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Isolation and Characterization of Antioxidants, Anti-Inflammatory Compound(s) from *Laggera mollis*

Abdu M. Ali[1], Rabiu Usman[1], Mustapha Isah[1*], Mujahid Ahmed[2], Zakariyya Dahiru[2]

[1]Department of Chemistry, Jigawa State College of Education, Gumel

[2]Department of Physics, Jigawa State College of Education, Gumel

*Email: abuabdallah99@mail.com

Abstract

Laggera mollis is used traditionally to manage inflammatory and infectious conditions, but systematic evaluation of its antioxidant and anti-inflammatory properties and chemical constituents is limited. Air-dried plant material (1kg) was percolated with ethanol (2.1 L) and fractionated by maceration into n-hexane (LM-HX), chloroform (LM-CF), ethyl acetate (LM-EA), and methanol (LM-ME) extracts. Antioxidant activity was assessed by DPPH radical scavenging, total antioxidant capacity, and ferric reducing antioxidant power (FRAP) assays. Total phenolic content was measured (µg gallic acid equivalents, µGAE mg). In vitro anti-inflammatory activity was evaluated using inhibition of egg albumin denaturation; diclofenac sodium served as reference. Bioactive constituents of the ethyl acetate fraction were isolated by chromatographic methods and characterized by FT-IR and NMR spectroscopy. LM-ME and LM-EA showed the highest total phenolic contents (298.11 and 389.43 µGAE mg, respectively) and produced the strongest antioxidant responses across assays. The LM-HX fraction and an isolated compound from LM-EA (LM-EA-25) exhibited notable anti-inflammatory activity in the albumin denaturation assay, with IC50 values of 0.25 µg/mL (LM-HX) and 15.65 µg/mL (LM-EA-25), comparable to diclofenac sodium. Spectroscopic analysis (FT-IR, NMR) confirmed the structure of the isolated bioactive constituent. Laggera mollis fractions, especially the ethyl acetate and methanol extracts, are rich in phenolics and display significant antioxidant activity, while LM-HX and an isolated LM-EA compound show appreciable anti-inflammatory effects. These results provide chemical and pharmacological support for the traditional use of L. mollis and identify it as a promising source for further isolation and pharmacological evaluation of natural therapeutic agents.

Key Words: *Laggera mollis*, Antioxidant activity, Anti-inflammatory activity, Bioactive Compound Isolation, FTIR and NMR Characterization, Total Phenolic content.

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1.0 Introduction

Medicinal plants have a long record history as therapeutic agents, with early documentation in Sumerian clay tablets and the Ebers Papyrus of ancient Egypt describing hundreds of plant-derived drugs. They remain central to healthcare in many non-industrialized societies because they are widely available and relatively inexpensive (Gravatto *et al.,* 2010). Plants synthesize diverse secondary metabolites that confer evolutionary advantagesserving as defenses against herbivores, pathogens, and environmental stressand many of these metabolites possess bioactive properties useful for human medicine (Gravatto *et al.,* 2010, Verma and Singh, 2008). Consequently, modern research increasingly focuses on the isolation, characterization, and pharmacological evaluation of phytoconstituents that traditional systems (e.g., Ayurveda) and ethnobotanical knowledge have long endorsed (Mudi and Ibrahim, 2009).

Phytochemicalsnon-essential dietary plant compounds such as alkaloids, saponins, phenols, flavonoids, steroids, and terpenoidsexert a broad range of biological activities including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. Alkaloids display cytotoxic and anti-inflammatory actions by modulating pro-inflammatory mediators and enzymes (Cassio *et al.,* 2020). Saponins exhibit anti-inflammatory and cytotoxic-enhancing properties (Francis et al., 2002). Phenolic compounds, including flavonoids, are notable antioxidants and contribute to antimicrobial and anti-cancer activities (Alu *et al.,* 2017, Martins *et al.,* 2016). Flavonoids such as quercetin act on multiple inflammatory pathways and show cardioprotective effects (Yang *et al.,* 2020). Terpenoids and plant steroids further diversify the therapeutic potential of plant-derived compounds (Zwenger and Basu, 2008, Bahar, 2007).

Oxidative stress, driven by reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals, contributes to cellular damage and is implicated in aging and disease (Wickens, 2001). Antioxidants bothendogenous (e.g., glutathione, catalase) and dietary (e.g., vitamins C and E, phenolics)neutralize free radicals and terminate oxidative chain reactions, thereby protecting biomolecules from damage (Sies, 1997). Given the central role of oxidative processes in numerous pathologies, assays that quantify antioxidant capacity are essential. Common in-vitro methods include DPPH, ABTS, FRAP, ORAC, CUPRAC, TPC/TAC, and TBARS, each based on different reaction principles and offering complementary information (Alam and Bristi, 2013).

The Folin–Ciocalteu assay remains the standard for estimating total phenolic content, reporting results as Gallic acid equivalents (GAE). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used for rapid screening of radical scavenging activity due to its simplicity, speed, and reproducibility; antioxidants reduce the stable, violet-colored DPPH radical to a non-radical form, producing a quantifiable color change (Sangar *et al.,* 2011).

Therefore, the objectives of the study include;

i. To extract and fractionate the plant material.
ii. To evaluate the antioxidant activities of the crude extract and solvents fractions.
iii. To assess the in-vitro anti-inflammatory activities of the crude extract, solvent fractions and isolated compound(s).
iv. To characterize and elucidate the chemical structure of the isolated bioactive compound(s).

2.0 Materials and Methods

2.1 Materials

Equipment: UV–visible spectrophotometer, rotary evaporator (RE 52A, England), UV lamp apparatus, Fourier transform infrared spectrometer (Agilent Technologies Cary 630 FTIR, USA), NMR spectrometer (JEOL Advance 600 MHz), and analytical balance.

