

Antioxidant and antibacterial activities of the leaf essential oil and its constituents furanodienone and curzerenone from *Lindera pulcherrima* (Nees.) Benth. ex hook. f.

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ABSTRACT

Background: *Lindera pulcherrima* (Nees.) Benth. ex Hook. f. (Family: Lauraceae), an evergreen shrub, is an important medicinal plant distributed in temperate Himalayan regions. The leaves and bark are used as spice in cold, fever, and cough. **Materials and Methods:** In this study, the terpenoid composition, antioxidant, and antibacterial activities of the leaf essential oil and its major constituents are being analyzed. **Conclusion:** The *in vitro* antioxidant activity showed a potent free radical scavenging activity for the essential oil as evidenced by a low IC₅₀ value for DPPH radical followed by furanodienone (0.087 ± 0.03 and 1.164 ± 0.58 mg/ml respectively) and the inhibition of lipid peroxidation for the oil and furanodienone also followed the same order (IC₅₀ 0.74 ± 0.13 and 2.12 ± 0.49 mg/ml, respectively). The oil and the constituents were also tested against three Gram negative (*Escherichia coli*, *Salmonella enterica enterica*, and *Pasturella multocida*) and one Gram positive (*Staphylococcus aureus*) bacteria. The essential oil was effective against *S. aureus* (IZ = 19.0 ± 0.34; MIC 3.90 µl/ml) while furanodienone showed potent activity against *E. coli* and *S. enterica enterica* (IZ = 18.0 ± 0.14 and 16.0 ± 0.10 respectively). On the other hand, curzerenone was found to be slightly effective against *E. coli* (IZ = 10.8 ± 0.52). The MIC value of the essential oil was least against *S. aureus* (MIC = 3.90 µl/ml) and that of furanodienone against *E. coli* (MIC = 3.90 µl/ml).

Key words: Antibacterial, antioxidant, curzerenone, furanodienone, lauraceae, *lindera*

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INTRODUCTION

Lindera pulcherrima (Nees.) Benth. ex Hook. f. (Family: Lauraceae), locally known as “Cher” is an evergreen shrub or often a tree. It occurs in the Himalayan region (1500-2700 m) in shady forests. The leaves and bark of *L. pulcherrima* are used as spice in cold, fever, and cough.^[1-3] Varieties of terpenoids have been reported from *Lindera* species.^[4-12] Sesquiterpene hydrocarbons were reported as major constituents from leaf oil of *L. queenslandica* with β-elemene, α-copaene, α-humulene, and β-caryophyllene as most representative constituents.^[11] β-Caryophyllene and (E)-nerolidol were reported as major constituents from

the leaf oil of *L. benzoin*.^[10] Among the *Lindera* species grown in Japan, the leaf oil of *L. umbellata* was shown to possess carvone, linalool, and 1,8-cineole as the major constituents while the leaf oil of *L. sericea* was mainly dominated by 1,8-cineole, limonene, and α-pinene. Bornyl acetate, -pinene, and camphene were reported as the major constituents of *L. sericea* var. *glabrata*.^[8] The essential oil from the leaves of *L. obtusiloba* contained camphor along with *cis*-ocimene, α-pinene, and camphene as the major constituents.^[9] The leaf essential oil of *L. erythrocarpa* was shown to possess β-caryophyllene, geranyl acetate, and geraniol as the major constituents. γ-Muurolene and β-caryophyllene were the major constituents of the leaf oil of *L. glauca*.^[9]

A wide range of furanosesquiterpenoids namely linderene, linderane, lindstrenene, lindenol, linderenone, linderalactone, isolinderalactone, linderoxide, and

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isolinderoxide etc. have been reported from *Lindera* species.^[4-6] Literature survey revealed very few reports on the biological activities of furanosesquiterpenoids, however, some of them are known for insecticidal, analgesic, and anti-inflammatory activities^[12-14] while biologically active alkaloids and flavones have been reported from various *Lindera* species.^[12-17] In continuation to our investigation on the bioactive plant constituents of Himalayan Laurels,^[18,19] the *in vitro* antioxidant and antibacterial activities of the leaf essential oil of *L. pulcherrima* and its constituents namely curzerenone and furanodienone have been taken in this study. This is the first report on the antioxidant and antibacterial activities of the curzerenone and furanodienone.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *L. pulcherrima* were collected from Bageshwar district of Uttarakhand, India. Plant herbaria were identified at Botanical Survey of India, Dehradun (No. BSD 101366) and the voucher specimen has been deposited in the Phytochemistry laboratory, Chemistry Department, Kumaun University, Nainital (No. Chem. / DST/ LP/01).

