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Research Article

Spectral signatures and biological efficacy of *Limonia acidissima* L. bark extracts in combating oxidative stress and inflammation

Rekka Raja[1*], Athira Prameela[2], Arunkumar Radhakrishnan[2], Priyanka Shanmugam[3], Sowmiya Eswaramoorthy[4], Abdul Kaffoor Habibulla[1], Thenmozhi Krishnasamy[1]

1. Assistant Professor, PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.
2. Research Scholar, PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.
3. Assistant Professor, Department of Medicinal Botany, Sivaraj Siddha Medical College, Salem, Tamil Nadu. India.
4. Student, PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.

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Abstract

Background: Medicinal plants are valuable reservoirs of bioactive molecules that continue to serve as leads for modern drug discovery. *Limonia acidissima* L. (Rutaceae), traditionally valued for its therapeutic uses, remains underexplored with respect to its bark-derived phytoconstituents. Materials and Methods: The ethanolic bark extract of *L. acidissima* was analyzed using High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FT-IR), UV-Visible spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS) to characterize phytochemical diversity. Antioxidant potential was assessed using the DPPH radical scavenging assay, while anti-inflammatory activity was evaluated through goat red blood cell (GRBC) membrane stabilization. Additionally, molecular docking was performed against the MYST acetyltransferase domain (PDB: 6OIO) to explore phytochemical-protein interactions. Results: HPLC revealed 14 peaks between 200-700 nm, with the highest peak at 13.598 minutes. FT-IR confirmed functional groups such as alkyl halides, aromatics, alcohols, carboxylic acids, alkanes, and amines. UV-Vis spectra indicated absorption peaks ranging from 500 to 1100 nm, characteristic of alkaloids, flavonoids, and phenolic compounds, while GC-MS detected 20 major metabolites, including 2-methoxy-4-vinylphenol, isopsoralen, scopoletin, braylin, marmesin, visamminol, and squalene. The extract exhibited moderate antioxidant activity in the DPPH assay (IC^{50} = 353.58 µg/mL) and 72% hemolysis inhibition in GRBC membrane stabilization at 1000 µg/mL, compared to 89% for acetylsalicylic acid. Docking studies revealed strong binding affinities of marmesin, braylin, and scopoletin, supporting their role in modulating inflammation pathways. Conclusion: The ethanolic bark extract of *L. acidissima* is rich in bioactive phytochemicals and exhibits appreciable antioxidant and anti-inflammatory potential. These findings validate its ethnomedicinal relevance and highlight its promise as a natural source of therapeutic agents.

Keywords: *Limonia acidissima,* Phytochemicals, Antioxidant activity, Anti-inflammatory activity, GC-MS analysis, Molecular docking.

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Introduction

Herbal medicine has long played a pivotal role in primary healthcare, particularly in regions with limited access to conventional treatments, owing to its historical use, affordability, and potential for fewer adverse effects compared to allopathic therapies (1,2,3). India, with its rich heritage of traditional knowledge and abundant diversity of medicinal plants, remains a hub of ethnomedicinal practices (4,5). Among these, *Limonia acidissima* L. from the Rutaceae family, commonly known as wood apple, elephant apple, monkey fruit, curd fruit, kath bel, or kaitha, is widely distributed across tropical and subtropical regions, including India, Sri Lanka, Pakistan, Southeast Asia, and parts of America (6). In recent years, there has been a surge in demand for underutilized fruit trees such as *L. acidissima*, owing to their potential applications in functional foods and sustainable healthcare products, as well as their therapeutic and ecological significance.

This plant has been valued in indigenous medicine for its diverse therapeutic properties, including antibacterial, hepatoprotective,

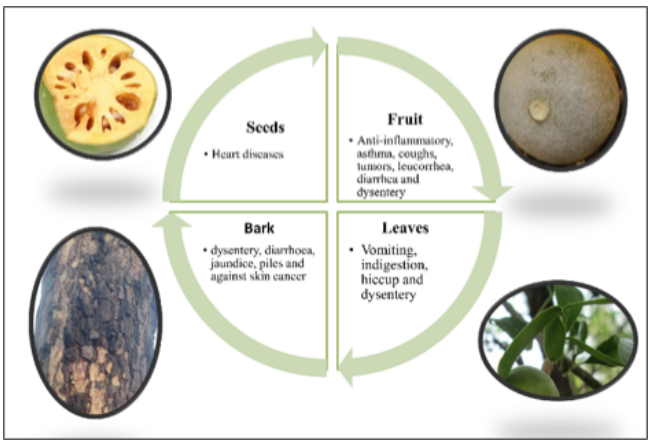
*** Corresponding Author:**
Rekka Raja
PG and Research Department of Botany,
Kongunadu Arts and Science College (Autonomous),
G.N. Mills Post, Coimbatore – 641029, Tamil Nadu, India.
Email Id: rekkar_bo@kongunaducollege.ac.in

antioxidant, nephroprotective, and neuroprotective activities (8). Various phytoconstituents, including alkaloids, flavonoids, tannins, coumarins, and anthocyanins, render *L. acidissima* pharmacologically significant (9). Different parts of the plant are used across traditional medical systems, the fruit pulp for diabetes, cancer, ulcers, and hypertension; root juice for snakebite; bark decoctions for malaria; and leaf extracts with honey for fever. The mashed seedless pulp is also reported to be effective in dysentery, diarrhoea, jaundice, piles, skin cancer, hiccups, and throat or gum disorders, while serving as a liver and cardiac tonic (10). This ethnobotanical adaptability emphasizes the plant's potential as a source of biologically active substances that could be widely utilized in modern pharmaceutical production applications (**Fig. 1**). Furthermore, its broad cultural applicability reinforces its significance as a 'crossover' medicinal plant, in which emerging pharmaceutical data complement traditional knowledge.