Chemicals and reagents

All chemicals were analytical grade and obtained from JHD, Sigma-Aldrich, Lobachem, and BDH Ltd (Poole). Solvents used included n-hexane, chloroform, ethyl acetate, diethyl ether, ethanol, and methanol. Other reagents were ascorbic acid, sodium citrate, sodium chloride, Folin–Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), potassium ferricyanide, ferric chloride, trichloroacetic acid, and reagents for the phosphomolybdenum assay.

Plant material

Fresh whole plants of *Laggera mollis* were collected from the waterlogged area of the Sport Complex, Bayero University Kano (old campus), Kano State, Plant identification and herbarium authentication were performed by Baha'uddeen Said Adam, Department of Plant Biology, Bayero University Kano. A voucher specimen was deposited (Herbarium accession number BUKHAN 0138, dated 25th May, 2026).

2.2 Preparation and extraction

Plant samples were screened, washed with clean water, and air-dried in the shade until constant weight. The dried material was ground to a fine powder using mortar and pestle. One kilogram of powdered *Laggera mollis* was soaked in 96% ethanol at room temperature in a brown 2.5L reagent bottle. The mixture was agitated twice daily for 14 days, filtered, and the filtrate concentrated under reduced pressure using a rotary evaporator. The concentrated crude extract was air-dried and stored for fractionation.

Fractionation

Twenty-five grams of the crude ethanolic extract were suspended in 50mL of n-hexane and mixed; an insoluble gelatinous material formed and settled. The soluble fraction was collected; the procedure was repeated three times. Sequential liquid–liquid partitioning was similarly performed using chloroform, ethyl acetate and methanol to afford n-hexane, chloroform, ethyl acetate (LM-EA) and aqueous methanol fractions. Each fraction was concentrated under reduced pressure, dried at room temperature, weighed, and percentage yield calculated.

2.3 Antioxidant Assays

DPPH Radical Scavenging Assay

Antioxidant activity was assessed using the DPPH assay (Brand-Williams *et al.,* 1995) with modifications. Stock solutions of each extract (15 mg in methanol) were serially diluted to prepare eight concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 µg·mL−1). Blank absorbances were measured at 517 nm. Then 200 µL of 100 µM DPPH solution was added to each well and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. Percentage inhibition was calculated as:

AA (%) = [(Abscontrol – Abssample) / Abscontrol] × 100,

where Abscontrol is absorbance of DPPH with solvent and Abssample is absorbance with extract. All assays were performed in triplicate and results reported as mean ± SD.

Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu microplate method with modifications of Gutfinger (1981). In each well, 100 µL Folin–Ciocalteu reagent and 100 µL saturated sodium carbonate were added, followed by 10 µL of sample. After incubation at room temperature for 30 min, absorbance was read at 765 nm. Results were expressed relative to a gallic acid standard curve.

Total Antioxidant Capacity (Phosphomolybdenum Assay)

Total antioxidant capacity was determined by the phosphomolybdenum method (Berk *et al.*, 2012) with slight modification. Sample (0.3mL) was mixed with 3.0mL reagent solution (0.6M H_2SO_4, 28mM NaH_2PO_4, 4mM $(NH_4)_6Mo7O24$). The mixture was incubated at 95°C for 90 min, cooled, and absorbance measured at 695nm. Results were expressed as ascorbic acid equivalents using a standard curve.

Ferric Reducing Antioxidant Power (FRAP Assay)

Reducing power was measured following Oyaizu (1986) with modifications. To 50µL extract in a 96-well plate, 50µL phosphate buffer (pH 6.8–7.0) and 50µL 1% (w/v) potassium ferricyanide were added. Plates were incubated at 50°C for 20 min, then 50µL 10% (w/v) trichloroacetic acid was added. A 100µL aliquot was transferred to a new plate, mixed with 100µL distilled water and 20µL 0.1% (w/v) $FeCl_3$, and absorbance read at 700nm. Assays were done in triplicate.

2.4 In Vitro Anti-Inflammatory Assay

Heat-induced egg albumin denaturation inhibition: Anti-inflammatory activity was evaluated by heat-induced egg albumin denaturation (Peiris *et al.,* 2025) with modifications. Sample solutions and egg albumin were mixed (1.5mL each) at concentrations ranging from 1000 to 7.8125µg·mL^{-1}. The mixtures were pre-incubated at 35°C for 5 min, then heated at 80°C for 15 min and cooled to room temperature. A 300µL aliquot was transferred to a microplate and absorbance measured at 660nm. Diclofenac sodium served as a positive control and distilled water as blank. Percentage inhibition of denaturation was calculated relative to control.

Chromatography and Isolation

Thin Layer Chromatography (TLC)

TLC of LM-EA was performed on pre-coated silica gel 60 F254 plates (0.2mm). Plates were spotted with dilute extract using capillary tubes, developed in appropriate solvent systems in a TLC chamber, air-dried, and visualized under UV light (254/365nm).

Column Chromatography

Eight grams (8g) of LM-EA were subjected to vacuum/flash silica gel column chromatography (silica 60–120 mesh) using a glass column (55 × 4.5cm) slurry-packed and equilibrated with n-hexane. The sample was adsorbed onto silica and loaded onto the column. Elution was performed with n-hexane followed by gradient mixtures of n-hexane:ethyl acetate of increasing polarity. Seventy-seven 20mL fractions were collected and monitored by TLC; similar fractions were pooled. Repeated column chromatography of pooled fractions

yielded forty fractions; fractions 25–41 were combined and yielded a dark yellow crystalline solid (single spoton TLC). Other single-spot fractions were observed in fractions 42-44.

2.5 Spectroscopic Analysis and Structure Elucidation

The isolated compound (LM-EA-25) was characterized by FT-IR and NMR spectroscopy. FT-IR spectra were recorded at the Instrumentation Laboratory, Department of Pure and Industrial Chemistry, Bayero University Kano. NMR spectra were obtained at King Fahd University of Petroleum and Minerals on a JEOL Advance 600 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to TMS and coupling constants (J) in Hz; $CDCl_3$ was used as solvent.

