Integrated Chemical and Biological Assessment of Lupine Seed Oil from Fatty Acid Derivatives to Potent Targeting of *Helicobacter pylori* and its Urease Inhibitory Activity

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The chemical composition and bioactivity of lupine seed oil were explored using gas chromatography-mass spectrometry (GC–MS). The analysis identified diverse constituents dominated by fatty acid derivatives, esters, and terpenoids. The major compounds were 9,12-octadecadienoyl chloride (23.6%), E-8-methyl-9-tetradecen-1-ol acetate (19.1%), and 2,3-dihydroxypropyl palmitate (8.83%), with moderate levels of tert-hexadecanethiol (6.97%) and 9,12,15-octadecatrienoic acid diacetate ester (6.58%). Minor components included caryophyllene (3.71%) and unsaturated fatty acids (< 5%). Antimicrobial evaluation revealed a larger inhibition zone for lupine seed oil (29.0 ± 0.5 mm) than the standard drug (28.0 ± 1.0 mm), a minimum inhibitory concentration (MIC) of 15.6 µg/mL, and a minimum bactericidal concentration (MBC)/MIC index of 2. Lupine seed oil exhibited potent antibiofilm activity, inhibiting 77.5%, 91.1%, and 97.1% of biofilm formation at 25%, 50%, and 75% MBC, respectively. Hemolysis inhibition ranged from 79.2 ± 2.1% to 98.1 ± 0.9% across 25 to 75% MIC. Urease inhibition reached 95.3% at 1000 µg/mL (IC_{50} = 9.56 µg/mL), and protein denaturation was inhibited. Cytotoxicity against Caco-2 cells was dose-dependent, with IC_{50} = 148.7 ± 2.3 µg/mL. These findings highlight lupine seed oil as a rich source of bioactive compounds with strong antimicrobial, anti-inflammatory, and anti-proliferative activities.

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INTRODUCTION

Lupine seed oil, like other plant-derived oils, is composed of bioactive lipid constituents that have been widely reported in the literature to display anti-inflammatory, antimicrobial, antioxidant, and anti-proliferative actions. Such biological properties have been documented in various plant oils and natural extracts, which are rich in fatty acid derivatives and related bioactive compounds (Al-Rajhi *et al.* 2025a; Alsalamah *et al.* 2025). Based on this general evidence, lupine seed oil was selected in the present study to evaluate its potential biological activities against *Helicobacter pylori* and related pathological mechanisms.

The chemical complexity of plant-derived oils allows them to interact with microbial membranes, inhibit enzyme activity, and modulate cellular pathways, making them valuable in pharmaceutical, food, and cosmetic applications (Al-Rajhi and Abdel Ghany 2023; Qanash *et al.* 2023a; Al-Rajhi *et al.* 2025b). Lupine seed oil, derived from *Lupinus* species, is rich in fatty acid derivatives, esters, and terpenoids, which contribute to its biological potential (Sotelo-Méndez *et al.* 2023; Georgieva *et al.* 2025). Human clinical studies indicate that incorporating 25 g of lupine protein into foods improves lipid profiles in hypercholesterolemic individuals by reducing cholesterol, triglycerides, and uric acid levels (Pihlanto *et al.* 2017). *Lupinus angustifolius* is a rich source of dietary fiber (41.5%), comprising both soluble (11%) and insoluble (30.5%) fractions, while *Lupinus albus* contains an even higher total fiber content (50.4%), primarily insoluble (Hall *et al.* 2005). Dietary fiber enrichment of food products supports intestinal health and may lower the risk of colorectal disorders (Písaríková and Zralý 2010).

Helicobacter pylori is a widespread gastric pathogen that is responsible for chronic gastritis, peptic ulcers, and is strongly associated with the development of gastric cancer, posing a significant global health challenge due to increasing antibiotic resistance (Yahya *et al.* 2022). *H. pylori* produces urease, a central enzyme that catalyzes the breakdown of urea to carbon dioxide and ammonia, neutralizing gastric acid and enabling the bacteria to colonize and survive and remain viable in the highly acidic conditions of the stomach. Colorectal cancer ranks among the leading causes of cancer-related morbidity and mortality worldwide. It is often associated with late diagnosis, limited treatment options, and the development of drug resistance (Mahdi *et al.* 2025). This underscores the urgent need for the development of safer and more effective therapeutic agents. Chronic inflammation is a major contributor to numerous diseases, including gastritis, ulcers, cardiovascular disorders, and cancer, often resulting from persistent infections or oxidative stress, and remains a significant challenge due to limited safe and effective anti-inflammatory therapies (Kapoor *et al.* 2025).

Natural bioactive compounds, such as those in lupine seed oil, offer a promising approach to overcome these challenges by exhibiting antimicrobial, anti-urease, anti-inflammatory, antibiofilm, and anti-proliferative activities, providing a multifunctional strategy for managing *H. pylori* infection, chronic inflammation, and colon cancer (Sbihi *et al.* 2013; Al-Amrousi *et al.* 2022). The present work aimed to comprehensively investigate the chemical make-up of lupine seed oil employing gas chromatography-mass spectrometry (GC–MS) analysis and to evaluate its biological potential through a series of *in vitro* assays against *H. pylori*. Specifically, antibiofilm, anti-hemolytic, anti-inflammatory (protein denaturation), urease inhibitory, and anti-proliferative activities, to

assess its probable as a natural source of multifunctional bioactive ingredients with medicinal relevance.

