ORIGINAL ARTICLE

In Vitro and *In Silico* Evidence for a Putative Role of *Lannea coromandelica* Bark's Methanolic Extract in the Treatment of Ulcerative Colitis

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Abstract

Introduction: Ulcerative colitis (UC) is a type of persistent inflammatory bowel disease that creates considerable challenges to its treatment with regard to both the lack of existing medicine efficacy and the raise in side effects. **Aim and Objectives:** The present paper discusses the *in vitro* anti-inflammatory, antioxidant, and antimicrobial properties of the methanol extract of *Lannea coromandelica* bark (LcBME) to manage UC. **Methodology:** The LcBME that was prepared was tested using gas chromatography-mass spectrum (GC-MS) determination of phytoingredients with previous screening to identify the presence of major phytochemicals in the LcBME. Further, the extract was subjected to membrane stability, protein denaturation, and inhibition of albumin denaturation studies to evaluate the anti-inflammatory activities and 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical scavenging assays to examine its antioxidant capacities. A resazurin microtiter method was followed to determine the minimal inhibitory concentration (MIC) effect against *Escherichia coli*. **Results and Discussion:** Flavonoids, diterpenes, and steroids were present in the LcBME. GC-MS analysis found several compounds, including 3-Methylene-1,6-heptadiene, phenol, 3-methyl, 2-ethyl alpha, 3-bis(acetyloxy) benzeneacetic acid, 3-pentadecanone, E,E-6,8-tridecadien-2-ol, acetate, undecane, furfural, 2-heptanone, and 5-methoxy-methyl ester. During anti-inflammatory studies, 500 µg/mL of the sample has membrane stability activity, protein denaturation inhibition, and inhibition of albumin denaturation activity up to 58.5%, 65%, and 62.5%, respectively. Similar concentration has 100% of DPPH activity and 45.6% of hydroxyl radical scavenging activity. In the resazurin microtiter assay, the extract produced an MIC at 125 µg/mL against *E. coli*. In a molecular docking study among the 9 GC-MS compounds based on the absorption, distribution, metabolism, excretion, and toxicity property, one compound (Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester) demonstrated the strongest binding affinity, with a binding energy of −5.66 kcal/mol. **Conclusion:** These findings suggest that LcBME contains significant bioactive chemicals and possesses potent anti-inflammatory, antioxidant, and antibacterial properties, making it a promising candidate for UC treatment after *in vivo* studies.

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INTRODUCTION

The kind of inflammatory bowel disease called ulcerative colitis (UC) belongs to the class of idiopathic inflammatory disorders and is known by chronic inflammation of the colonic mucosal layer.[1,2] Affecting millions around the globe, UC has a huge quality of life impact with the manifestation of symptoms like consistent abdominal discomfort, diarrhea, and rectal bleeding.[3,4] At present, available treatments, which include corticosteroids, immunosuppressants, and biologics, are effective in controlling the feelings; however, they do have some harsh side effects and are expensive as well.[5,6] In addition, these interventions are mostly aimed at relieving symptoms such as distress, instead of changing the causative cellular/molecular pathways of the condition. As a result, there is a substantial demand for safer and less expensive treatment alternatives that work on specific molecular targets. The ones that have shown some promise include the use of some natural products for the treatment of UC by focusing more on their antioxidant and anti-inflammatory properties.[7,8] Specifically, medicinal plants are promising agents because they are effective with a wide range of bioactive compounds. *Lannea coromandelica* is among those plants with rich bioactive compounds and has been ethnically used for the treatment of various types of gastrointestinal (GI) disorders, as it has the capacity to act as both an antioxidant and anti-inflammatory.[9,10] The bark extract of *L. coromandelica* has also been reported previously to have anti-inflammatory and antioxidant properties in *in vivo* models.[11] Yet its purported benefit as an anti-inflammatory does remain unexplored in detail, especially in regards to its efficacy in managing UC.[12,13] The available studies indicate the bioactive effectiveness of *L. coromandelica* in the inhibition of oxidative, anti-inflammatory, and microbial agents.[7] These activities, particularly oxidative stress and inflammation, warrant the need for more research on *L. coromandelica* for the remedy for UC and other inflammatory disorders. In terms of direct relationships of *L. coromandelica* to the treatment of UC, this remains unclear. In this respect, however, the several reports of the plant suggest that it could be helpful when combined with standard therapies or may even replace them. Further research investigation is needed to pinpoint the particular compounds responsible for these effects and to correlate and analyze their clinical effects managing UC. Hence, this study planned to determine the therapeutic efficacy of *L. coromandelica's* bark extract. In particular, it seeks to assess its antioxidant, anti-inflammatory, anti-bacterial, and molecular docking as a basis for its possible therapeutic use. In this regard, the research is intended to fill that gap by arguing for establishing a scientific rationale for the proposed future use. The resulted outcome of this study may offer a basis for the use of *L. coromandelica* for UC in a whole new way.

MATERIALS AND METHODS

Collection, authentication, and extraction of sample

The plant *L. coromandelica* (Houtt. Merrill) (common name: Indian Ash tree) was identified on the campus of Guru Nanak College in Velachery, Chennai, TN, India. (Latitude: 12.990822; Longitude: 80.219993). The bark specimen collected from the plant received authentication from the Central Ayurveda Research Institute, Ministry of AYUSH, Government of India, Chennai (letter number: F.NO: 1-29A/STP/CSMCARI/2022-23/864). Dated: March 07, 2023) [Figure 1]. The bark sample underwent drying in a hot air oven before being ground into coarse powder using a mixer grinder. 100mL of MeOH (methanol) was mixed with a total of 10 g of Indian ash tree powder and kept for 6 h of extraction using a Soxhlet apparatus. Following the extraction process, the methanol extract of *Lannea coromandelica* bark (LcBME) was sent through sterile Whatman filter paper, and the resultant content was subsequently dried in a desiccator. The sample was named LcBME. The LcBME yielded a final percentage that was calculated by,

$$\text{Percentage yield (\%)} = \text{plant extract (mL)/raw materials (g)} \times 100^{[14]}$$

Qualitative analysis of phytochemicals

Freshly prepared methanolic (crude) LcBME was subjected to qualitative determination of essential phytochemicals such as carbohydrates, proteins, alkaloids, saponins, flavonoids, and diterpenes in accordance with George and Shanmugam.[14]

Alkaloid

Two to three drops of Wagner's reagent were applied to 2 mL of the LcBME. Brown or crimson precipitate shows the alkaloids.