Pharmacological relevance arises from its bioactive components, including flavones, pectin, stigmasterol, volatile oils, and polyphenols, which also impart nutraceutical potential. While traditional knowledge highlights the medicinal efficacy of plants, only a small fraction (approximately 2%) of medicinal plants worldwide have been systematically investigated for their phytochemical and biological activities (11,12,13). Integrating indigenous knowledge with modern scientific approaches is important for the conservation, authentication, and sustainable use of medicinal plants, such as *L. acidissima*. Moreover, the increasing global inclination toward plant-derived treatments, coupled with consumer demand for holistic medicine, provides a robust platform to advance *L. acidissima* as a potential pharmacological resource.

To scientifically validate its ethnomedicinal applications and discover novel lead compounds, advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR), and UV-Vis spectroscopy have been employed to characterize secondary metabolites in *L. acidissima* bark extract (15,16). Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) offers a reliable platform for identifying bioactive compounds with therapeutic relevance (17). The combination of advanced analytical methods with traditional insights enables a comprehensive understanding of the pharmacological value of the plant. Therefore, the present study aims to explore the phytochemical composition of the ethanolic bark extract of *L. acidissima* using GC-MS analysis, thereby providing scientific evidence to support its pharmacological potential and ethnomedicinal value.

Figure 1: Ethnomedicinal uses of *L. acidissima* plant parts



Materials and methods

Collection and preparation of the sample

The bark of *L. acidissima* was procured from the Gurusimalai Hills, Pandalur, Nilgiri district, Tamil Nadu. It was thoroughly rinsed with tap water, shade-dried at ambient temperature, and finely ground into powder. The powdered bark was preserved in a light-resistant container for subsequent analysis. For extraction, the bark powder underwent cold maceration using an 80:20 ethanol-water mixture. The resulting extract was concentrated under reduced pressure to obtain a semisolid mass, which was further dried in a desiccator and meticulously stored in air-tight containers for future investigations (18).

Preliminary phytochemical screening

A qualitative assessment of the ethanolic bark extract of *L. acidissima* was performed to identify the presence of different phytochemical constituents, following the standard procedure described by Harborne (1998) (19).

HPLC analysis

For HPLC analysis, 100 mg of the ethanolic bark extract of *L. acidissima* was precisely weighed using an Afcoset electronic balance and dissolved in 1 mL of methanol. The mixture was centrifuged at 3000 rpm for 5 minutes, and the resulting supernatant served as the test solution. A 2 µL aliquot of this solution, along with 2 µL of the gallic acid standard, was carefully applied onto a 10 × 10 cm Silica gel 60F254 TLC plate using a Hamilton syringe fitted to a CAMAG LINOMAT 5 applicator. The chromatographic separation was carried out using a mobile phase composed of toluene, ethyl acetate, formic acid, and methanol in the ratio of 3:6:1.6:0.4. Before development, the twin-trough chamber was saturated with the mobile phase for 10 minutes. The TLC plate was then developed up to a migration distance of 85 mm to resolve phenolic and flavonoid constituents. Following development, the TLC plate was air-dried with hot air to eliminate residual solvents and subsequently photo-documented under UV light at 366 nm using a CAMAG REPROSTAR 3 system. The chromatogram was finally scanned with a CAMAG TLC SCANNER 3, and the corresponding peak tables, chromatographic profiles, and densitograms were generated for further interpretation (15).

FTIR Spectroscopic analysis

FT-IR spectroscopy was carried out to elucidate the characteristic functional groups present in the ethanolic bark extract of *L. acidissima*. The spectra were obtained using a Perkin-Elmer FT-IR spectrophotometer. For sample preparation, a trace amount of the dried extract was blended with potassium bromide (KBr) in an appropriate ratio and compressed into a translucent pellet. The infrared spectrum was scanned across the mid-infrared region (4000-400 cm^{-1}), enabling the identification of distinct absorption bands corresponding to functional groups such as hydroxyl (-OH), carbonyl (C=O), and aromatic rings. The spectral peaks were carefully documented, and the analysis was repeated twice to validate reproducibility and ensure analytical precision (16).

UV-Visible Spectroscopic analysis

UV-Visible spectrophotometric profiling was performed on the *L. acidissima* extract using a SpectraMax M3 spectrophotometer (Molecular Devices, California, USA). The extract was first centrifuged at 3000 rpm for 10 minutes to separate the supernatant and remove debris from the homogenate. The clear supernatant

was then diluted to a 1:10 ratio with the same solvent. The dilutions were aliquoted into 2 mL cryotubes and subsequently dispensed into 96-well plates for analysis. Spectral scanning was performed across the wavelength range of 500-1100 nm, and the distinctive absorption peaks were recorded for characterisation (17).

