and nematicide activity), myristic acid, antitumor and cancer preventive, and dioxolane

Table 6 Antifungal activity of the interaction between the methanolic extracted and their concentrations from several parts of *L. leucocephala*

Plant part extract	Concentration (mg/L)	*A. solani*	*F. solani*	*R. solani*
	Positive control	$51^{ef} \pm 1.42$*	$58.3^{b} \pm 1.02$	$100^{a} \pm 0.47$
	Negative control	0.00^{r}	0.00^{y}	0.00^{p}
Seeds	4000	$100^{a} \pm 1.42$	$63.83^{a} \pm 1.02$	$100^{a} \pm 0.47$
	2000	$48.2^{fg} \pm 1.42$	$34.23^{gh} \pm 1.02$	$76.6^{c} \pm 0.47$
	1000	$28.33^{jk} \pm 1.42$	$24.96^{mn} \pm 1.02$	$18.1^{j} \pm 0.47$
	500	$21.2^{lm} \pm 1.42$	$14.3^{r-t} \pm 1.02$	$5.86^{o} \pm 0.47$
	250	$13.43^{op} \pm 1.42$	$8.3^{uw} \pm 1.02$	0.00^{p}
Roots	4000	$74.43^{c} \pm 1.42$	$56.9^{bc} \pm 1.02$	$100^{a} \pm 0.47$
	2000	$36.8^{h} \pm 1.42$	$30.03^{jk} \pm 1.02$	$44.78^{g} \pm 0.47$
	1000	$18.36^{mn} \pm 1.42$	$11.56^{tu} \pm 1.02$	$10.73^{m} \pm 0.47$
	500	$16.23^{no} \pm 1.42$	$3.63^{x} \pm 1.02$	0.00^{p}
	250	$7.06^{q} \pm 1.42$	0.00^{y}	0.00^{p}
Leaves	4000	$78.76^{b} \pm 1.42$	$51.36^{e} \pm 1.02$	$88.06^{b} \pm 0.47$
	2000	$46.76^{g} \pm 1.42$	$30.5^{i-k} \pm 1.02$	$58.1^{e} \pm 0.47$
	1000	$34^{hi} \pm 1.42$	$25.86^{lm} \pm 1.02$	$17.33^{jk} \pm 0.47$
	500	$18.56^{mn} \pm 1.42$	$23.1^{m-o} \pm 1.02$	$12.2^{l} \pm 0.47$
	250	$11.3^{p} \pm 1.42$	$5.5^{wx} \pm 1.02$	0.00^{p}
Stem-wood	4000	$79.46^{b} \pm 1.42$	$55.03^{cd} \pm 1.02$	$100^{a} \pm 0.47$
	2000	$51.03^{ef} \pm 1.42$	$33.3^{hi} \pm 1.02$	$51.26^{f} \pm 0.47$
	1000	$24.8^{jk} \pm 1.42$	$16.13^{qr} \pm 1.02$	$21.46^{i} \pm 0.47$
	500	$19.83^{mn} \pm 1.42$	$12^{stu} \pm 1.02$	$7.70^{n} \pm 0.47$
	250	$6.367^{r} \pm 1.42$	$6.43^{wx} \pm 1.02$	0.00^{p}
Fruits	4000	$100^{a} \pm 1.42$	$53.16^{df} \pm 1.02$	$100^{a} \pm 0.47$
	2000	$54.56^{de} \pm 1.42$	$37.46^{f} \pm 1.02$	$59.96^{d} \pm 0.47$
	1000	$24.8^{kl} \pm 1.42$	$18^{pq} \pm 1.02$	$16.23^{k} \pm 0.47$
	500	$18.4^{mn} \pm 1.42$	$17.06^{qr} \pm 1.02$	0.00^{p}
	250	$15.53^{no} \pm 1.42$	$14.6^{rs} \pm 1.02$	0.00^{p}
Branches	4000	$79.4^{b} \pm 1.42$	$63.6^{a} \pm 1.02$	$100^{a} \pm 0.47$
	2000	$37.5^{h} \pm 1.42$	$31.9^{hij} \pm 1.02$	$60.36^{d} \pm 0.47$
	1000	$21.23^{lm} \pm 1.42$	$28^{kl} \pm 1.02$	$23.3^{h} \pm 0.47$
	500	$3.5^{qr} \pm 1.42$	$9.7^{uv} \pm 1.02$	0.00^{p}
	250	0.00^{r}	$6.9^{uw} \pm 1.02$	0.00^{p}
Stem-bark	4000	$100^{a} \pm 1.42$	$56.9^{bc} \pm 1.02$	$100^{a} \pm 0.47$
	2000	$56.63^{d} \pm 1.42$	$36.56^{fg} \pm 1.02$	$59.86^{d} \pm 0.47$
	1000	$31.13^{ij} \pm 1.42$	$22.2^{no} \pm 1.02$	$22.56^{hi} \pm 0.47$
	500	$14.13^{op} \pm 1.42$	$21.26^{o} \pm 1.02$	0.00^{p}
	250	$11.3^{p} \pm 1.42$	$2.76^{op} \pm 1.02$	0.00^{p}