Flavonoid

- Lead acetate test: 1–2 drops of a 10% lead acetate solution were mixed with 10 mg of LcBME. A yellowish color precipitate indicates the presence of flavonoids.

- Alkaline test: 1–2 drops of diluted sodium hydroxide were added to a 1–2 mL extract in a test tube. The extract will turn an intense yellow color. Include a few drops of diluted acid in the extract. The extract will turn colorless.

Steroids

1 mL of LcBME was taken; green color forms when 10% concentrated H_2SO_4 is added.

Diterpenes

2 mL of the LcBME was allowed to kept with 1 mL of aqueous copper acetate solution and examined for the formation of emerald green color.

Saponin

1 mL of LcBME was dissolved with 2 mL of H_2O and observed for foaming appearance after vigorous shaking.

Protein

- Biuret test: Detects proteins through peptide bonds. A violet or red color indicates the presence of proteins.
- Ninhydrin test: identifies amino acids and proteins through the -NH_2 present in their structures, pink or purple color formation while adding a few drops of ninhydrin solution.

Carbohydrate

- Fehling's test: Fehling's solution 5 mL mixed with 0.5 mg of LcBME, then warmed up in a water bath. Yellowish or red precipitate indicates a positive result.
- Benedict's test: Benedict reagent 5 mL mixed with 0.5 mg of LcBME, then placed in a water bath. Red, yellow, or green precipitate confirms the presence of reducing sugars.

Quantitative estimation of protein

The amounts of protein content of LcBME were determined by Lowry's method.[15,16] The standard used was Bovine Serum Albumin. The optical density (OD) value was read at 660 nm.

Quantitative estimation of total flavonoid (TF)

The TF content of LcBME was quantified as per Acharya *et al*.[17] In brief, different concentrations of samples and standards (quercetin) were taken. To this, 75 µL of 5% $NaNO_2$ (sodium nitrite) was mixed well. 150 µL of 10% $AlCl_3$ (aluminum chloride) was mixed after 5 min. Later, 750 µL of 1M NaOH (sodium hydroxide) was added and diluted with 2.5 mL distilled water. The quercetin was considered as a standard, and the final OD was read at 510 nm.

Gas chromatography-mass spectrum (GC-MS) analysis

The bioactive components available in the LcBME were determined through GC-MS analysis based on the protocol outlined by Enema *et al*.[18]

Anti-oxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH free radical scavenging test helps to measure the % radical scavenging activity (%RSA) to identify the antioxidant activity. Butylated hydroxytoluene was used as a control. At 517 nm, absorbance was measured 30 min after the process began. The formula below was used to compute the percentage of scavenging activity.

$$\% \text{ Antioxidant activity} = \{(\text{abs of blank}) - (\text{abs of test})/(\text{abs of blank})\} \times 100$$

A line equation was obtained from plotting concentration versus inhibition % to calculate the IC_{50}. All analyses were performed in triplicate.

Hydroxyl %RSA

The activity of scavenging of hydroxyl radicals by LcBME was tested based on the technique previously published by Wenli *et al*.[19] In this test, A standard was ascorbic acid, and the final reading was recorded at 532 nm.

Figure 1: Collection, authentication, and extraction of *Lannae coromandelica*

Inhibition (%) = (absorbance control – abs sample)/ (absorbance control) × 100

A line equation was obtained from plotting concentration versus inhibition % to calculate the IC_{50}. All analyses were performed in triplicate.

Anti-inflammatory activity

Membrane stabilizing property

The percentage of anti-inflammatory activity of LcBME was assessed utilizing its membrane-stabilizing property using methodology outlined by Navale *et al*.[20] The final absorbance values were measured at 560 nm. The standard was diclofenac (1 mg/mL), and the control is distilled water. The below mentioned formula was used to compute the percentage of membrane-stabilizing property.

% of Protection = 100 - [(OD of sample/OD of Control) × 100]

Protein denaturation inhibition assay

The protein denaturation inhibition method was performed according to Chandra *et al*.[21] Different concentrations of LcBME were filled in test tubes and made up to 11 mL with phosphate-buffered saline (pH 6.4). 4 mL of egg albumin was added to all the tubes, including the control tube. Then the solution was kept at 37°C for 15 min, followed by heating at 72°C for 5 min. Then the incubated samples and the control were allowed to cool for 10 min, and the absorbance values were recorded at 660 nm. Diclofenac (1 mg/mL) used as standard.

The following equation was used to calculate protein denaturation inhibition:

% of Inhibition = (absorbance of control – absorbance of sample/absorbance of control) × 100.

Inhibition of albumin denaturation

The proportion of anti-inflammatory action by means of albumin denaturation inhibition of LcBME was evaluated as prescribed by Dharmadeva *et al*.[22] At 660 nm, the OD value was measured. Diclofenac (1 mg/mL) acts as a control.

