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Evaluation of the antioxidant and gastroprotective effects of the aqueous extract of *Echinops spinosissimus* in an ethanol-induced gastric ulcer model

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"Sometimes the path you didn't choose ends up leading you exactly where you need to be "

dyna ♡

Dedication

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This journey might be complete but the pursuit of more knowledge continues.

Marwa Rayen Ouldlahoucine.

Evaluation of the antioxidant and gastroprotective effects of the aqueous extract of *Echinops spinosissimus* in an ethanol-induced gastric ulcer model

Abstract

Gastric ulcers pose a significant global health challenge, often exacerbated by factors such as alcohol consumption. This study investigates the gastroprotective potential of an aqueous extract from *Echinops spinosissimus* against ethanol-induced gastric ulcers in female *Albino Wistar* rats, along with its in *vitro* antioxidant and photoprotective properties.

Quantitative analysis revealed that the aqueous extract of *Echinops spinosissimus* contains 12 µg GAE/mg EXT of total polyphenols and 2.55 µg QE/mg EXT of total flavonoids. *In vitro* antioxidant assays demonstrated low activity: the extract exhibited DPPH and ABTS radical scavenging activity with (IC_{50} = 337.81 ± 12.98 µg/mL), (IC_{50} = 102.85 ± 2.48 µg/mL), respectively. The extract also showed the ability to reduce iron ions ($A_{0.5}$ = 640 ± 90.93 µg/mL). The *in vitro* sun protection factor (SPF) was found to be 10.94 ± 1.81, indicating weak photoprotective potential.

In the *in vivo* study, ethanol administration significantly increased malondialdehyde (MDA) levels in rat gastric tissue, a key biomarker of lipid peroxidation. Pre-treatment with *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) significantly reduced MDA levels to 59.25% and 44.44%, respectively, compared to the ethanol group (155.55%), demonstrating a preventive effect against lipid peroxidation.

Furthermore, ethanol treatment caused a significant reduction in glutathione (GSH) levels and glutathione peroxidase (GPx) activity. Pre-treatment with *Echinops spinosissimus* extract and omeprazole markedly restored GPx activity to 85.71% and 68.57%, respectively, and increased GSH levels to 110.57% and 91.34%, respectively, relative to the ethanol group, indicating an enhancement of the cellular antioxidant defense system. These beneficial effects were further supported by histological examination.

In conclusion, the aqueous extract of *Echinops spinosissimus* may serve as a natural and effective alternative to conventional drugs for the prevention and protection against gastric ulcer formation in humans.

Keywords: Ethanol, Gastric ulcer, *Echinops spinosissimus*, Polyphenols, Antioxidant activity, Photoprotective activity.

تقييم التأثيرات المضادة للأكسدة والمعدة الواقية للمستخلص المائي لنبات *Echinops spinosissimus* في نموذج قرحة المعدة المستحدثة بالإيثانول

ملخص

تشكل قرحة المعدة تحديًا صحيًا عالميًا كبيرًا، وغالبًا ما تتفاقم بسبب عوامل مثل استهلاك الكحول .تبحث هذه الدراسة في الإمكانات الواقية للمعدة لمستخلص مائي من نبات *Echinops spinosissimus* ضد قرحة المعدة المستحدثة بالإيثانول في إناث الجرذان البيضاء من سلالة ويستار ، بالإضافة إلى خصائصه المضادة للأكسدة والواقية من الضوء في المختبر .

كشف التحليل الكمي أن المستخلص المائي لنبات *Echinops spinosissimus* يحتوي (12 µg GAE/mg EXT) من البوليفينول الكلي و (2.55 µg QE/mg EXT) من الفلافونويدات الكلية، وأظهرت الاختبارات المضادات للأكسدة في المختبر نشاطًا ضعيفا: حيث أظهر المستخلص قدرت على اقتناص وازالة الجذور الحرة DPPH و ABTS وصلت حد (IC_{50}= 337.81±12.98 µg/mL) و (IC_{50}= 102.85±2.48µg/ml) على توالي وكما أظهرالمستخلص قدرة على اختزال ايونات الحديد($A_{0.5}$=640±90.63 µg/mL)، أما معامل الحماية من الشمس (SPF)في المختبر بلغ 1.81± 10.94، مما يشير إلى إمكانات ضعيفة للحماية من الضوء

في الدراسة داخل الكائن الحي ، أدى إعطاء الإيثانول إلى زيادة كبيرة في مستويات المالونديالدهيد (MDA) في أنسجة معدة الفئران، وهو مؤشر حيوي رئيسي لتأكسد الدهون .أدى العلاج المسبق بالمستخلص المائي لنبات *Echinops spinosissimus* (100 ملغ/كغ) والأوميبرازول (20 ملغ/كغ) إلى تقليل مستويات MDA بشكل ملحوظ إلى 59.25% و 44.44%، على التوالي، مقارنة بمجموعة الإيثانول (155.55%)، مما يدل على تأثير وقائي ضد تأكسد الدهون .

علاوة على ذلك، تسبب علاج الإيثانول في انخفاض كبير في مستويات الجلوتاثيون (GSH) ونشاط إنزيم الجلوتاثيون بيروكسيديز .(GPx) أدى العلاج المسبق بمستخلص نبات *Echinops spinosissimus* والأوميبرازول إلى استعادة ملحوظة لنشاط GPx إلى 85.71% و 68.57%، على التوالي، وزيادة مستويات GSH إلى 110.57% و 91.34%، على التوالي، بالنسبة لمجموعة الإيثانول، مما يشير إلى تعزيز نظام الدفاع المضاد للأكسدة الخلوي .وقد تم دعم هذه التأثيرات المفيدة بشكل أكبر من خلال الفحص النسيجي .

في الختام، قد يكون المستخلص المائي لنبات *Echinops spinosissimus* بديلاً طبيعيًا وفعالًا للأدوية التقليدية للوقاية والحماية من تكون قرحة المعدة لدى البشر.

الكلمات المفتاحية: الإيثانول، قرحة المعدة، *Echinops spinosissimus*، البوليفينول، النشاط المضاد للأكسدة، النشاط الواقي من الضوء.

Évaluation des effets antioxydants et gastroprotecteurs de l'extrait aqueux d'*Echinops spinosissimus* dans un modèle d'ulcère gastrique induit par l'éthanol

Résumé

Les ulcères gastriques représentent un défi majeur pour la santé mondiale, souvent exacerbés par des facteurs tels que la consommation d'alcool. Cette étude examine le potentiel gastroprotecteur d'un extrait aqueux d'*Echinops spinosissimus* (famille des Astéracées) contre les ulcères gastriques induits par l'éthanol chez des rates *Albinos Wistar* femelles, ainsi que ses propriétés antioxydantes et photoprotectrices in *vitro*.

L'analyse quantitative a révélé que l'extrait aqueux d'*Echinops spinosissimus* contient 12 µg GAE/mg EXT de polyphénols totaux et 2,55 µg QE/mg EXT de flavonoïdes totaux. Les tests antioxydants in vitro ont démontré une faible activité : l'extrait a montré une activité de piégeage des radicaux DPPH et ABTS avec (IC_{50} = 337,81 ± 12,98 µg/mL), (IC_{50} = 102,85 ± 2,48 µg/mL), respectivement. L'extrait a également montré une capacité à réduire les ions fer ($A_{0.5}$ = 640 ± 90,93 µg/mL). Le facteur de protection solaire (FPS) in vitro s'est établi à 10,94 ± 1,81, indiquant un faible potentiel photoprotecteur.

Dans l'étude in *vivo*, l'administration d'éthanol a significativement augmenté les niveaux de malondialdéhyde (MDA) dans le tissu gastrique des rats, un biomarqueur clé de la peroxydation lipidique. Un prétraitement avec l'extrait aqueux *d'Echinops spinosissimus* (100 mg/kg) et l'oméprazole (20 mg/kg) a significativement réduit les niveaux de MDA à 59,25 % et 44,44 %, respectivement, comparativement au groupe éthanol (155,55 %), démontrant un effet préventif contre la peroxydation lipidique.

De plus, le traitement à l'éthanol a entraîné une réduction significative des niveaux de glutathion (GSH) et de l'activité de la glutathion peroxydase (GPx). Un prétraitement avec l'extrait d*'Echinops spinosissimus* et l'oméprazole a nettement restauré l'activité de la GPx à 85,71 % et 68,57 %, respectivement, et augmenté les niveaux de GSH à 110,57 % et 91,34 %, respectivement, par rapport au groupe éthanol, indiquant une amélioration du système de défense antioxydant cellulaire. Ces effets bénéfiques ont été davantage confirmés par l'examen histologique.

En conclusion, l'extrait aqueux d*'Echinops spinosissimus* pourrait servir d'alternative naturelle et efficace aux médicaments conventionnels pour la prévention et la protection contre la formation d'ulcères gastriques chez l'homme.

Mots clés : Éthanol, Ulcère gastrique, *Echinops spinosissimus*, Polyphénols, Activité antioxydante, Activité photoprotectrice.

Abbreviations list

ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

$AlCl_3$: Aluminum chloride

BHA: Butylated hydroxyanisole

BHT: Butylated hydroxytoluene

b.w.: body weight

CagA: Cytotoxin-associated gene A

cAMP: cyclic AMP

CF: Correction factor

COX-1: Cyclooxygenase-1

COX-2: Cyclooxygenase-2

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DTNB: 5,5'-dithio-bis(2-nitrobenzoic acid)

***E. spinosissimus*:** *Echinops spinosissimus*

EGCG: Epigallocatechin gallate

EE(λ): Erythemal effect spectrum

EGF: Epidermal growth factor

EtOH: Ethanol

$FeCl_3$: Ferric chloride

FR: Free radical

FRAP: Ferric reducing antioxidant power

GA: Gallic acid

GI: Gastrointestinal

GPx: Glutathione peroxidase

GSH: Glutathione

***H. pylori*:** *Helicobacter pylori*

HCO_3^-: Bicarbonate

$H_3PMo_{12}O_{40}$: phosphomolybdic acid

$H_3PW_{12}O_{40}$: phosphotungstic acid

HO-1: Heme oxygenase-1

IC50: Half maximal inhibitory concentration

ICC: Interstitial cells of Cajal

I(λ): Solar intensity spectrum

IL-1β: Interleukin-1β

IL-8: Interleukin-8

iNOS: Inducible nitric oxide synthase

$K_2S_2O_8$: Potassium persulfate

$K_3fe(CN)_6$: Potassium Ferricyanide

LOOH: Lipid hydroperoxide

MDA: Malondialdehyde

MED: Minimal Erythemal Dose

MMC: Migrating motor complex

NF-κB: Nuclear factor kappa B

NSAIDs: Non-steroidal anti-inflammatory drugs

Nrf2: Nuclear factor erythroid 2–related factor 2

PG: Prostaglandins

PGE2: Prostaglandin E2

PPIs: Proton pump inhibitors

QE: Quercetin equivalent

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

rpm: rotations per minute

SOD: Superoxide dismutase

SPF: Sun Protection Factor

TBARS: Thiobarbituric acid reactive substances

TBS: Tris-buffered saline

TCA: Trichloroacetic acid

TFC: Total flavonoid content

TNF-α: Tumor necrosis factor-α

VacA: Vacuolating cytotoxin A

UV: Ultraviolet

Figures List

Figure **1:** Stomach regions. **4**

Figure 2: Structure of human stomach (left) and gastric wall (right). **5**

Figure 3: Mechanisms involved in the secretion of HCl by parietal cells. **6**

Figure 4: Functional phases of stomach motility. **8**

Figure 5: Peptic ulcer, human stomach. **9**

Figure 6: Classification of gastric ulcers based on their anatomic location **10**

Figure 7: Pathogenesis of *H. pylori* **12**

Figure 8 : COX-1 inhibition induced stomach ulcer. **13**

Figure 9: Schematic representation of peptic ulcer etiopathogenesis **15**

Figure 10: NSAIDs and ethanol damage the stomach by reducing prostaglandin and mucus production, causing ulcers. Medicinal plants help protect the stomach by boosting these protective substances. **19**

Figure 11: Illustrates the free radical (FR) generation by necrotizing agents like ethanol, NSAIDs, and stress. The scavenging activity of phenolic compounds extracted from plants in terminating FR formation is also depicted. **20**

Figure 12: Pharmacological properties of the Asteraceae family. **22**

Figure 13: Morphological aspect of *Echinops spinosissimus* **23**

Figure 14: Preparation of aqueous extract from *Echinops spinosissimus* Seeds **27**

Figure 15: General redox reaction in the Folin−Ciocalteu assay **28**

Figure 16: Reduction of 2,2-diphenyl-1-picrylhydrazyl using plant extract **30**

Figure 17: Serial dilution process **30**

Figure 18: Schematic representation of the DPPH assay for determining antioxidant activity **31**

Figure 19: ABTS chemical reaction with antioxidant compound **32**

Figure 20: Schematic representations of the ABTS assay for determining antioxidant activity **33**

Figure 21: Determination of antioxidant activity by Ferric Reducing Power method **34**

Figure 22: Experimental design for evaluating the effects of aqueous extract of *Echinops spinosissimus* on ethanol-induced gastric damage in rats **36**

Figure 23: Condensation reaction between TBA and MDA **37**

Figure 24: Reaction between GSH and DTNB **38**

Figure 25: Gallic acid calibration curve (mean ± SD from three trials)..... **41**
Figure 26: Quercetin calibration curve (mean ± SD from three trials). **42**
Figure 27: Polyphenols and flavonoids contents in the extract of *Echinops spinosissimus*. **42**
Figure 28: Percentage of inhibition of the free radical DPPH as a function of the concentration of *Echinops spinosissimus* extract. **43**
Figure 29: Percentage of inhibition of the free radical ABTS as a function of the concentration of *Echinops spinosissimus* extract. **45**
Figure 30: Iron reduction test by *Echinops spinosissimus* extract and standards (α-Tocopherol, ascorbic acid). **46**
Figure 31: The preventive effect of *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) on MDA levels in the stomach of rats treated with EtOH (5mg/kg).. .. .. **48**
Figure 32: The preventive effect of *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) on GSH levels in the stomach of rats treated with EtOH (5mg/kg).. ..... **49**
Figure 33: The preventive effect of *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) on GPx levels in the stomach of rats treated with EtOH (5mg/kg)... ..... **50**
Figure 34: Histopathological examination of gastric tissue **51**

Tables List

Table 1: Summary table of plants for treatment of gastric ulcer **7**
Table 2 : Botanical classification of *Echinops spinosissimus*.....**2**
Table 3: *Echinops spinosissimus* chemical composition **4**
Table 4: The antioxidant power of the DPPH radical (expressed by IC50 (in µg/ml)) of the reference antioxidants and the tested extract. **44**
Table 5: The antioxidant power of the ABTS•+ radical (expressed by IC50 (in µg/ml)) of the reference antioxidants and the tested extract. **45**
Table 6: The iron reducing power (expressed by A0.5 (in µg/ml)) of the reference antioxidants and the tested extract. **46**
Table 7: Sun protection factor calculation for aqueous extract obtained from *Echinops spinosissimus*.....**47**

Table of Contents

Acknowledgements
Abstract ..... **I**
الملخص.....**II**
Résumé……**III**
List of Abbreviations..... **IV**
List of Figures .....**VII**
List of Tables .....**IX**
Introduction.....**1**

Bibliographic synthesis

I. **Stomach** **3**

I.1. Structural components of the stomach **3**

I.1.1. Gross anatomy of the stomach **3**

- Cardia **3**
- Fundus **3**
- Body **3**
- Antrum **3**

I.1.2. Histological Structure of the Gastric Wall **4**

- Mucosa **4**
- Submucosa **4**
- Muscularis propria **4**
- Serosa **5**

I.2. Gastric physiology **5**

- Gastric acid and pepsin secretion **5**
- Gastric motility **6**
- Fasting period **7**
- Post-prandial Gastric Motility **7**
- Gastric emptying **8**

II. Gastric ulcers **8**

II.1. Classification of gastric ulcers **9**

- Type I **9**
- Type II **9**

- Type III **9**
- Type IV **9**
- Type V **9**

II.2. Gastric ulcer provoking factors **10**

II.2.1. Endogenous factors **10**

II.2.1.1. Pepsin and Gastric acid hypersecretion **10**

II.2.1.2. Genetic predisposition **10**

II.2.1.3. Aging **11**

II.2.2. Exogenous factors **11**

II.2.2.1. Helicobacter pylori infection **11**

II.2.2.2. Non-steroidal anti-inflammatory drugs (NSAIDs)…… **12**

II.2.2.3. Alcohol consumption **13**

II.2.2.3.1. Mechanisms of ethanol-induced gastrotoxicity ……. **13**

- Oxidative stress and inflammation **14**
- Effect of ethanol on gastric imbalance **14**