EXPERIMENTAL

Source of Lupine Seed Oil

The lupine seed oil used in this study was obtained as a commercially available fixed oil from a certified supplier (Attar Commercial Registration No. 7012821935) in Riyadh, Saudi Arabia. According to the supplier specifications, the oil was produced from *Lupinus* spp. seeds using cold pressing (mechanical expression) without the application of heat or chemical solvents, and subsequently it was refined to obtain a stable, food-grade fixed oil. The sample was supplied as a pre-extracted product and used directly without any further chemical treatment. The oil was kept in airtight amber glass containers at 4 °C to stop oxidation and maintain its chemical stability prior to analysis.

GC–MS Analysis of Oil

The chemical constituents of the lupine seed oil were analyzed using gas GC–MS. Briefly, the oil sample was diluted (1:100, v/v) in analytical-grade n-hexane and subsequently passed through a 0.22 µm syringe filter before analysis. The GC–MS analysis was performed using a GC system coupled with a mass selective detector (an Agilent 7890 GC system equipped with an Agilent 5975C MSD). Separation of the components was performed using an HP-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium served as the carrier gas at a constant flow rate of 1.0 mL/min. A sample volume of 1 µL was injected in split mode with a split ratio of 1:20, and the injector temperature was maintained at 250 °C. The oven temperature was initially set at 60 °C and held for 2 minutes, then increased to 200 °C at a rate of 5 °C/min, followed by a further increase to 280 °C at 10 °C/min, where it was held for 10 minutes, resulting in a total run time of approximately 40 minutes. The mass spectrometer operated under electron ionization (EI) conditions at 70 eV, with the ion source and quadrupole temperatures set at 230 °C and 150 °C, respectively. Mass spectra were acquired over a scan range of *m/z* 40–600. Compound identification was achieved by matching the obtained spectra with those available in the National Institute of Standards and Technology (NIST) and Wiley libraries, as well as by comparing calculated retention indices with previously reported values. The relative abundance of each constituent was determined using peak area normalization.

Antimicrobial Activity

For antimicrobial susceptibility assays, freshly grown *Helicobacter pylori* colonies were aseptically suspended in sterile physiological saline. The bacterial suspension turbidity was standardized to 2.0 McFarland, corresponding to approximately 1.0×10^8 CFU/mL, to ensure uniform inoculum density across all experiments. The inhibitory effect against *H. pylori* was evaluated *in vitro* by means of the agar well diffusion technique with minor modifications. Briefly, Mueller–Hinton agar plates complemented with 10% (v/v) sheep blood were uniformly injected with 100 µL of the standardized bacterial suspension. Wells measuring 6 mm were aseptically created in the agar by means of a sterile cork borer. A volume of 100 µL of the extract was added to each well at the designated concentration.

Dimethyl sulfoxide (DMSO) functioned as the negative control, while clarithromycin (0.05 mg/mL) was used as the positive control reference antibiotic. The plates were subsequently incubated at 37 °C for 72 h under microaerophilic situations with adequate humidity. Following incubation, antibacterial activity was evaluated by measuring the diameter of the clear inhibition zones surrounding each well, and results were recorded in millimeters (Qanash *et al.* 2023b).

Assessment of Minimum Inhibitory Concentration (MIC)

MIC of the tested agents against *H. pylori* was determined using a broth microdilution method. Stepwise two-fold serial dilutions of each test compound were prepared in Mueller–Hinton broth containing 10% fetal bovine serum. An equal volume of standardized *H. pylori* suspension (1.0×10^8 CFU/mL) was added to each well to achieve a final inoculum of approximately 5×10^5 CFU/mL (Al-Rajhi *et al.* 2023a). Incubation was carried out at 37 °C for 72 h under microaerophilic conditions. The MIC was defined as the lowest concentration of the tested compound that showed no visible bacterial growth compared with the growth control.

Assessment of Minimum Bactericidal Concentration

To assess the minimum bactericidal concentration (MBC), aliquots (10 µL) from wells viewing no noticeable development in the MIC assay were subcultured on top of Mueller–Hinton agar plates supplemented with 10% sheep blood. The plates were incubated under microaerophilic conditions at 37 °C for 72 h. The MBC was defined as the lowest concentration of the tested agent that resulted in no detectable bacterial growth on agar plates, indicating ≥ 99.9% bacterial killing (Al-Rajhi *et al.* 2023a).

Antibiofilm Assay

The antibiofilm activity against *H. pylori* was evaluated at sub-inhibitory concentrations ranged from to 25-75% of the verified MBC. A standardized bacterial suspension was inoculated into 96-well microtiter plates and treated with the tested agents at the indicated concentrations. The inoculated plates were maintained at 37 °C for 72 h under microaerophilic situations.