Following the below formula, the inhibition of albumin denaturation (%) was determined:

Inhibition (%) = (absorbance of control – absorbance of sample/absorbance of control) × 100

Determination of minimum inhibitory concentration (MIC)

To determine the least concentration of LcBME that inhibited *Escherichia coli* growth, the resazurin microtiter test was adopted. The MIC of the extract is represented as µg.[23]

Molecular docking

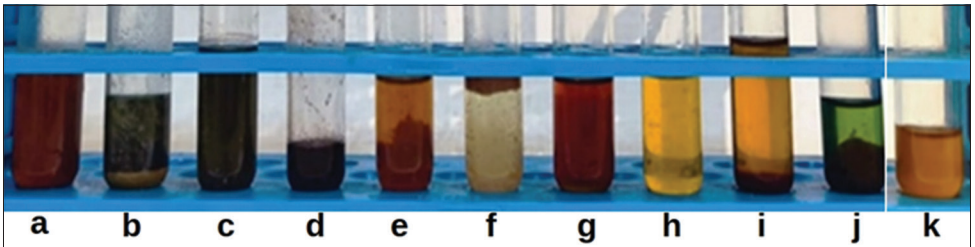
To conduct molecular docking, compounds identified through GC-MS analysis were initially evaluated for pharmacodynamic properties (absorption, distribution, metabolism, and excretion [ADME] characteristics) and compliance with Lipinski's rule of five. Bioavailability scores, P-glycoprotein, GI absorption, blood-brain barrier (BBB) permeability, logS, logP, hydrogen bond donors, hydrogen bond acceptors, topological polar surface area, and substrate properties were analyzed using Swiss-ADME and Data Warrior software. In addition, mutagenicity, tumorigenicity, and skin irritability potentials of the compounds were assessed using Data Warrior. Based on these preliminary evaluations, three compounds were selected for further analysis: Compound 1 (E, E-6,8-Tridecadien-2-ol, acetate), Compound 2 (Undecane), and Compound 3 (Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester). Their 3D structures were recovered from the PubChem database in structure data file format and reformed into protein data bank (PDB) format using OPEN BABEL software.

In this study, the target receptor protein was cyclooxygenase-2 (COX-2) (PDB ID: 1CX2). This protein's 3D structure was PDB formatted from the Research Collaboratory for Structural Bioinformatics PDB. Molecular docking of the selected ligands with the target protein was carried out using AutoDock 4.2, employing the Lamarckian genetic algorithm. Protein preparation implicated pull out water molecules, put on polar hydrogen atoms, and designates Kollman charges. The ligands were optimized for torsional flexibility, and a docking grid with dimensions of 12Å × 12Å × 12Å was established. Key docking results, including binding sites, binding energy, Van der Waals interactions, the number and distances of hydrogen bonds, and the involved amino acid leftovers, were examined using BIOVIA Discovery Studio Visualizer.[24]

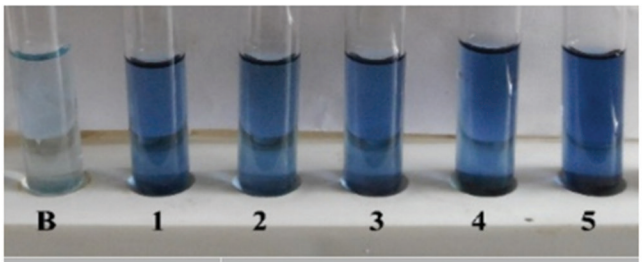
RESULTS AND DISCUSSION

Screening of phytochemicals

The present study proclaimed that the yield percentage for the LcBME was 10.44%, and Figure 2a confirmed the existence of phytoingredients. The qualitative phytoingredients analysis discloses the inhabitance of significant phytochemicals such as flavonoids, diterpenes, and steroids, which are known for their antioxidant and anti-inflammatory and properties. The extract also contains glucose and protein. Quantitative analysis showed a total protein content of 670 µg/500 µL of extract [Figure 2b] and a flavonoid content of 160 µg quercetin equivalents (QE)/500 µL of extract [Figure 2c]. In a previous study, Kumar and Jain have screened the TF (29.1 ± 0.08 mg QE/g) leaf portion of *L. coromandelica* leaf.[25] Previous study elucidated the structure of dihydroflavonols, (2R,3S)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol



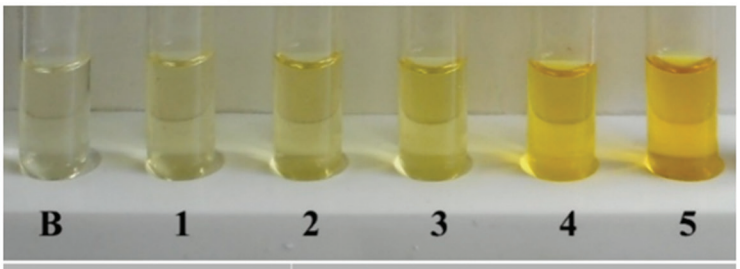
a



b

LcBME Sample	Concentration (µg/ml)				
	100µg	200µg	300µg	400µg	500µg
Protein Concentration (µg)	260	340	380	490	670

' B = Blank (without sample)



c

LcBME Sample	Concentration (µg/ml)				
	100µg	200µg	300µg	400µg	500µg
Flavonoid Concentration (µg)	50	75	100	120	160

* B = Blank (without sample)

Figure 2: (a) Presence of a. Alkaloid, b and c. Carbohydrate, d. Terpenoids, e and h. Proteins, f and i. Flavonoids, g. Steroids, j. diterpenes, k. saponins. (b) Quantitative estimation of proteins. (c) Quantitative estimation of total flavonoids

and (2R,3R)-(+)-4′,5,7-trimethoxydihydroflavonol, which were isolated from *L. coromandelica's* stem bark.[26] These previous study results confirm the presence of flavonoids in LcBME. Mya *et al*. studied indigenous Myanmar medicinal plants (*Carissa carandas, Heliotropium indicum, Hibiscus cannabinus,* and *Terminalia chebula*) to check their antioxidant activities.[27] All these extracts contain 36.05–184.2 mg rutin equivalent g^{-1} of flavonoid, where they showed excellent antioxidant activity.

GC-MS analysis

In the GC-MS examination of the LcBME, it was found that a number of bioactive compounds were present that had their pharmacological properties. The nine compounds identified were based on their retention time and mass spectral identifications with the standards of the National Institute of Standards and Technology library regulations [Figure 3]. The 3-methylene-1, 6-heptadiene, the main bioactive constituent, has been well-reported to have anti-inflammatory and antioxidant properties, and this compound aligns with the therapeutic objectives of treating UC. Further supports the extract's potential in reducing the oxidative stress by modulating immune responses. These findings suggest that the therapeutic potential of the extract can be associated with the synergistic effects of its bioactive constituents, particularly those with established roles in reducing oxidative stress and inflammation, which are imperative in the pathophysiology of UC.

