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RESEARCH ARTICLE

LCMS/MS analysis and evaluation of anti-inflammatory and antioxidant activities of the polyphenol fraction of *Litsea quinqueflora* (Dennst.) Suresh

Sumin Mary Jose & Anilkumar M*
Department of Botany, Union Christian College, Aluva 683 102, Kerala, India
*Email: drmakumar@gmail.com

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ABSTRACT
The main aim of the work was to scientifically prove the anti-inflammatory property of the polyphenol-rich fraction of hydro-alcoholic leaf extract of *Litsea quinqueflora* (Dennst.) Suresh by protein denaturation and free radical scavenging activity. The polyphenol-rich fraction of hydro-alcoholic leaf extract was obtained via acid-alkali hydrolysis, followed by fractionation with chloroform and ethyl acetate. HPTLC profiling of the finally obtained ethyl acetate fraction and consequent derivatisation with aluminium chloride revealed the presence of flavonoids in a more purified form. LCMS/MS analysis tentatively identified the presence of bioactive polyphenolic compounds such as gallocatechin, sinapic acid, pinocembrin, paeonol and umbelliferone in the separated fraction. The polyphenol-rich fraction of hydro-alcoholic extract of leaves showed anti-denaturing activity in heat-induced bovine serum albumin denaturation with an IC_{50} value of 23.59 µg/ml and was statistically significant at 0.1% level. The antioxidant property of the polyphenol-rich fraction determined by its free radical scavenging ability against DPPH and ABTS showed IC_{50} values 122.98 and 135.44 µg/ml respectively and was also statistically significant at 0.1% level. Hence, the traditional use of *Litsea quinqueflora* as an anti-inflammatory agent can be attributed to the presence of polyphenols.

Introduction

Plant-derived medicines attracted the attention of researchers due to the presence of different phytochemicals having a multitude of health benefits to humans (1). Among these phytochemicals, polyphenols are of much importance owing to their specific biological properties. They are the organic foods produced by plants as secondary metabolites. Polyphenols are involved in the physiological and other diverse functions such as lignification, growth, predator resistance etc. (2). These inevitable plant products play an important role in human health as they regulate metabolism, weight, cell proliferation and chronic diseases (3). Biological properties such as anti-inflammatory, immunomodulatory, antioxidant, cardioprotective and anticancer activities can be attributed to polyphenols (4). The diverse structure of phenolic compounds influences the anti-inflammatory activity of a drug. Even though a variety of non-steroidal anti-inflammatory drugs (NSAIDs) are available, natural phenolic compounds are also equally good and can inhibit the pro-inflammatory mediators such as interleukins, cyclooxygenase, lipoxygenase, nuclear factors etc. Traditional healers could identify the efficacy of plant-derived compounds, and they used them widely even before the advent of allopathy drugs (5).

The genus *Litsea* belongs to the family Lauraceae, which possess various pharmacological properties and have been used in traditional medicines to treat influenza, stomachaches, inflammatory diseases, bruises, insect bites etc. (6). Phytochemical analysis of different *Litsea* species identified flavonoids and terpenoids as their major constituents (7). Flavonoids obtained from different *Litsea* species such as *Litsea cubeba* Pers., *Litsea glutinosa* (Loureiro) Robinson and *Litsea coreana* H. Lev. act as anti-inflammatory and antioxidant compounds and inhibited different inflammatory pathways (8). Flavonoids like pinocembrin chalcone and kaempferol 3,4`-di-O-L-rhamnopyranoside were isolated from the leaves of *Litsea fruticosa* (Hemsl.) Gamble through column chromatography (9). Leaves of *Litsea quinqueflora* (Dennst.) Suresh was selected as the test material in this study as its leaf paste has been frequently used by the local healers of Kerala as an anti-inflammatory drug. The anti-inflammatory property of crude methanolic extract of *L. quinqueflora* leaves was