Following incubation, non-adherent cells were eliminated by washing with PBS, and the remaining biofilm biomass was measured using the crystal violet staining assay. Absorbance was measured at 570 nm, and biofilm inhibition was calculated relative to the untreated control (Al-Rajhi *et al.* 2023b). The percentage inhibition of biofilm formation was determined relative to the untreated control using the following Eq. 1:

$$\text{Inhibition of biofilm (\%)} = \left(1 - \frac{A_{\text{treated}}}{A_{\text{control}}}\right) \times 100 \quad (1)$$

Time-Kill Kinetic Assay

The bactericidal kinetics of the tested oil against *Helicobacter pylori* were evaluated using a time-kill assay. A standardized *H. pylori* suspension (1.0×10^6 to 10^7 CFU/mL) was exposed to the oil at concentrations equivalent to 1× and 2× MBC in supplemented Mueller–Hinton broth. The cultures were maintained at 37 °C under microaerophilic situations.

At scheduled time points (0, 2, 4, 8, 12, 24, and 48 h), aliquots were taken, serially diluted, and spread onto Mueller–Hinton agar supplemented with 10% sheep blood. Following 72 h of incubation, developed colonies were counted and expressed as $\log_{10}$ CFU/mL. Killing kinetics were determined by plotting $\log_{10}$ CFU/mL *versus* time.

Urease Inhibition Assay

The urease inhibitory potential of the tested oil was evaluated using a colorimetric assay based on a modified Berthelot reaction. Stock solutions of each extract were prepared at a concentration of 1 mg/mL. The formation of ammonia resulting from enzymatic urea hydrolysis was quantified spectrophotometrically at 625 nm. The IC_{50} values (half-maximal inhibitory concentrations) were subsequently calculated for each extract. Hydroxyurea, a well-established urease inhibitor, was used as the reference standard for comparative purposes. The reaction mixture was prepared by combining urea solution (850 µL), varying volumes of the extract solution (0 to 100 µL), and phosphate buffer (100 mM, pH 7.4) to obtain a final volume of 985 µL. Enzymatic activity was initiated by adding 15 µL of urease enzyme. The mixture was kept for 60 min to permit enzymatic hydrolysis. Upon completion of incubation, the released ammonia was detected by adding 500 µL of reagent A (a mixture of phenol and sodium nitroprusside in distilled H_2O) and 500 µL of reagent B (composed of sodium hydroxide and sodium hypochlorite solution). The mixture was further maintained at 37 °C for 30 min to allow the development of color. Absorbance readings were taken at 625 nm. A reaction mixture lacking any inhibitor was considered as the negative control and assigned 100% enzyme activity. Urease inhibition was calculated using Eq. 2,

$$\text{Inhibition (\%)} = \left[1-\left(\frac{T}{C}\right)\right] \times 100 \quad (2)$$

where T represents the absorbance of the test sample (extract or standard) in the existence of urease, and C corresponds to the absorbance of the control reaction containing enzyme and solvent only. The IC_{50} value, corresponding to the concentration producing 50% inhibition of urease activity, was determined by intrigue inhibition percentages versus extract concentrations and interpolating the concentration corresponding to 50% inhibition.

Anti-proliferative Assay on Caco-2 Cells

The cytotoxic potential of the tested samples was assessed using the human colorectal carcinoma Caco-2 cell line. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics under standard conditions at 37 °C in a humidified atmosphere containing 5% CO_2. For the assay, cells were seeded in 96-well plates and incubated for 24 h to allow adherence. Subsequently, the culture medium was replaced with fresh medium containing different concentrations of the tested samples. After 48 h of exposure, cell viability was evaluated using a colorimetric assay, and absorbance was measured using a microplate reader. Cell viability was calculated relative to untreated controls, and IC_{50} values were determined from dose–response curves (Qanash *et al*. 2025). The toxicity percentage is commonly calculated using Eq. 3,

$$\text{Toxicity (\%)} = \left(1-\frac{A_S}{A_C}\right) \times 100 \quad (3)$$

where A_s is the absorbance of cells which treated by the extract and A_c is the absorbance of

control cells (untreated). This equation expresses the percentage of cell death or growth inhibition relative to the control. The inhibitory concentration required to reduce cell viability 50% (IC_{50}) was determined from dose–response curves generated by plotting percentage viability against sample concentration.

Bovine Serum Albumin (BSA) Denaturation Evaluate

The anti-inflammatory potential of the tested samples was estimated *via* bovine serum albumin (BSA) denaturation technique. Briefly, 50 µL of each sample was mixed with 450 µL of a 1% (w/v) aqueous BSA solution to get final doses extending from 1.56 to 200 µg/mL. The reaction mixture pH was carefully adjusted to 6.3 employing 1 N hydrochloric acid. The prepared mixtures were initially incubated at ambient temperature for 20 min to ensure proper interaction between the protein and the test compounds. This was followed by thermal induction of protein denaturation by incubating the samples in a water bath at 55 °C for 30 min. Following heating, the mixtures were allowed to cool to temperature of room. The extent of protein denaturation was quantified by measuring the absorbance at 670 nm using a Biosystem 310 Plus spectrophotometer (BioSystems S.A., Barcelona, Spain). Diclofenac sodium was employed as the reference anti-inflammatory drug. The inhibitory effect on protein denaturation was calculated using the following Eq. 4,

$$\text{Inhibition (\%)} = \left(\frac{A_c - A_s}{A_c}\right) \times 100 \quad (4)$$

where A_c signifies the absorbance of the control reaction and A_s denotes the absorbance of the extract.

