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Antioxidant and antimicrobial properties of *Litsea elliptica* Blume and *Litsea resinosa* Blume (Lauraceae)

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PEER REVIEW

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Comments

Results of this study are important contributions to the body of knowledge. It has been shown that the methanol extracts of root and stem of both *L. elliptica* and *L. resinosa* showed significant scavenging activity and *L. resinosa* showed strong antibacterial activities compared to *L. eliptica* and less polar extracts such as hexane and dichloromethane extracts showed significant activities as compared to methanol extracts which is polar. Essential oil of both species also showed great potential in inhibiting fungus *F. oxysporum*.

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ABSTRACT

Objective: To investigate antioxidant and antimicrobial activities of two plant species, *Litsea elliptica* (*L. elliptica*) and *Litsea resinosa* (*L. resinosa*).

Methods: *In vitro* method -2,2-diphenyl-1-picrylhydrazyl radical scavenging assay was conducted for antioxidant activity determination while antimicrobial assay consisted of agar well diffusion assay and mycelial radial growth assay.

Results: Methanol extracts of root and stem of *L. elliptica* and *L. resinosa* exhibited the highest antioxidant activity with EC_{50} of 23.99, 41.69, 11.22 and 35.48 mg/L respectively. All methanol extracts of *L. resinosa* as well as root extracts from *L. elliptica* showed significant scavenging activity. Hexane extract from stem of *L. resinosa* presented the largest inhibition zone in Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* while chloroform extract from inner bark of *L. resinosa* showed major inhibition towards Gram-positive bacteria *Bacillus subtilis*. Essential oils from the root of both species showed significant antifungal activities which are 80.11% and 66.85% respectively.

Conclusions: Overall, methanol extracts from root and stem of both species showed antioxidant activity comparable to standard butylated hydroxytoluene. Extracts from *L. resinosa* demonstrated stronger antimicrobial properties compared to that from *L. elliptica*.

KEYWORDS

Litsea elliptica, *Litsea resinosa*, Antioxidant, Antifungal, Antibacterial, EC_{50}

1. Introduction

Litsea, which is an important genus from the Lauraceae family, is frequently found in regions such as tropical and subtropical Asia, Australia, and from North America to subtropical South America[1]. Indigenous plants like this have been widely utilized as traditional medicine in maintaining human health[2]. The increase in pervasiveness of multi-drug resistant microorganisms has raised the interest in natural product discovery from ethnomedicinal plants[3]. Thus, this species might have the potential to fulfill the increasing demands of antibiotics globally.

Secondary metabolites are produced by plants when they respond to environmental stress. Their production might also be a defense mechanism towards plant diseases[4]. They are selected by nature for specific biological interactions and possess drug-like properties. These metabolites usually retained antimicrobial characteristic

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and can be roughly classified into several classes. Major clusters of antimicrobial compounds including alkaloids[5], butanolide[6], flavonoids[7], lignans[8], sesquiterpenes, fatty acids[9] and essential oils[10] have been discovered through phytochemical investigations performed towards *Litsea* spp. These compounds have shown significant activities including antimicrobial[11], antitumor[12], anticancer[13], antioxidant[14], anti-inflammatory, wound healing[15], antidepressant[16] and anti hyperalgesic[17] properties in studies involving *Litsea* spp.

Litsea elliptica (*L. elliptica*) and *Litsea resinosa* (*L. resinosa*) originated from the Lauraceae family. *L. elliptica* was proven to be non-toxic towards female Sprague-Dawley rats in a study carried out by Siti Nor Ain *et al.* (2011)[18]. On the other hand, there is not much studies published regarding *L. resinosa* except from the large amount of essential oil constituents in it[19]. In fact, most essential oils from plants have been revealed to be vastly effective against food borne pathogens[20].

In determining antioxidant activities of plant compounds, *in vitro* assay including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay can be applied. This assay comprises reducing level of DPPH free radical, H_2O_2 scavenging activity by peroxidase/guaiacol and inhibition activity of lipid peroxidation by thiobarbituric acid. Commonly found synthetic antioxidants which are commercially available have been proven to be toxic and carcinogenic[21]. Hence, antioxidants from natural sources will be a considerable substitute to current synthetic antioxidants.