*Means with the same letter/s within the same column are not significantly different according to LSD at a 0.05 level of probability

Positive control: flutolanil

Negative control: 10% dimethyl sulfoxide

[50]. Quercetin, quercetin-3-O-α-rhamnopyranoside, and myricetin-3-O-α-rhamnopyranoside were the major flavonoids components in *L. leucocephala* leaves (Guangdong province in China) with potential anti-inflammatory, antidiabetic, and antioxidant activities [51]. The ethanol extract of *L. leucocephala* leaves at concentrations of 20, 40, 60, 80, and 100% showed IZs of 6, 6, 7.2, 10.2, and 15.4 mm, respectively, against the growth of *Staphylococcus aureus* by agar diffusion method [52]. While by disk diffusion method, the ethanol extract recorded IZs of 10.52, 11.47, 12.72, and 16.85 mm, at concentrations of 25, 50, 75, and 100%, respectively [53].

Caffeic acid in nature with its derivatives (caffeic acid phenethylester) is possessed several biological activities, such as antioxidant and anti-cancer [54]. Gallic acid and catechin identified from flowers of North-Eastern Portugal

Table 7 Minimum inhibition concentrations (MICs, mg/L) of extracts against the growth of *A. solani*, *F. solani*, and *R. solani*

Plant material	MIC (mg/L)		
	A. solani	*F. solani*	*R. solani*
Seeds	500	250	500
Roots	250	500	1000
Leaves	250	500	1000
Stem-wood	500	250	500
Fruits (pods)	250	250	1000
Branches	500	250	1000
Stem-bark	250	250	1000

showed potential effects against *Candida albicans* and *C. glabrata* [55]. Caffeic, 2,3,4-trihydroxybenzoic, *p*-coumaric, and pyrocatechuic acids identified in the late-ripening sweet cherries achieved the completely inhibition of *A. alternata* [56]. *Diospyros virginiana* fruits ME with its main compounds *m*-gallate, myricetin, gallic acid, luteolin, 3-O-α-rhamnoside, quercetin, myricetin, myricetin-3-*O*-*β*-glucoside, and myricetin 3-*O*-*β*-glucuronide showed significant antibacterial and antifungal activities [57].