Antioxidant activity

DPPH assessment

DPPH assessment is an analytical method that helps to measure the lipid-soluble antioxidants of a compound or plant extract. DPPH is an organic compound that is known as a stable free radical that has a dark blue color that turns colorless when lipid antioxidants reduce it. Protecting our

body against degenerative illnesses may be possible with the help of antioxidants, which neutralize reactive free radicals and stop other molecules from being oxidized. In this work, we studied the radical scavenging capabilities of LcBME by seeing how well it neutralized DPPH radicals. In this study, the %RSA of LcBME was dose-dependent. At 100 µg/mL, the extract achieved 38.9 ± 1% inhibitions, which increased to 100% at 500 µg/mL [Figure 4]. The IC_{50} value of LcMBE was observed to be 37.68 µg/mL. The antioxidant properties of plants may be due to the flavonoids found in the bark extract. The outcomes were highly in agreement with the determinations of Kumar and Jain's study on the total antioxidant capacity of scavenging activity by the crude methanolic acetate extract of the leaf of *L. coromandelica*.[25] The leaf extract significantly elevated the levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and GSH-peroxidase (GPx) levels and decreased malondialdehyde ratio in the liver and kidney of Ccl_4-intoxicated rats, which indicates its antioxidant activity. In biological systems, lipid peroxidation is the most significant form of oxidative radical damage due to its interaction with ferroptosis and its involvement in secondary damage to other biomolecules, including proteins.[28] The significant DPPH %RSA of LcBME highlights its potential to mitigate oxidative stress. This indicates that it can be used as a natural antioxidant in such disorders as UC, where oxidative damage is a significant determinant of disease severity.

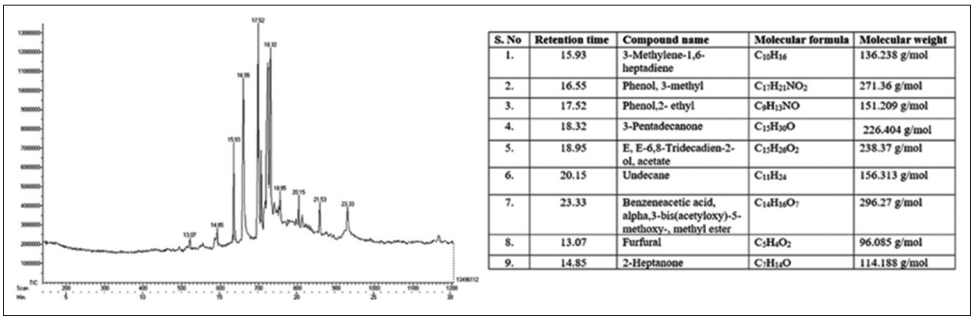
Hydroxyl %RSA (HRS)

The HRS activity assay evaluates the antioxidant power of a compound or extract to counteract the oxidative damage of hydroxyl radicals that are really reactive species with high reactivity. The HRS activity of LcBME was assessed using the deoxyribose degradation method. This assay evaluates the capacity of the LcBME to inhibit hydroxyl radicals generated through the Fenton reaction. Figure 5 confirming the %RSA of LcBME is dose-dependent. At 100 µg/mL, the LcBME exhibited 31 ± 1% inhibition of hydroxyl radicals, which increased to 75 ± 5% at 500 µg/mL. The values of the percentages of RSA of the bark extract were comparatively higher, by a factor of 2.3, than that of ascorbic acid, but the capability of the extract to have more than 80 distribute inhibition at a high concentration reveals its effectiveness in neutralizing the existence of hydroxyl radical.[29] This suggests the presence of potent antioxidant compounds within the extract. *L. coromandelica* (Houtt.) Merr. is held for treating cardiovascular issues and diabetes in some ethnic populations in Asian countries. LcBME also up-regulates the levels of mRNA and protein of CAT, SOD1, and GPx-1 and phase II detoxifying enzymes (heme oxygenase 1) through the up-regulations of the Nrf-2-mediated pathway in monocyte/macrophage-like RAW 264.7 cells.[30,31] The strong RSA of LcBME underscores its potential to mitigate oxidative stress by neutralizing one of the most damaging reactive oxygen species.[32] Such effects also enhance its therapeutic potential on conditions associated with oxidative stress, for example, UC.

Anti-inflammatory activity

Membrane stabilizing

The membrane-stabilizing property assay determines the capability of an extract to inhibit the lysis of erythrocyte



S. No	Retention time	Compound name	Molecular formula	Molecular weight
1.	15.93	3-Methylene-1,6-heptadiene	$C_{10}H_{16}$	136.238 g/mol
2.	16.55	Phenol, 3-methyl	$C_{13}H_{21}NO_2$	271.36 g/mol
3.	17.52	Phenol,2- ethyl	$C_9H_{13}NO$	151.209 g/mol
4.	18.32	3-Pentadecanone	$C_{15}H_{30}O$	226.404 g/mol
5.	18.95	E, E-6,8-Tridecadien-2-ol, acetate	$C_{15}H_{26}O_2$	238.37 g/mol
6.	20.15	Undecane	$C_{11}H_{24}$	156.313 g/mol
7.	23.33	Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester	$C_{14}H_{16}O_7$	296.27 g/mol
8.	13.07	Furfural	$C_5H_4O_2$	96.085 g/mol
9.	14.85	2-Heptanone	$C_7H_{14}O$	114.188 g/mol

Figure 3: Gas chromatography-mass spectrum profiling of *Lannea coromandelica* bark extract

