https://doi.org/10.48047/AFJBS.7.4.2025.1349-1363

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Advanced phytochemical insights and therapeutic potential of *Hyssopus officinalis* antibacterial action and gastric mucosal protection

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Volume 7, Issue 4,Apr 2025

Received: 15 Feb 2025

Accepted: 05 Mar 2025

Published: 05 Apr 2025

doi:10.48047/AFJBS.7.4.2025.1349-1363

Abstract:

Hyssopus officinalis (Lamiaceae) thrives in the mountainous regions of Algeria, where its aromatic properties and medicinal uses have been embraced by local. Traditionally used in herbal medicine, this aromatic plant has garnered attention for its potential benefits in treating infectious diseases and gastrointestinal issues, including ulcers. This study seeks to evaluate, *in vitro*, the antibacterial capacity of leaf η-butanol extract of *Hyssopus officinalis* (η-BLHE) against four pathogenic bacteria, investigate, *in vivo*, its protective effects on gastric mucosa, and identify its bioactive compounds. The agar well diffusion method was employed to assess the antibacterial effects. η-BLHE was orally administered to two experimental groups of rats, both before and after the induction of gastric ulcers using absolute ethanol. The extent of gastric mucosal lesions was assessed through macroscopic and histopathological examinations. Phytochemical compounds were recognized by UPLC-ESI-MS/MS. Among the 13 compounds identified in the η-BLHE, coumaric acid (45.26%) and rutin (18.93%) were found to be the most prevalent. η-BLHE demonstrated antibacterial activity comparable to gentamicin ($p < 0.05$) against *Staphylococcus aureus* strain. Our results indicated that pretreatment with η-BLHE significantly reduced the ulcerated and hemorrhagic areas in the gastric mucosa of rats subjected to ethanol-induced gastric ulcers. Macroscopic examination revealed a marked decrease in lesion size, while histopathological analysis demonstrated improved mucosal integrity and reduced inflammation. This dual functionality underscores the potential of *Hyssopus officinalis* in contemporary medicine, advocating for further investigation into its active constituents and their clinical applications in integrative health practices.

Keywords: *Hyssopus officinalis* L., gastroprotective effect, ethanol- induced gastric ulcers, antibacterial capacity.

1. Introduction

Gastrointestinal disorders, particularly peptic ulcers, are a significant global health concern, often exacerbated by bacterial infections such as *Helicobacter pylori* and factors like alcohol consumption and stress. These conditions can lead to severe complications, including hemorrhage, perforation, and gastric cancer if left untreated. Also, the growing resistance to conventional antibiotics necessitates the development of innovative treatment options. As bacteria evolve and become less susceptible to existing medications, there is an urgent need for alternative therapies that can effectively combat infections and provide additional benefits. Natural compounds with both antibacterial and gastroprotective properties offer a promising approach to addressing these critical health challenges [1].

Herbal medicines have gained increasing attention as viable alternatives to conventional pharmacological treatments, particularly in the context of long-term use. Sairam et al. [2], highlight the advantages of herbal remedies in reducing the need for prolonged administration of synthetic drugs. In recent years, significant research has focused on the potential of herbal-derived therapeutics for gastroprotection, the management of gastric ulcers and antibacterial treats. These natural products are often regarded as safe, cost-effective, and clinically efficacious. Furthermore, they tend to be better tolerated by patients compared to conventional medications, while offering global competitiveness in terms of both accessibility and affordability [3].

Numerous ethnobotanical surveys conducted worldwide have cataloged a diverse array of plants traditionally used in the treatment of gastrointestinal disorders and bacterial infections. Prominent examples include *Citrus medica* (citron), *Zingiber officinale* (ginger), *Mentha piperita* (peppermint), *Fumaria officinalis* (fumitory), and *Melissa officinalis* (lemon balm). These plants have long been incorporated into various cultural practices for their medicinal properties, particularly in the management of digestive issues. Their therapeutic effects are attributed to a range of bioactive compounds that exhibit antimicrobial, anti-inflammatory, and digestive-enhancing properties [4].

Hyssopus officinalis L., a distinguished herb from the Lamiaceae family, is indigenous to the Mediterranean basin and the Middle East, where it has been an integral component of traditional medicine for centuries. In addition to its medicinal uses, hyssop has a rich history in flavoring beverages, a testament to its aromatic and taste-enhancing properties [5]. Previous research has highlighted *Hyssopus officinalis* as a plant with a diverse array of medicinal properties, including potent antifungal, antibacterial, and antiviral activities. These

characteristics have made it a staple in traditional medicine, particularly for its ability to address a variety of health concerns. The plant is commonly used to support digestive health, relieve intestinal discomfort, and alleviate respiratory conditions, including tuberculosis, asthma, chronic catarrh, and bronchitis [6].