reported using the HRBC membrane stabilisation method under induced hypotonic conditions and preliminary screening revealed the presence of different biologically active phytochemicals (10). Different leaf extracts of *L. quinqueflora* possessed flavonoids and phenols and exhibited antioxidant properties also (11). All these studies used crude extracts of leaves, but a hydro-alcoholic solvent system was reported suitable for the extraction of polyphenol compounds (12). Hence, the present investigation was aimed to isolate, characterise and evaluate the anti-inflammatory and antioxidant properties of polyphenolic fraction (PLE) of the hydro-alcoholic leaf extract of *L. quinqueflora*. A more purified fraction of the hydroethanolic leaf extract was obtained via acid-alkali hydrolysis and fractionation using chloroform and ethyl acetate. This purified extract was then subjected to HPTLC followed by LCMS/MS analysis to get a clear picture of its phytochemical background.

Materials and Methods

Collection of plant and processing

Leaves of *Litsea quinqueflora* (Dennst.) Suresh was obtained from the Kurianad area of Kottayam district, Kerala, India. The flowering and fruiting twig of the plant is presented in Fig 1. The plant specimen was identified and authenticated by Dr Sujanapal. P, Kerala Forest Research Institute (KFRI), Kerala, India. The voucher specimen was deposited in the National Herbarium of KFRI (Accession No. 13057). Leaves from female plants were separated from twigs and washed thoroughly in water, and kept for shade drying. It was then powdered and used for further studies.

Isolation of polyphenols

Fig. 1. Flowering and fruiting twig of *Litsea quinqueflora* (Dennst.) Suresh.

Extraction of polyphenol was done by the acid-alkali hydrolysis method (13), followed by fractionation with chloroform and ethyl acetate (14, 15). Powdered plant material (10 gm) was added to 100 ml 80 % ethanol followed by agitation at 200 rpm for 5 hrs at room temperature in a shaking incubator (211DS, Labnet, NJ, USA). The filtrate thus obtained was concentrated at 40 ºC in a rotary evaporator (Hei-VAP Core, Heidolph, Germany). The pH changes were made by adding NaOH and HCl. The chloroform layer was separated and discarded. Ethyl acetate fraction of the acidified extract was collected and concentrated at 110 rpm. The concentrated fraction was blended at 120 rpm for 5 hrs with 50 ml NaOH. This alkaline fraction was acidified with HCl, and the separated ethyl acetate layer was concentrated to dryness (13).

HPTLC fingerprint profiling

High-Performance Thin Layer Chromatography (HPTLC) was performed with 5 ×10 cm aluminium plates pre-coated with silica gel F_{254} (Merck, Germany). The polyphenol sample thus isolated was dissolved in methanol, and 2 µl was spotted to the pre-activated plate using automatic TLC applicator Linomat-V with continuous N_2 flow (CAMAG, Switzerland). The plate was placed in the twin glass chamber for development in the mobile phase consisting of toluene, ethyl acetate and methanol in the ratio 7:3:1. Visualisation of the oven-dried plate was done in CAMAG TLC visualiser under UV 254 nm, 366 nm and visible light. HPTLC profiles were obtained after derivatisation using $AlCl_3$ (1 % ethanolic solution of aluminium chloride) (16, 17). Densitometric scanning of these plates was carried out in CAMAG TLC scanner III using a deuterium lamp with Camag WIN CATS software.

LC-ESI-MS/MS analysis

Liquid chromatography-mass spectroscopic analysis (18) was performed with Agilent 6520 accurate mass Quadrupole – Time of Flight (Q-TOF-LC/MS) attached with Agilent LC 1200 outfitted with Extend C-18 column of 1.8 µm, 2.1 × 50 mm size. Gradient elution was carried out with methanol (A) and 0.1 % acetic acid (B) in the ratio of 70:30 at a constant flow rate of 0.8 ml/min. LC-ESI-MS analysis was conducted in ESI negative mode. The optimisation for MS conditions was: drying gas (nitrogen) flow 5 L/min; nebuliser pressure 40 psig; drying gas temperature 325 ºC; capillary voltage 3000 V; fragmentor volt 125 V; Oct RF Vpp 750 V. Collision voltage of 4 V/100 Dalton with an offset of 6V was used for the Collision Induced Dissociation (CID) in the mass fragmentation.