II.3. Gastric ulcer treatment **15**

II.3.1. Treatment by surgery **15**

II.3.2. Chemical treatment **16**

II.3.2.1. Proton pump inhibitors (PPIs) **16**

III. Medicinal plants in ulcer protection **16**

III.1. Different protective mechanisms against gastric ulcer **18**

III.1.1. Cytoprotective mechanism **19**

III.1.2. Antisecretory action of acid mechanism **19**

III.1.3. Antioxidant mechanism **20**

IV. Presentation of the plant *Echinops spinosissimus* **21**

IV.1. Overview of the Asteraceae family **21**

IV.2. Genus *Echinops* **22**

IV.2.1. Plant systematics ……..**22**

IV.3. *Echinops spinosissimus* **23**

IV.4. Botanical description **23**

IV.5. Chemical composition of *Echinops spinosissimus* **24**

IV.6. Traditional use **24**

IV.7. Biological activities and mechanisms of action of the plant…….…… **25**

IV.7.1. Antioxidant activity **25**

IV.7.2. Anti-inflammatory activity **26**

IV.7.3. Antimicrobial activity **26**

IV.7.4. Cicatrisation **26**

Materials and methods

I. Materials **27**

I.1. Plant material **27**

I.2. Animal material **27**

I.2.1. Animals and housing conditions **27**

II. Methods **27**

II.1. Preparation of aqueous herbal infusions **27**

II.2. The phytochemical study **28**

II.2.1. Total phenolic content **28**

- ❖ Principle **28**
- ❖ Protocol **28**

II.2.2. Total flavonoid content .....….**29**

- ❖ Principle **29**
- ❖ Protocol **29**

II.3. Methods for assaying in *vitro* antioxidant activities **29**

II.3.1. DPPH free radical scavenging test **29**

- ❖ Principle **29**
- ❖ Protocol **30**

II.3.2. ABTS free radical scavenging test **32**

- ❖ Principle **32**
- ❖ Protocol **32**

II.3.3. Ferric reducing antioxidant power (FRAP) assay **33**

- ❖ Principle **33**
- ❖ Protocol **34**

II.4. Sun protection factor (SPF) determination **35**

- ❖ Principle **35**
- ❖ Protocol **35**

II.5. Methods for assaying in *vivo* antioxidant activities…….....**36**

II.5.1 Treatment of animals **36**

II.5.2 Animal sacrifice and dissection **36**

II.5.3 Evaluation of tissue antioxidant status **38**

II.5.3.1. Determination of Malondialdehyde (MDA) **37**

- ❖ Principle **37**
- ❖ Protocol **37**

II.5.3.2 Determination of glutathione (GSH) ……**38**

- ❖ Principle **38**
- ❖ Protocol **38**

II.5.3.3. Determination of glutathione peroxidase (GPX) ……**39**

- ❖ Principle **39**
- ❖ Protocol **39**

III.Anatomical-pathologicalexaminations……..……**39**

IV.Statistical analysis……..……..……..**40**

Results

I. Quantitative characterization of *Echinops spinosissimus* ……**41**

I.1. Total polyphenol and flavonoid content of the extract ……..**41**

- Total polyphenols content ……**41**
- Total flavonoids content ……...**41**

II. Evaluation of in *vitro* antioxidant activity **43**

II.1. DPPH scavenging activity ……... **43**

II.2. ABTS scavenging activity ……....**44**

II.3. Reducing power assay ……..... **46**

III. *In vitro* sun protection factor (SPF) determination…… **47**

IV. Results of the in *vivo* experimental Study ……**48**

IV.1. The effect of different treatments on cytosolic oxidative status ……**48**

IV.1.1. Effect on MDA level ……... **48**

IV.1.2. Effect on GSH level ……....**48**

IV.1.3. Effect on GPx activity ……..**49**

V. Histopathological study……..……**50**

Discussion

Discussion……**52**

General conclusion and perspectives……...**58**

Bibliographic references…….…….....**59**

Introduction

Introduction

Ethanol is an alcohol with a significant role as precursor in chemical synthesis and as a solvent. It is commonly utilized in the food industry and in pharmacology for drug dissolution. Even though ethanol's chemistry is well understood and the biological effects have been largely investigated, it is still considered a serious health problem, since it is strongly associated with roughly 200 different lifestyle diseases, including 14 different types of cancer, gastritis, chronic atrophic, hepatic steatosis and peptic ulcer disease (Pohanka, 2016; Caputo *et al.,* 2024).

Gastric ulcer or a stomach ulcer is essentially a break or an open sore in the protective lining of the stomach, it can be triggered by a variety of factors that penetrate through the muscular layer. nearly 10% of the world's population is affected by this disease. If left untreated, it can lead to serious complications like severe bleeding, perforation, and even obstruction, all of which can seriously endanger a person's health (Shen *et al*.,2025).

Although achievements and new drugs have been introduced to help cure gastric ulcerations, the mortality rate is still high, and drugs still not totally effective with high side effects, that limit patients' compliance. Accordingly, authentication of the effectiveness of more effective gastroprotective agents with fewer side effects is a promising way to improve treatment outcomes (Badr *et al.,* 2019; Sistani Karampour *et al.,* 2019)

Algeria has a rich and diverse flora that remains underexploited for its sensory and bioactive chemical potential. Medicinal plants, in particular, are promising and represent a valuable source of natural antioxidants and antibacterial agents for the use in the food and pharmaceutical industries (Gheffour *et al.,* 2015).

The use of medicinal plants to prevent and manage various health issues is on the rise around the world. This phytotherapy is becoming more important in both developed and developing countries due to the natural origins of these remedies and their lower risk of side effects. According to the World Health Organization, 80% of the global population turns to traditional medicinal plants, many of which are effective for treating gastrointestinal problems especially gastric ulcers due to the plants richness in a range of antiulcer compounds like terpenoids, saponins, phenolic compounds, flavonoids, and alkaloids where their effectiveness has been tested through pharmacological studies (Djanaev *et al.*,2023).

In this study, we focus on investigating a medicinal plant called *Echinops spinosissimus* from the Asteraceae family, it is a morphological diverse species that can be found in northern Africa, the Mediterranean area, and even in the Saharo-Arabian and Irano-Turanian regions. This plant is often dubbed a "complete pharmacy" due to its effectiveness in treating a range of issues such as infections, intestinal worm infestations, hemorrhoids, migraines, diarrhea, and heart pain since it contains a variety of biologically active compounds, including thiophenes, terpenoids, sterols, fatty acids, and alkanes, as highlighted in various phytochemical studies (Sanchez-Jimenez *et al.*, 2012 ; Al Masoudi &Hashim, 2023).

In this context, the overall objective of this work is to evaluate the antioxidant and gastroprotective activities of the aqueous extract from the seeds of *Echinops spinosissimus*. This study will be divided into four main parts:

- Quantitative evaluation of total polyphenols and flavonoids.
- Assessment of *in vitro* antioxidant activity using three tests: DPPH, ABTS, and ferric reducing antioxidant power (FRAP).
- Evaluation of photoprotective activity by determining the sun protection factor (SPF).
- Evaluation of the gastroprotective effects of the aqueous extract of *Echinops spinosissimus* in an ethanol-induced gastric ulcer model.

Bibliographic synthesis

I. Stomach

The stomach, located right in the upper abdomen and slightly to the left of the center, is a large, muscular, and hollow organ that plays a key role in the digestive system. It consists of four main parts: the cardia, fundus, body, and pylorus. One of its many jobs includes producing chyme, which is essential for breaking down food, synthesizing proteins that help with vitamin absorption, defending against microbes, and triggering the peristaltic reflex. Essentially, the stomach creates an environment where acidic solutions and digestive enzymes can effectively break down the food (Chaudhry *et al.*,2024; Hsu *et al.*, 2023).

I.1. Structural components of the stomach

I.1.1.Gross anatomy of the stomach

The stomach is a hollow, muscular organ located in the upper abdomen. It is divided into four main anatomical regions: the cardia, fundus, body, and antrum. These regions are distinguishable based on their location, histological features, and functional roles (Elzouki *et al.*, 2012; Ban, 2024) (Figure 1).

- **Cardia**

  Located near the gastroesophageal junction, the cardia is the proximal portion of the stomach. It contains mucus-secreting glands that protect the stomach lining from acidic gastric juices (Simpson, 2005; Elzouki *et al.*, 2012).

- **Fundus**

  The fundus is the dome-shaped region above the body of the stomach. It is rich in parietal cells, which secrete hydrochloric acid (HCl) and intrinsic factor, essential for protein digestion and vitamin B12 absorption (Elzouki *et al.*, 2012).

- **Body**

  The body is the main part of the stomach and contains oxyntic glands, which house parietal cells, chief cells, and mucous neck cells. These glands are responsible for acid secretion and the production of digestive enzymes (Elzouki *et al.*, 2012; Gyires & Fehér, 2017).

- **Antrum**

  The antrum is the distal portion of the stomach, leading to the pyloric sphincter. It contains pyloric glands that secrete mucus and gastrin, a hormone that stimulates acid secretion (Simpson, 2005; Elzouki *et al.*, 2012).

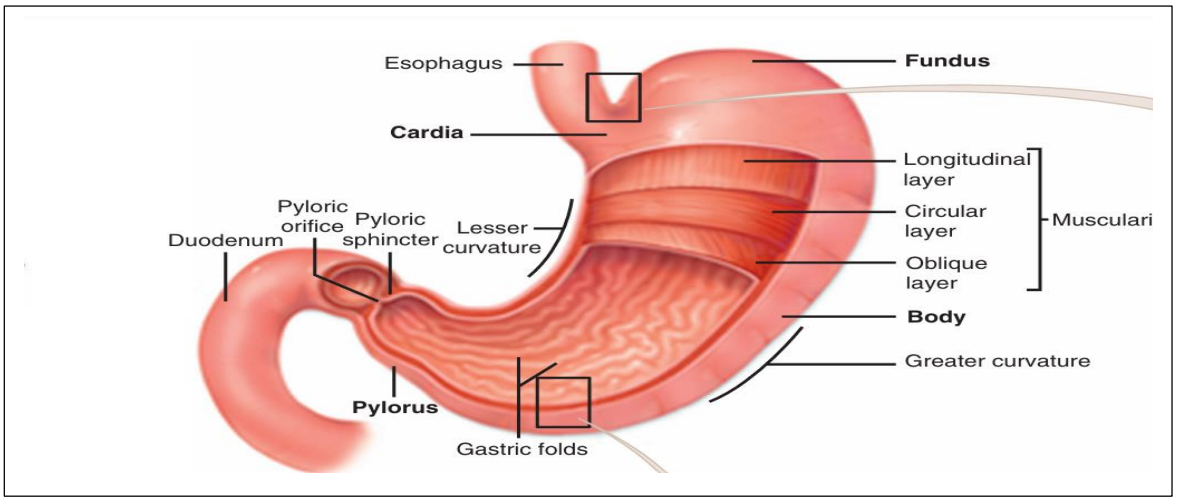

Figure 1: Stomach regions (Wilson & Stevenson, 2019).

I.1.2.Histological structure of the gastric wall

The stomach wall is composed of four layers: the mucosa, submucosa, muscularis propria, and serosa (Singh & Chanda, 2023; Ban, 2024) (Figure 2).

- **Mucosa**

  The innermost layer, the mucosa, is lined by a single layer of columnar epithelial cells. I t contains gastric pits and glands that secrete mucus, enzymes, and acids. The mucosa is rich in blood vessels and lymphatic vessels (Elzouki *et al.*, 2012; Singh & Chanda, 2023).

- **Submucosa**

  Beneath the mucosa lies the submucosa, a layer of connective tissue that contains blood vessels, lymphatic vessels, and nerves. It provides structural support and facilitates the exchange of nutrients and waste products (Mahadevan, 2014; Singh & Chanda, 2023).

- **Muscularis propria**

  This layer consists of three layers of smooth muscle: an inner oblique layer, a middle circular layer, and an outer longitudinal layer. These muscles generate the peristaltic contractions that mix food with gastric juices and propel it through the digestive tract (Fareé &Tack, 2013; Singh & Chanda, 2023).

❖ **Serosa**

The outermost layer, the serosa, is a thin membrane that covers the stomach. It produces a lubricating fluid to prevent friction between the stomach and adjacent organs (Mahadevan, 2014; Singh & Chanda, 2023).

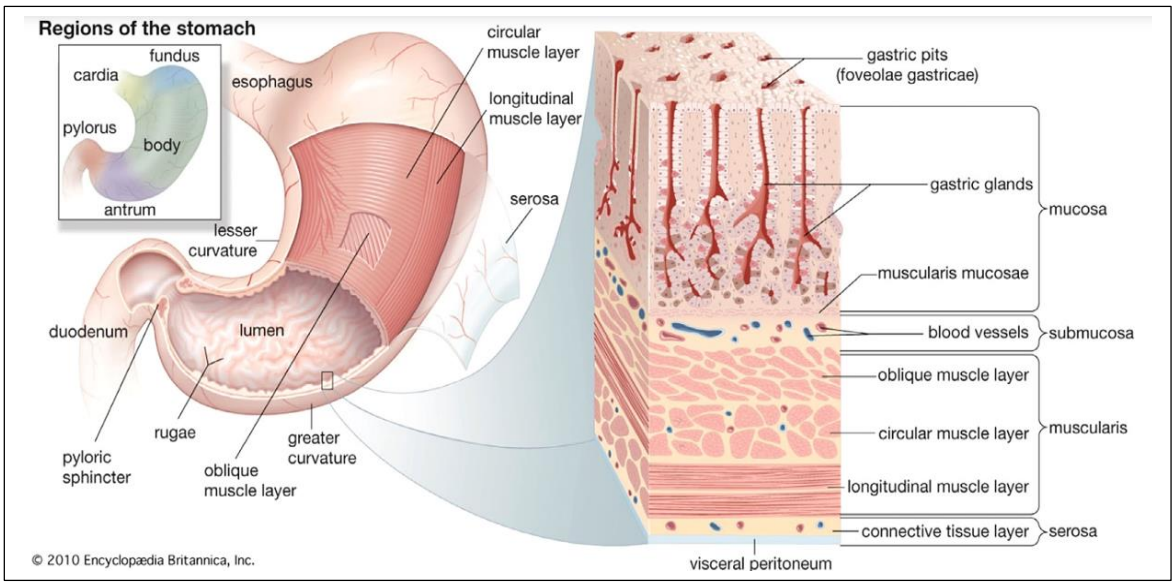

Figure 2 : Structure of human stomach (left) and gastric wall (right) (Brandstaeter et *al.*, 2019).

I.2. Gastric physiology

The core function of the human stomach is as an aid to digestion. This is an adaptive process that has had to modify itself many times in human history to adapt to changes in diet, lifestyle and microbiome. There are, however, four key components of gastric digestive function that are immutable, namely its function as a reservoir, acid secretion, enzyme secretion and its role in gastroin testinal motility (O'Connor & O'Moráin, 2014).

❖ **Gastric acid and pepsin secretion**

The secretion of hydrochloric acid (HCl) is a vital physiological process in the stomach that plays a central role in digestion and protection against pathogens. Hydrogen (H^+) and chloride (Cl^-) ions are secreted separately through hydrogen/potassium ATPase pumps and chloride channels in the stomach lining, resulting in a highly acidic environment. This acidity denatures dietary proteins, facilitates the activation of pepsinogen into its active form, pepsin an essential enzyme for protein digestion and helps eliminate ingested microorganisms (Hsu *et al.*, 2023; Vakil, 2025).

Pepsinogen is secreted by chief cells in the gastric lining in an inactive form and is subsequently activated by the acidic conditions created by hydrochloric acid secreted by parietal cells. This coordinated process between chief and parietal cells is crucial for effective protein digestion in the stomach (Heda *et al.*, 2023) (Figure 3).

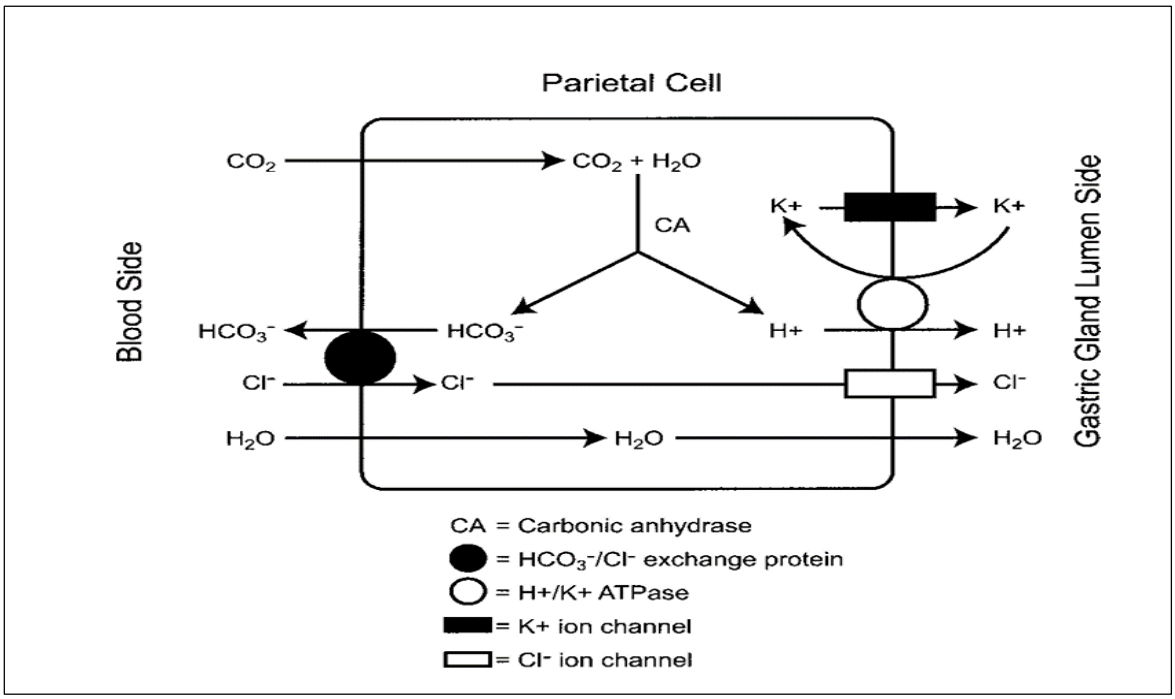

Figure 3 : Mechanisms involved in the secretion of HCl by parietal cells (Smith, 2003).