Study from Lin *et al.* (2007) stated that most extracts of plant species from Lauraceae genus showed antioxidant activity and revealed great free radicals of DPPH scavenger properties[22]. Methanol extract and fractions from *Litsea cubeba* showed remarkable antioxidant activity, and contained powerful natural antioxidant compounds[14]. However, antioxidant activity of majority of *Litsea* spp has not been reported.

The present study focused on the antioxidant activity of extracts from *L. elliptica* Blume and *L. resinosa* Blume by DPPH radical scavenging assay as well as antimicrobial properties through agar well diffusion method and mycelial radial growth assay.

2. Materials and methods

2.1. Plant materials

L. elliptica Blume and *L. resinosa* Blume were collected from the forest around Universiti Malaysia Sarawak, Kota Samarahan, Sarawak.

2.2. Sample preparation

Samples collected were washed, sorted out by different parts of the plants, shade air-dried, cut and grinded prior to sequential solvent extraction.

2.3. Crude extraction

The samples in powder form were used for solvent extraction following the polarity sequence from *n*-hexane, dichloromethane, chloroform, ethyl acetate to methanol (Merck EMSURE® ACS, ISO, Reag. Ph Eur) at room temperature. The sample was macerated in solvent in 5 L conical flasks at ambient temperature for 3 d, swirled three to four times per day. The extract was filtered with Advantec No. 1 filter paper and residue was kept for the subsequent extraction. Extraction of each solvent was carried out trice before the next solvent was used. Filtrates from the triplicate extractions were combined and concentrated using rotary evaporator (Heidolph Hei-VAP Advantage) at 40 °C. The concentrated filtrates were transferred into a pre-weighed beaker and left in a fume hood to completely dry out the solvent. Dried crude extracts were wrapped with foil and kept in freezer before use. The extraction yield was obtained in percentage using the weight of the extracts collected. The whole process was repeated and applied to different plant parts.

2.4. Essential oil extraction

Essential oils were extracted using hydrodistillation on a Clevenger-type apparatus. Samples were separated (root bark, root, inner bark of root, stem bark, twig, inner bark of stem and leaf) and cut into small pieces. Approximately 100-150 g of fresh samples was transferred into a 2 L flat-bottom round flask before 1.5 L of distilled water was added. The samples were hydrodistilled for 6 h continuously with the distillation rate of 1-2 drops per second. The essential oils were collected and left to cool to room temperature. The oil was separated from water and dried over anhydrous sodium sulphate. The hydrodistillation was repeated twice for each plant part. The essential oils were kept in vials and placed in 4 °C before analysis. The percentage yields were calculated.

2.5. Antioxidant assay -DPPH radical scavenging assay

DPPH radical scavenging antioxidant assay was modified from Wang *et al.* (2002)[23]. Samples of each extract including standard butylated hydroxytoluene (BHT) were prepared at different concentrations (0, 1, 10, 100 and 1 000 mg/L) in triplicates using methanol. Methanol (negative control) and 2 mL of reaction reagent were used as controls. Reaction reagent was methanolic solution of DPPH prepared by dissolving 5.9 mg of DPPH (Sigma-Aldrich) in 100 mL of methanol. Exactly 1 mL of each sample was mixed with 2 mL of the reaction reagent. The mixture was shaken thoroughly and left to stand in the dark for about 30 min at room temperature. Radical scavenging activity of the samples against DPPH free radical was measured according to the transmission of absorbance using UV-vis (ultraviolet visible) spectrophotometer (Jasco V-630). Distilled water (without reagent) was used as blank (solvent blank) in a cuvette for baseline. BHT was used as standard or positive control of this assay. Resultants absorbance of the controls and sample-reagent mixtures were recorded respectively

in cuvettes (Type 1Q, 10 mm, quartz cell 3.5 mL) at 517 nm. The percentage of DPPH radical scavenging activity (RSA) is obtained using the following equation:

$$RSA\ (\%) = [(A_c - A_s)/A_c] \times 100$$

Where, A_c is mean value of absorbance of the control, A_s is mean of absorbance values obtained in three replicates from reaction mixture of DPPH-methanol reagent and each sample.