Extraction of oils

The fresh leaves (6 kg) were chopped and steam distilled (2 h) in a copper still fitted with a spiral glass condenser. The distillate (12 l) was saturated with NaCl and the oil was extracted with *n*-hexane and dichloromethane, dried over sodium sulfate and stored at 4°C.

Isolation, characterization, and identification of constituents

The leaf essential oil was analyzed by GC and GC-MS, fractionated by column chromatography and HPLC and the constituents were characterized by IR, retention indices (RI), mass spectral data and library search (Nist and Wiley), ¹H NMR and ¹³C NMR spectral data in order to determine its chemical composition.^[20]

Antioxidant activity

β-Carotene/ linoleic acid bleaching assay

β-Carotene bleaching assay was carried out according to the standard method.^[21] *β*-Carotene (2 mg) was dissolved in CHCl₃ (20 ml). Its 3.0 ml solution was added to 40 μl linoleic acid and 400 μl tween 40. After removing CHCl₃ under reduced pressure, 100 ml of oxygenated water was added and mixed properly to obtain a stable emulsion which was mixed with 50 μl of sample and incubated for 1 h at 50°C. The absorbance was recorded at 0 min and after 60 min of incubation at 470 nm. Antioxidant activity was expressed as percent inhibition relative to control after a 60 min incubation period and calculated by the

following formula:

$$\% \text{AOA} = (D_c - D_s / D_c \times 100)$$

where D_c = degradation rate of control and D_s = degradation rate of sample. Antioxidative capacities of the oils were compared with those of butylated hydroxyl toluene (BHT) and blank.

Estimation of reducing power

Reducing power (RP) was determined using a ferric reducing-antioxidant power assay taking quercetin as standard.^[22] Different aliquots of sample maintained to 1 ml, followed by the addition of 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% w/v potassium ferricyanide in each reaction mixture thus obtained were incubated at 50°C for 20 min. After incubation, reaction was terminated by addition of 2.5 ml of 10% w/v trichloroacetic acid solution; 2.5 ml of above solution from each reaction was diluted with equal amount of distilled water. Aliquot of 0.5 ml FeCl₃ (0.1%) was added in each and absorbance was recorded after 10 min at 700 nm. The RP was expressed as ascorbic acid equivalent (1 mmol = 1 ASE).

DPPH radical scavenging assay

The DPPH radical scavenging activity was determined by using the standard method.^[23] Different aliquots were added to 2.9 ml of freshly prepared solution of DPPH (6×10^{-5} M in MeOH). The absorbance was recorded at 517 nm after 1 h of incubation. Percent inhibition of DPPH (I %) was calculated according to formula:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100,$$

where A_{blank} = absorbance of the control reaction (containing all reagents except the test sample), A_{sample} = absorbance of the test sample. The IC₅₀ was estimated and calculated as described by Kroyer.^[24] IC₅₀ value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from a calibration curve by a linear regression.

Lipid peroxidation inhibition

Rats were fasted overnight and killed by cervical dislocation, dissected, and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and weighted amount of liver processed to get 10% homogenate in cold phosphate buffer saline (pH 7.4). The degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances. Different concentrations of oils were added to 1 ml liver homogenate. Liver peroxidation was initiated by adding 100 μl of 15 mmol FeSO₄ solution to liver homogenate. After 30 min incubation at 37°C, 100 μl of this reaction mixture was taken in a tube containing 1.5 ml of 10% TCA. After 10 min, tubes were centrifuged and supernatant was mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated in a water bath for 30 min. The intensity of colored complex formed was

measured at 532 nm. The percentage of inhibition of lipid peroxidation was calculated by comparing the results of test samples with those of control.

Antibacterial activity

Test bacteria

The *in vitro* antibacterial activities of the essential oil and its major compounds were evaluated against four pathogenic microorganisms namely three Gram-negative: *Escherichia coli* MTCC 443, *Salmonella enterica enterica* MTCC 3223, and *Pasturella multocida* MTCC 1148 and one Gram-positive: *Staphylococcus aureus* MTCC 737 procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. All the strains were stored in the appropriate medium before use.

Inhibitory effect by the disc diffusion method

The disc diffusion method^[25] was used for the evaluation of antibacterial activity of essential oils using 100 µl of suspension containing 10⁸ CFU/ml of bacteria spread on the inoculated agar. Empty sterilized discs were impregnated with oil (100 µg) and test compounds with appropriate dilution in DMSO (negative control). Gentamicin (10 µg/disc) was used as a positive reference standard to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition (IZ) in mm against the test organisms. The experiments were repeated in triplicate, and the results were expressed as average values.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the essential oils was determined by a two-fold serial dilution technique.^[26] Dilutions of the test samples were prepared in Mueller–Hinton broth (Hi Media, Mumbai) ranging from 0.06 to 125 ml/ml. To each tube 0.5 ml of the inoculum containing approximately 10⁸ CFU/ml microorganisms was added. A control test was also performed containing inoculated broth supplemented with only DMSO under identical conditions with gentamicin as reference. All the tubes were then incubated at 37°C for 24 h and examined for evidence of the growth.