Statistical Analysis

Data processing was performed with three replicates to calculated standard deviation *via* GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

From the current investigation, the chemical constituents of lupine seed oil, along with their retention times (R.T.), molecular formulas, relative abundance (area %), and molecular weights were recognized *via* GC–MS (Table 1 and Fig. 1). The chromatographic profile shows a chemically diverse oil dominated by fatty acid derivatives, esters, and terpenoid-related constituents. It is imperative to note that the identified ingredients were predominantly long-chain fatty acid derivatives and esters, indicating that the sample represented a fixed oil. The most abundant component was 9,12-octadecadienoyl chloride (Z,Z), accounting for 23.6% of the total area, highlighting the prevalence of linoleic acid–derived chlorinated fatty acyl compounds in the oil. This was followed by E-8-methyl-9-tetradecen-1-ol acetate (19.1%) and 2,3-dihydroxypropyl palmitate (8.8%), demonstrating a substantial contribution of long-chain fatty acid esters that are often related with bioactivity and lipid functionality. Moderate levels of tert-hexadecanethiol (6.97%) and 9,12,15-octadecatrienoic acid diacetate ester (6.58%) were also detected, signifying the occurrence of sulfur-containing composites and polyunsaturated fatty acid derivatives. Minor constituents comprised caryophyllene (3.71%), reflecting a sesquiterpenoid

fraction, along with numerous unsaturated fatty acids and alcohols, such as oleic acid (Z), 17-octadecynoic acid, and 12-methyl-E,E-2,13-octadecadien-1-ol, each contributing less than 5%. Supporting these findings, Sotelo-Méndez *et al.* (2023) indicated that lupine seed oil represents a valuable edible oil option, because of its enhanced oxidative stability and extended storage potential, which were associated with reduced unsaturation levels and consistently low acid and peroxide indices.

Some of the identified components are backed by earlier studies concerning their biological functions. Caryophyllene is well-documented for its notable pharmacological effects, including anti-proliferative properties against breast, lung, gastrointestinal, bone, blood, endometrial, and bladder cancers by inducing apoptosis and inhibiting proliferation and metastasis (Gbadebo *et al*. 2025). Moreover, caryophyllene has shown significant antibacterial properties, attaining full bactericidal effects against Bacillus cereus within 2 hours at its lowest inhibitory concentration (Moo *et al*. 2020). Additionally, fatty acid derivatives such as 9,12-octadecadienoyl chloride (Z,Z)- have been identified as reactive bioactive compounds exhibiting possible antimicrobial activity (Soltan *et al*. 2023). These results indicate that the identified ingredients might together show a role in the noted antimicrobial and cytotoxic effects of lupine seed oil.

Table 1. GC–MS Profiles of Chemical Constituents in Lupine Seed Oil

*R.T.	Compound	Area (%)	Molecular Formula	Molecular Weight
16.58	Caryophyllene	3.71	$C_{15}H_{24}$	204
28.84	2,3-Dihydroxypropyl palmitate	8.83	$C_{19}H_{38}O_4$	330
31.89	9,12-Octadecadienoyl chloride, (Z,Z)-	23.60	$C_{18}H_{31}ClO$	298
36.14	12-Methyl-E,E-2,13-octadecadien-1 ol	4.45	$C_{19}H_{36}O$	280
36.27	17-Octadecynoic acid	2.55	$C_{18}H_{32}O_2$	280
37.00	9-Octadecenoic acid (Z)-	3.93	$C_{18}H_{34}O_2$	282
41.62	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	3.00	$C_{16}H_{28}O_3$	268
41.67	14-á-H-Pregna	2.85	$C_{21}H_{36}$	288
41.95	tert-Hexadecanethiol	6.97	$C_{16}H_{34}S$	258
42.32	E-8-Methyl-9-tetradecen-1-ol acetate	19.13	$C_{17}H_{32}O_2$	268
43.00	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]et hyl ester, (Z,Z,Z)-	6.58	$C_{25}H_{40}O_6$	436