3.0 Results and Discussion

Table 1: Weight and Physical Appearance of the *Laggera mollis* Extracts

Extract	Colour	Texture	Weight (g)
LM-HX	Dark green	Gummy	14.7
LM-CF	Dark Green	Gummy	4.0
LM-EA	Greenish	Oily	17.4
LM-ME	Brown	Oily	13.6

The extraction procedure yielded 61.6g of crude ethanolic extract from 1kg of plant material following repeated percolation with ethanol. Subsequent liquid-liquid partitioning of 50g of the crude extract with solvents of varying polarity produced four fractions: n-hexane, chloroform, ethyl acetate, and methanol, which were labeled LM-HX, LM-CF, LM-EA, and LM-ME, respectively.

DPPH Radical Scavenging Activity of *Laggera mollis*

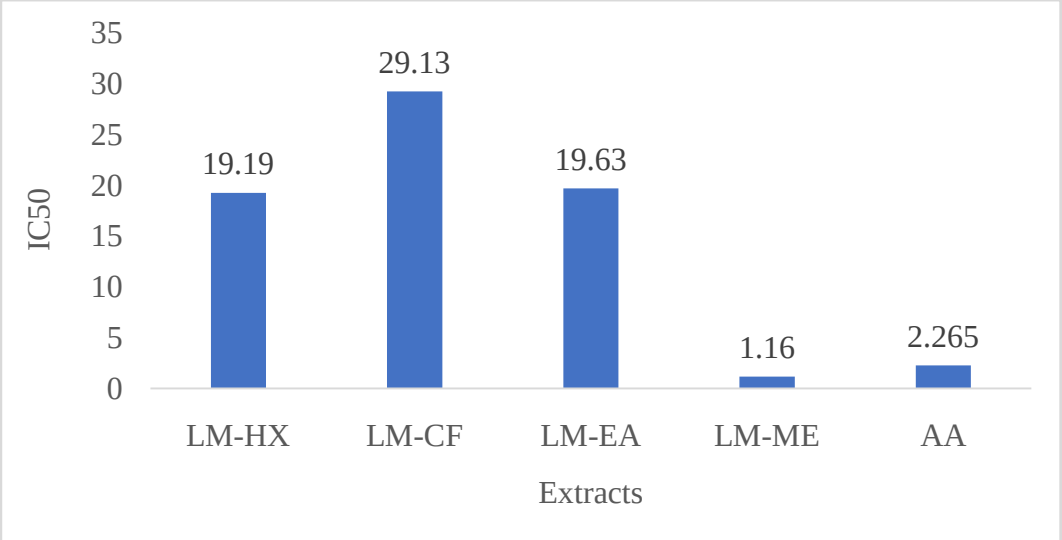


Figure 1: IC_{50} values of the *Laggera mollis* extracts and standard

The methanol extract of *Laggera mollis* (LM-ME) showed the strongest DPPH free-radical scavenging activity (IC_{50}= 1.16), comparable to the standard ascorbic acid (IC_{50} =2.26). This finding agrees with Ahmed et al. (2023), who reported potent antioxidant and ROS-scavenging effects for *Laggera pterodonta* methanol extract, and with Getahun *et al.*, (2020),

who observed high antioxidant activity for *Laggera tomentosa* aqueous extract at higher concentrations ($IC_{50} \approx 0.33–0.39$mg/ml). Together, these results indicate that Laggera species contain active constituents with significant free-radical scavenging potential.

Total Phenolic Content

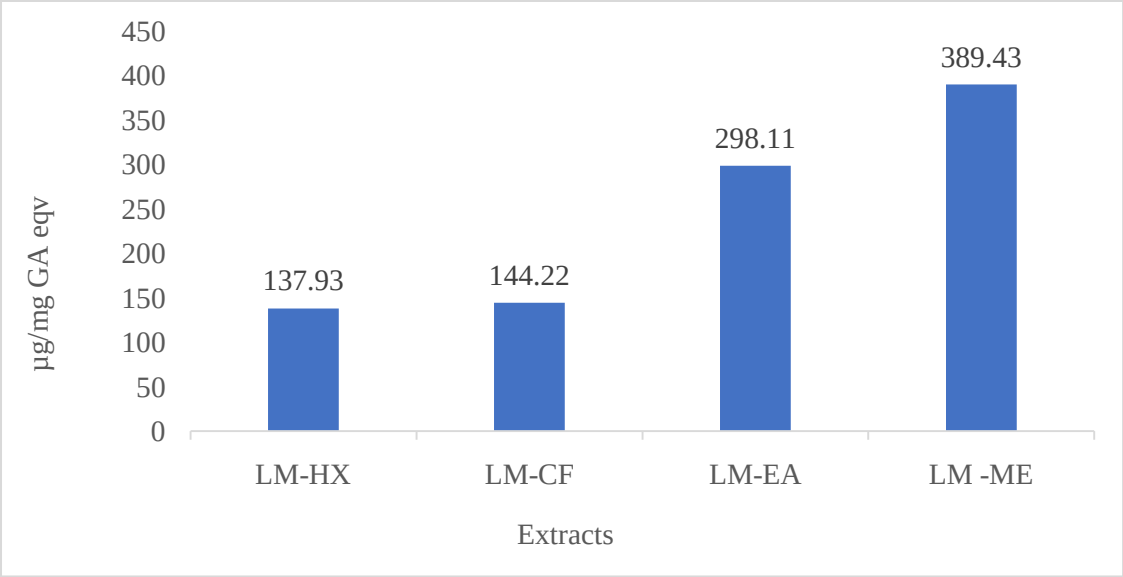


Figure 2: Phenolic Content of *Laggera mollis* Extracts

The total phenolic content (TPC) of the tested plant materials varied from 144.22 to 389.43µg GAE/g dry weight (Figure 2). The methanol extract (LM-ME) exhibited the highest phenolic concentration (389.43µg GAE/g dw), followed by the ethyl acetate extract (LM-EA) with 298.11 µg GAE/g dw. These results indicate that polar solvents, particularly methanol, more effectively extract phenolic compounds from the plant material than less polar solvents used in this study.