❖ **Gastric motility**

The stomach plays an important part in food digestion as it processes meals and distributes chyme to the small intestine. For proper gastric motility, the activity of the smooth muscles in the stomach is influenced by myogenic, neurological, and hormonal factors. At the heart of stomach motility is the intrinsic myogenic contraction, which happens on its own, without needing any outside influence. The myenteric plexus within the stomach is the main player in neural control, but it also gets some help from extrinsic inputs like the parasympathetic (vagal) and sympathetic (splanchnic) systems plus hormones like gastrin ghrelin and motilin. Gastric peristalsis primarily occurs in the lower section of the stomach and is regulated by the gastric slow wave. These slow waves are produced by specialized cells known as interstitial cells of Cajal (ICC), which are mainly located along the middle part of the stomach's greater curvature. The ICC play a crucial role in coordinating and

transmitting electrical signals within the smooth muscle cells of the stomach (Rostas *et al*., 2011).

- **Fasting period**

This process is characterized by the migrating motor complex (MMC), which is a rhythmic motor activity. The MMC consists of four distinct phases:

Phase I: Takes around 45 to 60mins, in this phase the peristaltic pump displays electrical slow waves independent of the muscle contraction.

Phase II: Linked to the electrical slow waves which are in turn associated to constant phasic contractions.

Phase III: Independent of the slow waves and marked by a series of contractions that have a significant amplitude, moving toward the pyloric sphincter and lasting anywhere from five to fifteen minutes.

Phase IV: The process involves the suppression of muscle contractions, which works hand in hand with the next phase of digestion. During periods of fasting and digestion, vagal stimulation quickly halts both gastric movement and neurohormonal activity (Goyal *et al.,* 2019).

- **Post-prandial gastric motility**

About five to ten minutes after food consumption, the MMC (migrating motor complex) shifts into a state of gastric muscle activity that signals being fed. The upper part of the stomach expands to accommodate the food and helps mix it with gastric juices, pepsin, and hydrochloric acid to start the digestion process. When the food is swallowed, the smooth muscle in the upper stomach relaxes in a response known as “receptive relaxation.” Similarly, as the volume of food increases, the upper stomach stretches in a process called “gastric accommodation.” These actions are triggered by vagal signals and various reflexes that respond to the stretching. In the end, this all leads to the upper stomach expanding, allowing it to temporarily store the food without increasing the pressure inside the stomach (Rostas *et al.,* 2011).

❖ **Gastric emptying**

Water leaves the stomach pretty quickly. Once food is broken down into chyme, which consists of tiny particles smaller than 2-3 mm, the digestible solids start to empty out. In the two to three hours after eating, both liquids and digestible solids are released. But during the time between meals, the stomach forcefully pushes larger food particles into the small intestine after holding onto them during digestion (Goyal *et al.*, 2019).

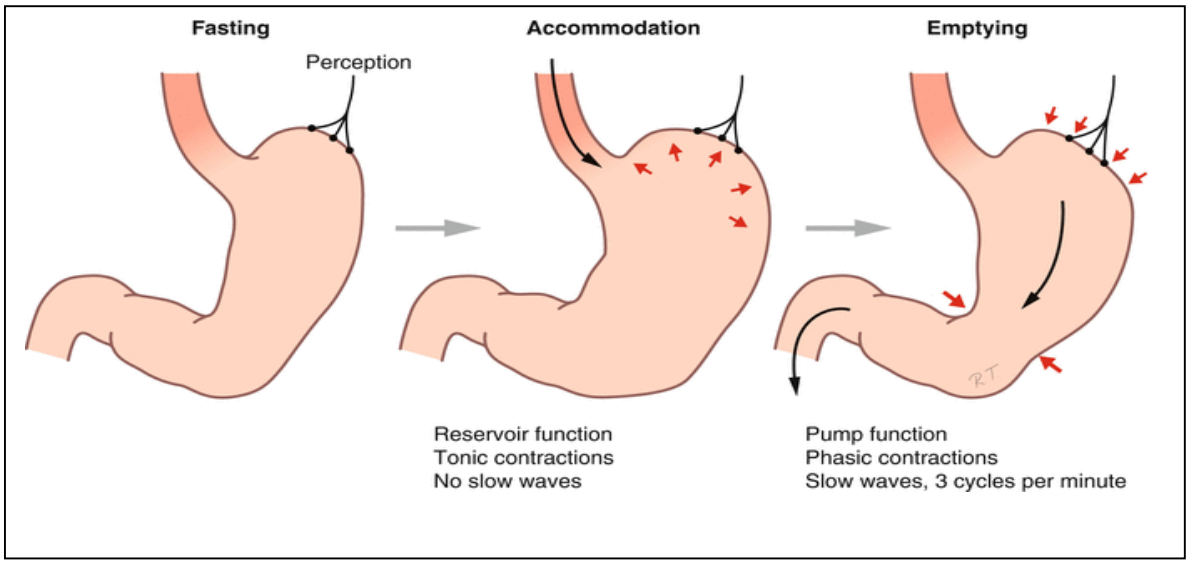

Figure 4: Functional phases of stomach motility (Bredenoord *et al.*, 2016).

II. Gastric ulcers

Gastric ulcers are open sores breaks or disruptions in the protective mucosal lining of the stomach measuring over 5 mm and penetrating the muscularis mucosa (Woolf & Rose, 2023), most commonly located on the lesser curvature (Malik *et al.*, 2023).

Almost 10% of the world's population sufers from this disease where it is concideder the most common inflammatory disease of the gastrointestinal tract (Anter *et al*., 2019). The characteristic feature of gastric ulcers are ruptures of the inner wall of the gastrointestinal (GI) tract caused by the release of pepsin or other gastric acids, where it crosses the muscularis propria membrane of the stomach epithelium.. It originates primarily in the stomach and the proximal duodenum, ulcers can also develop in the jejunum, distal duodenum, or lower esophagus (Khan *et al.*, 2023).

The interaction between the stomach's acids and the stomach's layers will lead into causing pain to the patient since the interaction helps with increasing the stomach's acids occurence which leads to the exposure of the capillaries underneath and causing bleeding,

on an empty stomach the majority of patients can not feel the pain or notice the symptoms because eating induces gastric acids (RaviKKumar, 2023),typically people with gastric ulcers experince epigastric pain from 15 to 30 mins after eating (Khan *et al.*, 2023) (Figure 5).

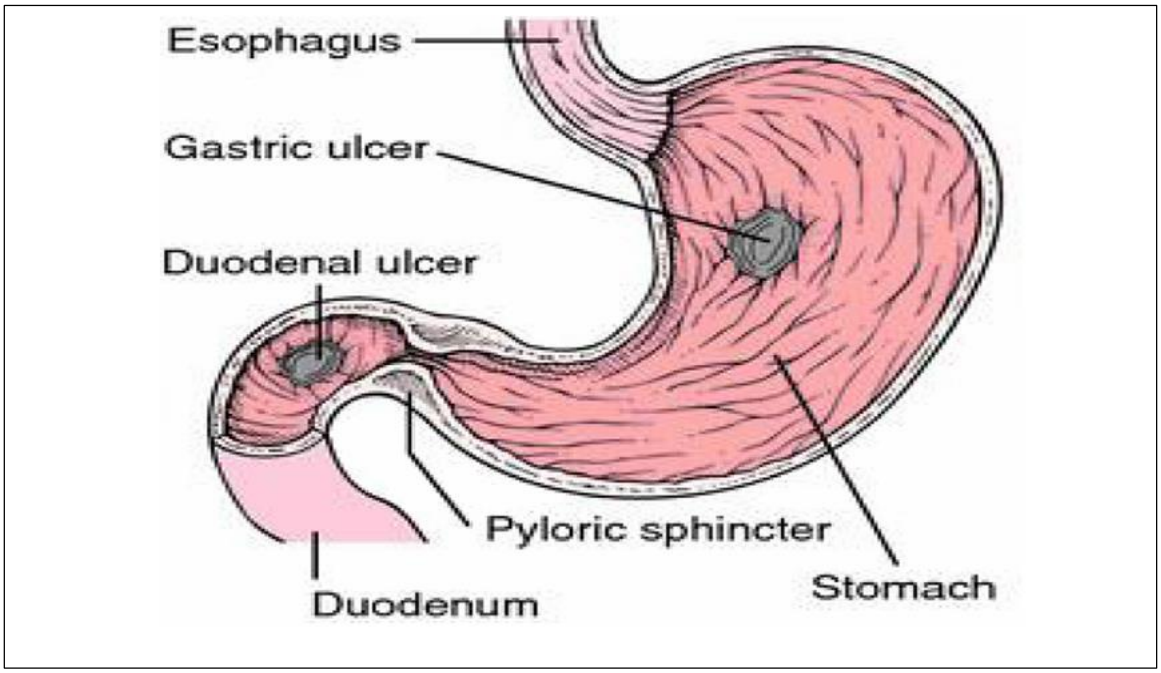

Figure 5 : Peptic ulcer, human stomach (Alazzouni *et al.*, 2020).

II.1. Classification of gastric ulcers

- **Type I**: Ulcers usually develop along the lesser curvature, at the junction between the fundic and antral mucosa near the incisura, and are associated with low acid secretion.
- **Type II**: Gastric ulcers are found in the pyloric channel and are associated with either a simultaneous duodenal ulcer or a previous duodenal ulcer that has healed with scarring.
- **Type III**: Ulcers are located in the prepyloric region or within the pyloric channel and are typically associated with elevated acid secretion.
- **Type IV**: Gastric ulcers are characterized by their anatomical position high on the lesser curvature, near the gastroesophageal junction.
- **Type V**: These lesions may develop at any location within the stomach and are typically caused by medications like NSAIDs (Ali *et al.*, 2019; Stern *et al.*, 2023) (Figure 6).

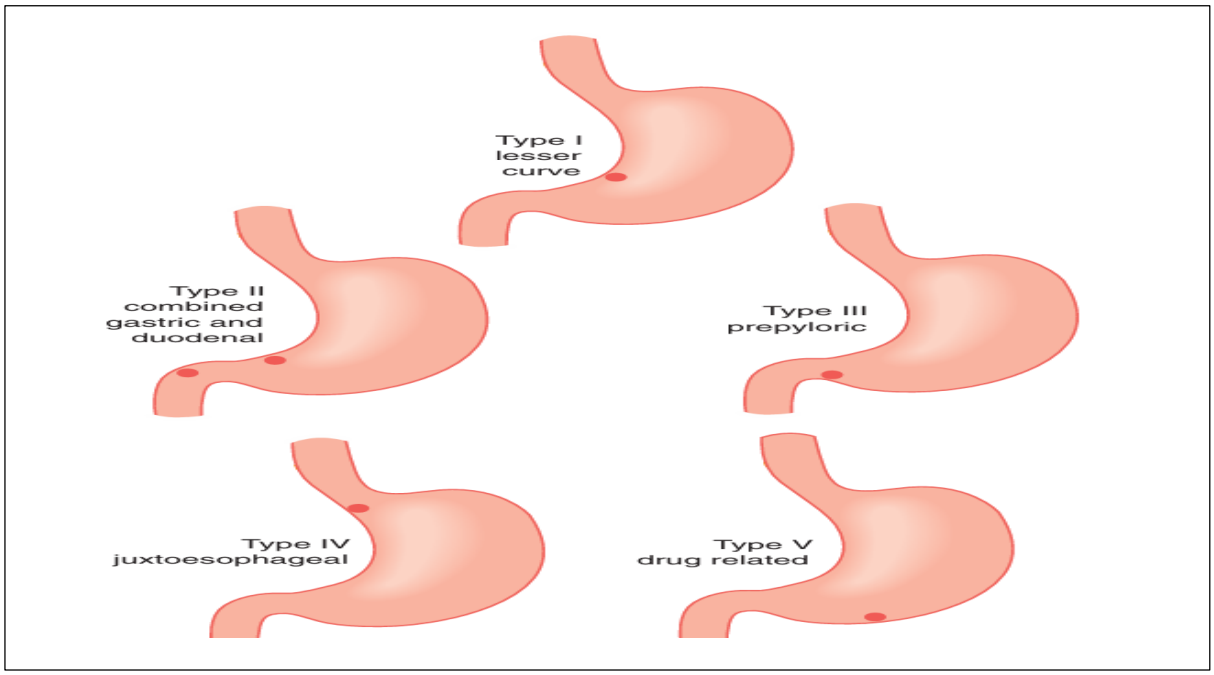

Figure 6 : Classification of gastric ulcers based on their anatomic location (Ali *et al.*, 2019).

II.2.Gastric ulcer provoking factors

II.2.1. Endogenous factors

II.2.1.1. Pepsin and gastric acid hypersecretion

Two key players in the development of ulcers are gastric acid and pepsin. For pepsinogen to transform into pepsin a proteolytic enzyme that helps break down proteins acid needs to be released from the stomach's parietal cells. Normally, a thick layer of mucus and bicarbonate protects the stomach lining and neutralizes the acid. However, when the balance between these protective factors and aggressive elements is disrupted, acid and pepsin can start to wear away at the mucosa, leading to ulcers formation (Agrawal, 2025).

II.2.1.2. Genetic predisposition

Ulcer formation is heavily influenced by genetic makeup. Some individuals are simply more susceptible to developing ulcers because of their genetic tendencies, which can lead to increased acid production or variations in the genes that help protect the stomach lining. Among the specific genetic factors at play we have family history where ulcers can be a bit of a family affair. If they seem to run in a family, it could mean all members have a higher risk, possibly hinting at some genetic factors at play. This might stem from shared environmental influences or inherited traits that families pass down (Agrawal, 2025).

II.2.1.3. Aging

The risk of developing peptic ulcer disease can increase with age due to the stomach lining becoming less resilient to damage. This is especially true for those who regularly take aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Research involving both humans and animals has shown that the aging gastric mucosa tends to have weakened defenses, leading to a reduction in the secretion of mucus and bicarbonate. Moreover, healthy older adults typically have lower levels of mucosal prostaglandins compared to their younger counterparts. Other studies have also indicated that the aging gastric mucosa exhibits decreased activity of nitric oxide synthase (NOS) and a changed response of sensory nerves to acid in the stomach (Kang *et al.,* 2010).

II.2.2. Exogenous factors

II.2.2.1. *Helicobacter pylori* infection

Helicobacter pylori is a Gram-negative, motile, flagellated bacillus that was first identified and isolated as a major cause of gastric and duodenal ulcers by Australian scientists Barry J. Marshall and J. Robin Warren in 1982 (Beiranvand, 2022).

A major virulence factor of *H. pylori* is its lipopolysaccharide, which significantly contributes to the bacterium's ability to adhere to host tissues. This adhesion is mediated through the secretion of specific adhesion molecules that recognize and bind to glycan structures expressed on the surface of gastric epithelial cells and within the mucus layer that lines the stomach (Lenka & Bhuyan, 2022).

Another important virulence factor of *H. pylori* is the enzyme on the surface known as urease is one of the most essential *H. pylori* virulence factors involved in bacterial metabolism and colonization within the gastric mucosa; it is the most abundantly ex pressed protein by this bacterium. *H. pylori* urease can be found in both the bacterial cytoplasmic compartment and on the surface of the bacteria; thus, two types of urease can be distinguished based on its localization internal and external.

The enzymatic reaction of urease is based on the hydrolysis of urea into ammonia and carbamate, which is further decomposed into another molecule of ammonia and carbonic acid that eventually induces the increase in gastric pH; the whole process is nickel-dependent (Baj *et al.,* 2020).

This bacterium also secretes exotoxins such as VacA and CagA. VacA is a potent toxin that induces apoptosis in host cells, while CagA disrupts cellular integrity and structure, promoting inflammation. CagA also stimulates the production of chemokines like IL-8, which attracts neutrophils to the site of infection. These neutrophils are highly inflammatory and contribute to tissue damage in the stomach. Together, the actions of VacA and CagA lead to the breakdown of gastric cells, ultimately resulting in ulcer formation (Lenka & Bhuyan, 2022) (Figure 7).

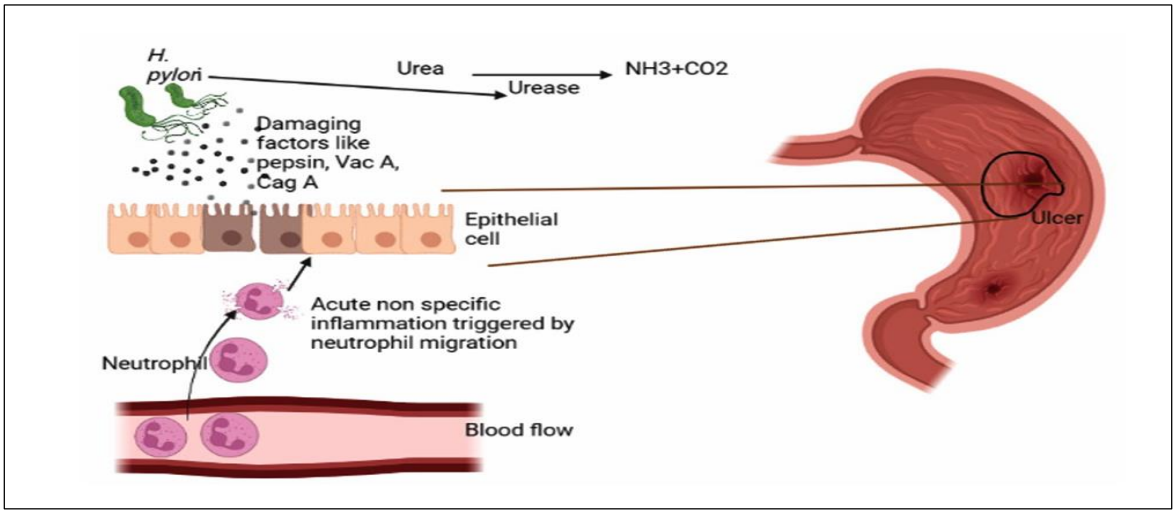

Figure 7 : Pathogenesis of *H. pylori* (Lenka & Bhuyan, 2022).