Gas Chromatography – Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of the sample was executed on a 451_GC system integrated with an Autosampler 8410, employing a Bruker capillary column (0.25mm) coupled to an FID detector. Helium was utilized as the mobile phase at a regulated flow of 1 mL/min. Injection was performed through a PTV injector, with the oven temperature programmed to hold at 40 °C for 3 minutes, followed by a gradual ramp-up to 280 ºC, completing the analysis within 30 minutes. Compound identification was achieved by comparing the obtained mass spectra with reference spectra from the NIST Library (Version 12) (20).

***In vitro* antioxidant evaluation of *L. acidissima* bark ethanolic extract**

The hydrogen-donating capacity was assessed through the stable DPPH method (21). A 0.1 mM DPPH solution was freshly prepared in methanol, and the samples (50- 250 µg/mL) were mixed with 5.0 mL of the DPPH solution. The reaction mixture was shaken and incubated at 27 °C for 20 minutes, after which the absorbance was recorded at 517 nm. The results were compared with the activity of rutin and BHA. The percentage of radical scavenging activity was determined using the equation:

DPPH radical scavenging activity (%) =
[(Control OD - Sample OD) / Control OD] × 100

The antioxidant efficiency of the extracts was expressed in terms of IC^{50} values, derived from the linear regression of percentage antioxidant activity (%) against extract concentration (22).

***In vitro* Anti-inflammatory activity through Membrane Stabilization assay**

The *in vitro* anti-inflammatory potential was evaluated using the goat red blood cell (GRBC) stabilization method, as the erythrocyte membrane is structurally analogous to the lysosomal membrane and can therefore serve as a model for lysosomal stabilization. The ability of standard drugs or test samples to prevent hypotonicity-induced hemolysis was taken as an indicator of anti-inflammatory activity. Hemoglobin release in the suspension was quantified spectrophotometrically at 560 nm. Fresh goat blood was collected and mixed with sodium oxalate to prevent clotting. The samples were stored at 4°C for 24 hours before experimentation. The blood was centrifuged at 2500 rpm for 5 minutes to remove the supernatant. The packed cells were washed three times with sterile saline solution (0.09% w/v NaCl), each wash followed by centrifugation at 2500 rpm for 5 minutes. After washing, the packed cell volume was measured, and the cells were reconstituted into a 40% suspension (v/v) in phosphate-buffered saline (10 mM, pH 7.4). The buffer was prepared by dissolving NaH2PO4 · 2H2O (0.26 g), Na^2HPO^4 (1.14 g), and NaCl (9 g) in 1 L of distilled water (16).

Molecular docking studies

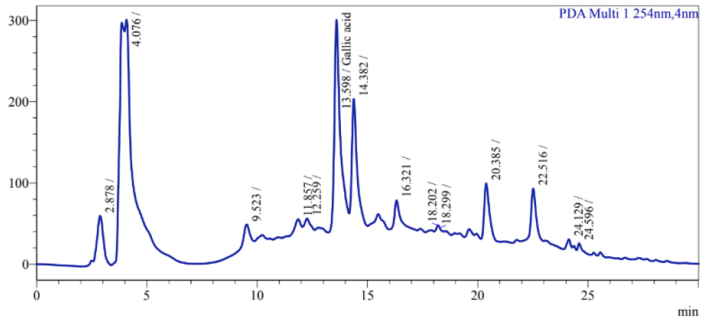
For the molecular docking evaluation, the three-dimensional crystal structure of MYST acetyltransferase domain in complex with inhibitor 60 (PDB ID: 6OIO) was sourced from the Protein Data Bank (PDB) (23). Non-essential water molecules and heteroatoms were meticulously eliminated using Molegro Molecular Viewer (MMV) to refine the target protein, which was subsequently saved in PDB format. Twelve bioactive compounds derived from *L. acidissima* were retrieved from the PubChem repository. The docking analysis was executed using PyRx 0.8, with ligand structures imported through Open Babel. A docking grid was generated, and molecular interactions were assessed through the Vina wizard. The docking outcomes were visualized and analyzed using Discovery Studio 2024, which provided an in-depth representation of 2D and 3D molecular interactions between the protein and ligand, offering insights into their binding affinities and structural compatibilities (^{24}).

Results and discussion

HPLC analysis for phenolic profile

The HPLC chromatogram of the ethanolic bark extract of *L. acidissima* revealed the presence of diverse phytoconstituents, as indicated by multiple peaks (**Fig. 2**). Gallic acid, a well-known polyphenol renowned for its potent antioxidant activity, exhibited the most prominent peak at 13.959 min, with major retention times spanning from approximately 2.878 to 25.429 min. Additional peaks observed at 4.076, 9.523, 11.853, 14.832, and beyond suggest the probable occurrence of other bioactive compounds, including flavonoids, tannins, or alkaloids, which may contribute to the extract's pharmacological potential. The relative abundance of these compounds is reflected in the peak intensities, expressed in milli-absorbance units (mAU). This finding aligns with earlier HPLC-densitometric studies, which confirmed the presence of phenolic acids, such as gallic, vanillic, and protocatechuic acid, along with quercetin, in *L. acidissima* (25,26). This further substantiates its antioxidant efficacy. The detection of coumarins and benzamide derivatives in previous investigations also supports the likelihood that several of these unidentified peaks could represent novel compounds with anti-inflammatory and neuroprotective properties (27,28). Collectively, the intricate chromatographic pattern highlights the metabolite richness of L. acidissima bark, lending scientific credence to its long-standing role in traditional medical applications.