Statistical analysis

Tests were carried out in triplicate and the results were calculated as mean ± SD.

RESULTS AND DISCUSSION

The identified constituents of the essential oil are given in Table 1 with their respective percentage. The oil was characterized by high percentage of sesquiterpenoids (91.6%) dominated by furanosesquiterpenoids (80.7%),

Table 1: Essential oil composition of *L. pulcherrima* leaves

Compounds*	RI ^a	RI ^b	Percent composition	Mode of identification*
Camphene	955	953	0.1	a, b
α-Terpinene	1020	1018	0.4	a, b
γ-Terpinene	1065	1062	0.5	a, b
α-Terpineol	1192	1189	0.4	a, b
δ-Elemene	1341	1339	0.2	a, b
α-Copaene	1379	1376	0.3	a, b
β-Cubebene	1392	1390	0.1	a, b
β-Elemene	1394	1391	0.4	a, b
β-Caryophyllene	1420	1418	0.9	a, b
β-Gurjurenene	1434	1432	1.0	a, b
Germacrene D	1482	1480	1.7	a, b
Isofuranogermacrene	1498	1496	2.9	a, b, c
γ-Cadinene	1516	1513	0.6	a, b
Spathulenol	1579	1576	3.1	a, b
Guaiol	1597	1595	0.9	a, b
Curzerenone	1604	1601	17.4	a, b, c
10- <i>epi</i> -γ-Eudesmol	1621	1619	1.9	a, b
<i>epi</i> -α-Cadinol	1642	1640	1.0	a, b
β-Eudesmol	1651	1649	1.6	a, b
Furanodiene	1680	-	3.5	a, b, c
Germacrene	1695	1693	0.7	a, b
Curcuphenol	1717	1715	0.8	a, b
Oplopanone	1735	1733	0.9	a, b
Furanosesquiterpenoid (M ⁺ 230)	1740	-	2.6	a, b
Furanosesquiterpenoid (M ⁺ 230)	1748	-	5.2	a, b
Furanodienone	1750	-	49.1	a, b, c
Total			98.2	

*Mode of identification: a = Retention Index (RI), b = MS (GC-MS), c = NMR (¹H and ¹³C NMR). ^a RI = Present work (Based on homologous series of *n*-alkanes (C₈-C₂₄)). ^b RI = Literature values.^[20]

namely furanodienone (49.1%), curzerenone (17.4%), furanodiene (3.5%), and isofuranogermacrene (2.9%) along with two unidentified furanosesquiterpenoids. Other constituents in significant amounts were spathulenol (3.1%), germacrene D (1.7%), 10-*epi*-γ-eudesmol (1.9%), and β-eudesmol (1.6%). These constituents are shown in Figure 1. Linderene furanosesquiterpenoids reported earlier from *Lindera strychnifolia*^[5,6,9,27] were not noticed in the leaf oil of *L. pulcherrima*. Furthermore, our analysis also revealed the trace presence of monoterpenoids in the leaf oil of *L. pulcherrima* as compared to earlier reports on several other species where monoterpenoids constitute the major part of oil compositions.^[7,9-11] Furanodienone is known to possess insecticidal activity by inducing contact toxicity against larvae of the polyphagous pest insects.^[28] Furanodienone, curzerenone, and their structural analogues have also been shown to have significant anti-inflammatory and analgesic activities.^[29,30] A recent report is also available on the cytotoxicity and antibacterial activity of *L. strychnifolia* essential oil and extracts.^[31] The high content

of furanodienone (49.1%) in the leaf oil of *L. pulcherrima* makes it useful for its commercial utilization.

The results of antioxidant activity determined by four complementary test systems namely β -carotene bleaching assay, RP, DPPH radical scavenging, and inhibition of lipid peroxidation are shown in Table 2. The essential oil of *L. pulcherrima* showed potent antioxidant activity for inhibition of β -carotene bleaching ($62.7 \pm 3.31\%$) followed by the compounds namely furanodienone and

curzerenone (46.1 ± 2.56 and 43.5 ± 1.72 , respectively). The RP (expressed as ascorbic acid equivalent; ASE/ml) of a compound serves as a significant indicator of its antioxidant activity. Essential oil showed the highest RP as evident from the lower ASE/ml (1.34 ± 0.32) as compared to the compounds.