II.2.2.2. Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are commonly prescribed to treat conditions like arthritis, musculoskeletal injuries, menstrual cramps, and migraines, owing to their anti-inflammatory and pain-relieving effects (Islam *et al.,* 2024). However, non-steroidal anti-inflammatory drugs are also among the leading causes of gastric ulceration. Individuals who use these medications have a relative risk approximately four times higher of developing gastric ulcers compared to non-users. NSAID-induced ulceration results from several mechanisms. These drugs, being weak acids, become activated upon exposure to gastric acid and tend to accumulate within epithelial cells, thereby increasing cellular permeability and causing direct cellular injury.

The principal mechanism, however, involves the inhibition of prostaglandin synthesis. NSAIDs suppress the activity of the cyclooxygenase-1 (COX-1) enzyme, which is normally responsible for promoting the synthesis of prostaglandins. These prostaglandins play a vital role in maintaining gastric mucosal integrity by stimulating bicarbonate secretion, enhancing

mucus production, improving mucosal blood flow, and facilitating the repair and regeneration of epithelial cells following injury. By impairing these protective processes, NSAIDs render the gastric mucosa more susceptible to injury from gastric acid and pepsin. Among the resulting pathophysiological changes, the reduction in gastric blood flow and the subsequent mild ischemia are considered the most detrimental (Danisman *et al.*, 2023; Woolf & Rose, 2023) (Figure 8).

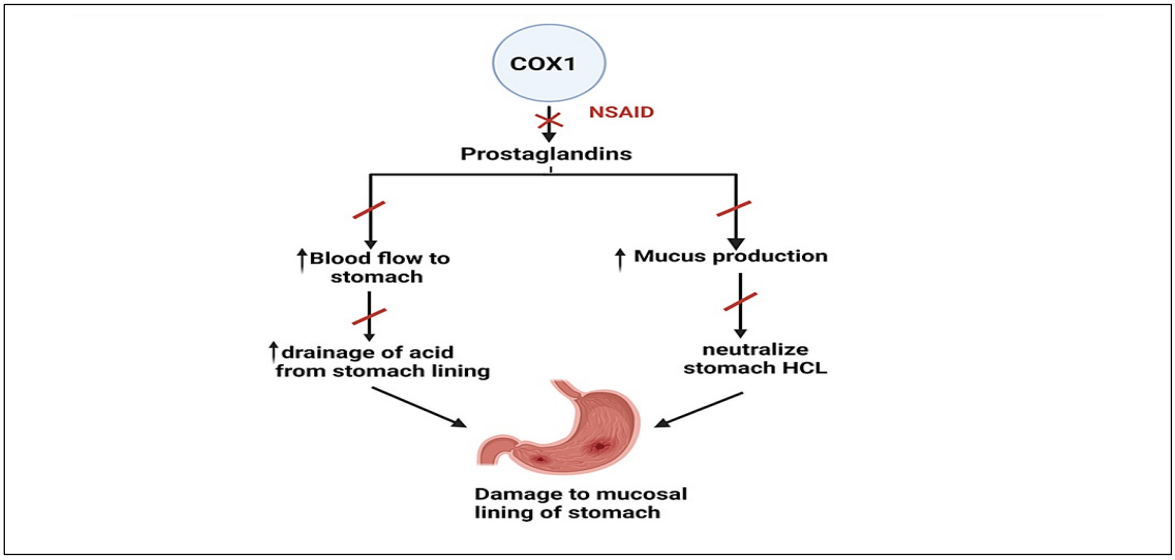

Figure 8 : COX-1 inhibition induced stomach ulcer (Sohail *et al.*, 2023).

II.2.2.3. Alcohol consumption

Ethanol, a type of alcohol, plays a major role as both a chemical precursor and a solvent. It is widely used in the food industry and in pharmacology for drug dissolution. Despite extensive knowledge of its chemical properties and biological effects, ethanol remains a significant public health concern (Pohanka, 2016).

Alcohol consumption is a notable risk factor for the development of gastric ulcers (Katary & Salahuddin, 2017). Chronic or excessive intake can lead to benign gastric lesions by damaging small blood vessels, impairing local blood flow, and triggering immune responses such as the rapid activation of neutrophils (Omer *et al.*, 2023).

II.2.2.3.1. Mechanisms of ethanol-induced gastrotoxicity

Ethanol is the major component of drinkable wine and alcoholic beverages. After drinking, alcohol is absorbed rapidly into the blood stream from the stomach and intestinal tract. High-concentration ethanol erodes directly the gastric mucosa and causes acute gastritis, leading to hyperemia, edema, hemorrhage, ulcer, etc.

It is well known that chronic alcohol abuse may induce gastrointestinal dysfunction, chronic atrophic gastritis and is closely related with gastric carcinoma. However, the detailed mechanism by which ethanol affects the gastrointestinal mucosa remains to be elucidated. Thorough research on how ethanol affects gastric mucosa will benefit the protection of gastric mucosa.

The effect of ethanol on gastric mucosa is a complicated and multifaceted process. It may be associated with disturbance of the balance between gastric mucosal defense and offensive factors (Pan et *al.,* 2008).

- **Oxidative stress and inflammation**

Oxidative stress and inflammation are considered to be the primary mechanisms underlying alcohol-induced gastric mucosal injury. Excessive production of reactive oxygen species (ROS) activates macrophages, which in turn release pro-inflammatory mediators such as nuclear factor kappa B (NF-κB) and tumor necrosis factor-α (TNF-α), thereby exacerbating tissue damage (Badr *et al.,* 2019).

In response to oxidative stress, the transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2) and its downstream effector heme oxygenase-1 (HO-1) play a crucial role in enhancing the antioxidant defense system and protecting the gastric mucosa. Nrf2 not only strengthens antioxidant defenses but also inhibits NF-κB activity, thus attenuating inflammatory responses and promoting cellular homeostasis.

In addition to promoting inflammation, ethanol directly damages the gastric mucosa by stimulating gastric acid hypersecretion, increasing ROS and reactive nitrogen species (RNS) generation, and further enhancing pro-inflammatory cytokine release. These factors synergistically contribute to mucosal apoptosis and reduce the production of two key protective mediators: nitric oxide (NO) and prostaglandin E2 (Raish *et al.,* 2021).

NO, a gaseous signaling molecule synthesized by inducible nitric oxide synthase (iNOS) from arginine, supports gastric microcirculation and promotes mucosal healing. However, ethanol-induced iNOS overexpression can lead to excessive NO levels, resulting in nitrosative stress and aggravating gastric ulcer development by disrupting the delicate balance between protective and harmful factors (Cho, 2001).

- **Effect of ethanol on gastric imbalance**

The effects of ethanol on the gastric mucosa are complex and multifaceted. They may be associated with a disruption of the balance between protective and aggressive factors

affecting the gastric lining. The gastric mucosa is exposed to gastric acid, pepsin, and other stimulants, while gastroprotective mechanisms maintain the integrity of the mucosal layer, the microcirculatory system, bicarbonate (HCO_3^-), prostaglandins (PG), epidermal growth factor (EGF) and epithelial cell renewing. Ethanol damages the vascular endothelial cells of the gastric mucosa and induces microcirculatory disturbances and hypoxia, associated with the overproduction of reactive oxygen species (Pan *et al.*, 2008; Mezdour *et al.*, 2017) (Figure 9).

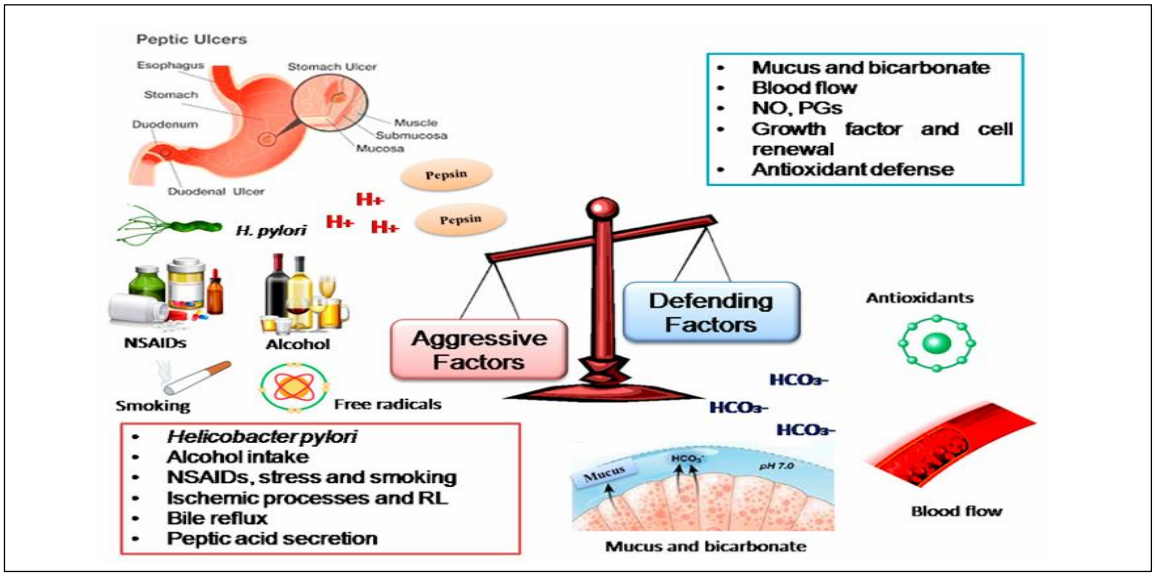

Figure 9 : Schematic representation of peptic ulcer etiopathogenesis (Serafim *et al.*, 2020).

II.3.Gastric ulcer treatment

II.3.1.Treatment by surgery

When it comes to peptic ulcers, surgery is often necessary due to complications like bleeding, perforation, penetration, or blockage. These procedures aim to prevent contamination of the peritoneal cavity, address the root cause of the ulcer, and ensure complete closure of any defects. Over time, various surgical techniques have been developed and refined, with the most common options being partial gastrectomy, omental patch repair (often referred to as the Graham patch), and simple closure. With simple sutures, often reinforced with nearby tissue. This straightforward and quick method is commonly employed in emergency situations, particularly for minor perforations (Pang *et al.*, 2025).

II.3.2.Chemical treatment

The chemical treatment of gastric ulcers involves several drug classes designed to reduce gastric acidity, eradicate Helicobacter pylori, and enhance mucosal protection. These therapies help restore the balance between aggressive factors like acid and pepsin and the protective mechanisms of the gastric mucosa (Katzung *et al.,* 2021).

II.3.2.1.Proton pump inhibitors (PPIs)

Proton pump is the ultimate mediator of gastric acid secretion by parietal cells. With the identification of H^+/K^+-ATPase as the primary gastric proton pump, it was proposed that activation of H^+ secretion occurred by incorporation of H^+/K^+-ATPase-rich tubulovesicles into the apical plasma membrane and that the pumps were re-sequestered back into the cytoplasmic compart ment on return to the resting state. Inhibition of the protons pumping H^+/K^+-ATPase as a means of control ling gastric pH has attracted considerable attention in recent years with the discovery of benzimidazole sulfox ide class of anti-secretory agents (Jain *et al*., 2007).

III. Medicinal plants in ulcer protection

Many medicinal plants are used in traditional medicine to treat peptic ulcers due to their ability to exert anti-ulcer effects through various mechanisms, including antioxidant activity, cytoprotection, and antisecretory action, these plants contain bioactive compounds like flavonoids, and terpenoids that can enhance the gastric mucosal barrier, inhibit acid secretion, and promote ulcer healing (Cherrada *et al.,* 2024).

A selection of such plants and their active constituents with anti-ulcerogenic properties is presented in Table 1.

Table 1 : Summary table of plants for treatment of gastric ulcer

Scientific Name	Common Name	Used Part	Principle Active(s)	Mechanism of Action (How the Used Part Inhibits Gastric Ulcer)
Phoenix dactylifera Family Arecaceae	Date palm	Fruit Seed	Flavonoids Phenolics Tannins	Enhances antioxidant activity (↑GSH), decreases acid/gastrin secretion, increases mucus content, reduces ulcer area, and alleviates gastric injury by reducing oxidative stress and lipid peroxidation (Hussein *et al.*, 2023)
Balanites aegyptiaca Family Zygophyllaceae	Desert date	Stem bark	Saponins, Flavonoids Alkaloids	Aqueous/ethyl acetate extracts reduce gastric lesions by antioxidant effects, decreasing acid secretion, and promoting mucosal protection (Ugwah-Oguejiofor *et al.*, 2023).
Origanum vulgare Family Lamiaceae	Oregano	Leaves	Carvacrol Thymol Flavonoids	Antioxidant and anti-inflammatory actions can protect the gastric mucosa by reducing oxidative stress and inhibiting pro-inflammatory cytokines (Sánchez-Campillo *et al*., 2013; Al-Snafi, 2015).
Punica granatum Family Lythraceae	Pomegranate	Peel Fruit	EllagitanninsPunicalagins	Neutralizes ROS, enhances endogenous antioxidants (SOD, CAT), suppresses TNF-α, IL-1β, increases mucosal protective agents, preserves gastric tissue architecture, and reduces ulcer severity (Zamanian *et al.*, 2025).
Zingiber officinale Family Zingiberaceae	Ginger	Rhizome	Gingerols Shogaols	Cytoprotective effect: enhances mucus production, reduces acid secretion, inhibits inflammatory mediators (e.g., TNF-α, IL-1β), promotes gastric motility, and enhances antioxidant defenses; protects against NSAID and ethanol-induced ulcers (El-Sayed *et al.*, 2020; Wang *et al.*, 2021).
Matricaria chamomilla Family Asteraceae	Chamomile	Flower	Apigenin Bisabolol	Reduces ulceration through anti-inflammatory and antioxidant properties, increases mucus secretion, and protects against aspirin-induced ulcers (Mubashir *et al.*, 2022; Wu *et al.*, 2023).

Aloe vera Family Asphodelaceae	Aloe	Leaf gel	Polysaccharides Anthraquinones	protects gastric mucosa by stimulating mucus and bicarbonate secretion, increasing prostaglandin synthesis, acting as an antisecretory, and enhancing mucosal repair; also reduces oxidative stress and inflammation (Ramos-Serpa *et al.*, 2024).
Camellia sinensis Family Theaceae	Green tea	Leaf	Catechins (EGCG), Polyphenols	Green tea extract promotes ulcer healing by increasing mucin content, restoring glutathione and SOD activity, and reducing inflammation and oxidative stress (Borato *et al.*, 2016).
Curcuma longa Family Zingiberaceae	Turmeric	Rhizome	Curcumin	Curcumin and turmeric extract promote ulcer healing through mucoadhesion, forming a physical barrier, and by antioxidant and anti-inflammatory actions (Gupta *et al.*, 2020).
Teucrium polium Family Lamiaceae	Felty germander	Aerial parts	Flavonoids, Terpenoids	Teucrium polium exerts anti-ulcer effects mainly through the antioxidant and cytoprotective actions of its flavonoids which promote mucosal healing, enhance mucin secretion, and modulate prostaglandin synthesis (Bahramikia,& Yazdanparast , 2012)
Myrtus communis Family Myrtaceae	Myrtle	Berries	Tannins, flavonoids	Inhibits gastric lesions, reduces acidity, prevents lipid peroxidation (Mansour *et al.*, 2022).

III.1. Different protective mechanisms against gastric ulcer

According to data gathered from the literature, the gastroprotective effects of medicinal plants may be attributed to four main defensive mechanisms: cytoprotective, molecular antisecretory, and antioxidant pathways. Each of these mechanisms will be examined in detail in the following subsections (Qader *et al.*, 2022).

III.1.1. Cytoprotective mechanism

The stomach secretes hydrochloric acid (HCl) to activate pepsin, which is essential for breaking down proteins, while protective mechanisms like mucus layers, epithelial cells, prostaglandins, and nitric oxide (NO) prevent damage to the stomach lining. Certain Malaysian medicinal plants exhibit strong gastroprotective properties. Notably, *Polygonum minus* extract showed the highest antiulcer activity by enhancing mucus production.

Prostaglandins, especially PGE2 produced via COX-1 and COX-2 enzymes, play a critical role in maintaining the mucus barrier and mucosal blood flow. NSAIDs and ethanol can inhibit prostaglandin production, leading to ulcers. Plant-derived phenolics and alkaloids, such as those from *Murraya koenigii*, also support gastric protection. Additionally, NO contributes to gastric defense by improving blood flow and preserving mucosal integrity. Several Malaysian plants promote NO production, offering protection against ulcer-inducing agents like ethanol and NSAIDs (Mani *et al.*, 2013 ; Qader *et al.*, 2022) (Figure 10).