This plant has long been prized in traditional medicine for its wide range of benefits. It helps ease rheumatic pain, bruises, and wounds. It also calms anxiety and hysteria, regulates blood pressure, soothes muscle tension and promotes relaxation [7]. The essential oils and extracts of *H. officinalis* have demonstrated a broad spectrum of bioactivities, including anti-inflammatory, antimicrobial, anti-diabetic, antioxidant, and anti-HIV properties. Moreover, studies in animal models have revealed that *H. officinalis* exhibits myorelaxant, antiplatelet, and α-glucosidase inhibitory activities [8]. Previous investigation has shown that the ethanolic extract of *H. officinalis* possesses gastroprotective properties, as indicated by an increase in gastric adhesion mucus content. The extract also demonstrates significant antiulcer and antioxidant potential, as evidenced by a reduction in reactive oxygen species (ROS) generation [9]. Although hyssop has shown promise as a medicinal plant, scientific investigation into its antibacterial and gastroprotective properties remains insufficient. The majority of existing research has been narrowly focused on the essential oil, leaving other potential therapeutic effects largely unexplored. Consequentially, this study is designed to identify and characterize the phytochemical constituents of the η-butanol extract from *Hyssopus officinalis* leaves (η-BLHE), harvested from eastern Algeria, using UPLC-ESI-MS-MS. It will also evaluate the *in vitro* antibacterial activity of the extract against four pathogenic bacterial strains and assess its potential as a gastroprotective agent, thereby advancing our knowledge of the plant's therapeutic properties, offering a natural bridge between ancient wisdom and contemporary wellness.

2. Material and methods

2.1. Plant sample:

Aerial parts of *Hyssopus officinalis* L. (Lamiaceae) were collected from the “Jijel” province (Eastern Algeria). Dr. Kameleddine Bazri of the Biology and Ecology department at the Faculty of Natural and Life Sciences Mentouri Brothers University Constantine 1, Algeria, carried out the botanical authentication of the samples and a voucher specimen (Ref No; UMC1/BED/2022/085) was deposited at the University Herbarium. In order to prepare the various extracts, the leaves were ground into a fine powder and preserved in the lab at room temperature until they were used.

2.2. Extraction process:

With a few minor modifications, the extraction procedure was performed by the technique outlined by Boudjada et al (10). The powdered leaves were macerated in a hydro-alcoholic solution (methanol /water: 70:30: v/v). This procedure was carried out three times using a renewal solvent. After evaporation, the remaining crude extracts were diluted with distilled water to obtain aqueous solutions ready for liquid-liquid extraction using η-butanol. The organic fraction was concentrated and weighed to determine the % yield of the soluble constituents using the following formula:

%Yield: Weight of dry extract /Weight taken or extraction x100.

2.3. Determination of phytochemical profile of leaf η-butanol extract (η-BLHE) using UPLC-ESI-MS/MS:

The procedure outlined by Akdeniz et al (11) was followed to get the chemical profile by ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry **(**UPLC-ESI-MS/MS) analysis. Shimadzu 8040 Ultra-High Sensitivity with UFMS Technology and a binary bump Nexera XR LC-20AD were used in the UPLC-ESI-MS-MS analysis. In this experiment, the column Restek Ultra AQ C18 (3µm, 150x4.6mm) was utilized. The mobile phase was constituted of 0.1% formic acid, methanol, and water. The ESI conditions were as follows: CID gas, 230 KPs; conversion dynode, −6.00 Kv; interface temperature, 350 ◦C; DL temperature, 250 ◦C; mobilizing gas flow, 3.00 L/min; heat block, 400 ◦C; drying gas flow, 15.00 L/min. The standards were made in methanol at a concentration of "500 µg/L". The ion trap mass spectrometer was used in both negative and positive ions with MRM mode (Multiple Reaction Monitoring).

2.4. Antibacterial activity

2.4.1. Antibacterial potential of the leaf η-butanol extract (η-BLHE) using the well diffusion assay

The selected method was agar well diffusion, as described by Chandur et al (12). The bacterial strains were obtained from the National Center of Biotechnology Research (C.R.Bt, Constantine) and comprised *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Salmonella typhi* ATCC 13076. These bacterial strains were reactivated by inoculating them on Petri dishes containing nutrient agar and incubated for 18 hours to obtain young, active cultures. The resulting bacterial colonies were transferred to tubes containing 9 ml of 0.9% saline solution and the suspension was adjusted to a turbidity equivalent to 0.5 McFarland. Samples were prepared at a concentration of 250 mg/mL in DMSO (Dimethyl sulfoxide**)**. The zones of inhibition were measured in mm. The

negative control was DMSO and the positive control was Gentamicin. All experiments were carried out in triplicate.