Total Antioxidant Capacity (Phosphomolybdenum Assay)

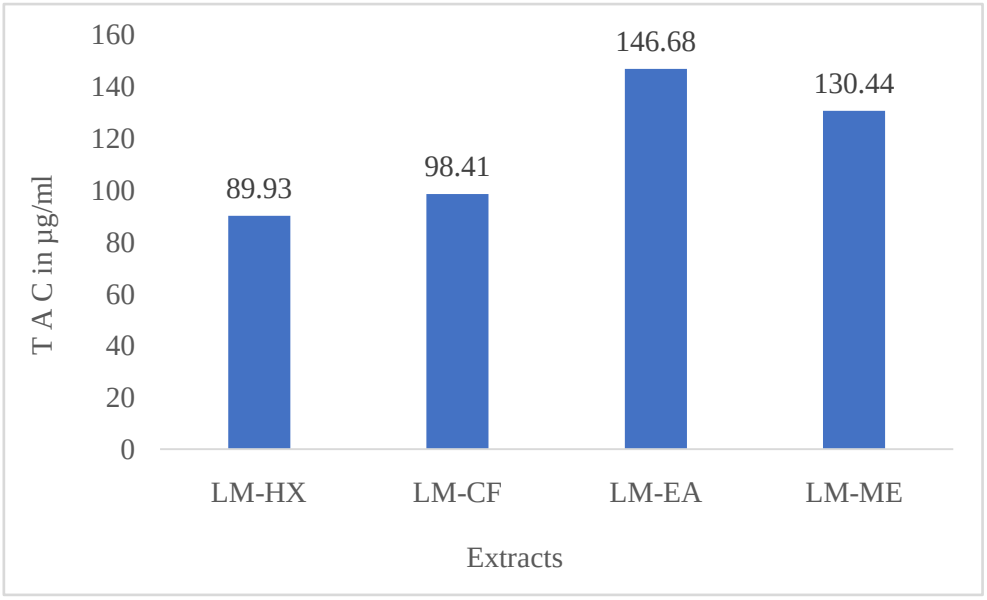


Figure 3: Phosphomolybdate Assay Capacity of *Laggera mollis* Extracts

The total antioxidant capacity (TAC) of the extracts was evaluated using the phosphomolybdenum assay, which is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds, forming a green phosphate/Mo(V) complex under acidic conditions. The results, expressed as ascorbic acid equivalents (gAAE/100g), revealed a concentration dependent increase in antioxidant activity across all tested samples. This trend suggests that the bioactive constituents responsible for antioxidant effects are present in sufficient quantities and exhibit dose responsive behavior.

Comparatively, the ethyl acetate extract (LM-EA) demonstrated higher total antioxidant capacity than the methanol extract (LM-ME). This observation is attributed to the differential solubility of antioxidant phytochemicals, particularly moderately polar compounds such as flavonoids, phenolics, and certain terpenoids, which are more efficiently extracted in ethyl acetate. The relatively lower activity observed in the methanol extract may indicate the presence of compounds with weaker reducing power or lower concentrations of active constituents. The appreciable antioxidant activity exhibited by both extracts supports the presence of redox-active phytochemicals in *Laggera mollis*. These compounds are known to act as electron donors, thereby neutralizing free radicals and mitigating oxidative stress. The findings are consistent with previous reports on medicinal plants rich in phenolic constituents, which are widely recognized for their antioxidant and therapeutic potentials.

Ferric Reducing Antioxidant Power (FRAP Assay)

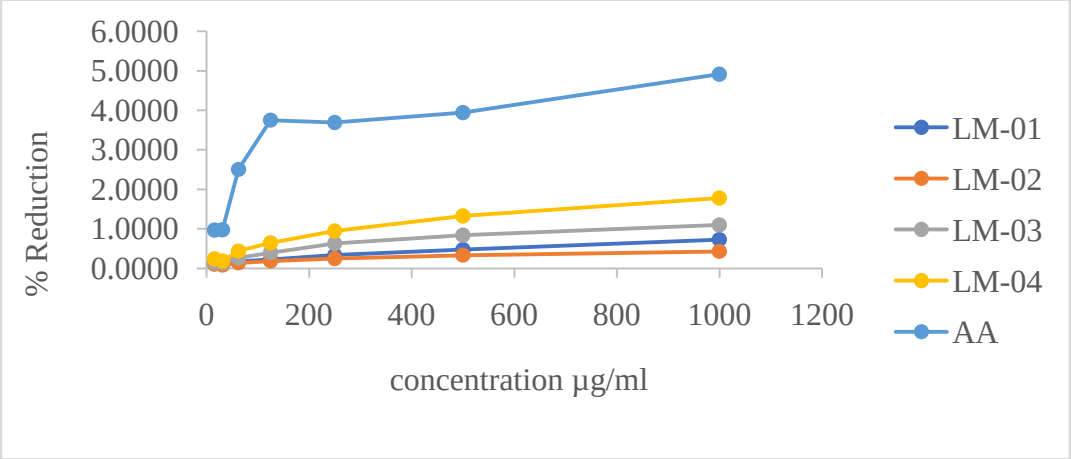


Figure 4: Graph of Percentage Reduction against Concentration

Figure 4 presents the ferric reducing antioxidant power (FRAP) of the evaluated extracts. Among all samples, the methanol extract (LM-ME) consistently exhibited the highest percentage reduction across the tested concentrations, demonstrating superior reducing capacity relative to the standard, ascorbic acid. This suggests a higher abundance of electron-donating constituents, likely phenolic compounds, in the methanol fraction. The ethyl acetate extract (LM-EA) showed the second-highest reducing activity, indicating a moderate presence of antioxidant constituents with intermediate polarity. In contrast, the hexane extracts (LM-HX) displayed lower reducing power, while the chloroform extract exhibited the least activity among the tested samples. The observed trend highlights the influence of solvent polarity on the extraction efficiency of antioxidant compounds, with polar solvents favoring the recovery of bioactive constituents responsible for ferric ion reduction.