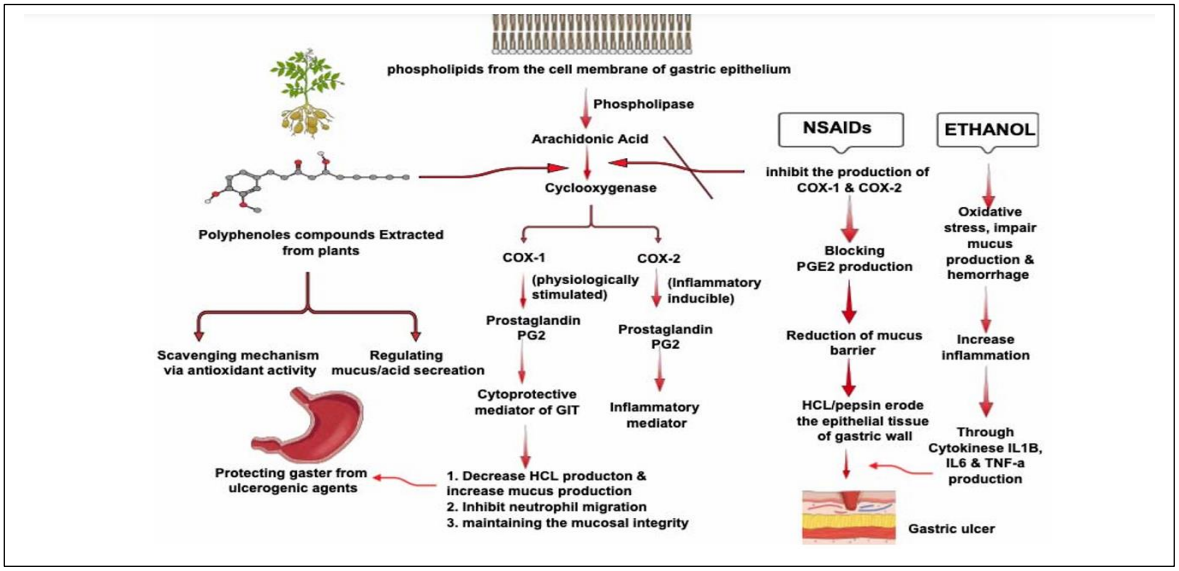

Figure 10: NSAIDs and ethanol damage the stomach by reducing prostaglandin and mucus production, causing ulcers. Medicinal plants help protect the stomach by boosting these protective substances (Qader *et al.*, 2022).

III.1.2. Antisecretory action of acid mechanism

Plants might have a unique way of reducing stomach acid, tapping into both the histaminergic and cholinergic pathways. While they operate differently, both histamine and acetylcholine play a role in boosting acid production. Acetylcholine works by attaching to M3-muscarinic receptors, which increases calcium levels inside parietal cells.

On the other hand, histamine connects to H2-receptors, raising both calcium levels and cyclic AMP an essential player in producing hydrochloric acid by activating protein kinase. The plants ability to lower gastric acidity could stem from its blocking action on M3 muscarinic receptors and H2-receptors. This might be due to the various bioactive substances or antisecretory compounds present in it (André Perfusion *et al.*, 2014).

III.1.3. Antioxidant mechanism

Oxidative stress is influential in causing illnesses, including gastric ulcers, resulting from a wide range of human pathogens. NSAIDs, alcohol, and stress are three primary sources disturbing mucosal resistance against free radicals (Suzuki *et al.*, 2011).

The antioxidant activity and protective effects against gastric ulcers of certain medicinal plants that contain standardized levels of flavonoids, tannins, polyphenols, vitamins, and minerals, have been explored through various mechanisms. These include scavenging free radicals like nitric oxide and the endogenous hydroxyl radical raising the pH in the gastrointestinal tract, and enhancing antioxidant enzymes such as catalase, superoxide dismutase, and ascorbic acid. Additionally, it help reduce lipid peroxidation and lipid hydroperoxide (LOOH), while also preventing a decrease in glutathione (GSH) (Altaf *et al.*, 2023) (Figure 11).

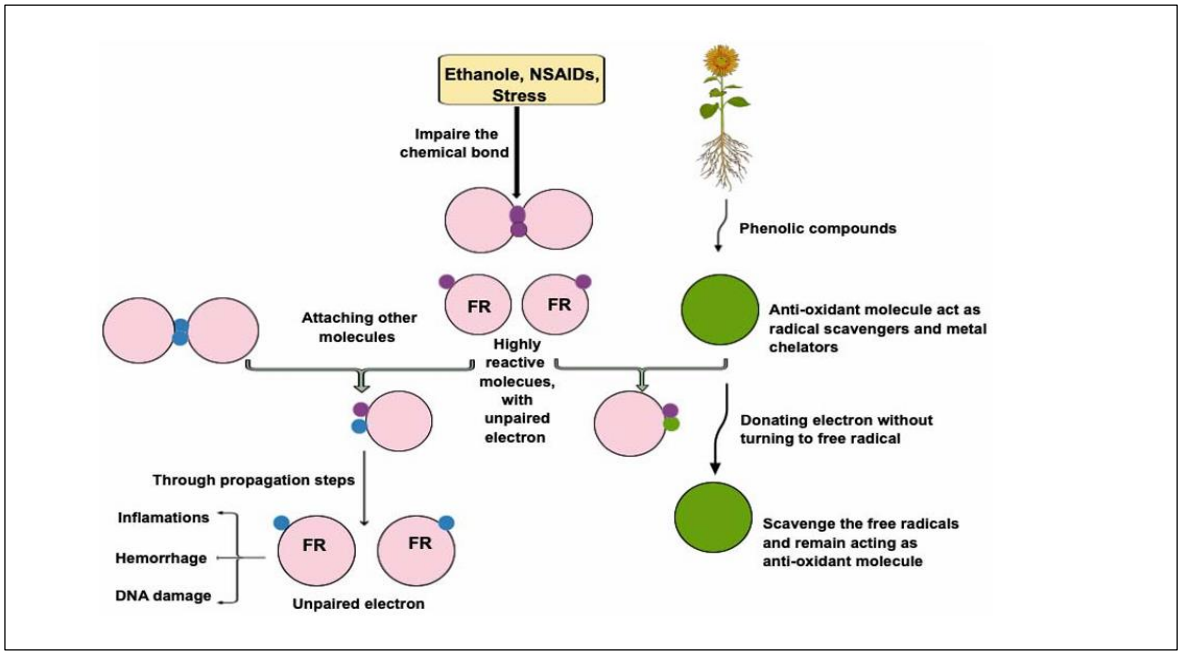

Figure 11: Illustrates the free radical (FR) generation by necrotizing agents like ethanol, NSAIDs, and stress. The scavenging activity of phenolic compounds extracted from plants in terminating FR formation is also depicted (Qader *et al.*, 2022).

IV. Presentation of the plant *Echinops spinosissimus*

IV.1. Overview of the Asteraceae family

The Asteraceae family, commonly known as the sunflower family, boasts over 1,600 genera and around 32,000 species across the globe, making it one of the largest families of flowering plants (Devkota, 2022). This diverse group includes familiar plants like chicory, sunflowers, lettuce, coreopsis, dahlias, and daisies, alongside several plants with medicinal properties, such as wormwood, chamomile, and dandelion. The family is distributed worldwide except in Antarctica and thrives in various natural settings. It can be spotted in urban parks, high-altitude meadows, and wooded areas, although it is less common in tropical climates (Rolnik & Olas, 2021).

The Asteraceae family showcases a fascinating range of shapes and sizes. While many of its species are shrubs, and most are perennial or short-lived annuals, there are also some tree species that can reach heights of over 30 meters. The leaves vary significantly: some are large and broad, while others are small and spiky. In fact, certain species don't even have leaves at all, relying instead on a green stem to carry out photosynthesis. Most leaves are covered in a mix of hairs and indumentum, which vary in length and color (Nadaf *et al*., 2025).

The family has a long history in traditional medicine, as many of its species possess pharmacological properties such as antioxidant, anti-inflammatory, anticancer, and antimicrobial activities, in addition to containing bioactive compounds like volatile components, phenolic acids, flavonoids, and terpenoids. The most well-known genera with these properties include *Achillea, Artemisia, Carthamus*, and *Echinops* (Nadaf *et al.*, 2025) (Figure 12).

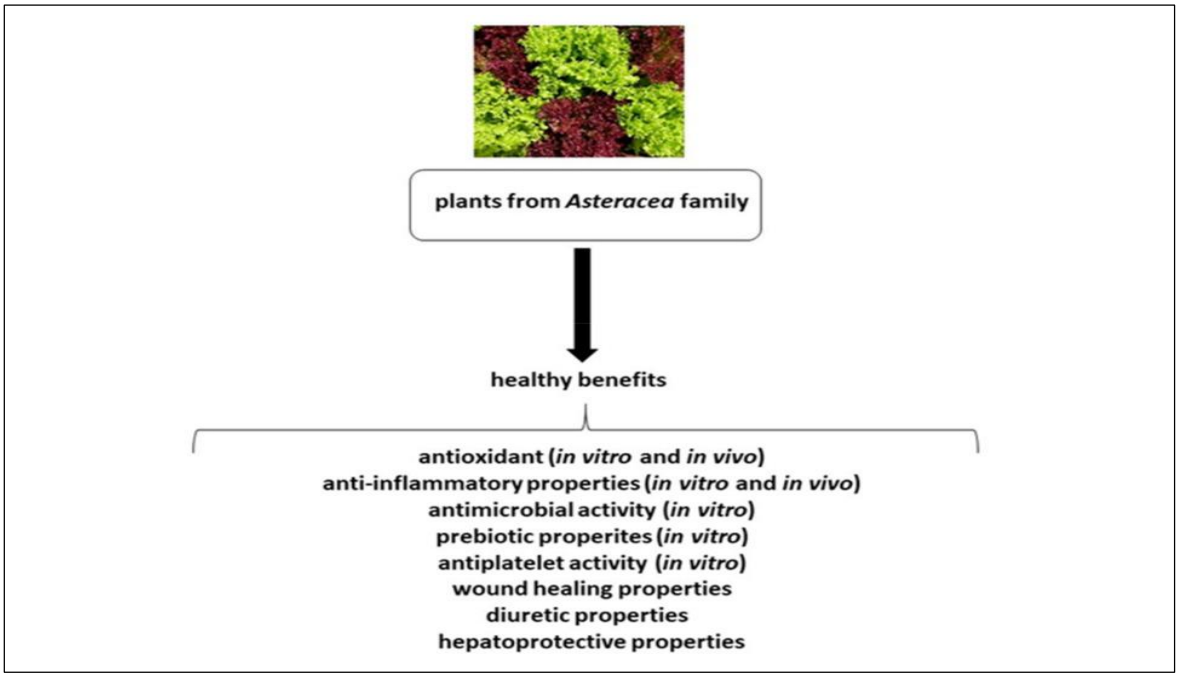

Figure 12: Pharmacological properties of the Asteraceae family (Rolnik & Olas, 2021).

IV.2. Genus *Echinops*

The *Echinops* genus boasts around 125 to 130 species, generally found thriving in the Mediterranean Basin, as well as in the temperate regions of Central Asia and the semi-arid areas of tropical and northern Africa. This genus contains a variety of phytochemical substances, such as thiophenes, terpenes, alkaloids, lipids, phenylpropanoids, flavonoids, and phenolic compounds. Flavonoids are found in the plant's aerial parts, while thiophenes are primarily located in the roots of *Echinops* (Diab *et al.*, 2025).

IV.2.1. Plant systematics

Table 2: Botanical classification of *Echinops* (Elseragy *et al.*, 2024)

Kingdom	Plantae
Division	Magnoliophyta (Flowering plants / Angiosperms)
Class	Magnoliopsida (Dicotyledons)
Subclass	Asteridae
Order	Asterales
Family	Asteraceae (Compositae)
Genus	*Echinops*

IV.3. *Echinops spinosissimus*

The genus *Echinops* is quite common in Algeria, particularly the *species Echinops spinosus L*. Interestingly, both the African Plant Database and the Plant List database refer to this species interchangeably with *Echinops spinosissimus Turra*. This plant thrives in arid desert conditions, where it receives between 20 to 100 mm of rainfall each year, and it can adapt to a variety of soil types, such as sandy, gravelly, rocky, coastal, and calcareous dunes. Botanists have classified *Echinops spinosus L*. into two subspecies: *E. spinosus ssp Maire* and *E. spinosus ssp. bovei (Boiss.)* (Bouzabata *et al.*, 2022).

IV.4. Botanical discerption

Echinops spinosus is a hardy perennial herb that can reach heights of up to 1 meter. It features upright stems that range in color from brownish to reddish, along with a few long leaves measuring between 10 to 15 cm in diameter. These leaves are hairy and have a unique arachnoid texture, complemented by very long spines. When it flowers, the inflorescence often takes the form of a single hemispherical globe, which can grow up to 5 cm across and is surrounded by many long spines. The small hermaphrodite flowers that cluster together in a dense head are tubular in shape, transitioning from green to white and yellowish as they fully bloom. The resulting fruits are tiny achenes topped with membrane scales that help them spread (Bouzabata *et al.,* 2022) (Figure 13).

Figure 13: Morphological aspect of *Echinops spinosissimus* (Zitouni-Nourine *et al.,* 2022).

IV.5. Chemical composition of *Echinops spinosissimus*

There hasn't been a lot of information about the phytochemistry of this species. However, studies on *E. spinosissimus* have uncovered a phytocomplex that includes compounds from various molecular classes. (Zitouni-Nourine *et al.*, 2022) (Table 3).

Table 3: *Echinops spinosissimus* chemical composition

Class of compounds	Example	Plant part	Refrerence
Sterols	Cholesterol	Flowers	(Bouzabata *et al.*, 2022).
Essential oils	Camphene, Limonene Alpha – pinene	Dried roots	(Majid *et al.*, 2024).
Phenolics	Rutin Gallic acid Rosmarinic acid	Flowers, leaves, stems	(Al Masoudi & Hashim, 2023).
Alkaloids	Echinopsine Echinorine	inflorescences	(Zitouni-Nourine *et al.*, 2022).
Thiophenes	α-Terthiophene	Not specified	(Zitouni-Nourine *et al.*, 2022).
Terpenes	Echinopine A Echinopine B	Roots	(Zitouni-Nourine *et al.*, 2022).
Sugars	Inulin Fructose Glucose	Ariel parts	(Abd El-Moaty, 2016).
Fatty Acids	Arachidic acid Oleic acid Pentadecylic	Ariel parts	(Abd El-Moaty, 2016).
Amino Acids	Asparagine Glutamine Glycine	Ariel parts	(Abd El-Moaty, 2016).

IV.6. Traditional use

Species of the *Echinops* genus have been used for ages to treat a variety of ailments.Their traditional uses can generally be grouped into three main categories. The most

frequently cited application is for alleviating symptoms like fever, pain and inflammation, the second category helps with respiratory issues like sore throats and coughs. This plant genus has also been utilized to address stomach, esophageal, uterine tumors, and even as an aphrodisiac.

In Chinese medicine and for the mongolian one the root of *Echinops* is utilized to address stomach tumors, blood disorders, and mental health conditions. Moreover, the powdered root is employed to help with angina, throat and lung ailments, liver echinococcus, esophageal cancer, and a host of other health concerns (Turgumbayeva *et al.,* 2023).

In Algeria, the roots or flower heads of *Echinops spinosus* have been used in the treatment of prostat ism and dysmenorrhea. This botanical remedy has also been used as a peripheral vasoconstrictor in the treatment of hemorrhoids, varicose veins, and varicocele, in various venous hemorrhages and in metrorrhagia. It is considered as a hypertensive drug (Bouzabata *et al.*, 2022).

IV.7. Biological activities and mechanisms of action of the plant

Traditional medicinal systems have long relied on this genus to address infections and inflammatory conditions. Scientific research has validated many of these traditional uses by revealing a rich phytochemical profile that includes thiophenes, flavonoids, terpenoids, alkaloids, and essential oils (Bitew and Hymete, 2019). These bioactive constituents contribute to a wide range of pharmacological effects through diverse molecular mechanisms such as enzyme inhibition, membrane disruption, oxidative stress reduction, and induction of apoptosis (Zheleva-Dimitrova *et al.,* 2023; Eid *et al.,* 2024).

The sections that follow summarize the major biological activities of *Echinops* species:

IV.7.1. Antioxidant activty

The antioxidant activity of the genus *Echinops* is primarily attributed to its rich content of bioactive compounds such as flavonoids, phenolic acids, coumarins, and thiophenes, which act through several complementary mechanisms (Sweilam *et al.,* 2021). These compounds directly scavenge reactive oxygen species (ROS) by donating electrons, as evidenced by strong DPPH and ABTS radical scavenging activities in extracts from species like *Echinops erinaceus* and *Echinops polyceras* (Al-Assaf & Khazem, 2021). The extracts also inhibit pro-oxidant enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing the formation of inflammatory ROS, and they chelate transition metals

(e.g., Fe^{2+}), which prevents the Fenton reaction and subsequent hydroxyl radical production (Bitew&Hymete,2019).