The lowest concentration from the MEs that caused 50% inhibition of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was as follows: roots (129.89 mg/L), fruits (131.40 mg/L), stem-wood (153.59 mg/L), leaves (153.59 mg/L), and bark (341.78 mg/L), branches (380.50 mg/L), and seeds (424.24 mg/L) compared to VC (6.88 mg/L). Previously, the antioxidant activity was observed from *L. leucocephala* seed extract measured by DPPH assay with EC_{50} 839.56 mg/L, which was considered to be related to the presence of the phenolic of content 37.38 mg GAE/g [58]. While leaf extract showed EC_{50} 296.1 mg/L [37]. The plant extract showed a DPPH scavenging activity of 59.68% at 1000 mg/L, whereas for ascorbate it was found to be 61.58% at 1000 mg/L. The EC_{50} of the plant extract and ascorbate was found to be 499 and 478 mg/L, respectively [50]. The EC_{50} value measured by the DPPH test showed that the leaf extract of *L. leucocephala* was 296.10 µg/mL [59]. The ethyl acetate fraction from the aerial parts extract and the isolated flavonoid compounds (caffeic acid, isorhamnetin, chrysoeriol, isorhamnetin 3-O-galactoside, kaempferol-3-O-rubinoside, quercetin-3-O-rhamnoside, and luteolin-7-glucoside) showed high antioxidant activity (84.18–90.31%) measured by DPPH compared to Trolox (95.06%) [35]. The antioxidant of quercetin glycosides from 20% of *L. leucocephala* dried leaf aqueous ME was not show cytotoxic effects at 200 µg/mL, while epicatechin-3-O-gallate observed slight cytotoxicity against Vero cells with LC_{50} of 92 µg/mL [22]. Health benefits like antioxidant and potential hepatoprotective effects were reported as the application with gallic acid was done [60]. Pharmacological, anti-inflammatory, and antioxidant properties were shown by naringenin, gallic acid, ellagic acid, and catechin

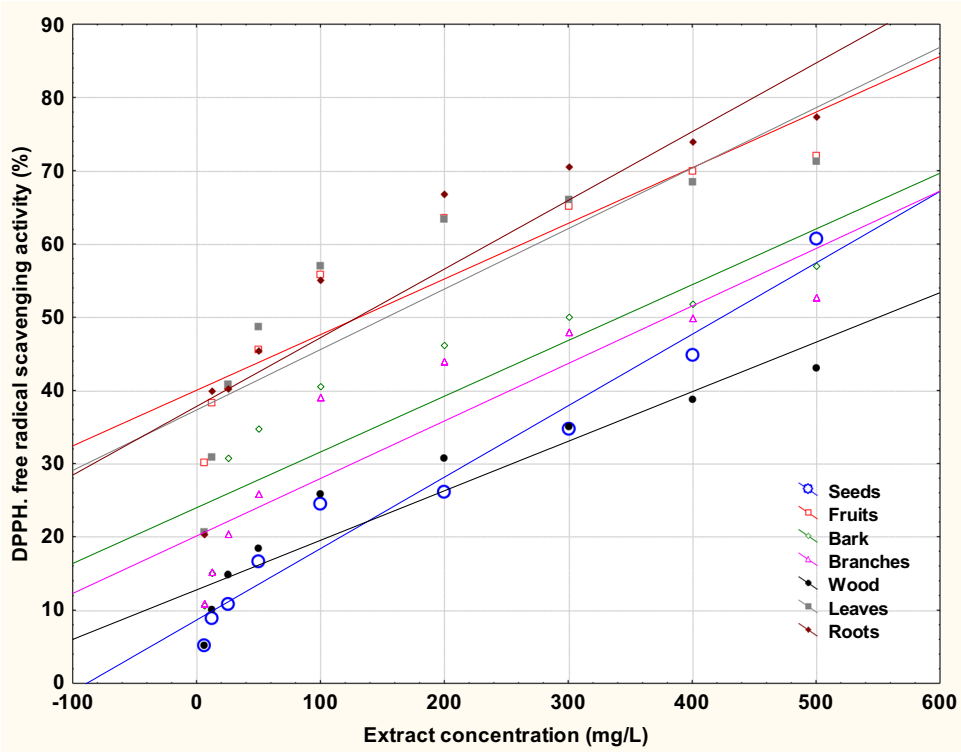

Fig. 4 Total antioxidant activity of the methanol extracts from several parts of *L. leucocephala*