Figure 2: HPLC analysis of ethanolic bark extract of *L. acidissima*



FT-IR Spectroscopic analysis

The FT-IR spectrum of the ethanolic bark extract of *L. acidissima* unveiled the presence of multiple functional groups, indicating a diverse array of bioactive compounds (**Fig. 3, Table 1**). The strong absorption bands at 3379.39 cm^{-1} and 3350.80 cm^{-1} corresponded to N-H stretching, suggesting the presence of amines, while a peak at 3162.10 cm^{-1} indicates O-H stretching, characteristic of carboxylic acids. The absorption at 2956.25 cm^{-1} was attributed to C-H stretching, typical of alkanes. A C=O stretching band observed at 1435.24 cm^{-1} further supported the existence of carboxylic acids. Additionally, the band at 1057.85 cm^{-1} corresponded to C-O stretching, indicative of alcohols, whereas the peak at 874.87 cm^{-1} suggests aromatic ring vibrations.

The absorption at 634.71 cm^{-1} was assigned to C-Cl stretching, indicating the presence of alkyl halides. Such FTIR findings are in agreement with earlier reports on *L. acidissima* fruit extract, which displayed characteristic absorption peaks for proteins, polyphenols, carboxyl groups, and saponins, all of which are essential for nanoparticle biosynthesis (29). Similar studies have shown that the FTIR peaks of *L. acidissima* leaf extracts remain stable under high temperatures, indicating that room-temperature extractions are optimal for preserving functional integrity in AgNP production (30). Together, these spectral features suggest the presence of alkaloids, flavonoids, tannins, and other phytochemicals that may contribute to the therapeutic efficacy of *L. acidissima*.

Figure 3: FT-IR analysis of ethanolic bark extract of *L. acidissima*

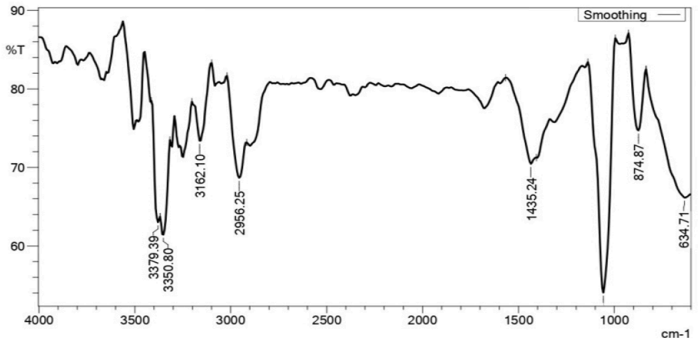


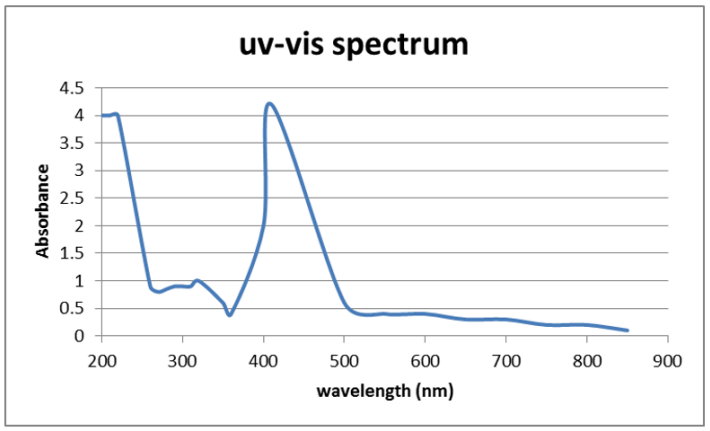
Table 1: FT-IR analysis of ethanolic bark extract of *L. acidissima*

S.no	Peak value	Molecular motion	Functional groups
1	634.71	C-Cl	Alkyl halide
2	874.87	Ar -C	Aromatic
3	1057.85	C-O	Alcohols
4	1435.24	C=O	Carboxylic acid
5	2956.25	C-H	Alkane
6	3162.10	O-H	Carboxylic acid
7	3350.80	N-H	Amine
8	3379.39	N-H	Amine

UV-Vis Spectroscopic analysis

The UV-Vis absorption profile of the ethanolic bark extract of *L. acidissima* revealed distinct spectral peaks, highlighting the abundance of phytoconstituents (**Fig. 4**). The strong absorbance in the UV region, particularly between 200-300 nm, suggested the presence of phenolic compounds and flavonoids, which characteristically absorb in this range due to $\pi \rightarrow \pi^*$ electronic transitions. A distinct peak observed around 400-450 nm further suggested the presence of extended conjugated systems, including flavonoids, tannins, and other polyphenolic metabolites, which are closely associated with antioxidant and therapeutic activities. The negligible absorption beyond 500 nm implied the absence of highly conjugated chromophores. This spectral evidence validates earlier findings, which showed that similar absorption patterns in *L. acidissima* fruit pulp correlated with polyphenolic richness, essential fatty acids, and organic acids such as citric and tartaric acids, all of which contribute to both nutritional and medicinal applications (31). Comparable UV-Vis profiles of *L. acidissima* and related medicinal plants have also been linked to the reduction and stabilization of nanoparticles, further underscoring the extract's multifunctional applications (32). Overall, these spectral features provide compelling evidence of bioactive constituents in the extract, consistent with the traditional use of *L. acidissima* as a therapeutic plant.