Overall, the genus *Echinops* demonstrates significant antioxidant activity through a combination of direct radical scavenging, enhancement of cellular antioxidant systems, inhibition of ROS-generating enzymes, and metal chelation, making it a promising source of natural antioxidants for therapeutic and functional applications (Bitew & Hymete, 2019; Sweilam *et al.,* 2021; Al-Assaf & Khazem, 2021).

IV.7.2. Anti-inflammatory activity

Echinops species possess notable anti-inflammatory properties. The mechanism of action involves the downregulation of pro-inflammatory mediators, notably nitric oxide (NO) and prostaglandin E2 (PGE2), through the suppression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes. This effect is predominantly linked to the presence of thiophenes and flavonoids in the plant extracts (Bitew & Hymete, 2019).

IV.7.3. Antimicrobial activity

The antimicrobial activity of *Echinops* is attributed to its thiophenes and essential oils, which disrupt microbial cell membranes, leading to leakage of cellular components and eventual cell death. These compounds may also interfere with microbial DNA and protein synthesis (Bitew & Hymete, 2019).

Recent research demonstrated that essential oil from *Echinops ritro* exhibits potent antibacterial effects against foodborne pathogens by compromising bacterial membrane integrity (Jiang *et al.,* 2017).

IV.7.4.Cicatrisation

Echinops spinosissimus blooms fully, producing tiny achenes topped with membranous scales that aid in their dispersion (Bouzabata *et al.,* 2022). This plant has also been reported to promote healing in excision wound models by stimulating cell growth, collagen formation, and epithelial tissue regeneration (Zitouni-Nourine *et al.,* 2022).

Materials and Methods

I. Materials

I.1.Plant material

Echinops spinosissimus was purchased from a herbalist in the Wilaya of Constantine. The extraction of bioactive compounds from this plant was carried out in the laboratories of the Faculty of Natural and Life Sciences at the University of Constantine.

I.2.Animal material

I.2.1.Animals and housing conditions

The experimental animals were female *Wistar albino* rats, weighing between 160 and 200g. They were raised at the brothers Mentouri University animal facility in Constantine, under controlled conditions (25°C, 12-hour light/dark cycle). The rats were housed in aluminum cages, five per cage, with free access to food and water.

II. Methods

II.1.Preparation of aqueous herbal infusions

The seeds of the plant were ground into a fine powder using a mechanical grinder. An aqueous infusion extract was prepared following the traditional method. Specifically, 6 g of the resulting powder of *Echinops spinosissimus* were infused in 500 mL of boiling distilled water for approximately 30 minutes. After infusion, the solution was filtered, and the filtrate was poured into multiple petri dishes and dried in a laboratory oven at 35 °C until completely dry. The resulting aqueous extract was then used for gastroprotective activity and antioxidant studies.

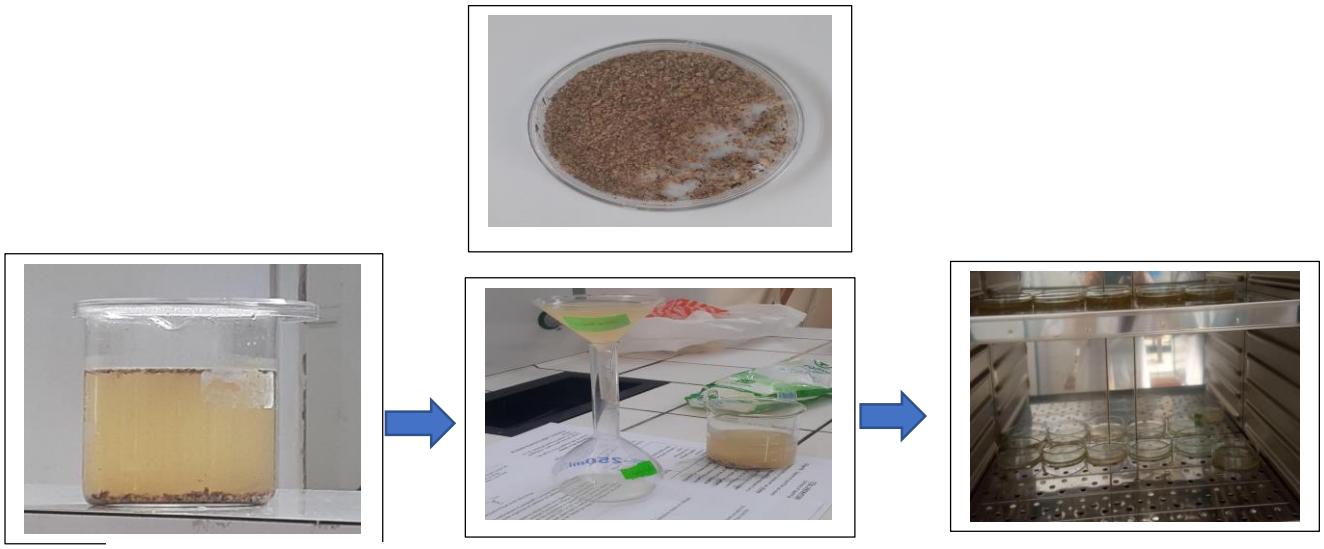

Figure 14: Preparation of aqueous extract from *Echinops spinosissimus* Seeds.

II.2.The phytochemical study

II.2.1. Total phenolic content

❖ **Principle**

The Folin–Ciocalteu reagent is composed of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). During the oxidation of phenolic compounds, this reagent is reduced, leading to the formation of a blue complex consisting of oxides of tungsten and molybdenum. The intensity of the resulting blue coloration, measured by absorbance at 765 nm, is directly proportional to the concentration of polyphenols present in the plant extracts (Boizot & Charpentier, 2020) (Figure 15).

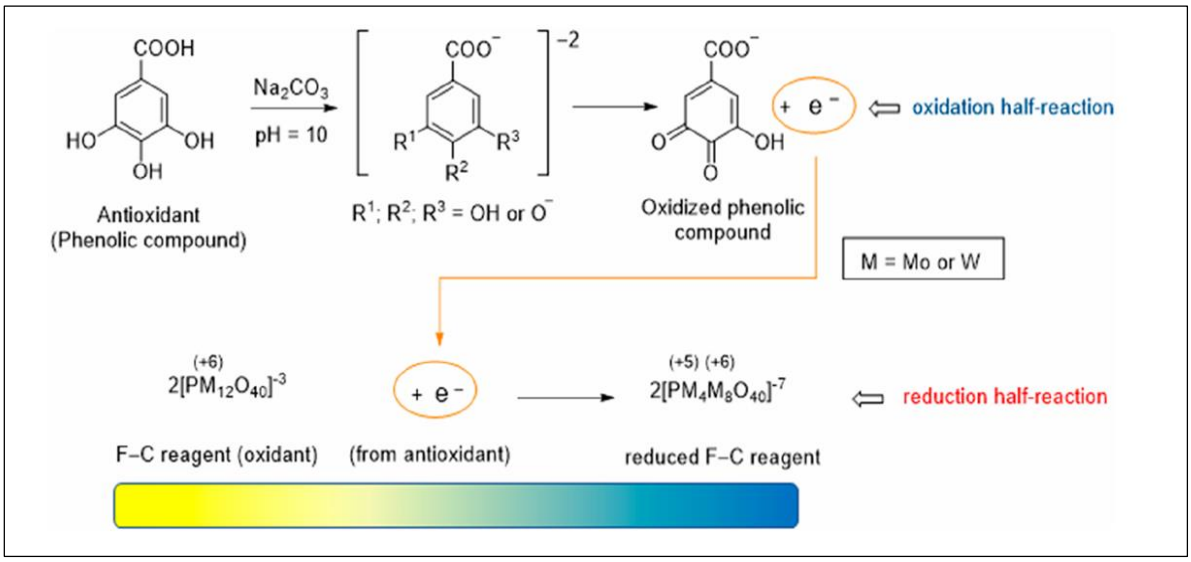

Figure 15: General redox reaction in the Folin−Ciocalteu assay (Pérez *et al.*, 2023).

❖ **Protocol**

The Folin–Ciocalteau assay was conducted to perform the colorimetric analysis, as was defined by Singleton *et al.* (1999). A volume of 20 µL of the sample was mixed with 1.58 mL of distilled water and 100 µL of Folin–Ciocalteu reagent. The mixture was left to stand for 8 minutes at room temperature. Subsequently, 300 µL of a 20% sodium carbonate solution were added, and the mixture was thoroughly vortexed. The reaction was then incubated in the dark for 2 hours to allow color development. Finally, the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid (GA) has been used as a standard, and total phenolic content concentration was expressed as µg GA equivalent per mg of extract. Tests were carried out in triplicate.

II.2.2.Total flavonoid content

❖ **Principle**

The quantification of flavonoids was carried out using a method based on the formation of a stable complex between aluminum chloride and the oxygen atoms located at carbons 4 and 5 of the flavonoids (Ali-Rachedi *et al.,* 2018).

❖ **Protocol**

The total flavonoid content (TFC) was determined using the method described by Wang *et al.* (2008), based on the aluminum chloride assay. In this procedure, 0.5 mL of the extract was mixed with 0.5 mL of a 2% aluminum chloride ($AlCl_3$) solution and incubated in the dark for 1 hour. The absorbance was then measured at 430 nm. Quercetin has been used as a standard, and TFC concentration was expressed as µg QE equivalent per mg of extract. Tests were carried out in triplicate.

II.3. Methods for assaying *in vitro* antioxidant activities

II.3.1. DPPH free radical scavenging test

❖ **Principle**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used and reliable method for evaluating the free radical scavenging activity of natural compounds, particularly plant extracts (Pyrzynska &Pękal, 2013; Chekuri *et al.,* 2018; Baliyan *et al.,* 2022). This colorimetric method is based on the reduction of the stable purple DPPH radical by antioxidant molecules, which donate hydrogen atoms or electrons. Upon reduction, the purple DPPH radical is converted into a yellow-colored non-radical form (Sirivibulkovit *et al.,* 2018; Baliyan *et al.,* 2022).

The degree of discoloration reflects the scavenging potential of the antioxidant and can be quantified by measuring the decrease in absorbance at 517 nm using a spectrophotometer (Wei *et al.,* 2014; Sirivibulkovit *et al.,* 2018; Baliyan *et al.,* 2022) (Figure 16).

The DPPH method is favored due to its rapidity, high sensitivity, ease of execution, and low reagent consumption. These advantages make it an efficient and cost-effective technique for screening antioxidant activity in various plant extracts, even with small sample volumes (Sirivibulkovit *et al.,* 2018; Baliyan *et al.,* 2022; El Babili *et al.,* 2022).

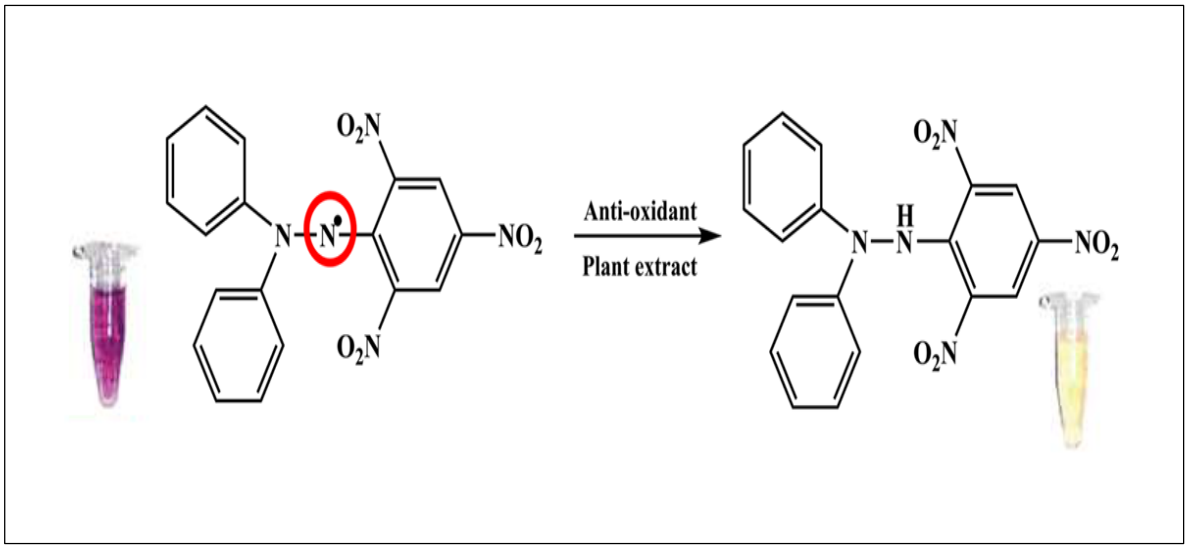

Figure 16: Reduction of 2,2-diphenyl-1-picrylhydrazyl using plant extract (Nanaei *et al.*, 2019).

❖ **Protocol**

To prepare the plant extract for analysis, 4 mg of the dried extract were dissolved in 1 mL of methanol to obtain the stock solution. Serial dilutions were performed using six Eppendorf tubes, each initially containing 500 µL of methanol. Then, 500 µL of the stock solution were transferred into the first tube and mixed thoroughly. Subsequently, 500 µL from the first diluted tube were transferred into the second tube and mixed, and the process was repeated sequentially through the sixth tube. This resulted in seven concentrations: the undiluted stock solution and six serial dilutions, each with a final volume of 1 mL (Figure 17).

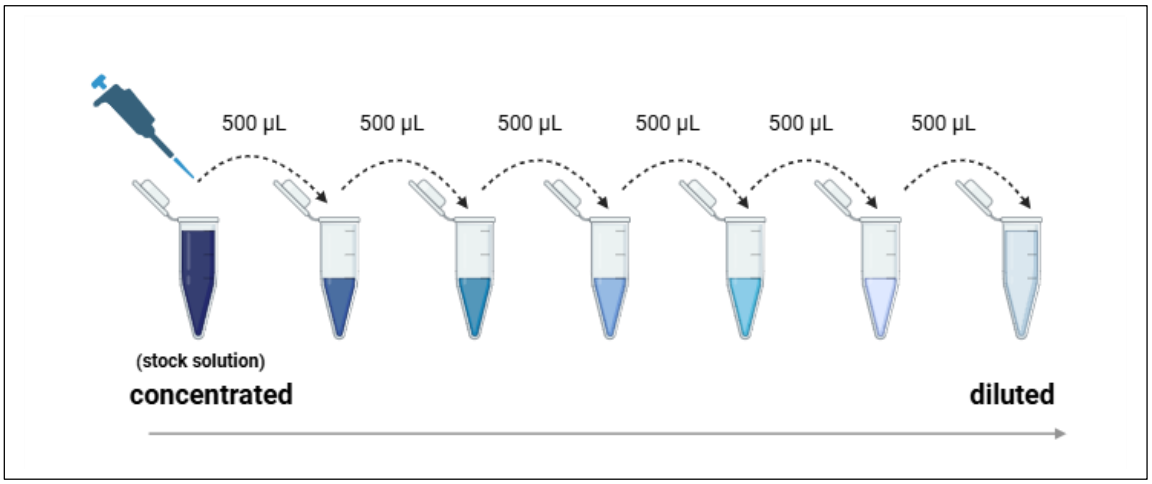

Figure 17: Serial dilution process

The DPPH test was performed using the method described by Blois, (1958). Briefly, in a 96-well microplate, 160 µL of DPPH solution (0.006%) was mixed with 40 µL of various dilutions of the plant extract. After 30 minutes of incubation in the dark at room temperature, the absorbance is measured at 517 nm (Figure 18).

BHA and BHT were used as control or positive standard. The % inhibition of anti-radical activity was calculated using the following equation:

$$\text{Inhibition } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

- A control: the absorbance of the negative control (DPPH + methanol)
- A sample: the absorbance of the extract-treated well.

We determined the IC_{50} parameter (inhibitory concentration value), which is the concentration of the extract that causes a 50% inhibition of DPPH activity (color change). It is calculated graphically by linear regression of the plotted graphs showing the percentage of inhibition as a function of the different concentrations of the fractions used. Thus, the IC_{50} of each extract is calculated:

IC50 = (Y- b)/a

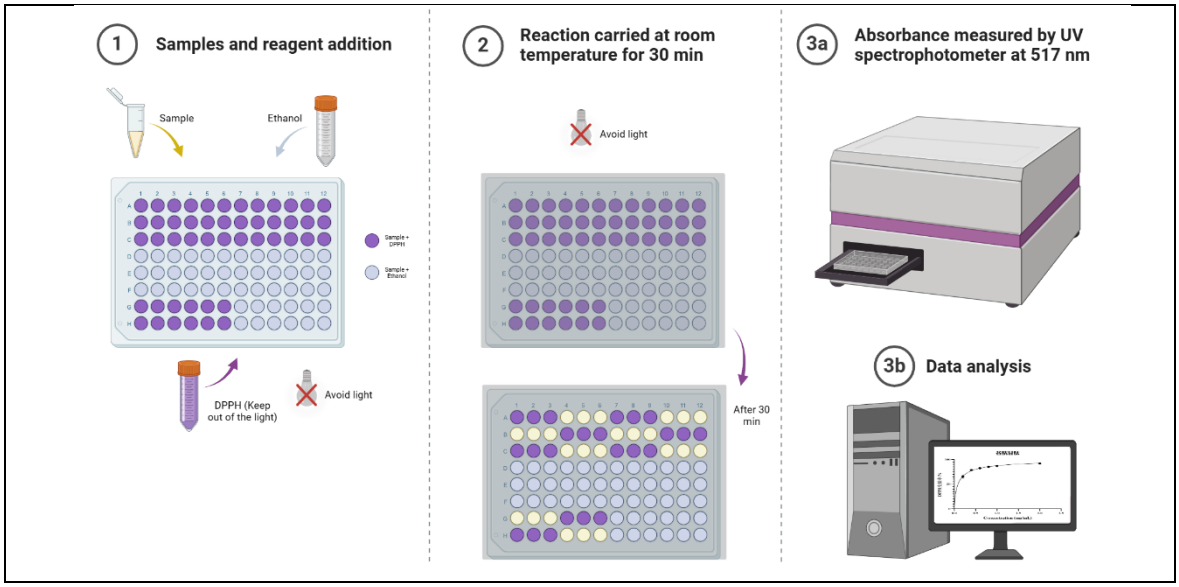

Figure 18: Schematic representation of the DPPH assay for determining antioxidant activity.