Table 8 Concentration of the chemical compounds identified in methanol extracts from different plant parts of *L. leucocephala*

Compound	Concentration (mg/kg ME)						
	Seeds	Roots	Leaves	Branches	Fruits	Stem wood	Stem bark
Pyrogallol	ND*	ND*	191.64	ND*	ND	319.85	101.33
Quinol	ND	ND	ND*	ND	ND	27.079	137.13
Gallic acid	ND	ND	104.49	ND	21.67	ND	27.93
3-Hydroxytyrosol	ND	ND	ND	ND	65.31	181.68	ND
Catechol	ND	ND	1134.56	66.79	ND	200.29	305.51
p-Hydroxybenzoic acid	ND	17.08	492.35	589.53	207.24	23.98	222.90
Catechin	39.42	2.23	116.24	ND	26.04	52.21	60.35
Chlorogenic acid	77.68	8.15	114.17	273.29	51.009	376.23	8.54
Vanillic acid	179.12	ND	37.06	ND	38.86	5.07	ND
Caffeic acid	ND	8.32	49.31	ND	74.17	7.05	90.67
Syringic acid	90.01	22.98	35.31	ND	ND	31.39	38.07
p-Coumaric acid	ND	ND	18.55	ND	ND	1051.59	6.34
Benzoic acid	1520.44	554.04	789.01	ND	ND	62.45	1071.11
Ferulic acid	20.59	5.804	255.13	37.306	25.62	512.45	76.35
Rutin	223.21	0.0001	ND	ND	ND	36.005	25.29
Ellagic acid	265.57	36.203	54.91	61.38	28.37	1319.75	68.93
o-Coumaric acid	247.98	40.32	55.103	691.16	37.53	15.69	32.92
Resveratrol	ND	44.44	2983.99	1605.3	261.07	10.83	1079.01
Cinnamic acid	ND	ND	54.741	ND	ND	229.84	ND
Quercetin	ND	ND	2052.43	ND	ND	446.94	ND
Rosmarinic acid	792.46	ND	4768.16	2230.26	431.43	405.23	234.75
Naringenin	ND	419.99	1182.39	ND	ND	98.28	ND
Myricetin	848.73	205.51	1432.63	681.93	174.38	ND	193.12
Kaempferol	ND	ND	931.85	ND	ND	ND	ND

**ND*, not detected

[61–64]. Tannins isolated from *Leucaena* are previously isolated and recognized to form from ellagic and gallic acids [65]. On the other hand, chlorogenic acid found in all parts-extracts has been found to own antioxidant, hypoglycemic, anti-inflammatory, and hypolipidemic properties [66].

The mechanisms of action of the identified phytochemicals against several microbial pathogens, cell wall degradation [67], the damage caused in membrane proteins and cytoplasmic membrane [68], contents leakage out of the cell, cytoplasm coagulation, and proton motive force depletion [69, 70] have been reported.

Caffeic acid showed potentiating antibacterial effect against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, while a synergistic effect of pyrogallol with two antibiotics only against *S. aureus* [71]. Pyrogallol, the hydroxylated compound, was proved to be an antimicrobial compound with its mechanism of action occurring through enzymatic inhibition by oxidized compounds [72]. Pyrogallol induced antibacterial effect and cell membrane disruption on methicillin-resistant *S. aureus* (MRSA) with MIC 15.6 µg/mL [73]. Functional antimicrobial low-density polyethylene (LDPE)/pyrogallol exhibited acceptable antimicrobial activity against *S. aureus* and *Escherichia coli* [74].