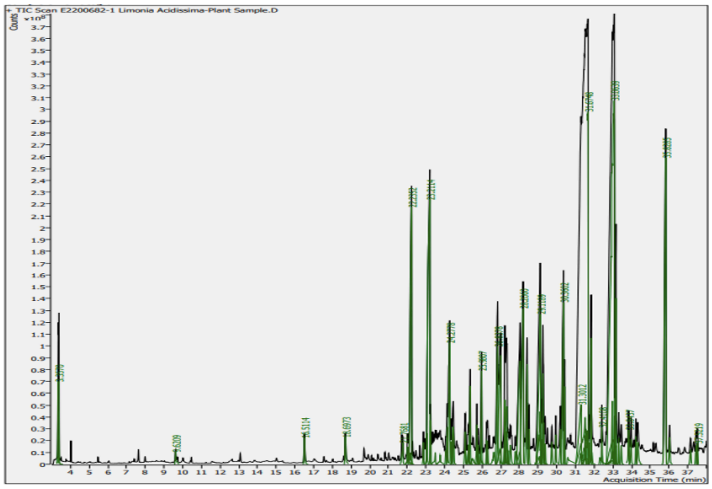
Figure 4: UV-VIS analysis of ethanolic bark extract of *L. acidissima*



GC-MS analysis

The preliminary phytochemical screening of the ethanolic bark extract of *L. acidissima* confirmed the presence of carbohydrates, proteins, phenolic compounds, alkaloids, flavonoids, volatile oils, fixed oils, and fats (**Fig. 5**).

Fig. 5: GC-MS profile depicting bioactive constituents of *L. acidissima* bark extract



The detailed results of this analysis are presented in **Table 2**. Further characterisation through GC-MS, a highly sensitive analytical tool for identifying bioactive molecules in medicinal plants (33), unveiled a complex phytochemical composition. The chromatographic analysis revealed 20 chemical compounds, out of which 11 are major phytoconstituents, including 2-methoxy-4-vinylphenol, caryophyllene oxide, coniferyl aldehyde, isopsoralen, 7,8-dimethoxycoumarin, scopoletin, linoleic acid ethyl ester, braylin, marmesin, visamminol, docosanoic acid ethyl ester, and squalene, manifested a match factor above 95 percent. The chromatograms are presented in **Fig. 6**, while the active principles, along with their retention times (RT), molecular formulas, areas, and reported biological activities, are summarized in **Table 3**. Several of these identified compounds have been widely documented for their pharmacological significance. Isopsoralen has been reported to significantly inhibit tumor growth in both animals and humans (34,35). Scoparone, a coumarin derivative found in *Artemisia capillaris*, exhibits a broad spectrum of pharmacological effects, including anti-

inflammatory, antitumor, and antifibrotic activities (36). Braylin has shown strong potential as a candidate drug for treating immune-inflammatory disorders (37). Marmesin interacts with the active site of HSULF-2, suggesting a mechanism for its inhibitory and antitumor activity, as reported in bael fruit extract studies (38). Likewise, squalene has exhibited robust antitumor and anticancer activity against ovarian, breast, lung, and colon cancers (39). Visamminol, derived from the seed extracts of *Ammi visnaga*, has demonstrated efficacy against breast cancer cells (40). 2-methoxy-4-vinylphenol, a phenolic compound, possesses anti-inflammatory properties and has been shown to induce cell cycle arrest, making it a promising candidate drug for pancreatic cancer treatment (41). Caryophyllene oxide exhibits broad-spectrum anticancer activity by inhibiting the growth and proliferation of various cancer cell types, while also suppressing tumor angiogenesis and metastasis markers (42). Coniferyl aldehyde, a non-cytotoxic phenylpropanoid, and cinnamaldehyde have established anticancer activity against the H1299 cell line (43). Furthermore, 7,8-dimethoxycoumarin, isolated from *Citrus decumana* peels, has been reported to attenuate gastric inflammation (44). Linoleic acid ethyl ester, detected in *Ficus carica* leaves, is known for its potent antioxidant and anti-inflammatory activities (45). The phytochemical diversity identified through GC-MS indicates that *L. acidissima* is a rich source of biologically active metabolites, providing scientific support for its ethnomedicinal significance.