Anti-Inflammatory Properties of the Extract and the Isolated Compound

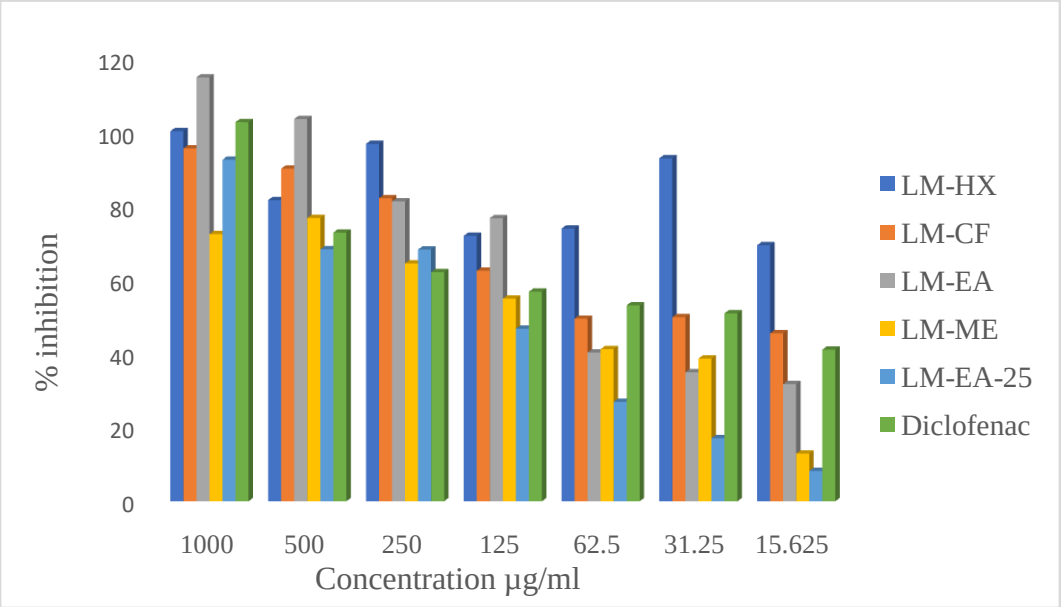


Figure 5: Percentage Inhibition against Concentration of *Laggera mollis* extracts, LM-EA-25 and standard.

Erythrocyte membranes share notable structural and functional similarities with lysosomal membranes, and are therefore often employed as experimental models for evaluating membrane stabilization (Kumar *et al.,* 2015). The stabilization of red blood cell (RBC) membranes by plant extracts is indicative of their potential to inhibit the release of lysosomal enzymes, which are key mediators in inflammatory processes. Consequently, the ability of medicinal plant extracts to prevent erythrocyte hemolysis reflects their capacity to exert anti-inflammatory effects through membrane stabilization. Furthermore, erythrocytes are rich in hemoglobin, a protein susceptible to denaturation under stress conditions. The inhibition of protein denaturation, as demonstrated using the egg albumin assay, serves as an additional indicator of anti-inflammatory activity. Plant extracts capable of protecting proteins from denaturation may similarly stabilize cellular proteins in vivo, thereby reducing inflammation.

In this study, the hexane extract (LM-HX), the isolated fraction (LM-EA-25), and diclofenac sodium (reference drug) exhibited notable anti-inflammatory activities, with IC_{50} values of 0.25µg/mL, 15.651µg/mL, and 39.234µg/mL, respectively. These results suggest that LM-HX demonstrated the highest inhibitory activity among the tested samples. However, despite the comparatively lower IC_{50} value of diclofenac reported, its overall anti-inflammatory efficacy was observed to be significantly higher than that of the ethyl acetate (LM-EA) and methanol (LM-ME) extracts, likely due to its well-established pharmacodynamic properties.