2.6. Antimicrobial assay

2.6.1 Antibacterial assay -agar well diffusion assay

Antibacterial activities of extracts were evaluated using agar well diffusion assay modified from Tan *et al.* (2008) [23]. Plant extracts were dissolved in methanol to a final concentration of 10 000 mg/L. Nutrient broth was used for the culturing of 6 different test strains including yeast strain which consist of *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*), *Bacillus megaterium* (*B. megaterium*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Saccharomyces cerevisiae* (*S. cerevisiae*). Test strains cultured for 24 h were spread on the surface of nutrient agar. Equidistant well was bored into the agar using sterile cork borer (5 mm) followed by the addition of 50 μL diluted extracts. The plates were incubated at 37 °C for 24 h. Benzylpenicillin potassium (10 000 mg/mL), tetracycline (30 000 mg/L) and thymol (10 000 mg/L) were used as positive controls while methanol as the negative control. Antibacterial activity was obtained by measuring the diameter of zone of inhibition of the triplicates.

2.6.2 Antifungal assay-mycelial radial growth inhibition assay

The antifungal activities of extract were examined using mycelial radial growth assay[24]. Extracts of volume of 250 mg were dissolved in 2.5 mL of methanol prior added into 250 mL sterile potato dextrose agar at 60 °C. Approximately 15 mL of the mixed medium was poured into Petri dish and the final concentration of extract in media was 1 000 mg/L. For negative control, blank methanol was added instead of the extracts. Cycloheximide of concentration 10 mg/L and 1 000 mg/L as well as thymol of concentration 10 mg/L were preferred as positive controls. Upon carrying out the assay, a 5 mm plug with fungus strain was placed in the centre of the media before incubating at 25 °C. The results of assay were obtained after 7 d for *Fusarium oxysporum* (*F. oxysporum*). The average measurements of the triplicates were used to calculate the percentage of mycelial radial growth (MRG) inhibition by applying the formula below:

$$\%\ \text{of MRG Inhibition} = [(D_c - D_s)/D_c] \times 100$$

Where, D_c is the average diameter of fungal strain in negative control, D_s is the average diameter of fungal strain in media with extract samples.

3. Results

Percentage yields of the essential oils of *L. elliptica* and *L. resinosa* as shown in Table 1 ranged from 0.11% to 2.28% (vol/wt of dry materials). The leaf oil of *L. elliptica* [(2.28±0.09)%] and the stem bark oil of *L. resinosa* [(2.27±0.38)%] gave the highest yields.

Table 1

Percentage yields and characteristics of the essential oils of *L. elliptica* and *L. resinosa*.

Species	Plant parts	Yields (v/w, %)	Characteristics
L. elliptica	Twig (MPTO)	0.36±0.02	Green oils
	Stem (MPSO)	1.50±0.03	Light green oils
	Inner stem (MPISO)	0.11±0.00	Yellow oils
	Small root (MPRSO)	0.46±0.05	Green oils
	Root (MPRO)	1.51±0.08	Yellow/Green oils
	Inner root (MPIRO)	0.14±0.02	Yellow oils
	Leaf (MPLO)	2.28±0.09	Light green/Yellow oils
	Twig (METO)	0.68±0.20	Pale yellow - yellow oils
L. resinosa	Stem (MESO)	2.27±0.38	Colourless oils/White solid when cool
	Inner stem (MEISO)	0.23±0.02	Pale yellow oils
	Small root (MERSO)	0.49±0.14	Pale yellow - yellow oils
	Root (MERO)	1.40±0.50	Colourless oils/White solid when cool
	Inner root (MEIRO)	0.27±0.04	Pale yellow oils
	Leaf (MELO)	1.19±0.12	Pale yellow oils

EC_{50} values of the extracts (Table 2) showed that methanol extracts from root and stem of both *L. elliptica* and *L. resinosa* exhibited the highest antioxidant activity among of the samples studied, with EC_{50} (50% DPPH free radical scavenging and effectiveness concentration) values of 23.99, 41.69, 11.22 and 35.48 mg/L respectively. The methanol extracts from both the root of *L. resinosa* as well as *L. elliptica* showed stronger scavenging activity with EC_{50} values lower than that of the standard BHT with EC_{50} value of 28.18 mg/L.

Table 2

Extraction yield and EC_{50} values of *L. elliptica* and *L. resinosa*.