Anti-inflammatory assay: Inhibition of protein denaturation

Inhibition of protein denaturation was done as per the method of Mizushima and Kobayashi (19). The reaction mixture (pH 6.3) contained 0.45 ml of 1 % aqueous solution of bovine serum albumin (BSA) with 0.05 ml of leaf extracts (62.5, 125, 250, 500 µg/ml). The reaction mixture was incubated at 37 °C for 20 min, and later the temperature was raised to 57 °C for 3 min. It was then allowed to cool at room temperature. Then added 2.5 ml of phosphate-buffered saline (PBS) of pH 6.3 and measured the optical density (OD) at 660 nm using UV-Vis spectrometer (Shimadzu - UV 1800) with PBS as blank. Diclofenac sodium was used as the standard anti-inflammatory drug. Test control was mixed with distilled water instead of extract, and in product control, distilled water was added instead of BSA.

The percentage of inhibition (20) was calculated by the formula,

$$100 - \left[\frac{OD\,of\,test\,solution - OD\,of\,product\,control}{OD\,of\,test\,control} \right] \times 100$$

Antioxidant assay

The reducing power of PLE against free radicals was analysed with DPPH (1, 1 diphenyl-2-picryl hydrazyl) radical scavenging assay and ABTS [2, 2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid)].

DPPH radical scavenging assay

Evaluation of radical scavenging activity of PLE against DPPH was done with 0.1 mmol/L of DPPH based on the standard method (21). The reaction was done with 1 ml DPPH, and 0.5 ml of PLE of various concentrations (12.5, 25, 50 and 100 µg/ml) and test control was taken without sample. The reaction solution was finally adjusted to 3 ml by adding ethanol and incubated in the dark for 20 min. Ascorbic acid was used as the standard drug to compare with the test, and absorbance was measured at 517 nm.

ABTS radical scavenging assay

This decolourisation assay was done according to the method of Re et al. (22). The reaction solution was prepared by mixing 7 mM ABTS and 2.45 mM Potassium persulfate (1:1) in distilled water. It was then incubated in the dark at room temperature for 12–16 hrs. The prepared ABTS solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm. Test solution (100 µl) was prepared with different concentrations of PLE (12.5, 25, 50, 100, 200 µg/ml) and was added to 900 µl of ABTS solution and incubated at room temperature for 1 min. The absorbance was measured at 734 nm, where methanol served as blank and ascorbic acid as standard.

The antioxidant activity was calculated using the formula,

$$\left[\frac{Absorbance\,of\,control\,solution - Absorbance\,of\,test}{Absorbance\,of\,control\,solution} \right] \times 100$$

Statistical analysis

The anti-inflammatory and antioxidant assays were performed in triplicates, and results were expressed in mean with standard deviation. IC_{50} values were calculated using Microsoft Excel 2013. The inhibitory percentages obtained with each concentration of each assay were statistically analysed through one-way analysis of variance (ANOVA) followed by post hoc Tukey test using IBM SPSS statistics 25 (23, 24).

Results and Discussion

The plant phenols exist mainly in conjugated forms, either as esters or as glycosides (25). Phenolic compounds, especially flavonoids, are mainly extracted through alcoholic solvents, and further pH changes of the extracts lead to the frequent hydrolysis of flavonoids and increased the separation of the maximum amount of phenolic constituents (25). Acid and alkaline hydrolysis of the extract leads to the separation or glycosylation of phenolic glycosides and resulted in the formation of aglycones. The easy absorption and enhanced solubility of phenolic aglycones than bound forms were evaluated and proved in earlier studies in *Ginkgo biloba* L. flavonoids in rat plasma (26). Methods of extraction of polyphenols included isolation of aglycones from different polyphenol compounds such as isoflavones, flavanones, anthocyanins etc. (27). Different hydrolytic methods like heating enabled to break down glycosides and separated aglycones from soybeans (28) and grapefruit (29).