Free-radical scavenging activity for the DPPH radical expressed as IC_{50} was also found to be highest for essential oil ($IC_{50} = 0.087 \pm 0.03$ mg/ml) which was found to be closer to the standard quercetin ($IC_{50} = 0.031 \pm 0.02$) followed by furanodienone and curzerenone ($IC_{50} = 1.164 \pm 0.58$ and 1.563 ± 0.33 , respectively). The inhibition of lipid peroxidation induced by $FeSO_4$ was assayed by measuring the lipid oxidation products such as thiobarbituric acid-reactive substances (TBARS). Results showed that the essential oil inhibited TBARS formation ($IC_{50} = 0.74 \pm 0.13$ mg/ml) as compared to the isolated compounds.

The results of antibacterial activity against four bacterial species are summarized in Tables 3 and 4. The essential oil exhibited highest zone of inhibition against *S. aureus* ($IZ = 19.0 \pm 0.34$) followed by *S. enterica enterica* ($IZ = 17.0 \pm 0.40$). Furanodienone was found to be effective against *E. coli* and *S. enterica enterica* ($IZ = 18.0 \pm 0.14$ and 16.0 ± 0.10 , respectively) while curzerenone was

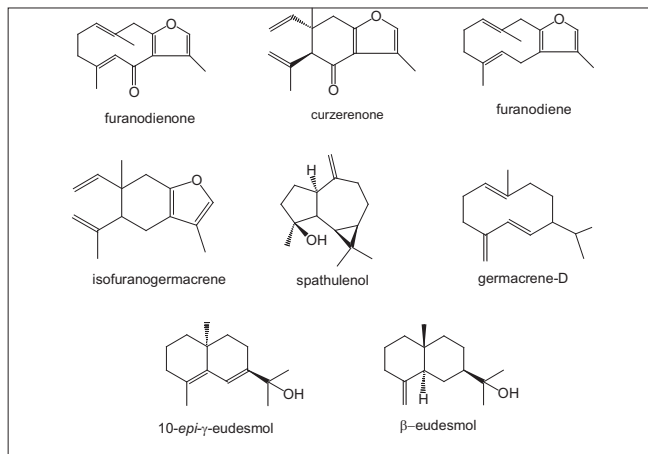


Figure 1: Structure of the constituents of *Lindera pulcherrima*

Table 2: Antioxidant activities of the leaf essential oil and major compounds

Essential oil/ Compounds	AOA%	DPPH (IC_{50} mg/ml)	RP (ASE/ml)	LPO (IC_{50} mg/ml)
Crude oil	62.7 ± 3.31	0.087 ± 0.03	1.34 ± 0.32	0.74 ± 0.13
Curzerenone	43.5 ± 1.72	1.563 ± 0.33	3.84 ± 0.56	2.38 ± 0.70
Furanodienone	46.1 ± 2.56	1.164 ± 0.58	3.26 ± 0.42	2.12 ± 0.49
BHT ^a	50.2 ± 3.46	nd	nd	nd
Quercetin	Nd	0.031 ± 0.02	0.52 ± 0.09	0.089 ± 0.04

Values are means of three replicates (+ SD). ^abutylated hydroxyl toluene. nd = not determined.

Table 3: Zone of inhibition of the leaf essential oil and major compounds

Essential oil/ Compounds	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella enterica enterica</i>	<i>Pasteurella multocida</i>
Crude oil	na	19.0 ± 0.34	17.0 ± 0.40	9.3 ± 0.22
Curzerenone	10.8 ± 0.52	na	na	na
Furanodienone	18.0 ± 0.14	na	16.0 ± 0.10	10.7 ± 0.23
Standard	26	22	19	21

Diameter of inhibition zones (mm) including the diameter of disc (6 mm), Values are means of three replicates (\pm SD), test samples= 100 Mg/disc (compounds > 95% pure; GC). na = not active. Standard, 10 μ g/disc (Gentamicin; Ranbaxy; 80 mg with 02 ml distilled water).

Table 4: Minimum inhibitory concentrations of the leaf essential oil and major compounds

Essential oil/ Compounds	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella enterica enterica</i>	<i>Pasteurella multocida</i>
Crude oil	na	3.90	7.81	15.62
Curzerenone	15.62	na	na	na
Furanodienone	3.90	na	7.81	15.62
Standard	-	-	-	-

Minimum inhibitory concentration, values are in ML/ml.

found to be slightly effective against *E. coli* (IZ = 10.8 ± 0.52). The MIC value of the essential oil was least against *S. aureus* (MIC = 3.90 µl/ml) and that of furanodienone against *E. coli* (MIC = 3.90 µl/ml).

CONCLUSION

The oil of *L. pulcherrima* showed a potent free radical scavenging activity (low IC₅₀ value for DPPH radical, 0.087 mg/ml) and inhibition of lipid peroxidation (IC₅₀, 0.74 mg/ml). Further, the essential oil and furanodienone were found to be good antibacterial agents against one or two tested strains. The presence of furanoids in such an amount in the essential oil makes it medicinally valuable.

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