* (R.T.) Retention Time

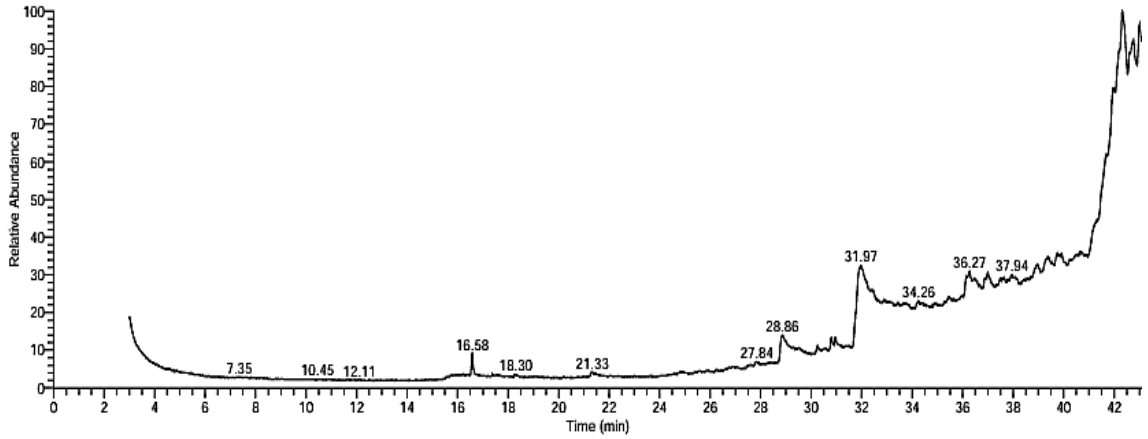

Fig. 1. GC–MS chromatogram of chemical constituents in lupine seed oil

The quantitative antimicrobial assessment, including inhibition zone diameters, MIC, MBC, and the MBC/MIC index of lupine seed oil were detected (Table 2). Lupine seed oil exhibited a larger inhibition zone (29.00 ± 0.5 mm) than the standard drug (28.00 ± 1.0 mm), indicating superior or comparable diffusion-based antibacterial efficacy. The MIC of lupine oil was 15.6 µg/mL, which is two-fold lower than that of the standard drug (31.25 µg/mL), reflecting higher potency. The MBC value for lupine seed oil (31.2 µg/mL) yielded an MBC/MIC index of 2, suggesting a bactericidal mode of action. In contrast, the standard drug showed an MBC/MIC index of 1. The quantitative inhibition zones were also demonstrated in Fig. 2, where the well containing lupine seed oil (O) produced a clear and pronounced zone of inhibition comparable to, and slightly larger than, that of the standard antibiotic (S), while no inhibition was observed for the negative control (NC). According to Al-Amrousi *et al.* (2022), lupine seed oil inhibited the growth of various bacteria such as *Staphylococcus aureus*, *Salmonella senftenberg*, *Bacillus subtilis*, and *Escherichia coli*. However, the activity of lupine seed oil against *H. pylori* was not reported previously. The biological activities reported for lupine seed derivatives appear to be highly dependent on their chemical composition, extraction fraction, and target organism or cell type. Al-Amrousi *et al*. (2022) reported a relatively low antibacterial effectiveness of lupine seed oils versus Gram-negative bacterial strains, including *Salmonella senftenberg* and *Escherichia coli*, whereas a moderate effect was observed versus Gram-positive strains such as *Staphylococcus aureus* and *Bacillus subtilis*. This variation was ascribed to differences in the structural organization of bacterial cell walls, which influence permeability and susceptibility to lipid-based bioactive compounds. In addition, lupine alkaloid extracts have demonstrated antibacterial activity against clinical isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Romeo *et al*. 2018), suggesting that non-lipid fractions may contribute more strongly to antimicrobial efficacy in certain cases.

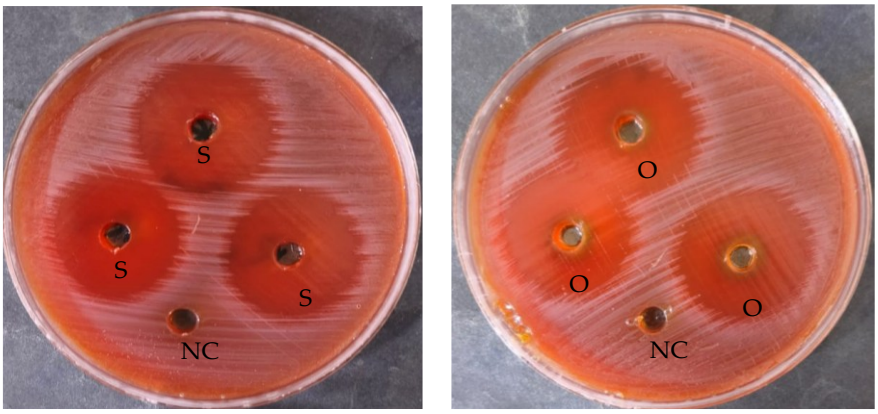

Fig. 2. Agar well diffusion assay showing antimicrobial activity against *H. pylori*; s = standard antibiotic, NC = Negative control, and O = Lupine seed oil

Table 2. Antimicrobial Examination of Lupine Seed Oil Compared with Clarithromycin (Standard Drug), Using Agar Diffusion Test

Treatment	Inhibition Zone (mm)	MIC (µg/mL)	MBC (µg/mL)	MBC/MIC Index
Seed oil	29.00 ± 0.5	15.62	31.25	2
Standard drug	28.00±1.0	31.25	31.25	1

The biofilm inhibition and the hemolysis inhibition assays revealed a concentration-dependent inhibitory effect of lupine seed oil (Fig. 3). As shown by the percentage inhibition values, lupine seed oil markedly reduced biofilm and hemolysis development at all tested sub-MBC and MIC, respectively. At 25% of the MBC, lupine seed oil achieved 77.5% biofilm inhibition, demonstrating substantial antibiofilm activity even at low concentration, although this effect was lower than that of the control (93.6%). Increasing the concentration to 50% of the MBC expressively enhanced the inhibitory effect, with lupine seed oil suppressing biofilm formation 91.10%. At the highest tested level, 75% of the MBC, lupine seed oil exhibited very strong antibiofilm activity (97.1%), which was nearly comparable to the control (98.8%). Biofilm formation represents a major survival strategy of *H. pylori*, enhancing bacterial adhesion, antibiotic resistance, and long-term persistence in the gastric environment; so, the observed antibiofilm activity of lupine seed oil indicates its potential to disrupt bacterial colonization and reduce infection recurrence. At 25% of the MIC, lupine seed oil inhibited hemolysis by 79.2 ± 2.1%, which was markedly greater than the control (66.1 ± 1.9%). Increasing the concentration to 50% of the MIC suggestively enhanced the protective effect, with hemolysis inhibition reaching 93.1 ± 1.6%, compared to 79.3 ± 2.2% for the control. At the highest concentration tested (75% of the MIC), lupine seed oil exhibited near-complete protection, achieving 98.1 ± 0.9% inhibition, slightly higher than the control (93.8 ± 1.1%). Hemolysis-protective effectiveness of lupine seed oil at increasing MIC doses in the existence of *H. pylori* was too visulised (Fig. 4). The hemolysis-protective effectiveness observed for lupine seed oil is consistent with previous studies on plant-derived bioactive compounds. For instance, rosemary extract has been reported to exhibit anti-hemolytic activity in the existence of *Helicobacter pylori*, which is likely due to its antioxidant properties and ability to stabilize erythrocyte membranes (Bakri *et al*. 2024). This suggests that the protective effect of lupine