Isolation of polyphenols and HPTLC fingerprint profiling

The hydroethanolic filtrate of *L. quinqueflora* leaves after acid-alkali hydrolysis and fractionation with chloroform and ethyl acetate yielded 0.04% polyphenol fraction of extract from leaf powder. The maximum separation of polyphenols was obtained with the mobile phase toluene, ethyl acetate and methanol in the ratio 7:3:1. The Rf values ranged from 0.01–0.92. The bands prominent at 254 nm were not so prominent at 366 nm. But no bands were obtained under visible light. There were four main peaks obtained under 366 nm. Densitometry estimation revealed that Rf value 0.09 was predominant with 42 % area followed by Rf values 0.17 and 0.33. HPTLC profiling with densitometric estimation and derivatisation is shown in Fig. 2 and peak values in Table 1. Derivatisation with aluminium chloride showed prominent yellow fluorescence between Rf values 0.1 and 0.2, indicating flavonoids in the extract.

HPTLC profiling of flavonoids of stem extract of *Cissus latifolia* Lam. was done with the mobile phase Toluene: ethyl acetate: formic acid (7:3:0.1), where eight separation spots were noticed (30), and this can be compared with the five separation spots of PLE. HPTLC profiling is quality control of herbal drugs. The HPTLC analysis of hexane, ethyl acetate and ethanol extracts of heartwood and small branches of *Litsea chinensis* Lam. produced a similar banding pattern in which ethanol extract showed clearer bands than others (31). The HPTLC fingerprint profiling of flavonoids of leaf and root extracts of *Hypochaeris radicata* L. (32), fruit extracts of *Carissa bispinosa* (L.) Desf. ex Brenan, *Ficus sycomorus* L. and *Grewia bicolor* Juss. (33) used aluminium chloride as a spraying reagent in the derivatisation of plates and reported the presence of flavonoids with the indication of yellow fluorescence. Hence, it can be assumed that the yellow colouration on the HPTLC plate of PLE indicates the presence of flavonoids in a more purified form. The yellow colour fluorescence obtained after derivatisation with spraying reagent can be due to the reaction between aluminium ion, Al (III), and carbonyl and hydroxyl groups of flavones and flavonols of PLE (34).

LC-ESI-MS/MS analysis

Liquid chromatography of PLE was carried out with mass analysis in negative polarity mode. This study revealed the presence of different polyphenolic compounds identified with m/z value and fragmentation pattern. Mass bank.eu data was used for the identification of compounds with different ions having different m/z. Total ion chromatogram (TIC) was extracted to obtain the base peak chromatogram (BPC) of separated molecular ions. The ion with m/z 305.0395 $[M-H]^-$ yielded a fragment of ms/ms at m/z 131.06, 199.13, 201.1, 215.1, 245.08,

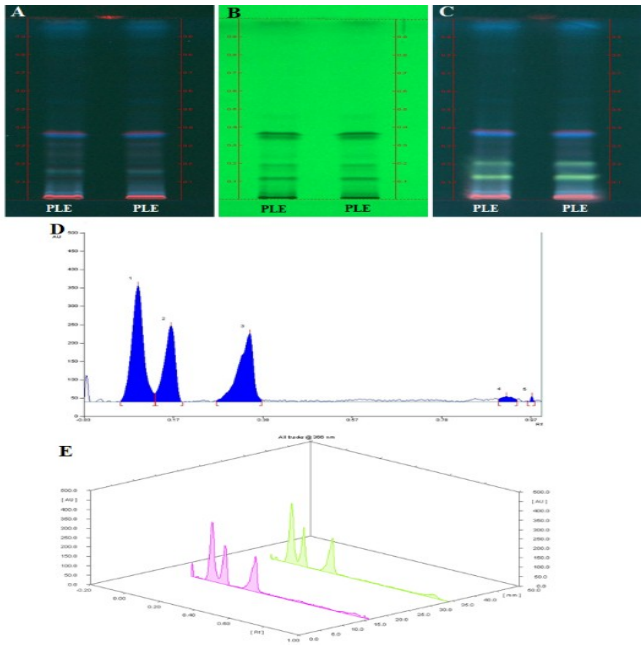

Fig. 2. HPTLC profile of PLE (A) Before derivatisation at UV 366 nm, (B) Before derivatisation at UV 254 nm, (C) After derivatisation at UV 366 nm, (D) Peak densitogram of PLE, (E) Three-dimensional representation of chromatogram.