2.4.2. Estimation of minimal inhibitory concentration

The Minimum Inhibitory Concentration (MIC) for a specific strain was determined as the lowest concentration at which there was no observable growth of the microorganism. The experiments for determining the MIC were carried out using the standard dilution method on the Mueller Hinton agar medium. Each extract was subjected to the double dilution method to create a concentration range spanning 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64, ranging from 125 to 3.9 mg/mL using distilled water. The inhibition zone was measured in mm [13].

2.5. Antiulcer activity of *Hyssopus officinalis* η-butanol extract (η-BLHE)

2.5.1. Animals

In this study, twenty-four healthy adult male albino rats (115-200 g) were obtained from the Animal Center of Constantine 1 University. The animals were meticulously randomized into four groups of six. Following the National Institutes of Health guidelines for laboratory animal care [14], the rats were housed under strictly controlled environmental conditions, with a temperature maintained at 23 ± 2 °C and humidity at 55 ± 15% in a pre-clinical facility. To ensure an empty stomach, they were fasted for 24 hours prior to experimentation while having continuous access to water, and were placed in mesh-bottomed cages to prevent coprophagia. All experiments were conducted in agreement with the guidelines of the Center of Animal Experimentation Constantine 1 and the National Institutes of Health and were approved by the Ethics Committee on Animal Use in Research of University of Constantine 1.

2.5.2. Acute toxicity

Acute toxicity studies were conducted on male Swiss mice (20-25 g), following the OECD 425 guidelines [15] with slight modifications. The mice were randomly assigned to four groups (n = 6) and fasted for 12 hours while having ad libitum access to water. All experiments were conducted in agreement with the guidelines of the Center of Animal Experimentation Constantine 1 and the National Institutes of Health and were approved by the Ethics Committee on Animal Use in Research of University of Constantine 1. η-BLHE were orally given at the doses of 500, 1000 and 2000 mg/kg. Observations were systematically conducted at 60, 120 and 240 minutes following the oral treatments, with comprehensive daily assessments extending over a 14-day period. Each day, data were meticulously collected on behavioral and body weight changes, food and water intake, clinical signs of toxicity, and mortality rates.

2.5.3. Experimental protocol and ethanol-induced gastric lesions method

A slightly modified method from Devaraj et al. (16) was employed for gastric ulcer induction in this study. The rats were randomly divided into four groups, each consisting of six animals. All treatment subjects received an experimental dose volume of 5 mL/kg. Group I (negative control) was gavage-fed sterilized distilled water, while Group II (positive control) received 80 mg/kg of omeprazole. Groups III and IV were gavage-fed 250 mg/kg and 500 mg/kg of η-BLHE, respectively. After one hour fasting period with free access to water, all rats were given a single oral gavage of absolute ethanol (1.5 mL/rat) to induce gastric ulcers. One hour later, the animals were euthanized using a high dose of intraperitoneal xylazine/ketamine (100/16 mg/kg) for anesthesia.

2.5.4. Macroscopical observations

The stomachs were carefully excised and opened along the greater curvature. The contents were removed, and the gastric mucosa was gently washed with 0.9% saline. Subsequently, mucosal lesions were meticulously examined with a 10x magnifier lens, allowing for precise counting and measurement of lesion areas. The ulcer index for each group was determined using the formula:

Ulcer Index= (Sum of lesion areas/Total stomach area) ×100

To assess the efficacy of the treatment, the preventive ratio was calculated with the equation:

Preventive Ratio= (Ulcer Index (negative control) −Ulcer Index (treated)/Ulcer Index (negative control)) ×100 [17].

The volume of gastric juice was measured with a graduated cylinder, and 10 mL of distilled water was added to facilitate pH evaluation and total acid secretion analysis. A pH meter was then utilized to accurately determine the acidity of the gastric juice.

2.5.5. Histopathological analysis

The gastric tissues collected from distinct groups of rats were fixed in 10% buffered formaldehyde for 24 hours to preserve cellular architecture. Following fixation, the samples were embedded in paraffin and sectioned into 5 μm slices using a microtome. These sections were then stained with hematoxylin-eosin, and high-resolution images were captured using a light microscope, enabling a detailed examination of the tissue morphology for comprehensive analysis [18].

2.6. Statistical analysis

In vitro, antibacterial data were analyzed in triplicate. Data were expressed as means ±SD. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by the Tukey test ($P < 0.05$).

In vivo gastroprotective data were expressed as the mean ± SEM (n = 6) for animal groups. The comparison was performed using one-way analyses of variance (ANOVA) used with SPSS. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Extraction yield

Using the established formula, the methanolic extract yield from the leaves was calculated at 5.68%, while the yield from the η-butanol extract was 1%.

