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Quantitative phytochemical analysis and antioxidant activity of three endemic species of *Litsea* Lam. Lauraceae

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Abstract
Litsea is a dominant genus in Lauraceae distributed in subtropical and tropical parts of the world. Most of the species possess significant phytoconstituents which contributes to the specific pharmacological properties applied for the curing of many diseases. The pharmacological properties like, antimicrobial, cytotoxic, and anti-inflammatory activities of many *Litsea* spp. are reported. The present study deals with the quantitative estimation of phytochemicals and free radical scavenging activity of leaf and bark of three selected species of *Litsea viz*., *L. bourdilloni*, Gamble, *L. coriacea* (Nees) Hook. f. and *L. wightiana* (Nees) Hook. f., which are endemic to Southern Western Ghats, Peninsular India, and the Western Ghats respectively. The total flavonoid estimation was carried out by Aluminium Chloride method. Total phenolic and tannin contents were estimated by Folin Ciocalteau reagent.Among bark samples, the flavonoid content was more in *L. wightiana* (32 µg/mg). Phenol, and tannin were found high in *L.bourdilloni (*79 µg/mg and 173 µg/mg respectively). When leaf samples were analyzed, the flavonoid, phenol, and tannin content were observed more in *L. wightiana* (37µg/mg, 119.14 and 294.66 µg/mg respectively). The antioxidant property of the samples was analyzed by DPPH Radical Scavenging Assay. The antioxidant property was found high in *L. wightiana* leaf (IC_{50} 30.32µg/ml) and low in *L. bourdilloni* leaf with IC_{50} 89.79µg/ml). In bark samples, there is no significant antioxidant activity. Results obtained from the present investigation indicates that the leaf extracts of *L. wightiana* Possess more phytoconstituents like flavonoids, phenols and tannins and has shown high antioxidant activity.

Keywords: *L. bourdilloni*, *L. coriacea*, *L. Wightiana*, flavonoid, phenol, tannin, DPPH assay

Introduction
The members of Lauraceae are economically very important and are the sources of perfumes, timber, medicine, fruis, spices etc. Traditional medicinal preparations were by using different parts of the plants [1]. *Litsea* Lam. is the largest medicinal genus under the Lauraceae family, which comprises more than 44 species, of which 18 are restriced in Peninsular India having pharmacological properties including antioxidant, antimicrobial, antidiuretic, cytotoxic effects, etc. The specific biological activity of these medicinal plants are due to the presence of significant secondary metabolites such as flavonoids (leaves of *L. coreana* and *L. japonica*) [2], butanolides (leaves of *L. acutivena*) [3], sesquiterpene (leaves and twigs of *L. verticillata*) [4], 1,3-diarylpropan-2-ol (bark of *L. rotundifolia*) [5], butanolide, coumarin and syringaldehyde (bark of *L. akoensis*) [6] Essential oil content in the leaves of *L. cubeba* and *L.glutinosa*, flowers and bark of *L. monopetala*, [7, 8, 9.] present in the various parts. The phytochemical screening of *L. glutinosa* bark revealed the presence of alkaloids, flavonoids, glycosides, phenols, and tannins [10]. Phytochemicals (secondary metabolites) are bioactive chemicals of plant origin. They are naturally synthesized throughout the plant body [11]. The presence of tannins and flavonoids confirmed the antioxidant property in many plants. Free radicals or highly reactive oxygen species are generated as a result of various metabolic processes in the human body. These molecules can oxidise the biomolecules like proteins, lipids and nucleic acids and can cause serious diseases like cancer, artherosclersis, emphysema, cirrhosis, etc. Antioxidants are compounds that neutrilise the reactive compounds and decrease the dangerous activity of these factors [12]. The present study is the first attempt to quantify the phytochemicals and revealing the antioxidant property of the three unexplored species of *Litsea viz*., *L. bourdilloni*, Gamble, *L. coriacea* (Nees) Hook. f. and *L. wightiana* (Nees) Hook. f.

Materials and Methods
Collection and Authentication of Plant Specimens
Conducted field explorations to Nelliampathy Hills of Nemmara Forest Division of Palakkad District, Kerala, India for the collection of leaf and bark samples of three species of *Litsea*, *viz*., *L.boudilloni*, Gamble, *L.coriacea* Hyne ex Nees (Hook.) f. and *L.wightiana* (Nees) Hook. f. The authentication of *Litsea* species was done by Dr. Robi John, Assistant Professor, BAM College, Pathanamthitta, Kerala, India.

Extraction
The collected leaf and bark samples were washed thoroughly, shade dried, powdered, and subjected to methanolic extraction by Soxhlet apparatus for 72 hours at a temperature of 60^0C. The solvent was evaporated by a rotary vacuum evaporator and the exract was separated and stored at -20^0C [13]. The calculation of extractive values was done by the fllowing formula

$$\% \text{ Extractive value} = \frac{\text{Weight of dry extract}}{\text{Weight of the plant sample taken for extraction}} \text{ x100}$$

Quantitative estimation

The quantitative phytochemical estimation of flavonoids, phenols, and tannins was conducted. Total flavonoid content was estimated by Aluminium Chloride colourimetric assay and Quercetin (20-100µg) as standard [14]. Folin-Ciocalteau method was adopted for the estimation of total phenol and Gallic acid as the standard [15]. The content of tannin in the sample estimated by Folin-Ciocalteau method by using tannic acid as the standard [16]. The concentration of phytochemicals calculated as mean ± SD. All the tests were carried out in triplicates.