Table 1. Peak list and Rf values of polyphenol fraction (PLE) of *Litsea quinqueflora*

Peak	Max Position (Rf)	Max Height (AU)	Max %	Area (AU)	Area %
1	0.01	16.1	2.12	141.0	0.77
2	0.09	322.8	42.57%	7742.4	42.34
3	0.17	210.7	27.78	4556.4	24.92
4	0.33	189.6	25.00	5043.8	27.58
5	0.92	19.2	2.53	801.1	4.38

which was tentatively identified as gallocatechin. Fragments obtained from m/z 135, 149.04, 179.12 and 223.06 point to the presence of sinapic acid with m/z 223.034. The presence of pinocembrin, a polyphenol, was identified by the ion with m/z 255.25 and corresponding fragments with m/z 106, 115, 125 and 255. The mass fragment pattern of the ion with m/z 165 showed m/z 119, 121 and 122 and denoted the polyphenol paeonol. The ion with m/z at 161 showed a mass fragment pattern of m/z 117 and 161 which can be recognised as umbelliferone (7- hydroxyl coumarin). The mass value and fragments of identified compounds are shown in Table 2 and the spectrum of each compound is displayed in Fig. 3.

The LCMS analysis revealed the presence of different polyphenolic compounds, which were considered natural anti-inflammatory agents. The ion with m/z 305.0395 $[M-H]^-$ was assumed as gallocatechin, a flavonol categorised under the flavonoid class of polyphenols (35). LC-ESI-MS/MS analysis of phenolics of different plants belonging to the family Lamiaceae reported the same m/z value for gallocatechin (36). Gallocatechin was identified in *Litsea glaucescens* Kunth through LCMS/MS analysis of its infusion and fermented beverage (37). Catechins are considered to be good antioxidant agents, and it was proved in the study of bark extracts of *Quercus sideroxyla* Humb. & Bonpl. (38).

Table 2. List of compounds tentatively identified by LCMS/MS analysis

Sl. No	m/z [M-H]	MS/MS	Tentative identification
1	305.0391	131.06, 199.13, 201.1, 215.1, 245.08	Gallocatechin
2	223.0339	135.12, 149, 179.1, 223.07	Sinapic acid
3	255.246	106.58, 117, 125, 255.24	Pinocembrin
4	165.0314	119.0626, 121.04, 122.04	Paeonol
5	161.0569	117.06, 161.0575	Umbelliferone