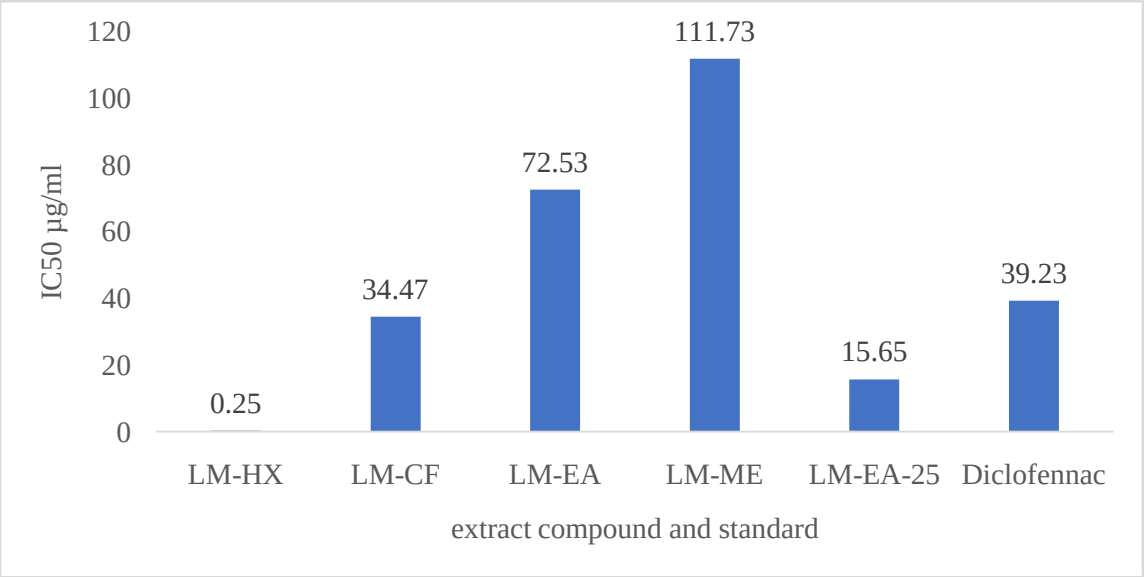


Figure 6: IC_{50} of the *Laggera mollis* extracts and LM-EA-25

Comparatively, the hexane extract (LM-HX) showed superior activity relative to the methanol extract (LM-ME) across all tested concentrations, indicating that non-polar constituents may play a more significant role in the observed anti-inflammatory effect. This suggests that bioactive compounds with anti-inflammatory potential are more concentrated in the non-polar fraction of *Laggera mollis*. The observed anti-inflammatory activity of the extracts can be attributed to the presence of bioactive phytochemicals such as steroids, phenols, tannins, saponins, terpenoids, flavonoids, and alkaloids. These classes of compounds are widely reported to exhibit anti-inflammatory mechanisms, including inhibition of protein denaturation, stabilization of lysosomal membranes, and suppression of pro-inflammatory mediators.

Overall, the findings from the egg albumin denaturation assay support the ethnomedicinal use of Laggera species in the management of inflammatory conditions. This includes traditional

applications in the treatment of bronchitis, leukemia, phlegm-related disorders, and bacterial infections. The results provide scientific validation for its use as a herbal anti-inflammatory agent and highlight its potential for further pharmacological investigation.

3.1 Isolation and Characterization

The ethyl acetate extract (LM-EA) was analyzed by thin layer chromatography (TLC) on silica gel 60 PF254 aluminum sheets (0.2mm thickness) using solvent systems of varying polarity n-hexane: ethyl acetate ratios. Under UV light (254nm), multiple spots were observed, indicating the presence of compounds with Rf values ranging from 0.15 to 0.72. Column chromatography of the 8g ethyl acetate extract was performed on a silica gel (60-120 mesh) column (55 × 4.5cm) using a gradient elution starting with 100% n-hexane, followed by n-hexane-ethyl acetate mixtures. A total of 77 extracts (20mL each) were collected. TLC profiling revealed similar spot patterns in extracts 25-41 upon pooling indicative of a pure isolate. Further purification via repeated micro-column chromatography on extracts 42-44 also produced single-spot purity (Rf = 0.28) in the same system, isolating an oily greenish solid (about 26mg).

Fourier Transform -Infra Red Spectroscopy (FT-IR)

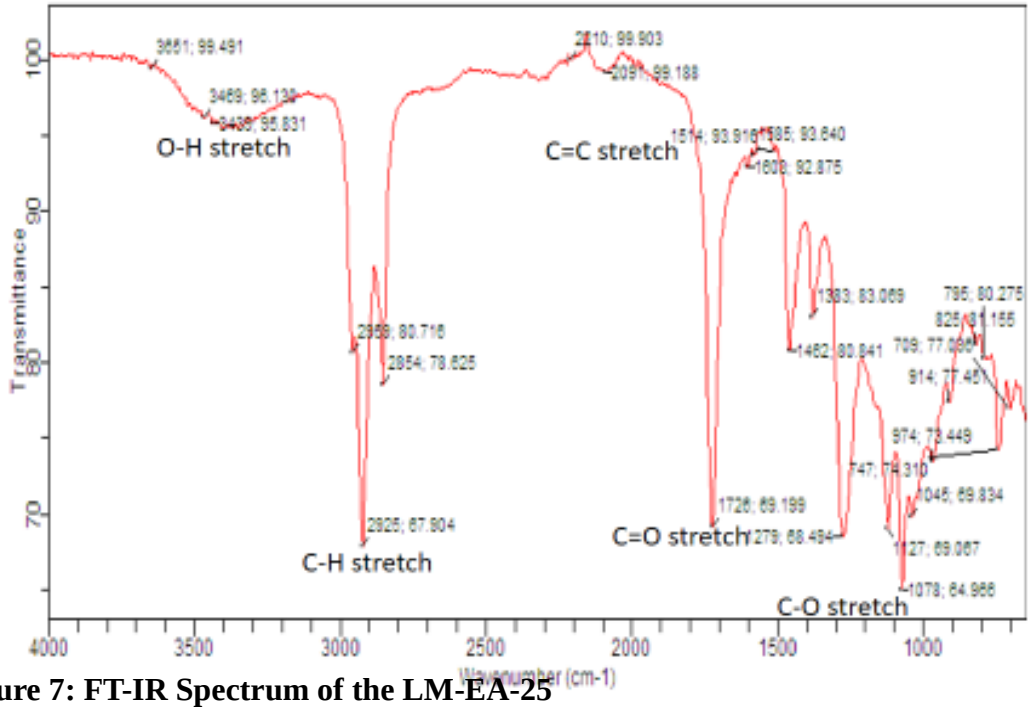


Figure 7: FT-IR Spectrum of the LM-EA-25

The FT-IR spectrum of the isolated compound reveals several characteristic absorption bands indicative of its functional groups. Absorption at 3651cm^{-1} is attributed to O–H stretching vibration, suggesting the presence of a hydroxyl group, possibly free or weakly hydrogen-bonded. The bands observed at 2959cm^{-1} and 2925cm^{-1} correspond to aliphatic C–H stretching vibrations, indicating the presence of saturated hydrocarbon moieties. A prominent absorption band at 1726cm^{-1} is characteristic of a carbonyl (C=O) stretching vibration, consistent with a ketone functional group. Additionally, the band at 1608cm^{-1} is assigned to C=C stretching within an aromatic ring, confirming the presence of an aromatic system in the compound. The absorption at 1279cm^{-1} is attributed to C–O stretching vibration, which

further supports the presence of an oxygenated functional group such as a phenolic or ether linkage.