II.3.2. ABTS free radical scavenging test

❖ **Principle**

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay is a widely used spectrophotometric method for evaluating the antioxidant potential of natural products by measuring the reduction of the ABTS radical cation ($ABTS^{\bullet+}$), a stable blue-green chromophore that absorbs at 734 nm. Upon interaction with antioxidant molecules, $ABTS^{\bullet+}$ is reduced, causing a decrease in absorbance that can be quantitatively monitored. The extent of decolorization is directly proportional to the antioxidant capacity of the sample (Prior *et al.,* 2005 ; Silva *et al.,* 2022) (Figure 19).

This assay is valued for its sensitivity, rapidity, and applicability to both hydrophilic andlipophilic antioxidants, making it a standard tool in antioxidant research (Kumar *et al.,* 2021; Zhang *et al.,* 2023).

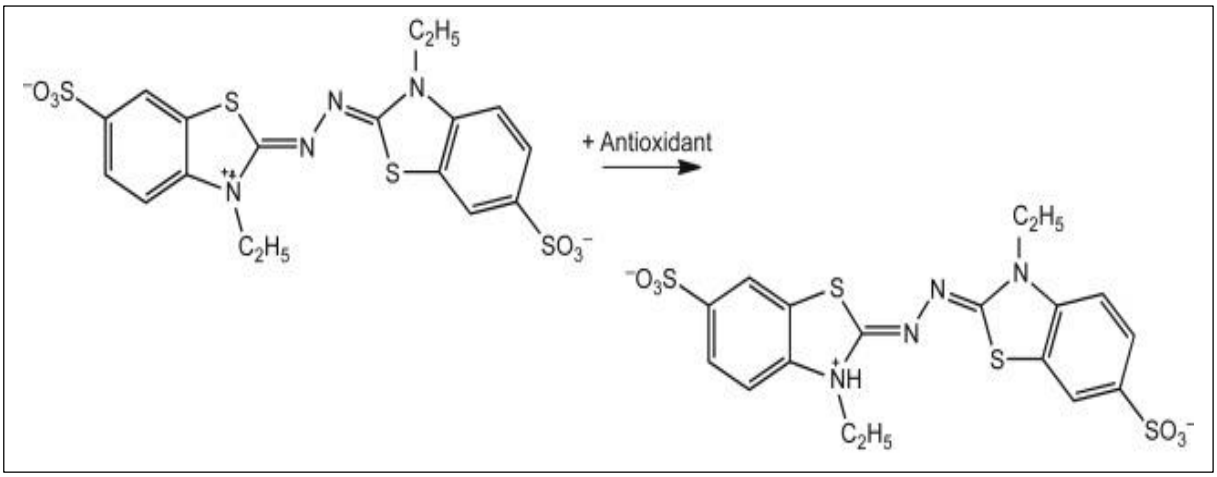

Figure 19: ABTS chemical reaction with antioxidant compound (Hernández-Rodríguez *et al.,* 2019).

❖ **Protocol**

The $ABTS^{\bullet+}$ radical cation decolorization assay was performed according to the method described by Re *et al*. (1999).

To generate the $ABTS^{\bullet+}$ radical solution, 19.2 mg of ABTS was dissolved in 5 mL of distilled water, and separately, 3.3 mg of potassium persulfate ($K_2S_2O_8$) was dissolved in another 5 mL of distilled water. The two solutions were then mixed and left to react for 16 hours at room temperature in the dark to allow complete formation of the stable $ABTS^{\bullet+}$

radical. Prior to use, the solution was diluted—typically in methanol or phosphate-buffered saline until it reached an absorbance of approximately 0.70 ± 0.02 at 734 nm.

In a 96-well microplate, 160 µL of ABTS solution was mixed with 40 µL of various dilutions of the plant extract. After 10 minutes of incubation in the dark at room temperature, the absorbance is measured at 734 nm using a microplate reader (Figure 20).

The antioxidant activity was expressed as a percentage of ABTS radical inhibition using the equation:

$$\text{Inhibition } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

- **A control**: the absorbance of the negative control (ABTS + methanol)
- **A sample**: the absorbance of the extract-treated well.

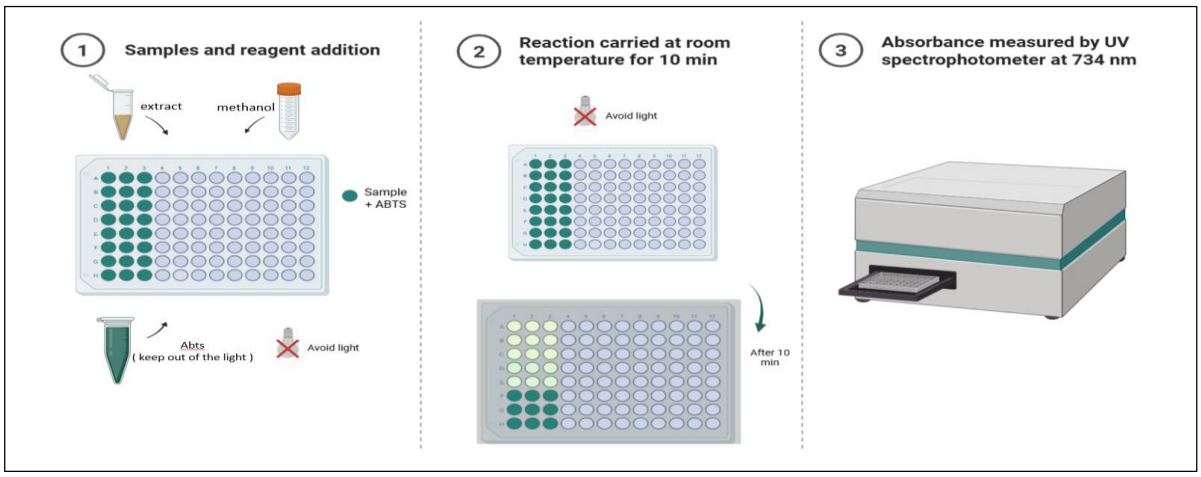

Figure 20: Schematic representations of the ABTS assay for determining antioxidant activity.

II.3.3.Ferric reducing antioxidant power (FRAP) assay

- **Principle**

Substances with reducing potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride to form a ferric-ferrous complex that has a maximum absorption at 700 nm (Jayanthi, 2011).

The FRAP assay, antioxidants in the sample reduce ferric ions, resulting in the formation of a colored complex whose intensity correlates directly with antioxidant capacity (Xiao *et al.*, 2020; Tang *et al.*, 2024).

$$\textbf{Potassium ferricyanide + Ferric chloride} \xrightarrow{\text{Antioxydant}} \textbf{Potassium ferrocyanide + Ferrous chloride}$$

❖ **Protocol**

The reducing power test was evaluated according to the method of Oyaizu, (1986). To 10 µL of sample solution at different concentrations were added 40 µL of phosphate buffer (pH 6.6) and 50 µL of potassium ferricyanide (1%) [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 minutes. Then, 50 µL of trichloroacetic acid (TCA, 10%), 40 µL of distilled water, and 10 µL of ferric chloride ($FeCl_3$, 0.1%) were added to the mixture, and the absorbance was measured at 700 nm using a 96-well microplate reader. Ascorbic acid and α-tocopherol were used as standards (Figure 21).

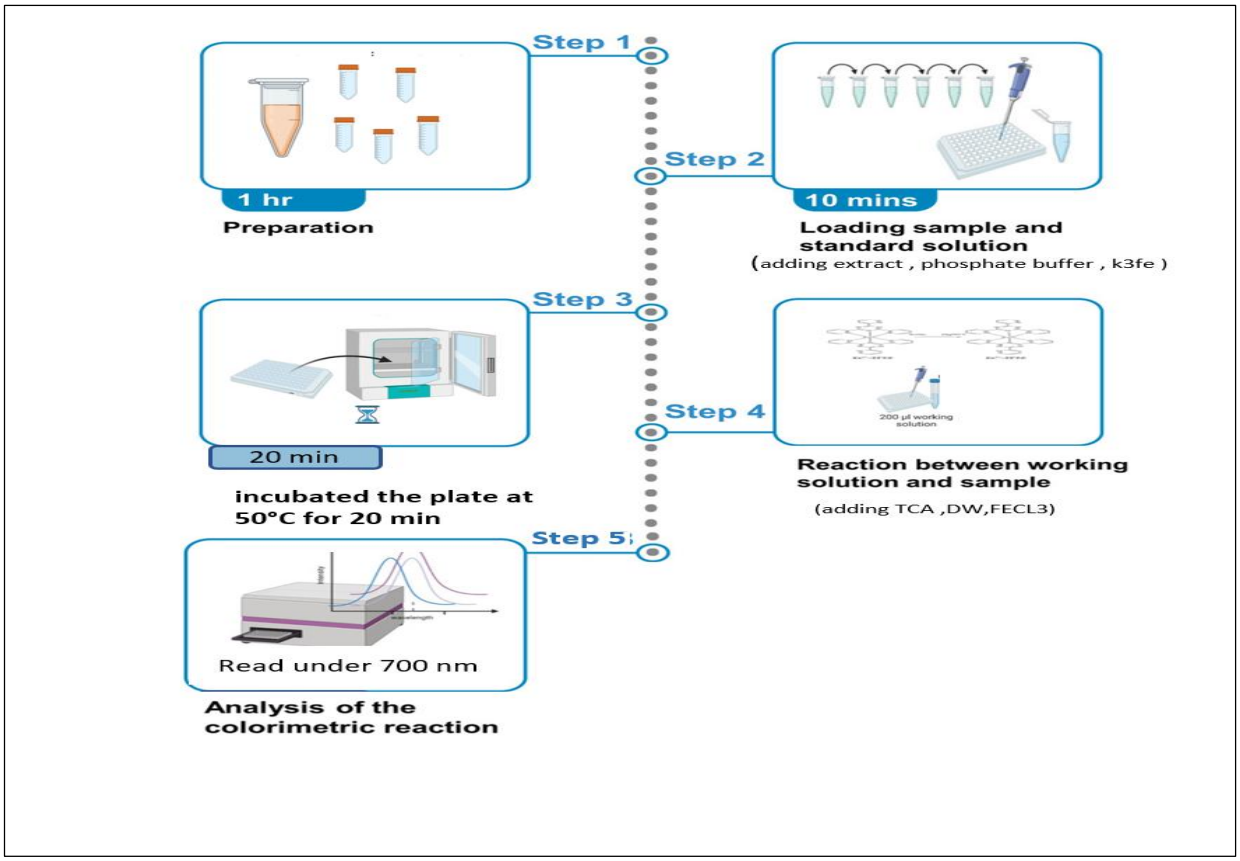

Figure 21: Determination of antioxidant activity by Ferric Reducing Power method.

II.4. Sun protection factor (SPF) determination

❖ Principle

The Sun Protection Factor (SPF) is a numerical indicator of a substance's ability to protect the skin from the harmful effects of ultraviolet B (UVB) radiation (290–320 nm), which is primarily responsible for sunburn and contributes to skin cancer. The spectrophotometric method, based on the Mansur equation (1986), is commonly used to estimate the SPF by measuring the absorbance of a sample across the UVB range. The SPF is then calculated using the following formula:

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where:

- ✓ **CF** is the correction factor (commonly 10),
- ✓ **EE(λ)** is the erythemal effect spectrum,
- ✓ **I(λ)** is the solar intensity spectrum,
- ✓ **Abs(λ)** is the absorbance of the sample at each wavelength (Mosa *et al.*, 2023).

❖ Protocol

The sun protection factor (SPF) was evaluated according to the method of Mansur *et al.* (1986). A stock solution of the extract was prepared by dissolving 2 mg of extract in 1 mL of methanol. The solution was vortexed until complete dissolution. Using a UV-transparent 96-well microplate, three wells were filled with the extract solution to ensure repeatability, while methanol was used as the blank control. The absorbance of the sample was measured using a microplate reader at 290, 295, 300, 305, 310, 315, and 320 nm, with measurements taken at 5 nm intervals across the UVB spectrum. The absorbance values obtained were then used to calculate the SPF using the Mansur equation.

II.5. Methods for assaying *in vivo* antioxidant activities

II.5.1. Treatment of animals

The 20 *wistar albino* rats were divided into 4 groups each consisted of 5 rats as the following:

- **Group 1**: Represents the untreated control group.
- **Group 2**: Ethanol group that received an oral dose of ethanol (5 mL/kg b.w.).
- **Group 3**: Rats received an oral administration of an aqueous extract of *E. spinosissimus* (100 mg/kg b.w.). One hour later, they were treated with absolute ethanol (5 mL/kg b.w.).
- **Group 4**: Rats received an oral administration of omeprazole (20 mg/kg b.w.). One hour later, they were treated with absolute ethanol (5 mL/kg b.w.). All animals were sacrificed one hour after ethanol administration (Figure 22).

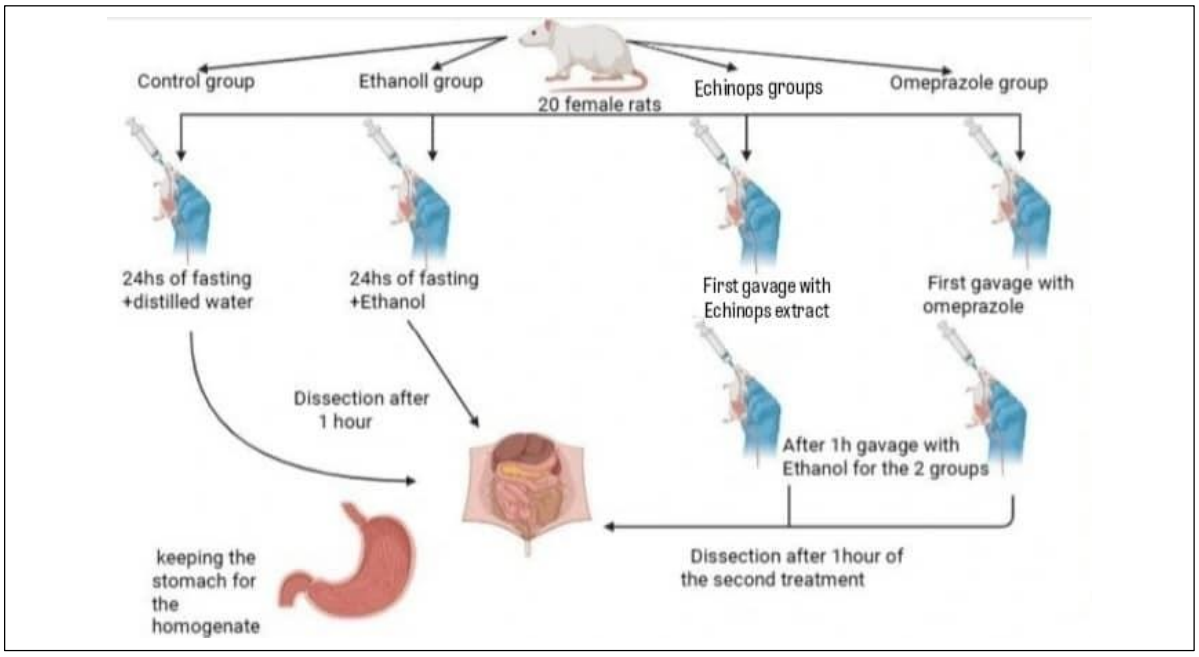

Figure 22: Experimental design for evaluating the effects of the aqueous extract of *Echinops spinosissimus* on ethanol-induced gastric damage in rats.

II.5.2. Animal sacrifice and dissection

After following the treatment protocol, the rats were anesthetized using chlorofome, and portal vein blood that was used for the biochemical analysis was col lected on heparin tubes.

The stomach of every rat was washed after being emptied with the NaCl 0.9% solution, dried then measured using a scale.

The stomach was divided into two parts, the first part was preserved in 10% formol, and the second was used to prepare a 10% homogenate after dipping in cold KCl (1.15%) solution. The resulting homogenate was centrifuged at 3000 rpm for 15 minutes at cold temperature. All antioxidant parameters were analyzed using the obtained supernatants.

II.5.3. Evaluation of tissue antioxidant status

II.5.3.1. Determination of Malondialdehyde (MDA)

- **Principle**

Malondialdehyde (MDA) is one the most commonly known biomarker of oxidative stress in various health conditions, the assay is based on a condensation reaction that occurs between two TBA molecules and one MDA molecule. Factors like temperature, pH, and the concentration of TBA influence the speed of this reaction (Khoubnasabjafari *et al.*, 2015) (Figure 23).