The antifungal action with significant results in plants that present caffeic acid exist was demonstrated through mycelial growth inhibition from *Barringtonia racemosa* extracts that have high concentrations of gallic acid [75]. The inhibition growth of saprobe fungi in terms of sporulation and germination can be observed by the application of gallic acid [76]. Gallic acid at 500 µg/mL had the greatest growth inhibition of three strains of *Candida albicans* [77].

p-Hydroxybenzoic acid isolated naturally from *Daucus carota*, *Elaeis guineensis*, grapes *Vitis vinifera*, *V. negundo*, *Fagara macrophylla*, *Xanthophyllum rubescens*, *Paratecoma peroba*, *Tabebuia impetiginosa*, *Pterocarpus santalinus*, *Catalpa bognoniooides*, *Areca catechu*, *Roystonea regia*, and *Mespilus germanica* [78], which have been observed potential antimicrobial activity against *E. coli*, *Bacillus aureus*, *S. aureus*, *P. aeruginosa*, *C. albicans*, *Lactobacillus paraplantarum*, *L. plantarum*, *L. fermentum*, *L. fermentum*, *L. brevis*, *L. cornyformis*, *Listeria monocytogenes*, *Fusarium culmorum*, and *Saccharomyces cervisae* [79].

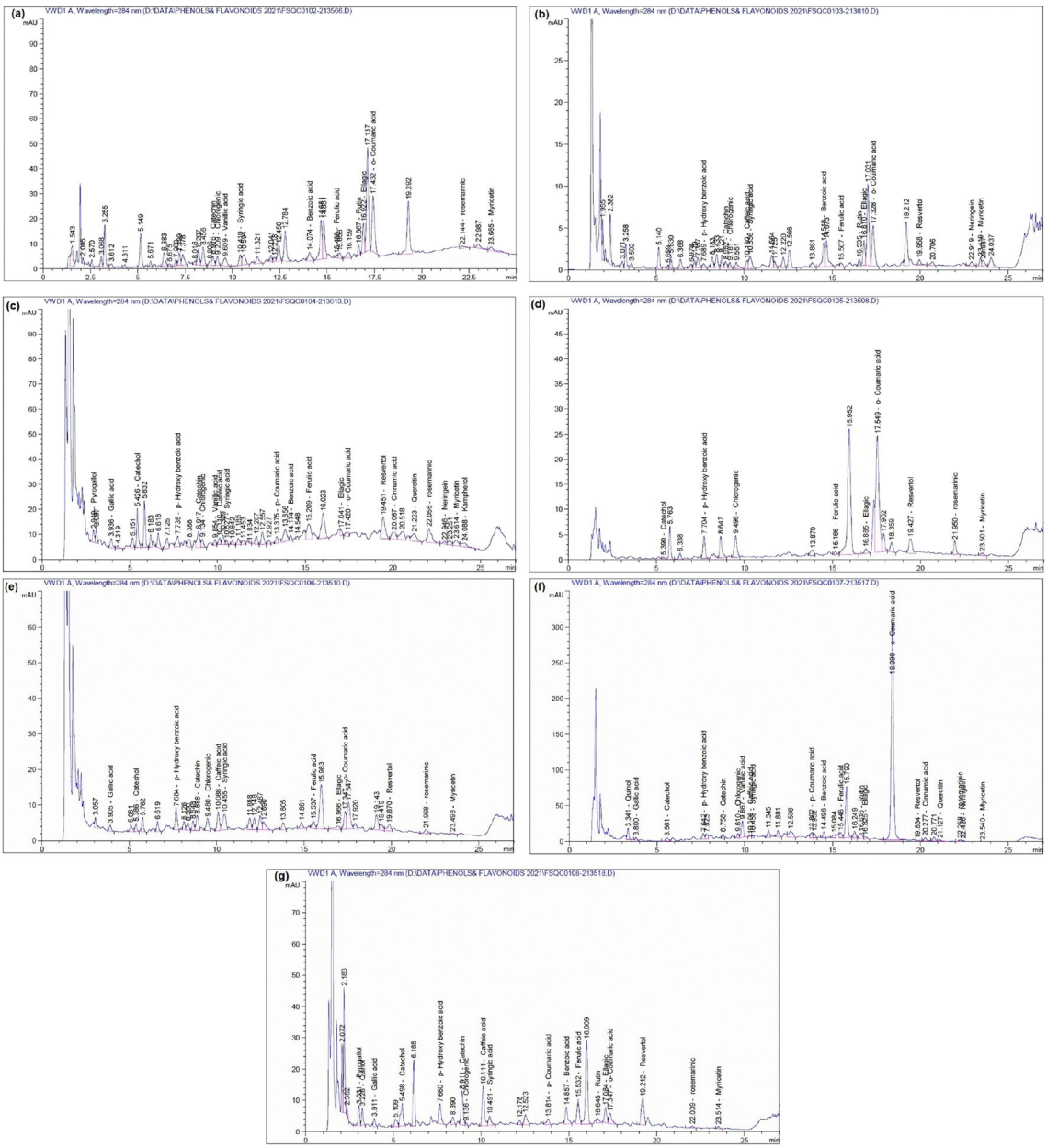

Fig. 5 HPLC chromatograms of the phytochemical identified in the methanolic extracts from various parts of *L. leucocephala*: **a** seeds; **b** roots; **c** leaves; **d** branches; **e** fruits; **f** stem wood; **g** stem bark

The antimicrobial activity of p-hydroxybenzoic acid can be detected as it crosses the cell wall of microorganisms [80, 81]. Rosmarinic acid identified from the extract *Ocimum basilicum* and *Rosmarinus offcinalis* exhibited damaged cytoskeletons of *Aspergillus niger* hyphae with broken intercepts and convoluted cell surfaces [82, 83]. Rosmarinic acid showed killing activity on planktonic forms of *S. aureus* as well as suppressing the activity of biofilm development in the early stages [84].

The antimicrobial mechanism of flavonoids like quercetin, naringenin, myricetin, and kaempferol is involve in membrane disruption, inhibition of the synthesis of the nucleic acid, inhibition of the synthesis of cell envelope, quorum sensing, and bacterial virulence inhibition, which impairs their ability to form biofilms, efflux pumps inhibition, and inhibition of NADH-cytochrome C reductase activity and ATP synthase [85–87]. Kaempferol, myricetin, naringin, and rutin, the major flavonoids present in the *Phaleria macrocarpa* extract, showed weak to moderate antibacterial activity [88].