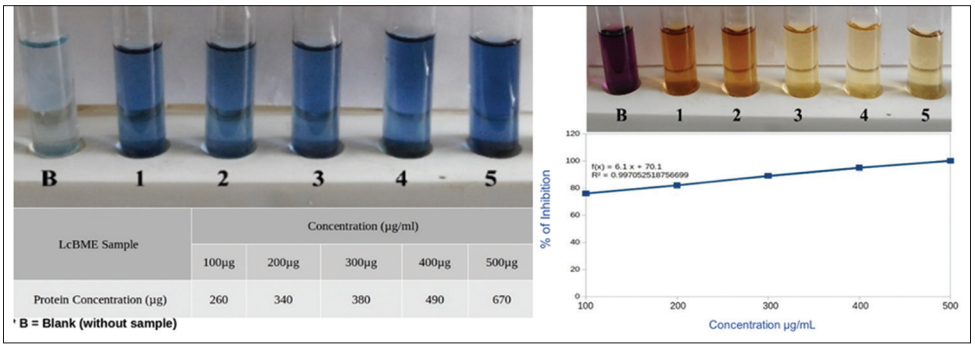


Figure 4: 2,2-diphenyl-1-picrylhydrazyl assay

membranes under stressful conditions, such as the stress needed to stabilize the lysosomal membranes during inflammation. Stabilization of the membrane of LcBME was determined by knowing the sensitivity of the human erythrocytes in the hypotonic solution-induced hemolysis. The extract stabilizes the erythrocyte membranes, which means that it can stabilize lysosomal membranes and thus lead to the reduction of proinflammatory mediators released during disease goods. The bark extract also showed a dose-response stabilizing effect of the membrane, with 24 ± 2% inhibition of the hemolysis at 100 ug/mL and 53 ± 6 at 500 ug/mL [Figure 6]. Diclofenac sodium is regarded as a standard. The percentage of inhibition is very great at the higher concentrations, which indicates that LcBME has huge potential to stabilize the membrane. The transition is most likely to be attributed to the existence of bioactive compounds that enhance the integrity of the membrane under hypotonic stress. The outstanding capability of LcBME to stabilize the lysosomal membranes by preventing the damage and inflammation of the cells is evidenced by its excellent membrane-stabilizing effect. It justifies its use in the treatment of inflammatory diseases, including UC, in which lysosomal destabilization contributes to the tissue damage.[33]

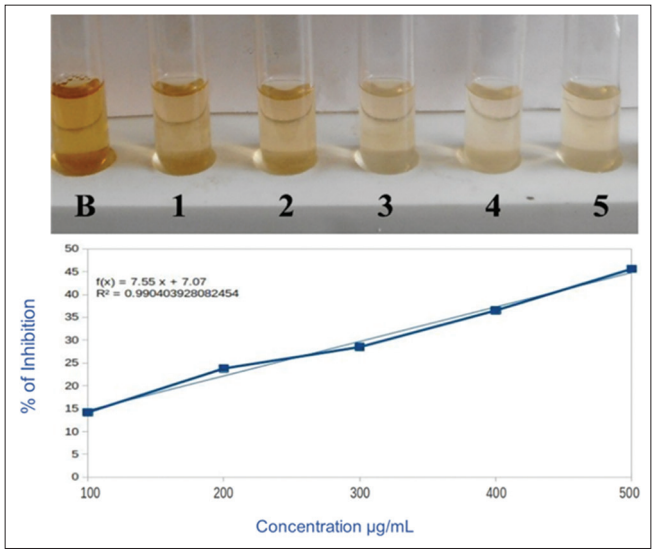


Figure 5: Hydroxyl % radical scavenging activity assay

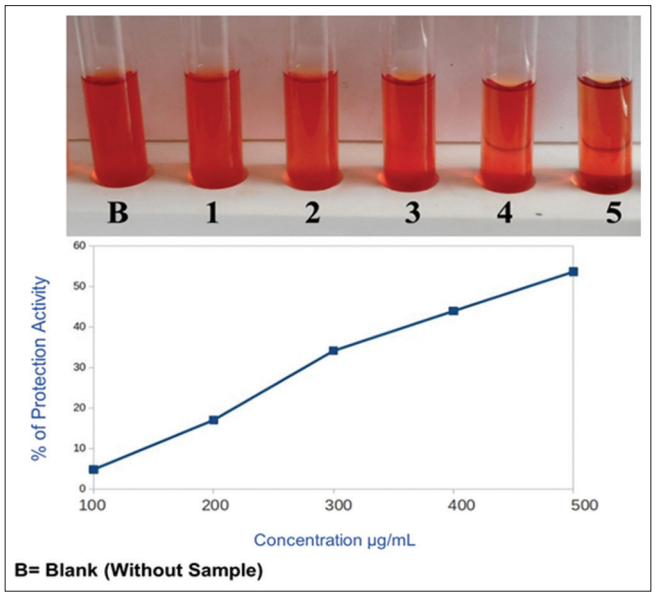


Figure 6: Membrane stabilization activity

Inhibition of protein denaturation

A frequently applied technique to investigate the anti-inflammatory capacity of the extracts is the suppression of the protein denaturation assay. Denaturation of proteins implies a sequence of steps where proteins drop their tertiary and secondary structures, and is a hallmark of inflammation. LcBME's anti-inflammatory action was evaluated by its capacity to stop heat-induced protein denaturation. The occurrence of protein denaturation is linked to inflammatory diseases, and anti-inflammatory agents could involve the prevention or inhibition of the process [Figure 7]. The denaturation of proteins caused by the LcBME depends on the dose. At 100 µg/mL concentration, the extract inhibited denaturation by 38 ± 4%, which increased to 84 ± 6% at 500 µg/mL.