Antioxidant Assay by DPPH Radical Scavenging Activity

DPPH free radical scavenging activity of selected samples was carried out [17]. DPPH reacts with antioxidant compounds, which can donate hydrogen and reduce DPPH. The change in colour (from deep violet to light yellow) was determined at 515 nm on a UV Visible Spectrophotometer. Ascorbic acid was used as standard for DPPH Assay, The ascorbic acid stock solution was prepared in distilled water (1mg/ml:w/v). A 60µM DPPH solution in methanol was produced 3.9ml of it was combined with 100µl of the test sample at various concentrations (6.25, 12, 25, 50, 100 µg/ml). The tubes were incubated in the dark for 15 minutes at room temperature and the in absorbance was measured at 515nm. Control was prepared by DPPH only without any extract. 95% of methanol was used as a blank.

Radical Scavenging activity was measured by the following formula

$$\text{Percentage of Inhibition} = \frac{(\text{Absorbance of Control at 0 min} - \text{Absorbance of the test})}{\text{Absorbance of control at 15 min}} \times 100$$

All the determinants were conducted in triplicate. IC_{50} values that is the concentration of plant extracts (µg/ml) required to decrease the radical scavenging by 50% were derived by plotting the DPPH radical scavenging activity against the concentration of extracts.

Statistical studies

Statistical analysis of Data was expressed as mean± SD and one-way ANOVA was performed, which were analysed using SPSS 20 software.

Results and Discussion

Determination of Extractive value

When bark and leaves of *Litsea* spp. were extracted with methanol, maximum extractive percent was obtained for *L. wightiana* bark (43.5%) followed by *L. bourdilloni* leaf (35.65%) All the samples yielded extract in methanol (Table: 1).

Table 1: Extractive value of leaf and bark samples of *Litsea* spp.

Sample	Weight of the Sample(g)	Weight of dry extract(g)	Extractive value (%)
L. bourdilloni leaf	20	7.13	35.65
L. coriacea leaf	20	7.4	37
L. wightiana leaf	20	4.86	24.3
L. bourdilloni bark	20	0.96	4.8
L. coriacea bark	20	2.25	11.25
L. wightiana bark	20	8.7	43.5

Quantitative estimation of Phytochemicals

The quantitative phytochemical estimation of methanolic extracts of the leaf and bark of selected *Litsea* samples *viz*., *L. bourdilloni*, *L. coriacea*, and *L. wightiana* showed that the flavonoid content was high in the leaf of *L. wightiana* (37µg/mg) and lesser in the bark of *L. coriacea* (15µg/mg). The phenol content was observed higher in *L. wightiana* leaf (119.14µg/mg) and lower in the bark of *L. coriacea* (8.14µg/mg). The higher amount of tannin recorded in the leaf of *L. wightiana* (294.66µg/mg) and a lesser amount in the bark of *L. coriacea* (30µg/mg) (Table.2).

Table 2: Comparative quantitative analysis of the methanolic extracts of the samples

Samples	Mean Concentration of Flavonoid in µg/mg of samples ± SD	Mean Concentration of Phenol in µg/mg of samples ± SD	Mean Concentration of Tannin in µg/mg of samples ± SD
L.bourdilloni bark	4±0.005	79±0.0043	173±0.0025
L.bourdilloni leaf	25±0.0046	82.42±0.0030	163.5±0.0036
L.coriacea bark	15±0.0052	8.14±0.0020	21.5±0.0032
L.coriacea leaf	29±0.0032	19.2±0.0015	30±0.0042
L.wightiana bark	32±0.0037	21.4±0.0015	30.5±0.0030
L.wightiana leaf	37±0.0041	119.14±0.0026	294.66±0.0042

Values are mean±SD,n=3;P<0.005 significant against control

Antioxidant Activity

The IC_{50} values of leaf and bark methanolic extracts have the potential to scavenge DPPH radicals according to the results of the antioxidant investigation in selected *Litsea* plants. (Table.3). In leaf samples, the antioxidant property was high in *L. wightiana* (IC_{50} = 30.32µg/ml) and low in *L. bourdilloni* leaf (IC_{50} = 89.79µg/ml). The bark samples of L. *bourdilloni*, *L. coriacea*, and *L. wightiana* show no or little antioxidant activity. The IC_{50} values obtained from DPPH Assay indicates that the leaf extracts have good antioxidant activity compared to Ascorbic acid (IC_{50} = 54.53µg/ml) as the standard. The lowest IC_{50} values indicate high antioxidant activity in the leaf of *L.wightiana.* (Table 3).