Species	Plant part	Weight of sample (kg)	Crude extract	Yield (%)	EC_{50} (mg/L)
L. elliptica	Stem	2.435	*n*-Hexane (MPSH)	2.82	>1000.00
			Dichloromethane (MPSD)	4.30	794.33
			Chloroform (MPSC)	1.12	457.09
			Ethyl acetate (MPSE)	1.40	794.33
			Methanol (MPSM)	2.88	41.69
	Root	0.960	*n*-Hexane (MPRH)	0.56	>1000.00
			Dichloromethane (MPRD)	1.47	>1000.00
			Chloroform (MPRC)	1.83	346.74
			Ethyl acetate (MPRE)	0.75	288.40
			Methanol (MPRM)	2.51	23.99
	Inner bark	5.413	*n*-Hexane (MPIH)	0.30	>1000.00
			Dichloromethane (MPID)	0.81	288.40
			Chloroform (MPIC)	0.63	263.03
			Ethyl acetate (MPIE)	0.13	114.82
			Methanol (MPIM)	0.94	199.53
L. resinosa	Stem	2.520	*n*-Hexane (MESH)	1.52	>1000.00
			Dichloromethane (MESD)	3.44	549.54
			Chloroform (MESC)	1.26	794.33
			Ethyl acetate (MESE)	0.37	398.11
			Methanol (MESM)	4.00	35.48
	Root	0.601	*n*-Hexane (MERH)	1.99	>1000.00
			Dichloromethane (MERD)	4.33	>1000.00
			Chloroform (MERC)	0.98	549.54
			Ethyl acetate (MERE)	0.32	354.81
			Methanol (MERM)	5.09	11.22
	Inner bark	1.298	*n*-Hexane (MEIH)	2.21	>1000.00
			Dichloromethane (MEID)	0.92	288.40
			Chloroform (MEIC)	0.30	281.84
			Ethyl acetate (MEIE)	0.13	70.79
			Methanol (MEIM)	4.90	83.18
BHT (standard)				28.18	

In comparison of the different parts of plant, the

inhibitory activities of both plants decreased from stem, root to inner bark and extracts from *L. elliptica* showed better inhibitory result as compared to that of *L. resinosa*. The inhibition zones support the results achieved through well diffusion assay. Plant extracts showed the strongest antimicrobial activities towards skin pathogen *P. aeruginosa*, followed by food-borne pathogens *E. coli* and *B. subtilis* (Table 3). The inhibition activities of the three positive controls were evaluated as shown in Table 4.

Table 3

Summary of antimicrobial activities of sample extracts.

Test strains		Amount of extracts effective towards test strains (44 samples)
Gram negative bacteria	*P. aeruginosa*	42
	E. coli	38
Gram positive bacteria	*B. subtilis*	39
	S. aureus	23
	B. megaterium	21
Yeast	*S. cerevisiae*	33
Fungus	*F. oxysporum*	38

Table 4

Inhibition of positive controls towards test strains.

Test strains		Zone of inhibition (mm)		
		Benzyl penicillin potassium	Tetracycline (TE)	Thymol
Gram-negative bacteria	*P. aeruginosa*	25.00±0.00	34.67±0.58	12.33±0.58
	E. coli	26.00±1.73	35.00±0.00	13.00±1.73
Gram-positive bacteria	*B. subtilis*	22.67±1.15	33.67±1.15	14.00±1.73
	S. aureus	18.00±1.00	29.00±1.00	13.33±1.53
	B. megaterium	29.00±0.00	39.33±1.15	12.33±0.58
Yeast	*S. cerevisiae*	25.33±1.15	34.67±0.58	12.67±0.58

Extracts from *L. resinosa* demonstrated stronger antibacterial properties than *L. elliptica* especially in inhibiting Gram negative bacteria as shown in Table 5 and Table 6. Hexane extract from stem of *L. resinosa* presented the largest inhibition in Gram-negative *E. coli* [(19.33±1.15) mm] while chloroform extract from inner bark of *L. resinosa* showed major inhibition [(15.33±3.21) mm] towards Gram-positive *B. subtilis*. Major crude extracts from *L. resinosa* showed higher efficacy towards *E. coli* with inhibition zones larger than standard thymol [(13.00±1.73) mm].

Table 5

Inhibition zones of *L. elliptica* and *L. resinosa* towards Gram-negative bacteria strains and yeast strain.