3.2. Phytochemical profile of *Hyssopus officinalis* η-butanol extract (η-BLHE) using UPLC-ESI-MS/MS

In this study, bioactive compounds present in the η-BLHE were characterized and semi-quantified using the ultrasensitive UPLC-ESI-MS/MS method, applied in both positive and negative ion modes. A total of thirteen metabolites were identified from the analyzed plant parts out of twenty tested phytochemical standards, representing a wide range of classes including phenolic acids, flavanones, flavonol glycosides, vitamins, and quinones (Table 1).

Table 1: Ultra performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) analysis of *Hyssopus officinalis* η-butanol extract (η-BLHE)

N°	Analytes	Retention time (min)	η-BLHE Area (%)
1	Maleic acid	46	0.69
2	Lawson acid(2-hydroxy-1,4-naphthoquinone	0.95	14.80
3	Gallic acid	2.29	0.08
4	Beta-carotene	43.5	0.19
5	Butylated hydroxytoluene	18.5	1.88
6	Rutin	14.85	18.93
17	Ascorbic acid	47.75	0.09
18	Chlorogenic acid	19	1.42
19	Caffeic acid (3,4-dihydroxy-cinnamic acid)	7.4	0.26
10	Cinnamic acid	11.25	0.33
11	Folic acid	35.15	15.92
12	Hespertin acid	37.6	0.09
13	Hydroxy-coumarin acid	47.30	45.26

Notably, the η-BLHE exhibited a rich profile of bioactive compounds, featuring substantial concentrations of lawson acid (14.8%) and folic acid (15.92%). This extract was

particularly dominated by hydroxy-coumarin acid at 45.26% and rutin at 18.93%. Additionally, the η-BLHE contained trace amounts of ascorbic acid, chlorogenic acid, caffeic acid, cinnamic acid, maleic acid, and hesperetin acid.

3.3. Antibacterial potential of *Hyssopus officinalis* η-butanol extract (η-BLHE) using a well diffusion assay

The results in Table 2 demonstrate that *Staphylococcus aureus* showed greater susceptibility to the η-butanol leaf extract of *Hyssopus officinalis* (η-BLHE) at a concentration of 250 mg/mL, revealing significant antibacterial activity comparable to gentamicin, with an inhibition zone measuring 23 ± 1.01 mm. In contrast, η-BLHE exhibited no antibacterial activity against *Salmonella typhi, Escherichia coli, and Bacillus cereus*, indicating its selective antibacterial properties. These findings call for further investigation into the minimal inhibitory concentration (MIC) of the extract, especially concerning the more sensitive *Staphylococcus aureus* strain. Notably, η-butanol extract of *Hyssopus officinalis* (η-BLHE) showed no effect at lower concentrations, firmly establishing the MIC against *Staphylococcus aureus* at 250 mg/mL.

Table 2: Antibacterial screening of *Hyssopus officinalis* η-butanol extract (η-BLHE) (250 mg/mL) against selected pathogenic bacteria

Plant extracts	**Inhibition zones (mm)**			
	Gram(+ve) pathogenic bacteria		**Gram(-ve) pathogenic bacteria**	
	Bacillus cereus	***Staphylococcus aureus***	***Echerichia coli***	***Selmonella typhi***
η-BLHE	NA	23± 1.01	NA	NA
Gentamicin (PC)	30± 0.03	31± 0.02	30± 0.04	29± 0.02

NA: No activity; **η-BLHE**: η-butanol leaf extract.
Data are means of three replicates (n = 3) ± standard error with statistical significance set at $p < 0.05$ according to the Tukey test and comparable to gentamicin as positive control (PC).

3.4. Antiulcer activity of *Hyssopus officinalis* η-butanol extract (η-BLHE)

3.4.1. Safety of *H. officinalis* leaf extract

Male mice were subjected to an acute toxicity test following the OECD 425 guideline. Throughout the 24-hour evaluation period, the animals exhibited no behavioral or physical abnormalities, and no mortality was observed at the administered dose of 500 mg/kg over the 14-day investigation period. Based on this safety assessment of the plant, doses of 250 mg/kg and 500 mg/kg were chosen for further experimentation. Similar results were recorded at the 1000 mg/kg dose, although a few signs such as drowsiness, irritation, and tremors were noted during the initial two hours. However, these effects were transient, as the mice quickly regained

their normal responsiveness afterward. Mice administered the 2000 mg/kg dose displayed some behavioral symptoms in the first 24 hours, but these effects diminished over time. Additionally, there were no significant weight changes among any of the treated groups. Overall, the findings suggest that the lethal dose exceeds 2000 mg/kg.