Table 3: DPPH Free Radical Scavenging activity and IC50 Values of leaves in *Litsea* spp.

Sample	Conc.µg/ml	Mean % DPPH scavenging activity ± SD	IC50 µg/ml
L. bourdilloni	6.25	5.218±0.001	89.79
	12.5	7.160±0.057	
	25	15.16±0.006	
	50	34.83±0.015	
	100	53.03±0.015	
L. coriacea	6.25	14.05±0.031	67.5
	12.5	17.41±0.020	
	25	25.99±0.052	
	50	44.52±0.011	
	100	66.54±0.073	
L. wightiana	6.25	10.16±0.01	30.32
	12.5	25.15±0.07	
	25	46.63±0.051	
	50	89.45±0.165	
	100	97.58±0.020	
Ascorbic Acid	6.25	25.18±0.095	54.53
	12.5	39.80±0.01	
	25	44.93±0.025	
	50	72.67±0.55	
	100	94.98±0.068	

The quantitative analysis of methanolic extracts of leaf samples indicated that the amount of flavonoids content was in the order *L. wightiana>L.bourdilloni>L.coriacea* and in the bark samples was in the order *L.wightiana>L.coriacea>L.bourdilloni*. The concentration of tannin in the leaves was in the order *L.wightiana> L.bourdilloni> L.coriacea* and in bark samples, it was *L.bourdilloni> L.wightiana> L.coriacea* and the amount of phenols in leaf and bark samples in the order *L.wightiana> L.bourdilloni> L.coriacea*. The antioxidant activity of plant exracts always correlated to the phenolic content. The present study also justifies the statement. The reducing power of extracts is the main indicator of antioxidant activity and is indicated by the polyphenols causing reduction [19]. In *L. floribunda* the reducing power of leaf and stem bark is caused by the presence of tannins and flavonoids [20]. The result is comparable with the antioxidant activity of four *Litsea* plants *viz., L. monopetala, L. glutinosa, L. assamica, and L. leata* [21]. The total flavonoid and tannin content was higher in leaf than the bark extracts. The phenol and flavonoid content shows a positive correlation with antioxidant activity. Four different kinds of phenolic compounds were identified in *Litsea monopetala* bark [22]. Plant flavonoids which have antioxidant activity *in vitro*,have been suggested to act as antioxidants *in vivo* in various investigations. [23, 24]. Polyphenols and flavonoids found in nature can help to reduce peroxidation, low density lipoprotein oxidation and causes artheroslerosis and heart diseases [25]. The genus according to Agrawal *et al*., [26] contains many secondary metabolites. Our research also supported the assertion. (Fig1). Many therapeutic plants have significant level of phenolic compounds and there is a positive linear relationship between total phenolic content and plant antioxidant activity. [27].

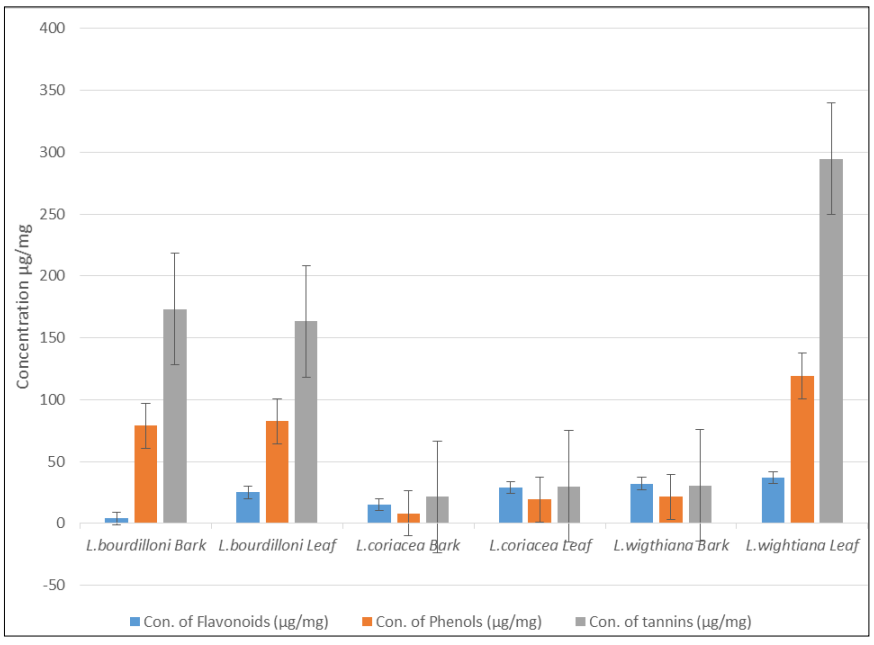

Fig 1: Quantitative Estimation of Phytochemicals in *Litsea* spp.

Conclusion

The study concludes that the data obtained from the quantitative estimation and antioxidant studies inferred that the selected *Litsea* plants contain the flavonoids, tannins, and phenols with significant antioxidant activities. These findings indicated that these plants are the potential antioxidant sources and medicinal agents of tremendous value.

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