The standard is diclofenac sodium. It has a high percentage of inhibition at a high concentration rate, which means the existence of bioactive compounds, which have the potential

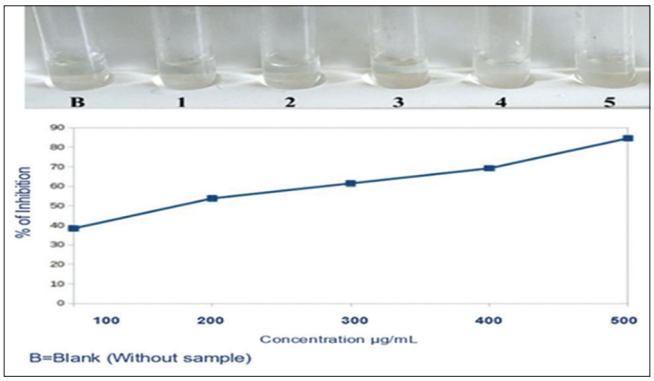


Figure 7: Protein denaturation inhibition activity

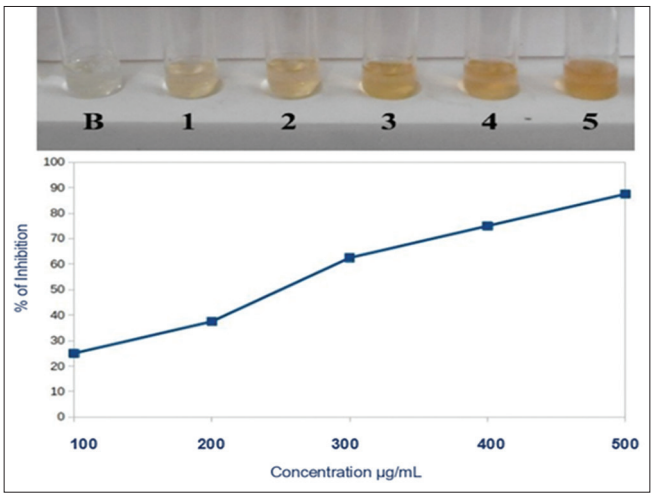
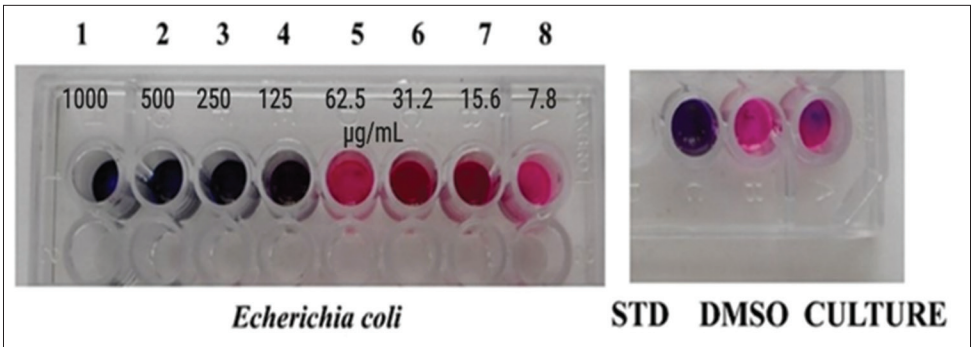


Figure 8: Inhibition albumin denaturation



S. No	Bacteria	Growth of inhibition										
		1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	Std. Steptomycin 10 µg	DMSO	Culture
1	*E. coli*	-	-	-	-	+	+	+	+	-	+	+

Figure 9: Minimum inhibitory concentration of *Lannea coromandelica* bark extract against *Escherichia coli*