Plant part	Crude extract/Essential oil	Inhibition zone (mm)		
		PA	EC	SC
L. elliptica				
Stem	*n*-Hexane (MPSH)	10.00±0.00	13.00±1.73	10.33±0.58
	Dichloromethane (MPSD)	12.00±0.00	16.33±1.15	10.00±0.00
	Chloroform (MPSC)	10.00±0.00	14.67±1.15	11.00±0.00
	Ethyl acetate (MPSE)	9.00±0.00	9.33±0.58	12.00±1.00
	Methanol (MPSM)	NI	NI	NI
Root	*n*-Hexane (MPRH)	9.00±0.00	9.67±1.53	NI
	Dichloromethane (MPRD)	9.00±0.00	12.67±0.58	11.00±0.00
	Chloroform (MPRC)	8.33±0.58	11.33±0.58	10.33±0.58
	Ethyl acetate (MPRE)	7.67±0.58	9.33±2.52	10.67±0.58
	Methanol (MPRM)	NI	NI	NI
Inner bark	*n*-Hexane (MPIH)	9.67±1.15	NI	10.00±1.00
	Dichloromethane (MPID)	10.00±2.00	7.67±1.15	NI
	Chloroform (MPIC)	9.00±1.00	7.33±0.58	NI
	Ethyl acetate (MPIE)	10.00±2.00	NI	10.00±0.00
	Methanol (MPIM)	9.00±1.00	NI	NI
L. resinosa				
Stem	*n*-Hexane (MESH)	15.00±0.00	19.33±1.15	13.67±1.15
	Dichloromethane (MESD)	14.00±0.00	18.00±0.00	12.67±0.58
	Chloroform (MESC)	13.67±1.15	18.67±1.15	13.33±1.15
	Ethyl acetate (MESE)	11.33±0.58	15.00±1.00	13.33±1.53
	Methanol (MESM)	9.67±1.15	NI	NI
Root	*n*-Hexane (MERH)	10.33±0.58	15.00±0.00	12.67±0.58
	Dichloromethane (MERD)	10.33±0.58	11.67±1.15	11.33±0.58
	Chloroform (MERC)	10.00±1.00	15.00±2.65	11.00±1.00
	Ethyl acetate (MERE)	9.67±0.58	15.00±2.00	11.00±1.00
	Methanol (MERM)	9.67±1.53	9.00±0.00	NI
Inner bark	*n*-Hexane (MEIH)	13.67±1.53	13.33±2.31	11.00±0.00
	Dichloromethane (MEID)	13.33±0.58	13.67±2.08	11.33±1.53
	Chloroform (MEIC)	13.33±0.58	13.33±1.53	10.00±1.00
	Ethyl acetate (MEIE)	13.00±1.73	10.00±1.00	10.00±0.00
	Methanol (MEIM)	10.33±0.58	9.00±0.00	NI
L. elliptica				
Twig	Essential oil (MPTO)	14.00±1.00	13.00±0.00	10.67±1.15
Stem	Essential oil (MPSO)	12.00±1.73	12.33±0.58	10.67±1.15
Inner stem	Essential oil (MPISO)	12.00±1.00	11.33±1.53	10.00±0.00
Root	Essential oil (MPRO)	12.33±0.58	11.67±0.58	11.00±0.00
Small root	Essential oil (MPRSO)	11.00±1.00	11.00±0.00	10.00±0.00
Inner root	Essential oil (MPIRO)	8.33±0.58	9.00±0.00	9.67±0.00
Leaf	Essential oil (MPLO)	10.00±0.00	9.00±0.00	NI
L. resinosa				
Twig	Essential oil (METO)	12.67±1.15	10.33±0.58	11.33±0.58
Stem	Essential oil (MESO)	11.33±1.15	11.00±0.00	10.67±1.15
Inner stem	Essential oil (MEISO)	11.33±1.15	11.67±1.15	11.67±1.15
Root	Essential oil(MERO)	8.00±0.00	8.00±0.00	NI
Small root	Essential oil (MERSO)	14.00±1.73	12.33±0.58	11.00±1.73
Inner root	Essential oil (MEIRO)	10.67±1.15	12.00±0.00	9.33±0.58
Leaf	Essential oil(MELO)	12.33±1.15	14.67±0.58	11.00±1.15
Control	Negative	NI	NI	NI

NI: No inhibition; PA: *Pseudomonas aeruginosa*; EC: *Escherichia coli*; SC: *S. cerevisiae*.

Table 6

Inhibition zones of *L. elliptica* and *L. resinosa* towards Gram-positive bacteria strains.