Table 2: Preliminary phytochemical screening of bark ethanol extract of *L. acidissima*

S.no	Constituents	Test	Observation	Reaction
1	Carbohydrate	Fehling's test	Red precipitate	+
2	Protein	Millon's test	White precipitate	+
3	Phenolic compounds	Ferric chloride test	Violet precipitate	+
4	Alkaloids	Mayer's test	White precipitate	+
5	Flavonoids	Conc. H_2SO_4 test	Yellow color	+
6	Saponins	Foam test	Foam	-
7	Volatile oil	Fluorescence test	Bright pinkish	+
8	Fat and oil	Spot test	Oil stain on paper	+

Table 3: Biological compounds identification of *L. acidissima* ethanolic bark extract

S.no	RT	Compound name	Area	Biological activity
1	16.5114	2-Methoxy-4-vinylphenol	82082664.6	Anti-inflammatory anticancer
2	22.0258	Caryophyllene oxide	44459638.9	Anti-inflammatory and anticancer
3	24.1547	Coniferyl aldehyde	103168724.5	Anticancer
4	25.3725	Isopsoralen	221501768.1	Anti-inflammatory, antiviral, Psoriasis, and vitiligo
5	25.9807	7,8-Dimethoxycoumarin	241108058.2	Anti-inflammatory
6	27.3067	Scopoletin	129270089.5	Anti-inflammatory, anticancer, and anti- diabetic
7	29.1939	Linoleic acid ethyl ester	41299907.3	Antioxidant, anticancer, and anti-inflammatory
8	30.2017	Braylin	98948609.4	Anti-inflammatory and anti-asthmatic
9	31.6748	Marmesin	88950416.3	Anticancer and antimalarial drug
10	33.0639	Visamminol	4273983715.4	Anticancer and antioxidant
11	33.3141	Docosanoic acid, ethyl ester	43400331.5	Antiandrogenic and hypocholesterolemic effects
12	35.5285	Squalene	1362976528.3	Anticancer and antioxidant

Antioxidant potential

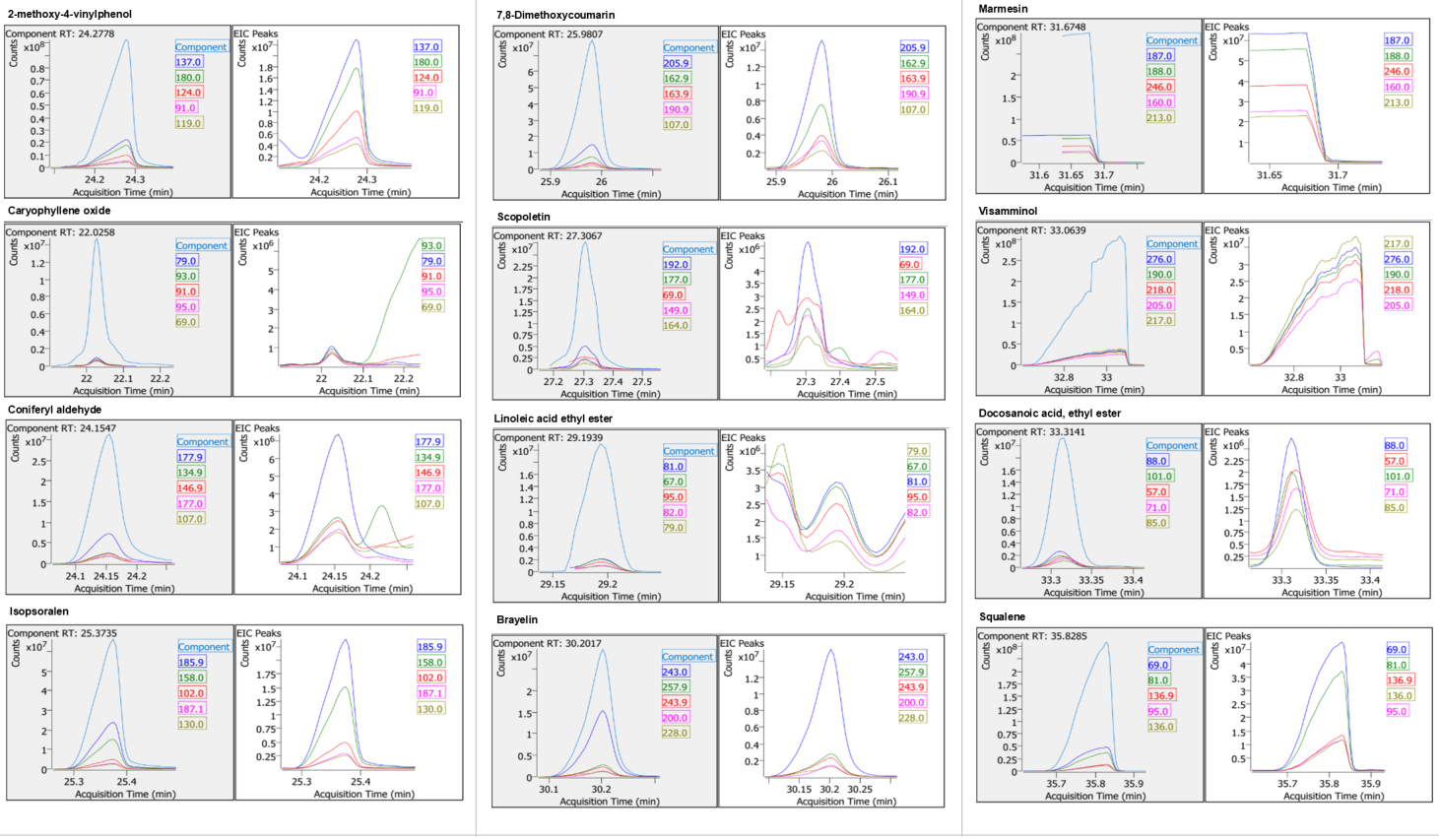
The ethanolic bark extract of *L. acidissima* exhibited moderate antioxidant potential in the DPPH assay, with an IC^{50} value of 353.58 µg/mL, indicating a concentration-dependent but relatively weak free radical scavenging effect. Previous studies on different parts of the *Limonia* and *Feronia limonia* species have reported substantially stronger activities. For instance, methanolic bark extracts of *F. limonia* showed IC^{50} values ranging from 15.8-17.4 µg/mL (46), while ethanolic leaf extracts of *L. acidissima* from Indonesia displayed an IC^{50} as low as 10.44 µg/mL (47). Similarly, ultrasound-assisted ethanolic leaf extractions yielded a high polyphenolic content (85.8 mg GAE/g dry weight) and a strong antioxidant capacity (235.01 µmol TEAC/g dry weight), underscoring the solvent- and method-dependent variations (48). *In vivo* studies also corroborate these findings; the methanolic fruit pulp extract enhanced antioxidant enzymes, such as superoxide dismutase and catalase, in rat models, thereby promoting wound healing (49). Comparable hepatoprotective effects were observed in CCl^4-induced hepatic damage, where methanolic pulp extracts significantly improved hepatic antioxidant status (50). Additional studies on *L. acidissima* fruit extracts have confirmed significant free radical scavenging and metal-chelating capacities (51,52). Collectively, these reports suggest that the comparatively lower radical scavenging observed in the present ethanolic bark extract may be due to differential phytoconstituent distribution between bark and fruit or due to solvent polarity effects on phenolic and flavonoid recovery.