seed oil against hemolysis may be attributed to similar mechanisms involving membrane stabilization and reduction of oxidative stress induced by bacterial infection.

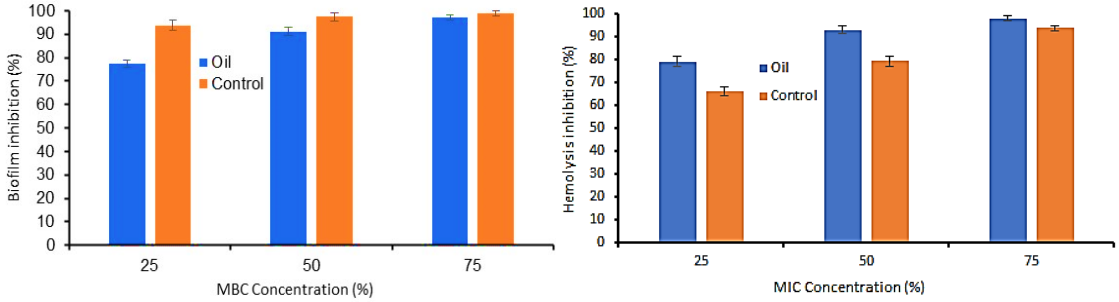

Fig. 3. Concentration-dependent effects of lupine seed oil on biofilm inhibition (25 to 75% MBC) and hemolysis inhibition (25 to 75% MIC) in comparison with the control

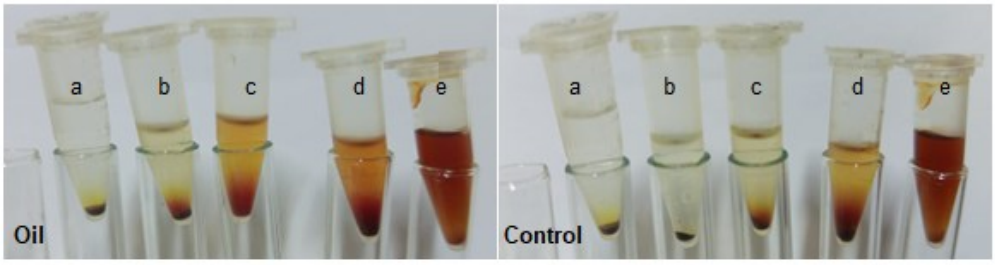

Fig. 4. Hemolysis-protective efficacy of lupine seed oil at increasing MIC fractions in the presence of *H. pylori*: Treatments: (a) standard drug, (b) 75% MIC, (c) 50% MIC, (d) 25% MIC, and (e) untreated

A rapid, time-dependent killing effect of lupine seed oil against *H. pylori* was documented in Table 3. Bacterial counts declined from 72×10^5 CFU at 0 h to 14×10^3 CFU at 30 min, and further to 26×10^2 CFU after 1 h. A sharp reduction was observed at 2 h (134 CFU), reaching 23 CFU at 2.5 h, and complete bacterial elimination (0 CFU) by 3 h. On the other hand, the control presented a slower reduction, with 114×10^3 CFU at 30 min, 189×10^2 CFU at 1 h, and 132 CFU at 2.5 h, with residual growth (25 CFU) still detected at 3 h. These quantitative results confirm the faster and stronger bactericidal activity of lupine seed oil compared to the control.

Table 3. Killing Kinetic Time of *H. pylori* Affected by Lupine Seed Oil Compared with Control

Killing Kinetic Time (min)	CFU For Lupine Seed Oil	CFU For Control
0	$72 \times 10^5 \pm 1.0$	$72 \times 10^5 \pm 1.0$
30	$14 \times 10^3 \pm 1.0$	$114 \times 10^3 \pm 0.4$
60	$26 \times 10^2 \pm 0.5$	$189 \times 10^2 \pm 1.5$
120	134 ± 1.5	286 ± 0.8
150	23	132
180	0	25
360	0	0