Plant part	Crude extract/Essential oil	Inhibition zone (mm)		
		BS	SA	BM
L. elliptica				
Stem	*n*-Hexane (MPSH)	10.67±1.15	NI	NI
	Dichloromethane (MPSD)	11.00±1.00	NI	NI
	Chloroform (MPSC)	12.00±1.00	NI	NI

NI: No inhibition; BS: *Bacillus subtilis*; SA: *S. aureus*; BM: *B. megaterium*.

Table 6

Inhibition zones of *L. elliptica* and *L. resinosa* towards Gram-positive bacteria strains.

Plant part	Crude extract/Essential oil	Inhibition zone (mm)		
		BS	SA	BM
	Ethyl acetate (MPSE)	9.33±0.58	NI	NI
	Methanol (MPSM)	NI	NI	NI
Root	*n*-Hexane (MPRH)	8.33±0.58	7.00±0.00	NI
	Dichloromethane (MPRD)	10.50±0.71	7.33±0.58	NI
	Chloroform (MPRC)	10.67±0.58	7.00±0.00	NI
	Ethyl acetate (MPRE)	NI	NI	NI
	Methanol (MPRM)	NI	NI	NI
Inner bark	*n*-Hexane (MPIH)	9.33±0.58	NI	NI
	Dichloromethane (MPID)	9.33±1.53	NI	NI
	Chloroform (MPIC)	8.33±0.58	NI	NI
	Ethyl acetate (MPIE)	8.67±0.58	NI	NI
	Methanol (MPIM)	9.00±0.00	NI	NI
L. resinosa				
Stem	*n*-Hexane (MESH)	14.33±2.08	12.00±1.00	9.00±1.73
	Dichloromethane (MESD)	15.00±2.65	14.33±1.53	7.67±0.58
	Chloroform (MESC)	13.00±0.00	9.00±2.00	NI
	Ethyl acetate (MESE)	13.00±3.46	7.67±0.58	NI
	Methanol (MESM)	7.67±0.58	NI	NI
Root	*n*-Hexane (MERH)	12.33±0.58	9.33±0.58	8.00±0.00
	Dichloromethane (MERD)	11.00±0.00	11.33±1.15	8.00±0.00
	Chloroform (MERC)	11.00±0.00	9.33±0.58	8.33±0.58
	Ethyl acetate (MERE)	11.67±1.15	10.33±0.58	8.00±1.00
	Methanol (MERM)	11.00±0.00	NI	NI
Inner bark	*n*-Hexane (MEIH)	13.00±0.00	7.00±0.00	NI
	Dichloromethane (MEID)	15.00±1.73	10.67±0.58	7.67±1.15
	Chloroform (MEIC)	15.33±3.21	10.67±0.58	7.67±1.15
	Ethyl acetate (MEIE)	12.00±2.00	9.33±0.58	7.00±0.00
	Methanol (MEIM)	9.33±0.58	10.00±0.00	NI
L. elliptica				
Twig	Essential oil (MPTO)	12.00±0.00	10.33±0.58	10.33±0.58
Stem	Essential oil (MPSO)	12.00±0.00	17.33±1.15	11.33±0.58
Inner stem	Essential oil (MPISO)	12.33±0.58	10.00±0.00	10.00±0.00
Root	Essential oil (MPRO)	13.67±1.15	10.00±0.00	11.00±1.00
Small root	Essential oil (MPRSO)	11.33±1.15	10.00±0.00	10.00±1.00
Inner root	Essential oil (MPIRO)	9.33±0.58	9.00±0.00	NI
Leaf	Essential oil (MPLO)	NI	8.00±0.00	9.00±0.00
L. resinosa				
Twig	Essential oil (METO)	13.67±1.15	12.00±1.00	14.33±1.15
Stem	Essential oil (MESO)	13.33±1.53	11.00±0.00	11.33±0.58
Inner stem	Essential oil (MEISO)	9.33±0.58	10.67±2.08	10.67±1.15
Root	Essential oil(MERO)	NI	NI	NI
Small root	Essential oil (MERSO)	13.67±0.58	16.67±0.58	15.00±0.00
Inner root	Essential oil (MEIRO)	10.67±1.15	10.67±0.58	10.67±1.15
Leaf	Essential oil (MELO)	13.00±1.73	11.33±0.58	10.67±2.08
Control	Negative	NI	NI	NI

NI: No inhibition; BS: *Bacillus subtilis*; SA: *S. aureus*; BM: *B. megaterium*.