3.4.2. Assessment of gastroprotective properties

3.4.2.1. Macroscopical effects

Exposure to absolute ethanol led to significant gastric damage, characterized by severe mucosal lesions, including prominent hemorrhagic bands and petechial lesions. Upon gross examination, these hemorrhagic bands were found to vary in size and extended along the longitudinal axis of the glandular region of the stomach, highlighting the extent of the injury (Figure 1). Animals pretreated with *H. officinalis* η-butanol leaf extract exhibited only mild lesions when compared to the distilled water-treated group, as detailed in Table 3. The groups receiving the η-BLHE at doses of 250 and 500 mg/kg showed a significant decrease in the ulcer index, recorded at 0.014± 0.67 and 0.004± 0.00, respectively ($p \leq 0.05$). In comparison, the distilled water-treated rats had a much higher ulcer index of 0.15± 0.05. Furthermore, η-BLHE at a dose of 500 mg/kg displayed a significantly lower ulcer index than the omeprazole-treated rats at 80 mg/kg, which had an index of 0.020 ± 1.03 ($p \leq 0.05$). This finding indicates that η-BLHE may provide even greater gastroprotective benefits than omeprazole. Moreover, the pH levels of the η-BLHE at doses of 250 mg/kg (3.9± 0.68) and 500 mg/kg (6.5± 0.09) were significantly higher compared to the negative control ($p \leq 0.05$)**.** However, there was no significant difference between the pH of the η-BLHE (500 mg/kg) group and the positive control (omeprazole-treated group), which measured 5.7± 0.05 (p value ≤ 0.05). This indicates a dose-dependent response for the *H. officinalis* leaf extract.

Table 3: Impact of *H. officinalis* extract on ulcer index, ulcer area prevention, and gastric pH in stomach tissue (n = 6)[a].

Animal groups	Dosing (mg/kg)	Ulcer index	Prevention (%)	pH
η-BLHE[b]	500	0.004± 0.00	97	6.5± 0.09
η-BLHE	250	0.014± 0.67	90	3.9± 0.68
Omeprazole	80	0.020± 1.03	86	5.7± 0.05
NC[c]	-	0.15± 0.05	-	2.1± 0.14

[a]: Data are presented as means of six replicates (n = 6) ± standard error with statistical significance set at $p \leq 0.05$.
[b]: η-BLHE refers to *Hyssopus officinalis* η-butanol leaf extract. [c]: NC refers to negative control distilled water.

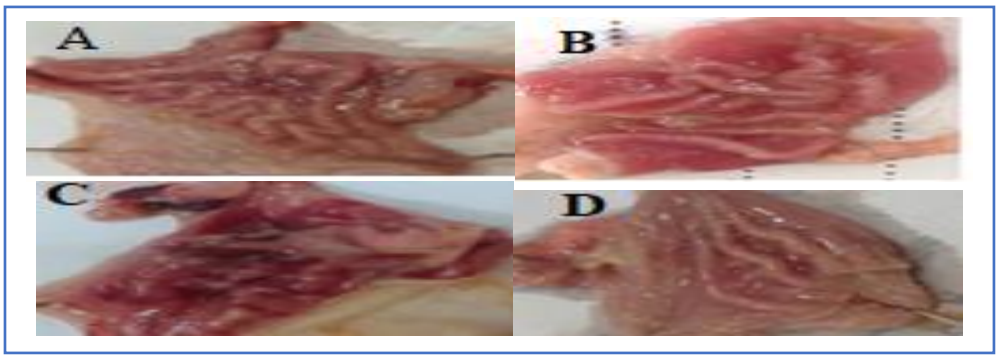

Figure 1: Effect of *H. officinalis* on macroscopic appearance of the gastric mucosa in rats (n=6) ($p \leq 0.05$). A: *Hyssopus officinalis* treated group (500mg/kg), B: *Hyssopus officinalis* treated group (250 mg/kg), C: Distilled water treated group (negative control), D: Omeprazole treated group (positive control, 80 mg/kg). Red arrows indicate the injury due to absolute ethanol administration.

3.4.2.2. Histopathological evaluation

The group receiving 250 mg/kg of η-BLHE showed moderate disruptions in the surface epithelium, including inflammatory cell infiltration, and submucosal edema. In contrast, the η-BLHE (500 mg/kg) group displayed only mild damage. Notably, both *H. officinalis*-treated groups demonstrated significantly less microscopic damage compared to the distilled water-treated group. *H. officinalis*-treated group (500 mg/kg) showed a more pronounced gastroprotective effect compared to omeprazole highlighting the potential of *H. officinalis* as a powerful natural alternative for gastric protection. Table 4 and figure 2 present the results of stomach autopsies across the various pretreated groups.

Table 4: Comparative effect of *H. officinalis* extract and omeprazole on histological changes in gastric ulcers induced by ethanol in rats (n=3)[a]

Animal groups	Dosing mg/kg	Submucosal edema	Epithelial erosion and disruption	Inflammatory cells		Mucosal necrosis
				Lymphocyte	Polynuclear	
η-BLHE[b]	500	0	0	2	0	0
η-BLHE	250	1	0	2	0	0
Omeprazole	80	0	1	2	0	0
NC[c]	-	3	3	2	2	3

[a]: Data are presented as means of three replicates (n = 3) ± standard error with statistical significance set at $p \leq 0.05$.
[b]: η-BLHE refers to *Hyssopus officinalis* η-butanol leaf extract. **[c]**: NC refers to negative control distilled water.
Absent :**0**, Mild :**1**, Moderate :**2**, Severe: **3**.