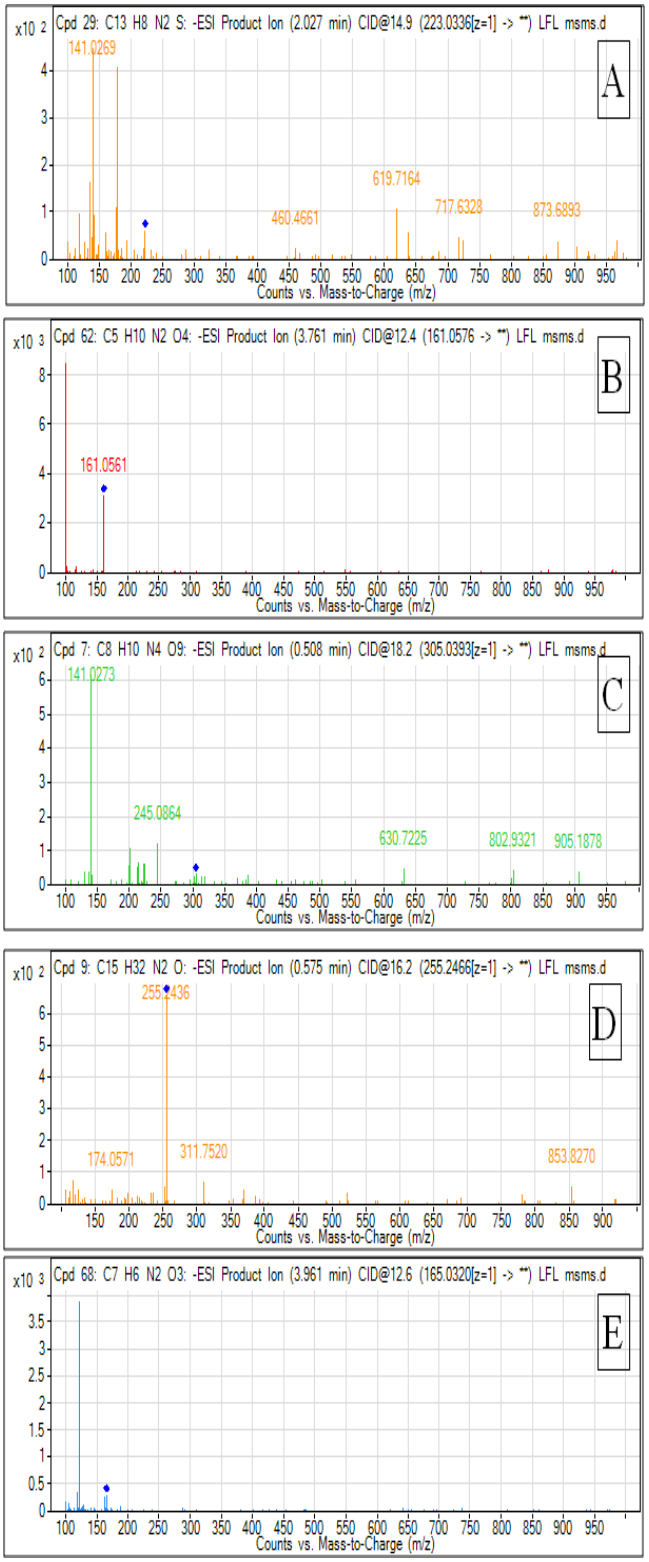

Fig. 3. LCMS spectrum indicated the presence of (A) Sinapic acid, (B) Umbelliferone, (C) Gallocatechin, (D) Pinocembrin, (E) Paeonol.

Mass fragments of m/z value 223.034 $[M-H]^-$ can be identified as sinapic acid (39) by referring to the LCMS studies on phenolics of red wine (40) and fruits of *Melicoccus bijugatus* Jacq. (41). Sinapic acid is a polyphenol compound coming under the class phenolic acids and subclass hydrocinnamic acid and reported as an antioxidant, anti-inflammatory, antimicrobial and antianxiety agent prevalent in medicinal plants (42). The presence of sinapic acid was noted in methanol extract of fruits of *Litsea cubeba* (Lour.) Pers., which is effective against free radicals, DNA damage and toxicities (43).

Flavonol pinocembrin, a member of class flavonoids of polyphenols, was tentatively identified in PLE with mass value 255 $[M-H]^-$ (Pinocembrin mass spectrum) (44). MS/MS analysis of propolis (natural resin) also identified pinocembrin with the same m/z value (45), and HPLC-PDA-ESI-MS analysis of three *Nolana* species displayed its $[M-H]^-$ as 255 and revealed its antioxidant activity (46). Pinocembrin and pinocembrin chalcone were isolated and identified from leaves of *L. fruticosa* (Hemsl.) (47). The ion with 165 $[M-H]^-$ was identified as paeonol, a polyphenol compound and fragments were identified with European MassBank (48). MS analysis of the dried root of *Paeoniae suffruticosa* Andr. (49) and an anti-Parkinson botanical drug DA-9805 confirmed the m/z value of paeonol (50). Commercially obtained paeonol was proved as an anti-inflammatory, antioxidant and anti-apoptotic agent in rats (51). The ion with $[M-H]^-$ 161 was identified as umbelliferone (7- hydroxyl coumarin) using the mass bank (52). MS/MS analysis of extracted phenolic compounds of fruits of *Rhus coriaria* (L.) Sumac reported the presence of umbelliferone with $[M-H]^-$ 161 (53) and roots and stem bark of *Aegle marmelos* Correa. obtained a mass value of $[M+H]^+$ as 163 (54). Umbelliferone was isolated from the rhizome of *Potentilla evestita* L. and evaluated its anti-inflammatory and antinociceptive properties in animals (55). Hence, the tentatively identified polyphenolic compounds in the present study are efficient biomolecules with antioxidant, anti-inflammatory, anti-apoptotic, antinociceptive, antimicrobial and antianxiety activities and can be considered the contributing factor for the traditional use of *L. quinqueflora* leaves.