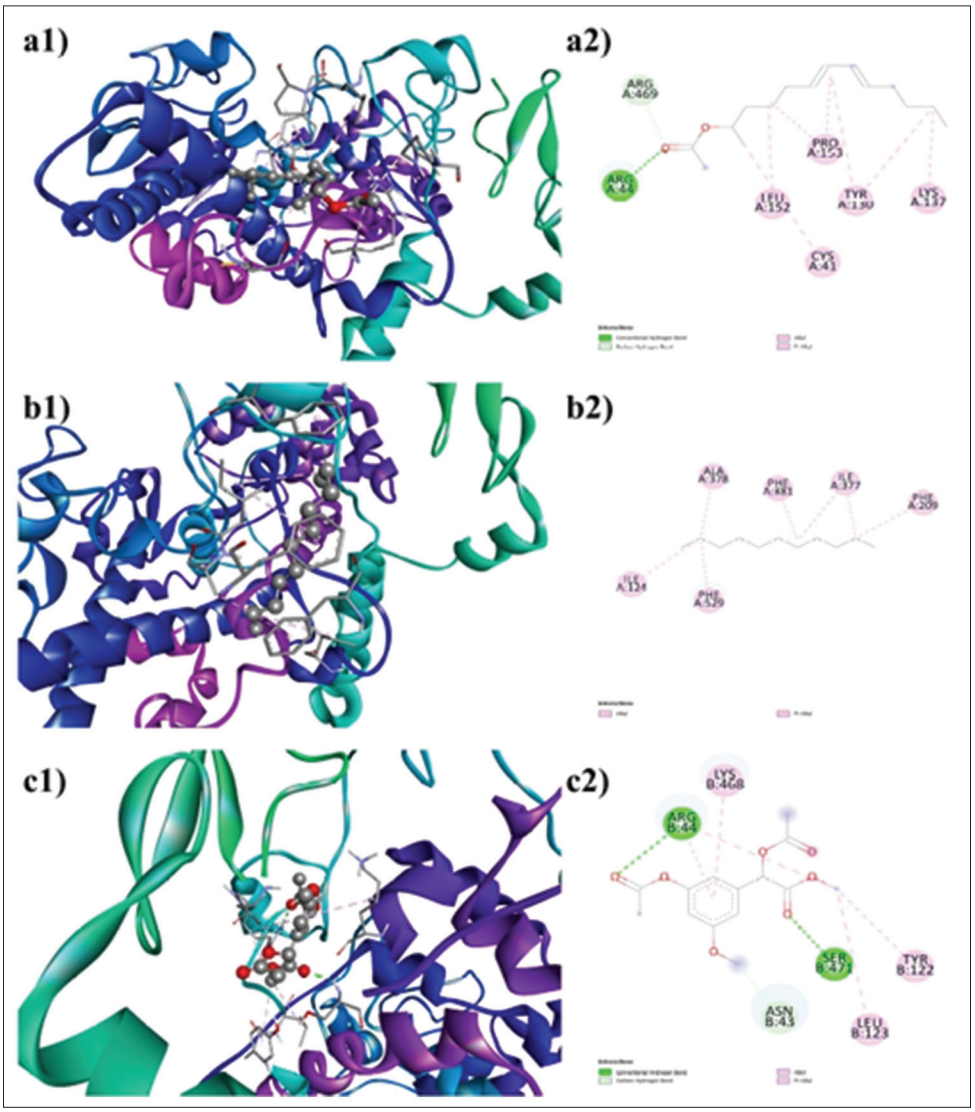


Figure 10: Molecular docking of phytochemical compounds (legend) and inflammatory protein cyclooxygenase-2 (receptor)

to prevent denaturation of proteins. The capacity of LcBME in preventing the protein denaturation effect emphasizes its anti-inflammatory effect with regard to natural properties investigated by Chaiya *et al*.[34] This justifies its potential use in the treatment of inflammatory disorders like UC, where the denaturation of proteins has been shown to have a major role in the pathology of the disease.

Inhibition of albumin denaturation

In this analysis, the ability of an extract to prevent the denaturation of protein in the presence of heat or chemicals, which is a characteristic of inflammation, is assessed. The anti-inflammatory effect of LcBME was tested in the present research by inhibiting the albumin denaturation test. The test is used to determine the properties of an extract to stabilize the albumin in the presence of heat stress. The extract of the bark showed a considerable dose-dependent inhibition of the denaturation of albumin. At 100 µg/mL, the extract inhibited denaturation by 25%, which increased to 87 ± 0.5% at 500 µg/mL. A standard is diclofenac sodium [Figure 8]. This could be attributed to the high percentages of inhibition at the high concentrations of the seeds, which shows the existence of bioactive compounds that can stabilize the proteins during stress. The ability of LcBME to prevent denaturation of albumin is an indication that the substance has potential in use as a natural anti-inflammatory drug. This property can help it to be effective in the management of inflammatory diseases like UC, in which the denaturation of proteins increases tissue inflammation and damage. The anti-inflammatory activities of organic solvents were investigated by Chaiya *et al*. to prevent the thermal denaturation of egg albumin[34] by organic solvents.

Determination of MIC

This is a technique that establishes the minimum percentage quantity of a substance that disrupts the functionality of the microbes. This paper employs the resazurin microtiter test to identify the MIC of LcBME against *E. coli*. Resazurin is a redox-sensitive dye that changes color under the influence of the metabolic activity of the microorganism and can be employed to determine the growth of bacteria. The bacterial growth can be verified by the color change as the blue resazurin turns to pink resorufin, which can be quantitatively detected in 96-well plate [Figure 9]. LcBME had high antibacterial activity, wherein the MIC was 125 ug/mL at which no color change appeared. When the concentration was below 62.5 ug/mL, the solution turned pink, which was an indication of bacterial growth. The streptomycin (positive control) has MIC of 10 ug/mL. LcBME MIC determined as 125 ug/mL, was lower relative to LcBME MIC of streptomycin (10 ug/mL), which demonstrated that although the extract can be used to show antibacterial activity, it was not as strong as the standard antibiotic. Nevertheless, the observation suggests that the extract can be a good prospect in future antimicrobial studies. Its antibacterial potential is demonstrated by the MIC of LcBME against *E. coli*. This activity can be accredited

Table 1: Molecular docking of ligands against selected receptors

Ligand	PDB ID	Receptor	Binding energy (kcal/mol)	Inhibition constant (µm)	Intermolecular energy (kcal/mol)	No of hydrogen bond formed	H-bond Amino acids	Distance Å	Conventional Amino acids
E, E-6,8 Tridecadien-2-ol, acetate	1CX2	COX-2	–5.62	76.28	–8.60	1	ARG A: 44	2.055	ARG A: 469, LEU A: 152, CYS A: 41, TYR A: 130, PRO A: 153, LYS A: 137
Undecane	1CX2	COX-2	–4.64	397.86	–7.03	0	-	-	ILE A: 124, ALA A: 378, PHE A: 529, PHE A: 529, ILE A: 377, PHE A: 209
Benzeneacetic acid, alpha, 3-bis (acetyloxy)-5- methoxy-, methyl ester	1CX2	COX-2	–5.66	71.44	–8.04	2	ARG B: 44 SER B: 471	2.461 2.247	LYS B: 468, TYR B: 122, LEU B: 123, ASN B: 43

COX-2: Cyclooxygenase-2, PDB: Protein data bank

to bioactive compounds found in the bark, which can be investigated further in application in the treatment of illnesses such as GI infections, such as *E. coli*.[35] Further studies are required to determine the specific antimicrobial compounds and their effect of action. According to Ha *et al*., methanol and aqueous extracts of the *L. coromandelica* fruit are very useful against *Proteus vulgaris*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas* spp., *Staphylococcus aureus* and *Streptococcus pyogene*.[7]

Molecular docking

A methodology of revealing the binding affinity of the compounds with biological applications is based on molecular docking technique.[36-38] The toxicity profiles and the pharmacokinetic properties of the chosen compounds, as described in Table 1, were screened against Lipinski rule of 5, which is a prerequisite of drug-likeness on important criteria in this research. The Compound 1 (E, E-6,8-Tridecadien-2-ol, acetate), Compound 2 (Undecane), and Compound 3 (Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester) corresponded to the Lipinski criteria, which indicates their possible positive bioavailability. These compounds exhibited high theoretical passive absorption and were not permeable across the BBB, as they were consistent with bioavailability scores of 0.55. In addition, all the compounds have not been found to be mutagenic, tumorigenic, or irritants, highlighting the appropriateness of the compounds in further pharmacological investigations [Table 2].