Table 7

Antifungal activity of *L. elliptica* and *L. resinosa* towards *F. oxysporum*.

Species	Plant part	Crude extract	Percentage of mycelial radical growth (%)
			F. oxysporum
L. elliptica	Stem	*n*-Hexane (MPSH)	49.17±1.91
		Dichloromethane (MPSD)	24.31±2.53
		Chloroform (MPSC)	18.23±0.96
		Ethyl acetate (MPSE)	12.71±1.91
		Methanol (MPSM)	0
	Root	*n*-Hexane (MPRH)	43.65±0.00
		Dichloromethane (MPRD)	27.07±0.00
		Chloroform (MPRC)	17.13±0.00
		Ethyl acetate (MPRE)	25.97±1.91
		Methanol (MPRM)	0
	Inner bark	*n*-Hexane (MPIH)	14.36±0.96
		Dichloromethane (MPID)	3.31±0.96
		Chloroform (MPIC)	8.84±0.00
		Ethyl acetate (MPIE)	17.13±0.00
		Methanol (MPIM)	0

NA: Not available due to limited amount of sample.

Table 7

Antifungal activity of *L. elliptica* and *L. resinosa* towards *F. oxysporum*.

Species	Plant part	Crude extract	Percentage of mycelial radical growth (%)
			F. oxysporum
L. resinosa	Stem	*n*-Hexane (MESH)	43.09±0.96
		Dichloromethane (MESD)	19.34±0.96
		Chloroform (MESC)	21.55±0.96
		Ethyl acetate (MESE)	27.07±0.00
		Methanol (MESM)	0
	Root	*n*-Hexane (MERH)	38.12±3.45
		Dichloromethane (MERD)	12.15±0.00
		Chloroform (MERC)	18.78±2.87
		Ethyl acetate (MERE)	9.94±0.96
		Methanol (MERM)	0
	Inner bark	*n*-Hexane (MEIH)	3.87±1.66
		Dichloromethane (MEID)	20.44±0.00
		Chloroform (MEIC)	24.31±2.53
		Ethyl acetate (MEIE)	23.20±1.91
		Methanol (MEIM)	0
L. elliptica	Twig	Essential oil (MPTO)	55.25±1.66
	Stem	Essential oil (MPSO)	59.67±0.96
	Small root	Essential oil (MPSRO)	58.01±1.91
	Leaf	Essential oil (MPLO)	59.67±0.96
	Root	Essential oil (MPRO)	66.85±0.00
	Inner root	Essential oil (MPIRO)	NA
	Inner stem	Essential oil (MPISO)	NA
L. resinosa	Twig	Essential oil (METO)	72.93±1.91
	Stem	Essential oil (MESO)	70.17±1.66
	Small root	Essential oil (MESRO)	NA
	Leaf	Essential oil (MELO)	53.04±0.96
	Root	Essential oil (MERO)	80.11±0.00
	Inner root	Essential oil (MEIRO)	60.77±1.91
	Inner stem	Essential oil (MEISO)	NA
Controls		Negative with MeOH	0±0.00
Standards		Thymol	100±0.00
		Cycloheximide (10 mg/L)	45.86±0.96
		Cycloheximide (1 000 mg/L)	100±0.00

NA: Not available due to limited amount of sample.

Both extracts of *L. resinosa* and *L. elliptica* showed different level of antifungal activities against test strains as stated in Table 7. Unlike the crude extracts, essential oils from the root of *L. resinosa* and *L. elliptica* have the highest inhibition activities in this assay, which is (80.11±0.00)% and (66.85±0.00)% respectively.

4. Discussion

From the DPPH assay, the absorbance of sample-reagent mixture varied inversely with the free radical scavenging and antioxidant activity. This is due to the fact that absorbance decreases when antioxidant donates proton to DPPH radical. Results showed that methanol extracts from root of *L. resinosa* and *L. elliptica* showed stronger scavenging activity than standard BHT with EC_{50} value of 28.18 mg/L. This indicates that methanol extracts of root and stem from both species might contain

antioxidant agents which are useful as potential sources for natural antioxidants that are comparable to the synthetic antioxidant, BHT.