4. Discussion

The findings indicating both gastroprotective and antibacterial effects of *H. officinalis* suggest that these dual actions make *H. officinalis* a promising candidate for therapeutic use in both ulcer prevention and treatment, possibly reducing reliance on antibiotics and proton pump inhibitors.

The extraction method used plays a crucial role in determining both the yield and the bioactive profile of the compounds extracted. Traditional extraction techniques often lead to a decrease in extract quality, primarily due to the degradation or loss of volatile compounds, suboptimal extraction efficiency, long processing times, and the breakdown of solid residues. To enhance the efficacy and quality of the extract, future studies should focus on exploring more advanced or alternative extraction methods. Techniques such as ultrasound-assisted, microwave-assisted, or supercritical fluid extraction may offer improved efficiency, reduced degradation, and a better preservation of the bioactive components, ultimately leading to more potent and reliable therapeutic applications [19].

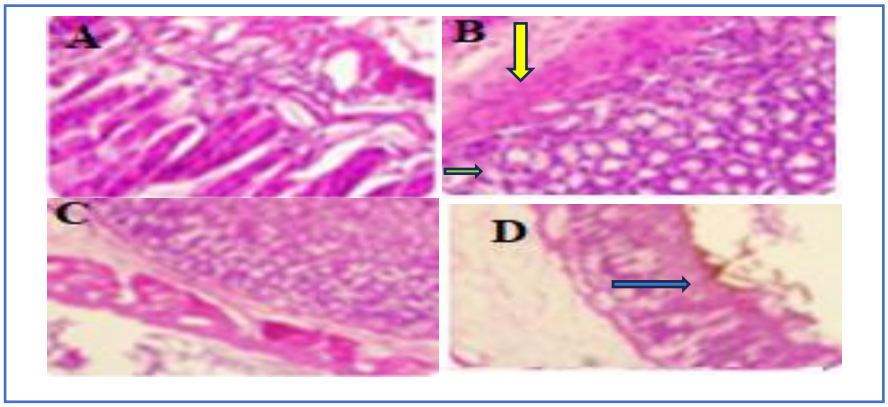

Figure 2: Effect of *H. officinalis* on microscopic appearance of the gastric mucosa in rats. Histopathological tissue stained by hematoxylin-eosin stain. A: *Hyssopus officinalis* treated group (500 mg/kg) B: *Hyssopus officinalis* treated group (250 mg/kg). C: Omeprazole group (positive control, 80 mg/kg), D: Distilled water treated group (negative control). Photomicrographs obtained from Hematoxylin Eosin-stained specimen examined under light microscope ($_x$100). Blue arrows indicate epithelia erosion, green arrows indicate submucosal edema, and yellow arrows indicate inflammatory cells.

The chemical profile of *Hyssopus officinalis* varies significantly across different global regions, such as Poland, Spain, Turkey, Italy, Serbia, India, Iran, and Romania, due to factors like climate, soil composition, and cultivation practices. This variability affects the plant's bioactive compounds, such as essential oils, flavonoids, and phenolic. The chloroform fraction of *Hyssopus officinalis* subsp. *angustifolius* cultivated in Turkey is rich in bioactive compounds,

including chlorogenic acid, its quinic acid isomer, and flavonoid glycosides such as isoquercitrin, rutin, and quercitrin. Additionally, quercetin and luteolin, two flavonoid aglycones, were identified. These findings highlight the diverse range of bioactive compounds in this Turkish variety [20]. Furthermore, HPLC analysis of the methanolic extract from *Hyssopus officinalis* leaves revealed key phenolic compounds, including p-coumaric acid, benzoic acid, coumaric acid, ferulic acid, and quercetin [21]. Proestos et al. (22) performed reversed-phase high-performance liquid chromatography with UV detection and identified the major phenolic acids in *H. officinalis* from Greece, including ferulic acid (13.2 mg/100 g), caffeic acid (6.5 mg/100 g), and chlorogenic acid (166.21 µg/g).

While previous studies on *H. officinalis* have primarily focused on the bioactive properties of its essential oils, particularly their gastroprotective and antibacterial effects [23], this narrow focus has limited exploration of the plant's broader phytochemical potential. Notably, the pharmacological activities of its non-volatile extracts remain underexplored. This study seeks to fill this gap by investigating the biological effects of *H. officinalis* extracts, thereby contributing new, critical insights into the plant's full spectrum of bioactive compounds and their potential therapeutic applications.