Anti-inflammatory potential

In terms of anti-inflammatory activity, the bark extract exhibited dose-dependent erythrocyte membrane stabilization, achieving 72.62% inhibition at 1000 µg/mL, relative to 89% inhibition by the standard drug. This finding is consistent with previous reports where aqueous and methanolic fruit extracts of *L. acidissima* displayed significant protein denaturation and membrane stabilization inhibition exceeding 70% at comparable concentrations (53,52). In another study, combined pulp and rind extracts inhibited protein denaturation by 74.55% at 1000 µg/mL, nearly mirroring the present observation (52). Furthermore,

pharmacological reports have demonstrated the relevance of these effects *in vivo*, where methanolic fruit extracts alleviated inflammatory markers and modulated oxidative stress enzymes in animal models (49,50). The bioactivity is likely attributable to phenolic acids, flavonoids, and tannins, which are widely reported in *Limonia* spp. and are known to confer both membrane-stabilizing and antioxidant functions (54,55). Overall, the ethanolic bark extract of *L. acidissima* exhibits appreciable anti-inflammatory activity, but its antioxidant potential is comparatively weak when compared to more polar extracts or fruit-based preparations. These findings highlight the influence of plant part, extraction solvent, and method on bioactivity outcomes, supporting the ethnopharmacological relevance of *Limonia* species in managing oxidative stress and inflammation.

Fig. 6: Individual chromatogram of identified compounds



Molecular docking analysis

Molecular docking analysis of bioactive compounds from *L. acidissima* bark extract against the inflammation protein 6OIO revealed significant interactions and binding affinities (**Fig. 7-10**). Marmesin exhibited the strongest binding affinity (BA: -8.0 kcal/mol), followed by braylin (-7.6 kcal/mol), scopoletin (-7.2 kcal/mol), isopsoralen (-7.1 kcal/mol), and visamminol (-7.0 kcal/mol), suggesting robust interaction and potential inhibitory activity. These compounds also demonstrated favourable re-rank scores and extensive interactions with crucial amino acid residues in the active site, particularly Arg 655, Arg 656, Ser 690, Ser 693, and Gly 659. Notably, marmesin and linoleic acid ethyl ester both displayed high docking scores and low re-rank values, reinforcing their potential as strong protein-ligand complex stabilizers (**Table 4**). These findings suggest that several constituents from *L. acidissima* may serve as promising leads for the development of anti-inflammatory drugs.

A comparative analysis with the published literature supports these findings. Previous *in silico* studies on *L. acidissima* and related *Limonia* species have demonstrated that phytochemicals, such as hexadecanoic acid and various fatty acid esters, interact significantly with estrogen receptor-related proteins associated with breast cancer, with binding affinities in a similar range to those observed in this study. Other studies also identified compounds from *L. acidissima* that interact with key amino acid residues and exhibit stable protein-ligand binding, corroborating the current results (56,57,58). Furthermore, nanoparticle and protease inhibitor derivatives from *L. acidissima* have demonstrated *in vitro* and *in silico* cytotoxic effects against breast cancer cell lines, further substantiating it as a rich source of anticancer agents (57,59). Collectively, these results highlight the therapeutic promise of *L. acidissima* bark phytoconstituents against inflammatory protein, justifying further preclinical and clinical investigation.