^{1}H Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR)

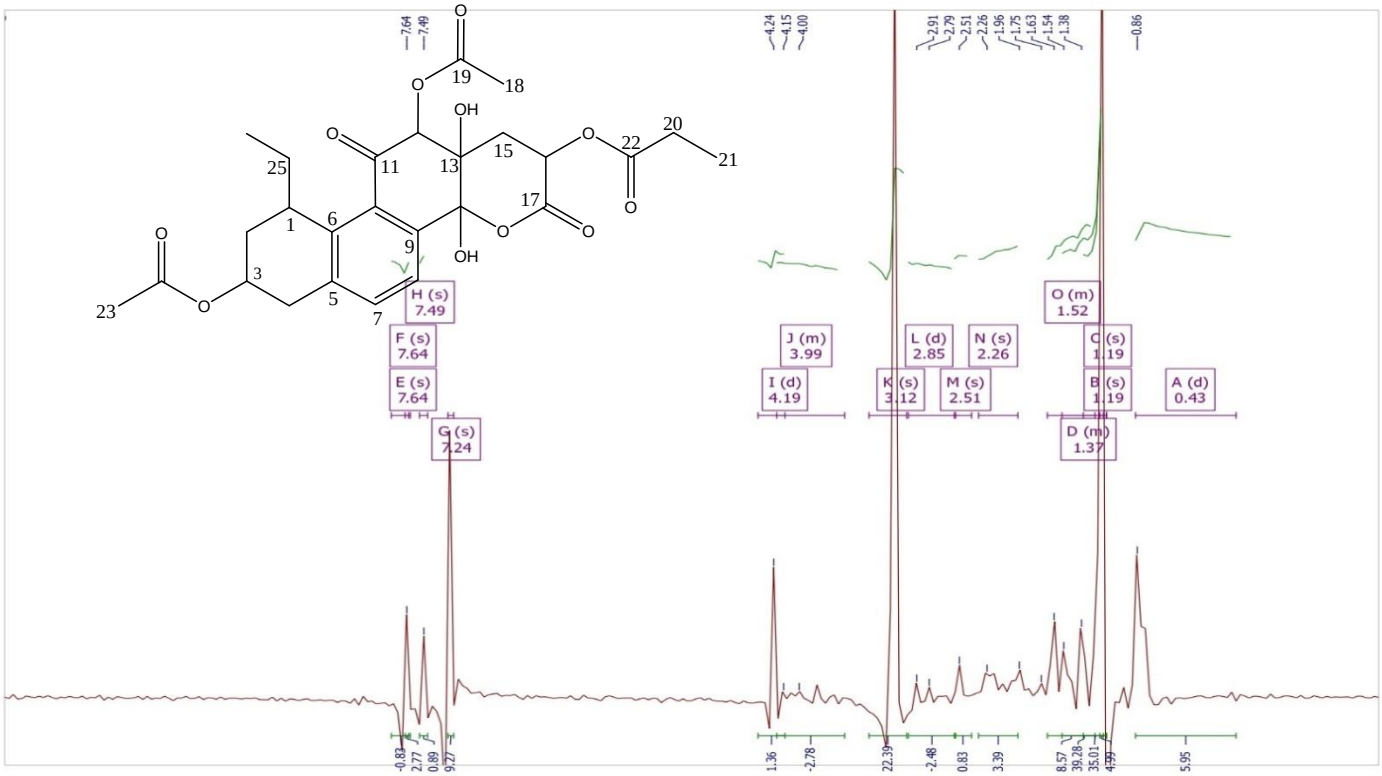


Figure 8: H^{1} NMR Spectrum of the LM-EA-25

The^{1}H NMR spectrum of compound LM-EA-25 was recorded in deuterated chloroform $CDCl_3$ at 700 MHz. The spectrum revealed multiple proton environments, indicating structural complexity within the molecule. The observed chemical shifts ranged from 0.86 to 7.64ppm, suggesting the presence of both aliphatic and aromatic proton systems. Signals in the up field region (0.86-2.50ppm) are characteristic of methyl, methylene, and methine protons in saturated hydrocarbon chains, while resonances appearing in the downfield region (6.00–7.64ppm) are indicative of aromatic or olefinic protons. The multiplicity and integration of these peaks further support the presence of distinct proton environments within the structure. The overall spectral profile is consistent with a compound containing both aliphatic and unsaturated (aromatic) moieties, supporting the proposed structural features of LM-EA-25.

^{13}C Nuclear Magnetic Resonance Spectroscopy (NMR)