Inhibition of urease was selected as one of a target of the current study because it is a key virulence factor of *H. pylori* and directly controls the bacterium's ability to survive in the acidic gastric environment. Figure 5 shows a concentration-dependent urease inhibitory activity of lupine seed oil compared with the control. Urease inhibition increased to 43.8% at 7.81 µg/mL, reaching 73.8% at 62.5 µg/mL and a maximum of 95.3% at 1000 µg/mL for lupine seed oil. The control exhibited lower inhibition at all concentrations, achieving 93.2% at 1000 µg/mL. Notably, lupine seed oil displayed a lower IC_{50} value (9.56 µg/mL) than the control (17.85 µg/mL), indicating stronger urease inhibitory potency. The lupine seed oil inhibited protein denaturation in a dose-dependent manner, with inhibition ranging from 43.7% at 1.56 µg/mL to 91.9% at 200 µg/mL. Diclofenac sodium showed slightly higher inhibition of protein denaturation (46.6 to 95.8%) than lupine seed oil. The IC_{50} of the lupine seed oil was 2.60 ± 0.05 µg/mL, compared to 1.70 ± 0.02 µg/mL for diclofenac, indicating noteworthy anti-inflammatory potential (Fig. 6). Chronic inflammation is a well-established contributor to the progression of *H. pylori*–associated gastric disorders. In this context, Keenan *et al.* (2012) reported that persistent inflammation significantly increases the risk of gastric atrophy and intestinal metaplasia, which are key precancerous lesions in *H. pylori*–related disease. The pronounced anti-inflammatory activity observed for lupine seed oil in the present study, as evidenced by its strong inhibition of protein denaturation, may therefore play a crucial role in mitigating inflammation-driven gastric tissue damage. Coupled with its potent urease inhibitory activity (95.3% inhibition, which directly targets a critical virulence factor of *H. pylori*, lupine seed oil demonstrates a dual mechanism that could suppress both bacterial survival and inflammation-mediated pathological progression. Consistent with the current findings, Mohamed and Farid (2024) reported that lupine seed oil exhibits strong gastroprotective activity against indomethacin-induced gastric ulcers by suppressing inflammation. Lupine seed oil markedly reduced gastric mucosal damage, including hemorrhage, inflammatory cell infiltration, and edema, supporting its role as an effective anti-inflammatory and gastroprotective agent. Furthermore, lupine seed–derived bioactive peptides have been reported to possess multiple biological properties, including antioxidant and anti-inflammatory (Garmidolova *et al*. 2022), highlighting the multifunctional nature of lupine constituents.

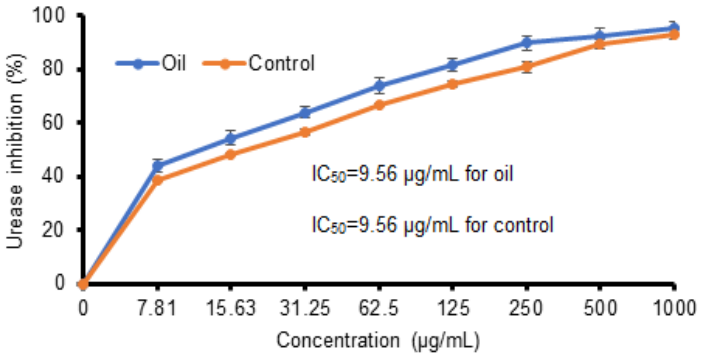

Fig. 5. Urease inhibition at different doses of lupine seed oil compared to control

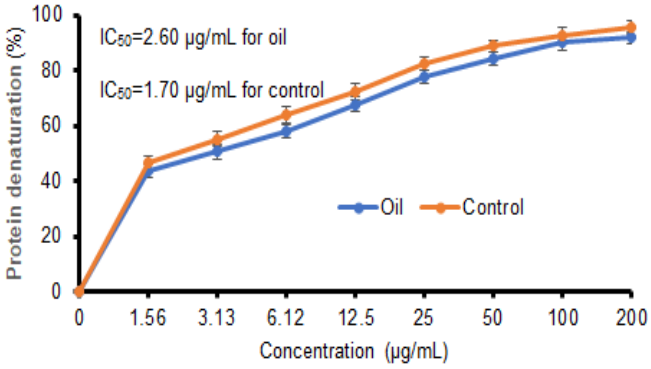

Fig. 6. Anti-inflammatory activity of lupine seed oil and diclofenac *via* protein denaturation assay

Helicobacter pylori causes chronic gastric infection that drives persistent inflammation and may influence downstream gastrointestinal epithelial damage. To extend the evaluation beyond antibacterial effects against *H. pylori*, Caco-2 cells were used as a representative intestinal epithelial (colorectal) model to investigate the potential cytotoxic and inflammation-related effects of lupine seed oil within the gastrointestinal system.