Conclusion

The ethanolic bark extract of *L. acidissima* demonstrated a rich phytochemical composition, comprising phenolics, flavonoids, coumarins, and fatty acid derivatives, as revealed by HPLC, FT-IR, UV-Vis, and GC-MS analyses. The extract exhibited appreciable antioxidant and anti-inflammatory potential, while molecular docking highlighted several constituents with strong affinities toward inflammation- and cancer-related targets. These findings validate the traditional medicinal use of *L. acidissima* and emphasize its promise as a source of novel therapeutic agents.

Table 4: Molecular docking against the inflammatory protein (6OIO)[1]

Compound	BA (kcal/mol)	HB	Av. Bond length	Amino acid	Docking score	Re-rank score
2-Methoxy-4-vinylphenol	-5.9	3	3.003	Ile 647(A) Ser 684 (A) Ile 649 (A)	-77.342	-67.667
Caryophyllene	-6.5	0	0	0	-66.902	-52.516
Coniferaldehyde	-6.3	5	2.986	Tyr 658 (A) Ser 693 (A) Arg 656 (A) Gly 657 (A) Gly 659 (A)	-81.980	-71.734
Isopsoralen	-7.1	2	3.382	Gly 657 (A) Arg 656 (A)	-70.890	-61.660
7,8-Dimethoxycoumarin	-6.4	4	3.306	Arg 656 (A) Arg 655 (A) Gly 659 (A) Gly 657 (A)	-64.268	-58.055
Scopoletin	-7.2	3	2.827	Ser 690 (A) Ile 647(A) Ser 684 (A)	-86.706	-75.984
Linoleic acid ethyl	-6.1	1	3.074	Ser 693 (A)	-99.097	-76.123
Braylin	-7.6	5	2.974	Arg 656 (A) Ser 693 (A) Gly 659 (A) Tyr 658 (A) Gly 657 (A)	-81.353	-70.801
Marmesin	-8	2	3.086	Arg 655 (A) Ser 690 (A)	-97.443	-84.179
Visamminol	-7	2	3.089	Ser 690 (A)	-64.388	-61.095
Docosanoic acid,	-5.7	1	2.911	Ser 693 (A)	-89.198	-69.226
Squalene	-5.1	0	0	0	-109.30	-64.415

Fig. 7: (a) Ramachandran plot of protein 6OIO showing residues in favored, allowed, and disallowed regions; (b) Predicted binding pockets with the largest pocket highlighted in pale green

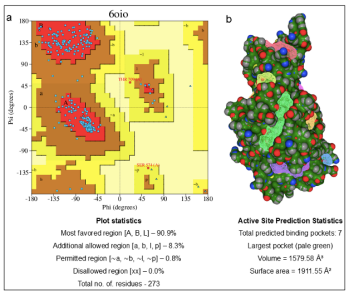


Fig. 8: Docking interaction between the target protein and bioactive phytocompounds: a 6OIO-2-methoxy-4-vinylphenol; b 6OIO-caryophyllene oxide; c 6OIO-coniferyl aldehyde; d 6OIO-Isopsoralen

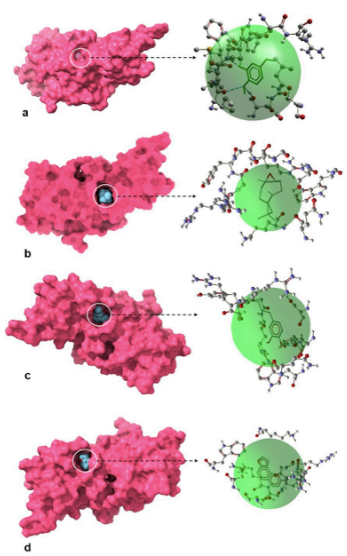


Fig. 9: Docking interaction between the target protein and bioactive phytocompounds: a 6OIO-7,8-dimethoxycoumarin; b 6OIO-scopoletin; c 6OIO-linoleic acid ethyl ester; d 6OIO-braylin

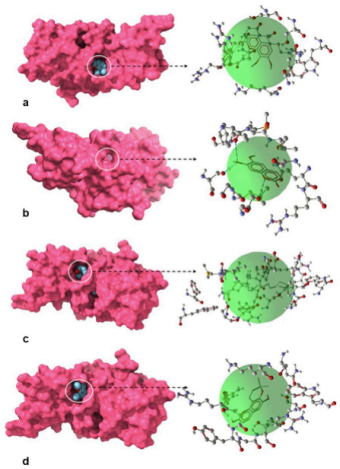
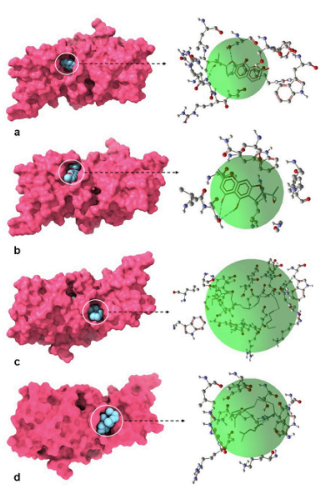


Fig. 10: Docking interaction between the target protein and bioactive phytocompounds: a 6OIO-marmesin; b 6OIO-visamminol; c 6OIO-docosanoic acid, ethyl ester; d 6OIO-squalene



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