All *n*-hexane and most dichloromethane extracts of both species possessed weak antioxidative properties with EC_{50} values higher than 1 000 mg/L. The activity increases as the concentration of the samples increases but is varied inversely to the EC_{50} values. Polarity and characteristics of solvent used in extraction influence the antioxidant activity due to the variation in chemical composition, compounds solubility and content of the extracts obtained. In addition, scavenging behavior is affected by concentrations, types of extracts, plant parts used and species tested.

Generally, Gram positive bacteria have higher susceptibility than Gram negative bacteria due to the lack of lipopolysaccharides and protein cell wall structures. However, in this study, Gram negative bacteria tested showed greater susceptibility in the plant extracts antibacterial assay. Gram positive *P. aeruginosa* showed the most notable results (> 95% of the plant extracts) towards bacteria through the inhibition as shown in Table 3 due to its restrictive external membrane barrier[25]. This might be due to the difference in concentrations of extracts, plant types[26] or permeability barrier by cell wall[27]. This is supported by the studies carried out on major Australian native plant by Palombo and Semple (2001) which indicated significant results towards Gram negative bacteria instead of Gram positive bacteria[28].

Test strains showed higher sensitivity towards hexane, dichloromethane and chloroform extract than methanol extract. There might be some active compounds present in these plants that are less polar and readily dissolved in these solvents. Of all the plant parts, extracts from stem of both species showed the most significant activity towards test strains. Negative results do not mean that the bioactive compound is absent or not bioactive. This phenomenon can be explained by the insufficient amount or concentration of active phytocompounds present in that particular plant extracts[4,29]. Thus, these compounds can be further accumulated and studied. Besides, the plant extracts might be active towards other bacterial strains rather than those tested[29].

Essential oils from both species showed greater activities as compared with crude extracts in antifungal assay. This matched with the studies carried out on essential oils of *Litsea* spp. such as *Litsea kostermansii*[30] and *Litsea akoensis*[31] demonstrating excellent antibacterial or antifungal activities. Mycelial radial growth assay for both species showed that hexane extracts showed the strongest inhibitory activities whilst methanol extracts from every part of the plants have weak or no antifungal activities towards the test strain *F. oxysporum*. Noteworthy, it was the essential oil from root and stem extracts from both plants exhibiting greater antifungal activities.

The overall results suggest that the less polar compounds in both species have the potency to be attributed as novel antimicrobial products while extracts with higher polarity may be an alternative in substituting the current synthetic antioxidant which will be harmful to health in long term. Further studies such as identification and accumulation of phytocompounds and antioxidative agents can be performed to observe their mechanism of activities.

In conclusion, the methanol extracts of root and stem of both *L. elliptica* and *L. resinosa* showed significant scavenging activity in comparison with the standard BHT. The extracts can be the potential sources for natural antioxidants that are comparable to the synthetic antioxidant. *L. resinosa* showed strong antibacterial activities compared to *Litsea eliptica* and less polar extracts such as hexane and dichloromethane extracts showed significant activities as compared to methanol extracts which is polar. Essential oil of both species showed great potential in inhibiting fungus *F. oxysporum*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Microorganisms are responsible for many diseases and there is a need for discovery of products from natural sources with anti-microbial properties. In addition, antioxidants from natural sources could be a substitute to current synthetic antioxidants which may have undesirable side effects.

Research frontiers

The present research shows different solvents extracts of different parts of *L. elliptica* and *L. resinosa* in antioxidant activity, anti-bacterial activities and anti-fungal activity of essential oil of both species.

Related reports

The toxicity effects of *L. elliptica* were studied (Siti Nor Ain *et al.*, 2011)[17]. Large amount of essential oil constituents of *L. resinosa* has been studied but not their activities[18].

Innovations and breakthroughs

Authors have demonstrated extracts of root and stem of both *L. elliptica* and *L. resinosa* which showed significant scavenging activity in modified DPPH radical scavenging antioxidant assay.

Applications

L. elliptica was shown to be not toxic (Siti Nor Ain *et al.*,

2011)[17] and the present study supported the use of this plant extract as an antioxidant.

Peer review

Results of this study are important contributions to the body of knowledge. It has been shown that the methanol extracts of root and stem of both *L. elliptica* and *L. resinosa* showed significant scavenging activity and *L. resinosa* showed strong antibacterial activities compared to *L. eliptica* and less polar extracts such as hexane and dichloromethane extracts showed significant activities as compared to methanol extracts which is polar. Essential oil of both species also showed great potential in inhibiting fungus *F. oxysporum*.

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