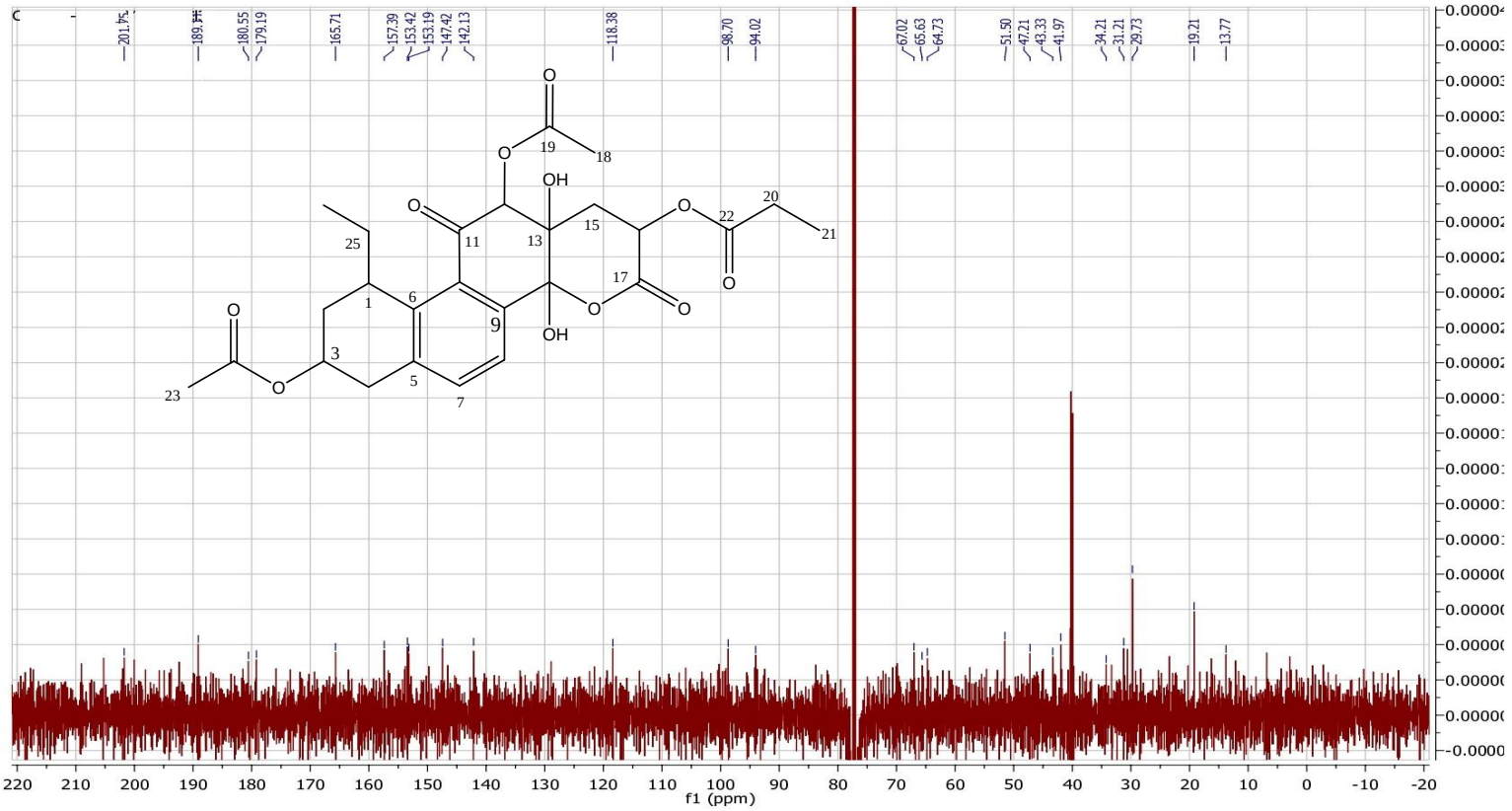
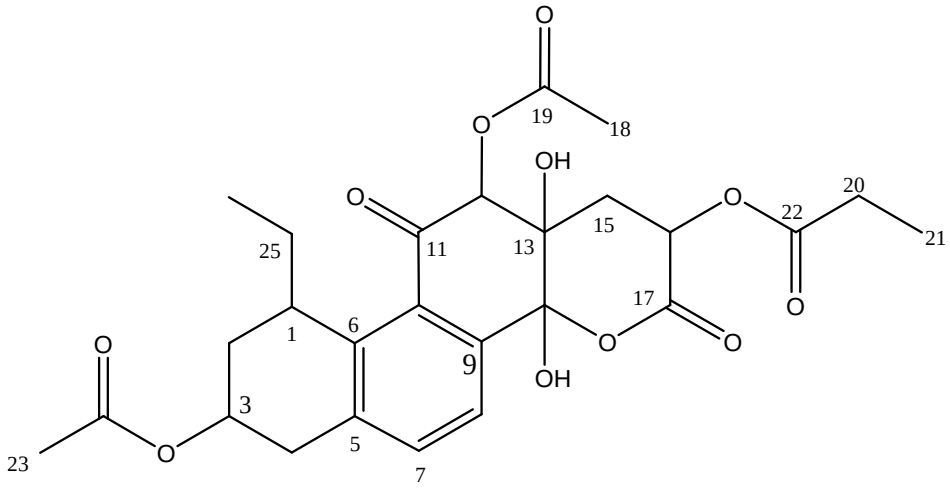


Figure 9: ^{13}C NMR Spectrum of the LM-EA-25

The 13C NMR spectrum of LM-EA-25 shows twenty-six carbon resonances (Figure 8). Resonances in the aromatic region between 118.38 and 157.39ppm (118.38, 142.13, 147.42, 153.19, 153.42 and 157.39ppm) are assigned to SP^2 carbons of aromatic C=C bonds. Downfield signals at 201.50, 189.10, 180.55, 179.19 and 165.71ppm are characteristic of carbonyl carbons and are attributed to ketone and ester functionalities. Signals in the 51.50–98.70ppm range (51.50, 64.73, 65.63, 67.02, 94.02 and 98.70ppm) are consistent with carbons bonded to oxygen (C–O), indicating the presence of alcohols, ethers or ester-linked methine/methylene carbons. The markedly deshielded carbon signals above 160 ppm likely reflect carbon atoms bearing additional electron-withdrawing substituents (other than oxygen), which further support substitution patterns on the aromatic system. Together, the observed chemical shifts are consistent with a molecule containing substituted aromatic rings, multiple carbonyl groups (ketone and ester), and several oxygen-bearing aliphatic carbons.

Combining all the above spectral information data, the compound was propose as Proponic acid-8, 12-diacetoxy-10-ethyl-4a, 12a-dihydroxy-3, 11-dioxo-2, 3, 4a, 7, 8, 9, 10, 11, 12, 12a-decahydro-1H-4-oxo-chrysen-2-yl ester.



Conclusion

The present study demonstrates that extracts of *Laggera mollis* exhibit significant antiphlogistic activity, supporting their traditional use in treating inflammation-related disorders such as bronchitis and leukemia. Bioassay and chemical characterization indicate the extracts mitigate oxidative stress and inflammatory responses, and the isolation and structural elucidation of a previously unreported compound represents the first documentation of this metabolite in the literature. These findings expand the phytochemical and pharmacological profile of L. mollis and identify a novel natural product with potential for development as an anti-inflammatory therapeutic lead. Further work including mechanism-of-action studies, in vivo efficacy and safety evaluations, and structure activity relationship analysis will be necessary to advance this compound toward preclinical development.

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