Table 2: Pharmacokinetic properties and the toxicity analysis LcBME extract

S. No.	Compound name	Formula	PubChem ID	H-bond acceptors	H-bond donors	TPSA	Log P	Log S	GI absorption
1.	3-Methylene-1,6-heptadiene	$C_{10}H_{16}$	556452	0	0	0.00	2.47	−2.33	Low
2.	Phenol, 3-methyl	$C_{17}H_{21}NO_2$	64695	3	0	29.54	3.24	−3.72	High
3.	Phenol, 2-methyl	$C_9H_{13}NO$	247477	2	2	46.25	1.85	−1.57	High
4.	3-Pentadecanone	$C_{15}H_{30}O$	545972	1	0	17.07	3.98	−4.35	High
5.	E, E-6,8-Tridecadien-2-ol, acetate	$C_{15}H_{26}O_2$	5363764	2	0	74.56	3.78	−3.66	High
6.	Undecane	$C_{11}H_{24}$	14257	0	0	0.00	3.59	−3.78	Low
7.	Benzeneacetic acid, alpha, 3-bis (acetyloxy)-5-methoxy-, methyl ester	$C_{14}H_{16}O_7$	592636	7	0	88.13	3.12	−2.28	High
8.	Furfural	$C_5H_4O_2$	7362	2	0	30.21	1.03	−1.16	High
9.	Heptanal	$C_7H_{14}O$	8130	1	0	17.07	2.01	−1.68	High

S. No.	Compound name	BBB permeant	Pgp substrate	Mutagenic	Tumorigenic	Irritant	Bioavailability score	Lipinski rule
1.	3-Methylene-1,6-heptadiene	Yes	No	None	Low	High	0.55	Yes
2.	Phenol, 3-methyl	Yes	No	None	None	High	0.55	Yes
3.	Phenol, 2-methyl	Yes	No	High	Low	Low	0.55	Yes
4.	3-Pentadecanone	Yes	No	None	None	High	0.55	Yes
5.	E, E-6,8-Tridecadien-2-ol, acetate	Yes	No	None	None	None	0.55	Yes
6.	Undecane	No	No	None	None	None	0.55	Yes
7.	Benzeneacetic acid, alpha, 3-bis (acetyloxy)-5-methoxy-, methyl ester	No	No	None	None	None	0.55	Yes
8.	Furfural	Yes	No	High	High	High	0.55	Yes
9.	Heptanal	Yes	No	High	None	High	0.55	Yes

LcBME: *Lannea coromandelica* bark extract, TPSA: Topological polar surface area, GI: Gastrointestinal, BBB: blood-brain barrier, Pgp: P-glycoprotein

Molecular docking analysis showed that there was a difference in the interaction of the three ligands with the COX-2 (PDB ID: 1CX2). Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester was found to have the best binding affinity with a binding energy of −5.66 kcal/mol, and a low inhibition constant (71.44 uM). It has been hydrogen bonded to ARG B:44 and SER B:471 at 2.461 A and 2.247 A, and also interacted with some conventional amino acids such as LYS B:468 and TYR B:122, indicating its stability at the binding pocket [Figure 10 and Table 1]. E, E-6,8-Tridecadien-2-ol, acetate also exhibited significant binding, with a binding energy of −5.62 kcal/mol, an inhibition constant of 76.28 μM, and a single hydrogen bond with ARG A:44 (distance: 2.055 Å), supported by interactions with amino acids such as ARG A:469 and LEU A:152 [Figure 10 and Table 1]. In contrast, Undecane showed the weakest interaction, with a binding energy of −4.64 kcal/mol, the highest inhibition constant (397.86 μM), and no hydrogen bonds, although it formed hydrophobic interactions with residues like ILE A:124 and PHE A:529 [Figure 10 and Table 1]. The docking of several compounds with the COX-2 protein has been reported in the earlier literature with the essential oil of *Solanum lyratum* Thunb. containing the GC-MS-identified compounds listed below: 7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione that was found to have a binding energy −5.14 kcal/mol with COX-2.[39] This was another experiment on marine macroalgae where the hexadecanal and neophytadiene compound (GC-MS) were found to be the most binding affinity compounds with the COX-2 having a binding energy of −5.3 kcal/mol.[40] Thus, it can be mentioned that Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester appeared most promising as COX-2 inhibitor, compared to other ligands, which possess better binding properties, and can be investigated in the further therapeutic research.

CONCLUSION

The findings also endorse the notion that UC may be treated with *L. coromandelica* bark methanolic extract. The results derived in the study implicate that the LcBME has value for development into products for treatment of UC and for other inflammatory diseases. The LcBME is effective owing to its free radical scavenging activities and membrane stabilizing abilities. The data suggest that *L. coromandelica* bark methanolic extract can prevent tissue damage due to inflammation. As far as we know, this is the first study emphasising the *in vitro* antibacterial, anti-inflammatory, and antioxidant qualities of *L. coromandelica* isolated from Tamil Nadu. Furthermore, there have been no empirical studies conducted to reveal the antibacterial, antioxidant, and anti-inflammatory potential in a single study. In molecular docking, benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester demonstrated the strongest binding affinity, with a binding energy of −5.66 kcal/mol. The LcBME could be promising and effective in modifying UC disease; however, further research is required to expand on its anti-inflammatory capacity in a clinical setting. Detailed research is also recommended to be done where the bioactive components causing these effects are isolated and identified. To sum up, there is a need for further studies around the LcBME, bearing in mind that it is a beneficial natural source of drugs with antioxidative, anti-inflammatory, and antimicrobial properties.

AUTHOR'S CONTRIBUTION

Ramesh T- Idea conceive and supervisor, Jesinthal Gnanamani C- Workplan development and experiment conduction, Macdalen Rubavathy M- Workplan development and experiment conduction, Keerthana K- Literature search, Lokesh E- Image and molecular docking, Ganesh Kumar A- Review and Edit, Amuthavalli A- Data curing, Sankarganesh P- Statistical, Discussion, manuscript preparation.

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