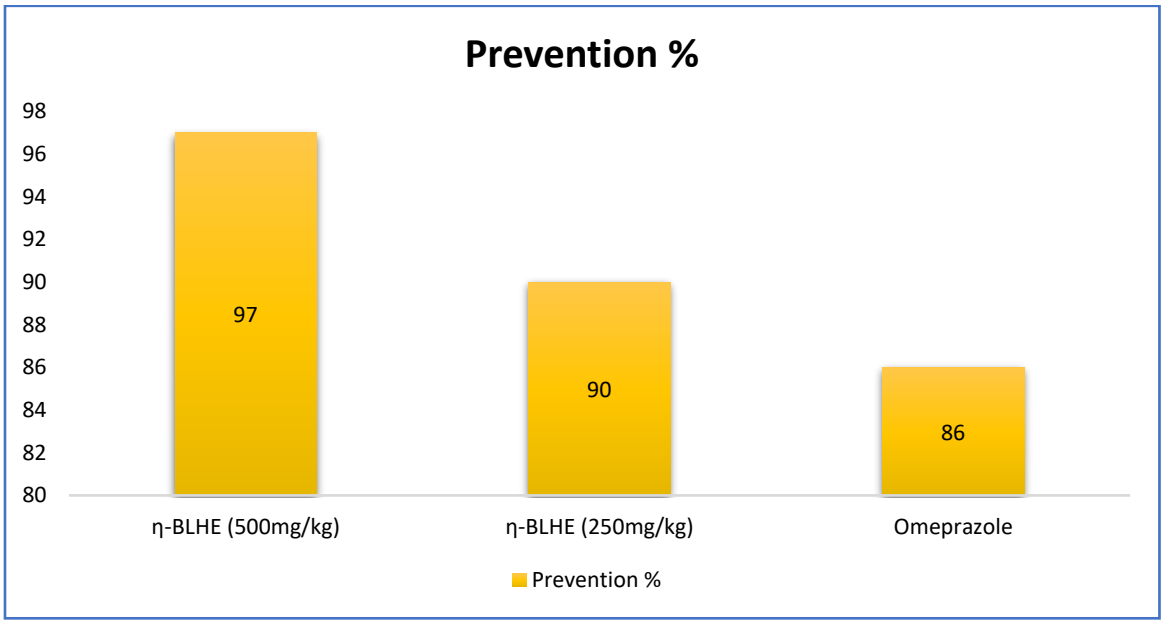

Figure 3: Effects of *H. officinalis* on ulcer area prevention (%) in ethanol-induced gastric ulcer (n = 6). The results were expressed as mean ± SEM with statistical significance set at $p \leq 0.05$.

The results of this study highlight the significant antibacterial activity of *H. officinalis* ŋ-butanol leaf extract (ŋ-BLHE) against *Staphylococcus aureus*, a pathogen known to exacerbate endocarditis, bacteremia, and infections of soft tissues and the respiratory tract. These findings are in agreement with earlier research of Vlase et al. (24), who demonstrated that 5 µL and 10

µL of *H. officinalis* essential oil from Anatolia, Turkey, exhibit significant antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes*. Similarly, research indicates that hyssop essential oil exhibits notable antimicrobial activity against a range of pathogenic microorganisms, including *Streptococcus pyogenes*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* [25]. Marino et al. (26) investigated the antimicrobial activity of the primary constituents of hyssop essential oil against six gram-positive and nine gram-negative bacterial strains. Their findings highlighted that phenolic compound, particularly carvacrol and thymol, are highly effective in inhibiting the growth of these microorganisms. Moreover, the findings of a recent study highlight the significant antimicrobial activity of *Hyssopus officinalis* essential oil against *Escherichia coli* and *Listeria monocytogenes* [27]. However, Özer et al. (28), reported that the methanolic extract of *Hyssopus officinalis ssp. Angustifolius* exhibited no antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*.

Given the adverse effects of modern medicines, there is increasing interest in medicinal plants for gastrointestinal disorders. Our study provides the first evidence that the η-butanol leaf extract of *Hyssopus officinalis* (η-BLHE) effectively prevents ethanol-induced gastric ulceration in rats. η-BLHE exhibits potent gastroprotective activity, significantly reducing the extent of gastric ulceration in rats.