Anti-inflammatory assay: Inhibition of protein denaturation

Polyphenol portion of leaf extract showed inhibition against protein denaturation, a well-documented process during inflammation. The isolated phenolic portion (PLE) showed an increase in inhibitory percentage with an increase in concentration and was statistically significant at 0.1% level ($p<0.001$) (Table 3). It inhibited protein denaturation with an IC_{50} value of 23.59 µg/ml, almost near the standard drug diclofenac.

Denaturation of proteins can be induced due to different stimuli such as heat, acid, alkali, detergents, alcohol etc. (56). In the present investigation, heat was used as a stimulus. Denatured protein can act as an antigen, affect our system's immunity, and lead to chronic inflammatory conditions like rheumatoid arthritis (57) and amyloidosis (58). Recent studies in ethanolic fraction of fruits of *Spondias mangiferra* Willd. and aqueous extract of bark of *Ficus benghalensis* L. reported protein denaturation under *in vitro* conditions (59, 60). Denaturation of proteins is as a serious cause of rheumatoid arthritis and other inflammatory conditions and their activity against protein denaturation considered them as anti-arthritic drugs. Hence, a drug with anti-denaturant activity can be considered an anti-inflammatory agent. Many plant extracts exhibit anti-denaturant activity, where polyphenols, especially flavonoids, are mainly considered as anti-inflammatory agents (61). Anti-inflammatory study of a polyphenol-rich extract of *Petroselinum crispum* (Mill.) Fuss, *Apium graveolens* L. and *Coriandrum sativum* L. showed 50 % inhibition of protein denaturation at 118, 227 and 247 µg/ml concentrations, respectively (62). Comparing those IC_{50} values with PLE depicted the better efficacy of polyphenolic fraction of leaf extract of *L. quinqueflora* as an anti-inflammatory agent. The inhibitory potential of PLE in protein denaturation assay was noticeably higher than that of sequentially isolated leaf extracts (63) and silver nanoparticles capped with methanolic extract (64) of *L. quinqueflora*. So, polyphenols can be considered as more potent phytoconstituent of *L. quinqueflora.* Phenolic compounds are demonstrated as thermal stability enhancers of proteins. Protein-phenol interaction enabled proteins to withstand heat-induced denaturation for a certain time (65). This corroborated the anti-denaturation activity of PLE, especially the polyphenol compounds present in PLE and thus the anti-inflammatory activity.

Table 3. Percentage of inhibition of protein denaturation by polyphenol fraction (PLE) of *Litsea quinqueflora*

Concentration	PLE	DS
62.5	48.58±1.44***	39.18±1.54***
125	60.36±1.49***	74.02±1.19***
250	77.59±1.55***	83.48±1.35***
500	90.2±1.14***	93.83±1.63***

***P≤0.001. Values represent mean ± standard deviation of triplicate experiments.

Antioxidant properties

Polyphenol portion of PLE exhibited effective radical scavenging activity against free radicals. The IC_{50} value in the DPPH assay was 122.98 µg/ml, and the ABTS assay was 135.44 µg/ml. The gradient of radical scavenging activity is proportional to the increase in concentration. The inhibitory percentages obtained with different concentrations of DPPH (Fig. 4) and ABTS (Fig. 5) were statistically significant at 0.1% level ($p<0.001$) (represented with asterisks).