The lupine seed oil exhibited dose-dependent cytotoxicity against Caco-2 cells. Minimal toxicity was observed at lower concentrations (0% at 31.2 µg/mL), which increased to 49.9% at 125 µg/mL, and reached 96.4 to 97.2% at 500 to 1000 µg/mL. The calculated IC_{50} value was 148.7 ± 2.3 µg/mL, indicating moderate *in vitro* cytotoxic and anti-proliferative activity. The cytotoxic activity of lupine seed oil was also evaluated against normal human fibroblast cells (HFB4) over the same concentration range (0 to 1000 µg/mL), yielding an IC_{50} value of 342.7 ± 2.66 µg/mL. This higher IC_{50} compared to Caco-2 cells suggests relatively lower toxicity toward normal cells. When both cell lines are considered together, the results indicate partial selectivity toward cancer cells. The microscopic assessment of anti-proliferative activity on Caco-2 cells is illustrated in Fig. 7. It revealed a dose-dependent cytotoxic effect of the lupine seed oil on Caco-2 cells. At the lowest concentration (31.25 µg/mL, panel A), cells appeared largely normal, while at 62.5 µg/mL (panel B), mild morphological alterations were visible. Shrinkage and detachment of treated cells were recorded at 125 µg/mL (panel C) and 250 µg/mL (panel D). At higher doses (500 to 1000 µg/mL, panels E and F), most cells were rounded, shrunken, and detached, representative extensive cell death. Control cells (panel G) maintained normal morphology and confluency. These notes confirm the concentration-dependent anti-proliferative activity of the lupine seed oil against Caco-2 cells. Lupines contain several bioactive compounds, including oligosaccharides, phenolics, and alkaloids, which are associated with protective effects against chronic diseases, particularly cancer (Jayasena and James 2013). The observed anti-proliferative activity may be attributed to lupine-derived flavonoids, including flavones, flavonols, and flavanols, which are known for their antioxidant capacity and their role in scavenging reactive oxygen and nitrogen species, thereby potentially contributing to the modulation of oxidative stress–related cancer pathways. These mechanisms contribute to the suppression of oxidative stress–mediated tumor progression, supporting the cytotoxic effects detected in our cancer assay (Ruiz-López *et al.* 2019). A limitation of this study is that all biological evaluations were conducted in vitro against only *H. pylori*, without *in vivo* validation. In addition, the anti-proliferative assessment was performed using a single cancer cell line. Although these

models are widely accepted and provide valuable preliminary insights into antimicrobial and cytotoxic activities, they do not fully replicate the complexity of *in vivo* biological systems. Therefore, further *in vivo* studies and expanded cellular investigations, are necessary to comprehensively confirm the safety profile of lupine seed oil.

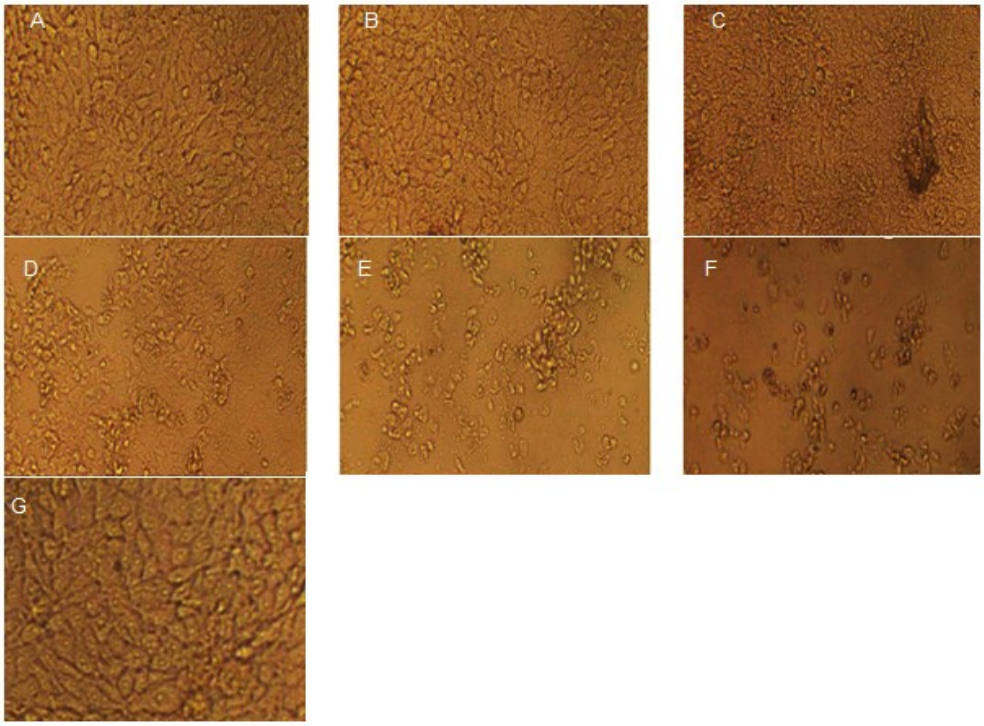

Fig. 7. Microscopic assessment of anti-proliferative activity of against Caco-2 cells at different tested concentrations: (A) 31.25, (B) 62.5, (C) 125, (D) 250, (E) 500, (F) 1000 µg/mL, and (G) Control cells

CONCLUSIONS

1. The gas chromatography-mass spectrometry (GC–MS) profiling revealed that lupine seed oil is dominated by biologically relevant fatty acid derivatives, esters, and terpenoids, with 9,12-octadecadienoyl chloride, E-8-methyl-9-tetradecen-1-ol acetate, and 2,3-dihydroxypropyl palmitate as the principal constituents, underpinning its multifunctional bioactivity.
2. Lupine seed oil demonstrated notable anti-*Helicobacter pylori* activity (29.0 ± 0.5 mm inhibition zone), along with antibiofilm, antihemolytic, urease inhibitory, and anti-inflammatory effects (IC_{50} 2.60 ± 0.05 µg/mL). In several assays, its activity was comparable to standard controls, suggesting its potential to target multiple pathogenic mechanisms, including biofilm formation and enzyme-mediated virulence.
3. The dose-dependent cytotoxicity observed in Caco-2 cells (148.7 ± 2.3 µg/mL), suggests that lupine seed oil may serve as a potential natural source for possible anti-proliferative relevance

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