The oral acute toxicity test demonstrated that η-BLHE is safe at doses of up to 500, 1000, and 2000 mg/kg, with no observed toxicity during the experiment. No significant changes in physical behavior or mortality were observed throughout the observation period. Our findings are consistent with those of Ma *et al*, (29) who reported that a dose of 1.6 g/10 g of body weight in mice is a safe dose and poses no risk. The aerial part extract of *H. officinalis* (η-BLHE) exhibited robust gastric protective activity against the harmful effects of absolute ethanol, with a clear dose-dependent response. At the highest dose (500 mg/kg), η-BLHE provided superior protection, significantly mitigating ethanol-induced gastric damage (0.004 ± 0.00, $p \leq 0.05$). This resulted in a markedly lower ulcer index compared to both the distilled water control group (0.15 ± 0.05) and the omeprazole (80 mg/kg) pretreated group (0.020 ± 1.03), highlighting the potent gastroprotective potential of the extract. Moreover, the plant's preventive efficacy was further confirmed by a significant increase in pH levels at the higher concentration (500 mg/kg), with a notable difference observed compared to the distilled water-pretreated group. Our results align with those obtained by Tahir et al. (30), who reported similar protective effects of *H. officinalis* extract in an indomethacin-induced gastric ulcer animal model.

Histopathological and macroscopic analysis of gastric tissue from the distilled water-pretreated rat group revealed severe ethanol-induced damage, characterized by marked

congestion in the lamina propria, submucosa, and inter-villus regions, along with widespread red blood cell extravasation into the mucosal villi. Such alterations are likely a result of ethanol toxicity, which interferes with normal coagulation pathways, thereby promoting persistent hemorrhage [31]. In a similar investigation, Hu et al. (32) observed extensive coagulative necrosis of the gastric mucosa in animals subjected to hemorrhagic shock following ethanol exposure.

The η-BLHE (250 mg/kg) pretreated group displayed only mild ethanol-induced gastric damage, characterized by minimal epithelial ulceration, submucosal edema, and inflammatory cell infiltration, when compared to the negative control group (distilled water-pretreated). In stark contrast, the η-BLHE (500 mg/kg) pretreated group exhibited complete protection, with no visible signs of ethanol-induced injury, demonstrating superior gastroprotective efficacy compared to omeprazole. Our results are consistent with those of Saini and Sharma (9), who reported that pretreatment with ethanolic extract of *H. officinalis* exhibits significant ulceroprotective activity in ethanol-induced ulcers. This effect is linked to a reduction in ROS production, improved gastric integrity, and increased nitric oxide levels.

Chromatographic analysis of η-BLHE reveals that it is primarily composed of esculin (27.77%) and hesperetin (33.29%). Both hesperidin and its aglycone form, hesperetin, have demonstrated protective effects against ulcerogenesis by boosting the levels of key antioxidants such as superoxide dismutase, glutathione, and catalase in the gastric mucosa. These flavonoids not only aid in the regeneration of ulcerated gastric tissue but also prevent the formation of hemorrhagic lesions in the mucosa [33]. Furthermore, esculin has shown significant gastroprotective properties, mitigating gastric injury through multiple mechanisms, including the stimulation of endogenous prostaglandin synthesis, enhancement of nitric oxide production, activation of K_{ATP} channels, reduction of free radicals, and modulation of antioxidant enzyme systems [34]. Together, these bioactive compounds contribute to the overall protective and regenerative effects on the gastric mucosa.

Conclusion

In conclusion, our study provides robust evidence that pretreatment with η-BLHE significantly alleviates ethanol-induced gastric ulcers in rats, as demonstrated by a marked reduction in ulcerated and hemorrhagic areas of the gastric mucosa. Both macroscopic and histopathological analyses revealed a substantial decrease in lesion size, enhanced mucosal integrity, and reduced inflammation, highlighting the gastroprotective potential of *Hyssopus officinalis*. Additionally, the extract exhibited notable antibacterial activity against

Staphylococcus aureus, suggesting its broad-spectrum therapeutic efficacy. Among the 13 compounds identified in the η-BLHE, coumaric acid (45.26%) and rutin (18.93%) were found to be the most prevalent, likely contributing to the observed biological effects. These results underscore the dual functionality of *Hyssopus officinalis* as both a gastroprotective agent and an antimicrobial remedy. The identification of key bioactive compounds provides a strong foundation for further research aimed at isolating and characterizing these constituents, which could lead to the development of novel therapeutic strategies for managing gastric disorders and infections, particularly those caused by antibiotic-resistant *S. aureus*. Future studies should focus on the mechanistic pathways underlying these effects to fully harness the clinical potential of *Hyssopus officinalis* in integrative health practices.

Declaration of interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors have no relevant financial or non-financial interests to disclose.

No funding was received to assist with the preparation of this manuscript.

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Contribution of authors:

All authors contributed to the study conception and design.

Hamoud Nour-El-Houda.: Material preparation, data collection and analysis, writing the first draft of the manuscript, methodology, collection of plant, performing the experiments, and formal analysis.

Maammeri Zineb: Supervision, conceptualization and visualization.

All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability statement:

The authors declare that all data generated or analyzed during this study and supporting the obtained findings are available within the article

Ethical approval

All experimental methods were carried out incompliance with ethical protocol no.23/100, the institutional rules established by the Animal Research Committee of the Mentouri Brothers University Constantine1

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