The plant extracts with polyphenol contents exhibit good radical scavenging activities. The polyphenolic extracts of leaves of *Ichnocarpus frutescens* (L.) R.Br. inhibited free radicals at IC_{50} value of 163.38 µg/ml (66), and polyphenol-rich extract of fruits of *Mallotus philippensis* (Lam.) Müll.Arg. showed effective scavenging activity against ABTS and DPPH radicals (67). The radical scavenging activity of PLE was found to be higher than these earlier reports on other plants. Bark and leaf extracts of *Litsea glutinosa* (Lour.) Robinson,

Litsea monopetala (Roxb.) Persoon, *L. assamica* Hook. f. and *L. laeta* (Nees) Hook. f. reported the

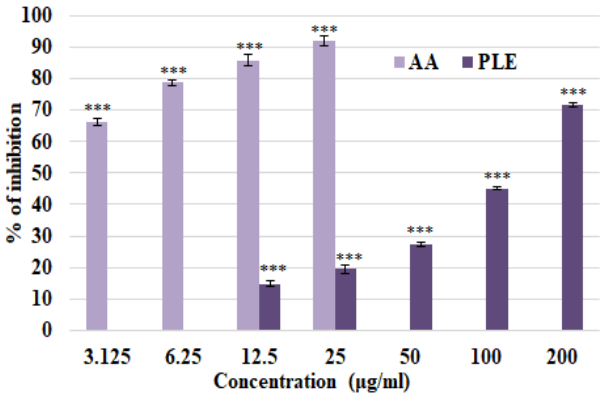

Fig. 4. Percentage of inhibition of ascorbic acid (AA) and polyphenol fraction of leaves of *L. quinqueflora* against DPPH with values represent mean ± standard deviation of triplicate experiments. ***p≤0.001.

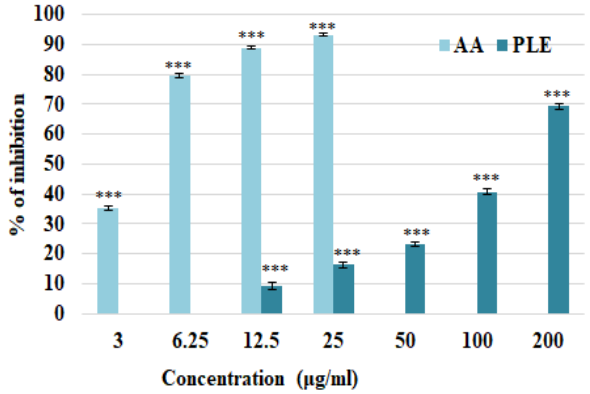

Fig. 5. Percentage of inhibition of ascorbic acid (AA) and polyphenol fraction of leaves of *L. quinqueflora* against ABTS with values represent mean ± standard deviation of triplicate experiments. ***p≤0.001.

antioxidant activity, especially the scavenging activity against DPPH free radical (68). Based on IC_{50} value, the antioxidant activity of the polyphenol fraction of *L. quinqueflora* was higher than that of these four species. When compared with the free radical scavenging activities of root extract of *Litsea martabanica* (Kurz) Hook.f. and stem bark extracts of *Litsea petiolata* Hook.f. (69,70), PLE exhibited less inhibitory activity against free radicals. Polyphenol compounds isolated from leaves of *L. quinqueflora* can be considered as a potential natural antioxidant source. The hydroxyl groups present in the phenolic constituents of plant extract assumed to be the active centre behind the radical scavenging activity.

Conclusion

Hydro-alcoholic extract of *L. quinqueflora* (Dennst.) Suresh leaves yielded polyphenols in the form of aglycones. They exhibited higher anti-inflammatory and antioxidant activities than that of crude extracts, as evidenced by previous publications. The compounds present in the polyphenol fraction were tentatively identified as gallocatechin, sinapic acid, pinocembrin, paeonol and umbelliferone and are known for their anti-inflammatory property. Polyphenol extract of *L. quinqueflora* exhibited anti-inflammatory and antioxidant properties in protein denaturation and free radical scavenging assays, respectively and the results were statistically significant. This provides strong support for its dose-dependent inhibitory activity. Hence, the present investigation can be assumed as preliminary scientific evidence for the traditional use of *Litsea quinqueflora* leaves as an anti-inflammatory drug.

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Authors' contributions

AM designed the experiment and corrected the manuscript. SM carried out the research and analysis part. Both authors read and approved the final manuscript.

Conflict of interests

The authors report no conflicts of interest.

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