Phenolic compounds extracted from vegetables, fruits, herbs, and spices such as chlorogenic acid, myricetin,

quercetin, rutin, curcumin, (−) epicatechin, eugenol, thymol, thymoquinone, and xanthohumol have severe physical damage and significant alteration in the morphological patterns of some bacterial isolates [89]. Phenolic compounds might bind to the cell surface and penetrate the target sites (membrane-bound enzymes and the phospholipid bilayer of the cytoplasmic membrane) [69].

5 Conclusions

Here, the biomass of *L. leucocephala* tree have several phytochemical compounds with potential bioactivity as antimicrobial and antioxidant agents. The methanol extracts from the various botanical parts: seeds, roots, leaves, stem wood, fruits, branches, and stem-bark were subjected to the bioactivity measurements. It was concluded that the highest antibacterial activity of MEs against *E. amylovora*, *A. tumefaciens*, and *S. aureus* were observed from stem-wood, leaves, and stem-bark, while the highest antifungal activity against the growth of *A. solani*, *F. solani*, and *R. solani* was from seeds, seeds/branches, and all parts, respectively. The highest antioxidant activity was observed in the ME of roots. The findings of this work confirmed that with several bioactive compounds the seeds, leaves, and bark, extracts have promising antimicrobial properties of *L. leucocephala*, and the extract from roots with good antioxidant activity.

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Author contribution N. E.: conceptualization, investigation, data curation, methodology, software, writing (original draft); A-S.: data curation, methodology, validation, supervision; M. S.: resources, methodology, data curation, supervision; A. M.: conceptualization, methodology, supervision. All authors writing—review, editing, read, and approved the final manuscript.

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Data availability Not applicable.

Declarations

Ethical approval Not applicable.

Conflict of interests The authors declare no competing interests.

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