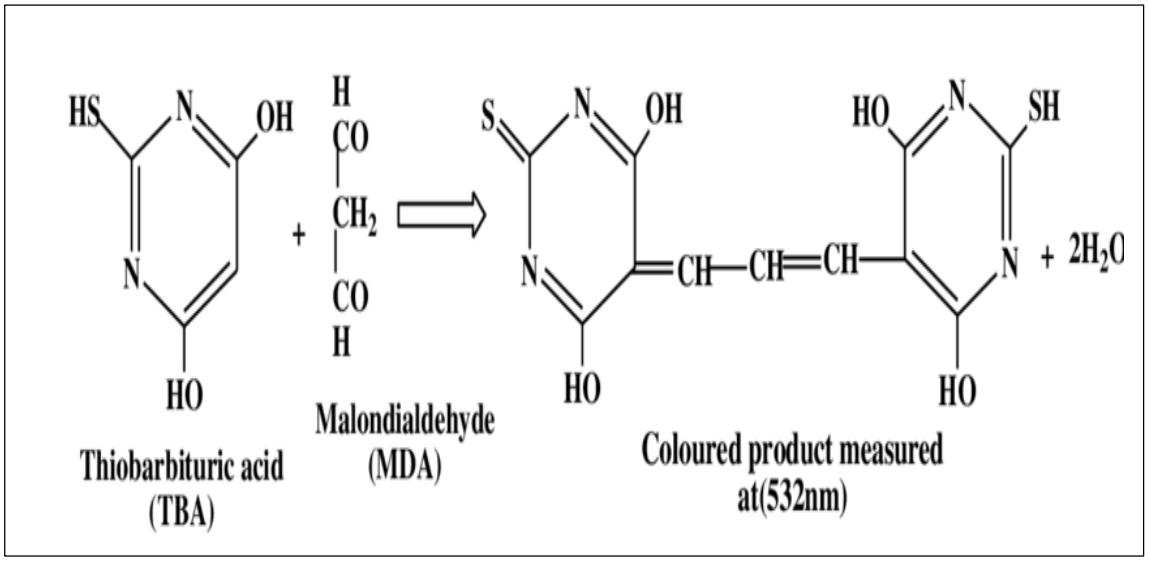

Figure 23: Condensation reaction between TBA and MDA (Al-Hamadany, 2019).

- **Protocol**

Lipid peroxidation was measured in the supernatants of all homogenates using the thiobarbituric acid reactive sub stances (TBARS), a colorimetric method of Uchiyama & Mihara (1978).

A volume of 250 µL of stomach homogenate was mixed with 1.5 mL of 1% phosphoric acid and 500 µL of 0.67% thiobarbituric acid (TBA). the mixture was then incubated in a boiling water bath for 45 minutes. After cooling, 2 mL of butanol were added to each sample.

The samples were subsequently centrifuged at 3000 rpm for 15 minutes. After that, the absorbance was measured at 532 nm using a spectrophotometer.

II.5.3.2. Determination of glutathione (GSH)

- **Principle**

GSH gets oxidized to create the yellow compound known 5'-thio-2- nitrobenzoic acid (TNB) through the action of the sulfhydryl reagent 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB)(Rahman, 2006) (Figure 24).

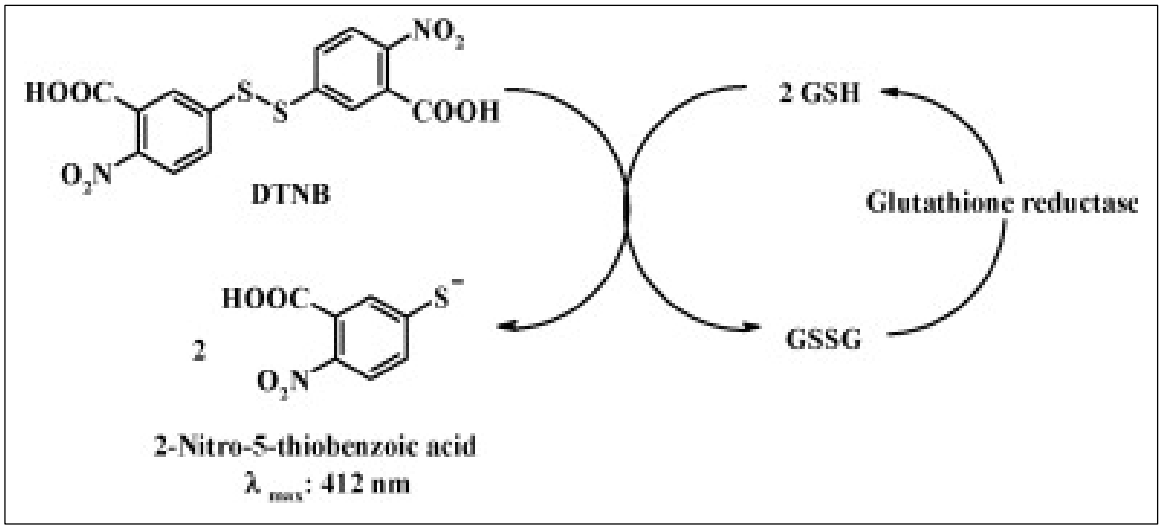

Figure 24: Reaction between GSH and DTNB (Hassan *et al.,* 2023).

- **Protocol**

GSH is a co-factor of many enzymes, a powerful antioxidant, and an important scavenger of harmful oxygen radicals, which aids in the maintenance of normal cell functions (Laraba *et al.,* 2022).

Glutathione (GSH) levels were determined using the method described by Ellman, (1959). The reaction mixture consisted of 0.5 mL of tissue homogenate and 0.5 mL of 10% trichloroacetic acid (TCA). The mixture was centrifuged at 2000 rpm for 5 minutes. Next, 200 µL of the resulting supernatant was added to 1.8 mL of phosphate buffer solution (0.1 M, pH 8.0), followed by the addition of 100 µL of Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid), also known as DTNB]. After the yellow color developed,

the absorbance was immediately measured at 412 nm using a spectrophotometer. GSH concentrations were expressed as nmol of GSH per mg of protein. All measurements were performed in triplicate.

II.5.3.3.Determination of glutathione peroxidase (GPX)

- **Principle**

Glutathione peroxidase (GPx), present in the tissue homogenate, catalyzes the oxidation of reduced glutathione (GSH) while simultaneously reducing hydrogen peroxide (H_2O_2) to water (H_2O). The remaining GSH then reacts with DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) to form a yellow-colored compound, which is measured spectrophotometrically at 420 nm, according to the following reaction:

$$\mathbf{H_2O_2 + 2GSH} \xrightarrow{\mathbf{GPx}} \mathbf{GSSG + 2H_2O}$$ (Flohé & Günzler, 1984)

- **Protocol**

GPx activity was evaluated using Flohé and Günzler (1984). A volume of 200 µLof tissue homogenate was mixed with 400µL of GSH solution (0.1 mM) and 200 µL of TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.4). The mixture was incubated at 25 °C for 5 minutes. Subsequently, 200 µL of hydrogen peroxide (H_2O_2, 1.3 mM) was added and allowed to react for 10 minutes. To stop the reaction, 1 mL of 1% trichloroacetic acid (TCA) was added, and the tubes were placed in an ice bath (0–5 °C) for 30 minutes.

The reaction mixture was then centrifuged at 3000 rpm for 10 minutes, and 480 µL of the resulting supernatant was transferred to a fresh tube containing 2.2 mL of TBS buffer and 320 µL of Ellman's reagent (DTNB, 1 mM).After 5 minutes, the absorbance was measured at 412 nm using a spectrophotometer. GSH activity was expressed as nmol GSH per mg of protein, and all experiments were conducted in triplicate.

III. Anatomical-pathological examinations

The histological study was performed on the stomach at the Deksi Constantine Hospital Pathology Laboratory. It was based on a semiological analysis comparing normal and pathological tissues, with the objective of identifying possible changes in the architecture of the organ following the administration of *E. spinosissimus* and ethanol.

IV. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 software. The results of in *vitro* tests are expressed as mean ± standard deviation. IC_{50} values (for DPPH and ABTS activity) and $A_{0.5}$ values (for reducing power) were calculated using linear regression analysis. For in *vivo* tests, results are also expressed as mean ± standard deviation. Correlations between test data were determined using Pearson correlation coefficients (r^2). Statistical significance was assessed using Student's t-test, with differences considered statistically significant at $p < 0.05$.

Results

I. Quantitative characterization of *Echinops spinosissimus*

I.1. Total polyphenol and flavonoid content of the extract

- **Total polyphenols content**

The total polyphenols content of *Echinops spinosissimus* was determined using the Folin–Ciocalteu colorimetric method. Gallic acid was used as the standard reference compound for the calibration curve. The absorbance values obtained from various concentrations of gallic acid (µg/mL) were used to generate the calibration curve shown in (Figure 25). The regression equation of the curve was ($y = 0.001x$) with a coefficient of determination $R^2 = 0.991$, indicating a strong linear relationship. Results were expressed as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg EXT).

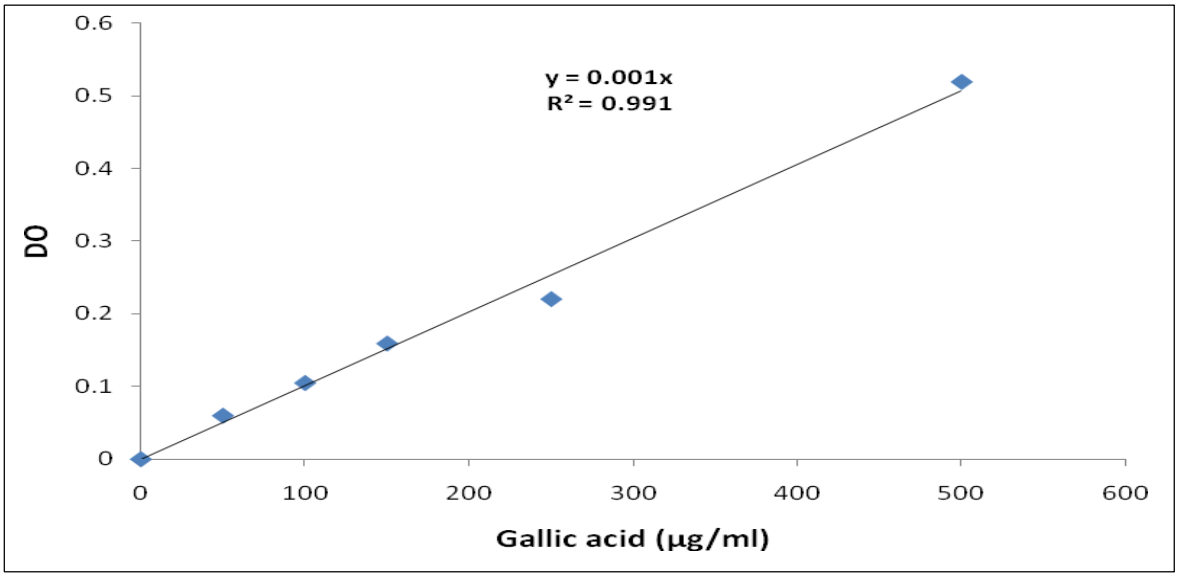

Figure 25: Gallic acid calibration curve (mean ± SD from three trials).

- **Total flavonoids content**

The determination of flavonoids in *Echinops spinosissimus* is based on the formation of a complex between Al^{3+} and flavonoids (Sultana *et al*., 2024). Quercetin was used as the standard in this method. The results are expressed as micrograms of quercetin equivalent per milligram of extract (µg QE/mg EXT), based on a calibration curve with the following equation: $y = 0.034x + 0.015$ ($R^2 = 0.983$) (Figure 26).

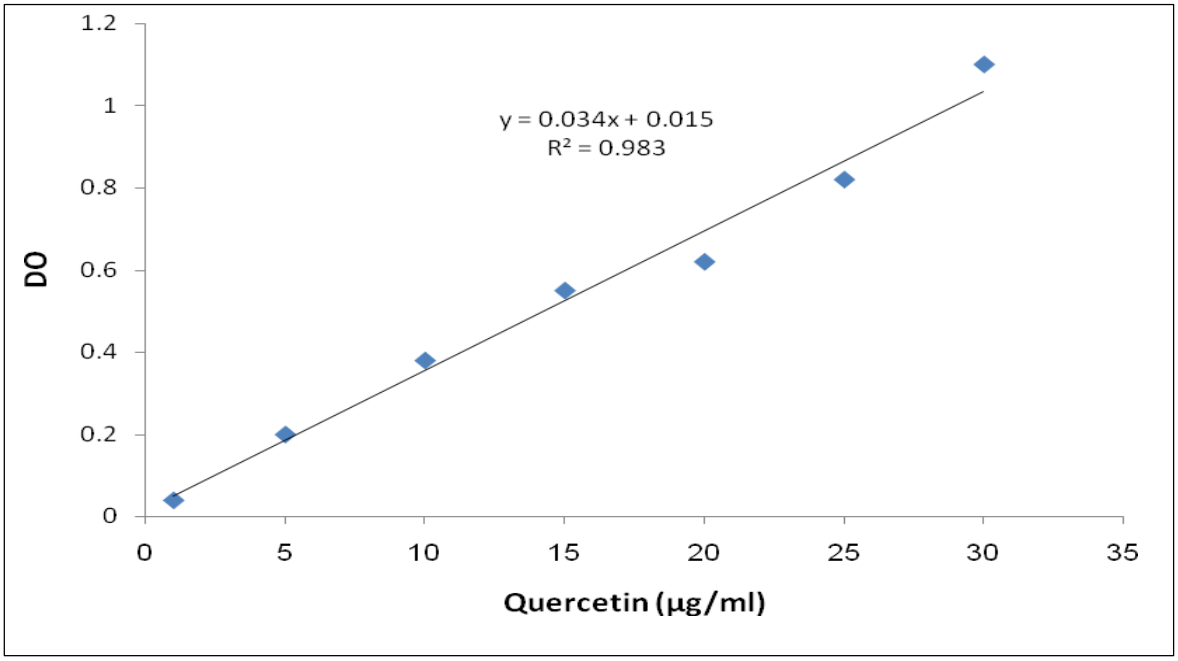

Figure 26: Quercetin calibration curve (mean ± SD from three

According to the results shown in Figure 27, the extract of *E. spinosissimus* contains a polyphenol content of 12 µg EAG/mg EXT and a flavonoid content of 2.55 µg QE/mg EXT.

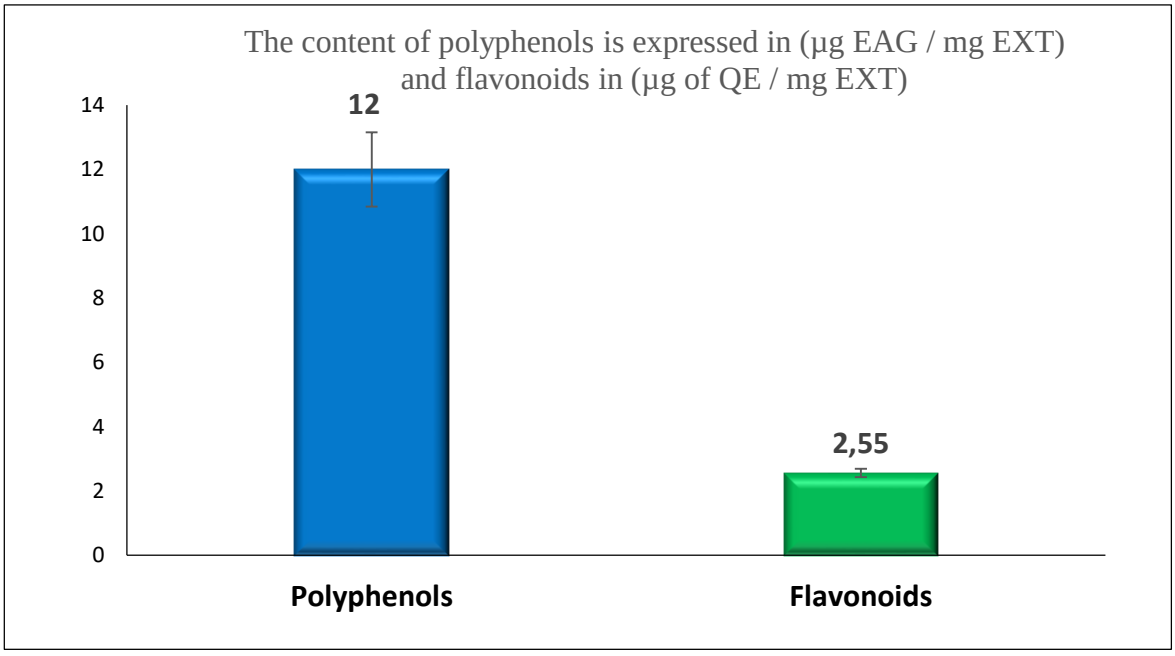

Figure 27: Polyphenols and flavonoids contents in the extract of *Echinops spinosissimus*.

II. Evaluation of *in vitro* antioxidant activity

The evaluation of antioxidant activity was carried out by three tests in which the antioxidant power of the aqueous extract of the *Echinops spinosissimus* plant is compared with that of reference molecules.

II.1. DPPH scavenging activity

The chemical compound DPPH was one of the first free radicals used to study the structure–antioxidant activity relationship of phenolic compounds (Popovici *et al.*, 2009). According to the results shown in Figure 28, it appears that the DPPH radical inhibition rate increases proportionally with the increase in concentration, whether for the standards BHA, α-Tocopherol, BHT, or for the tested extract.

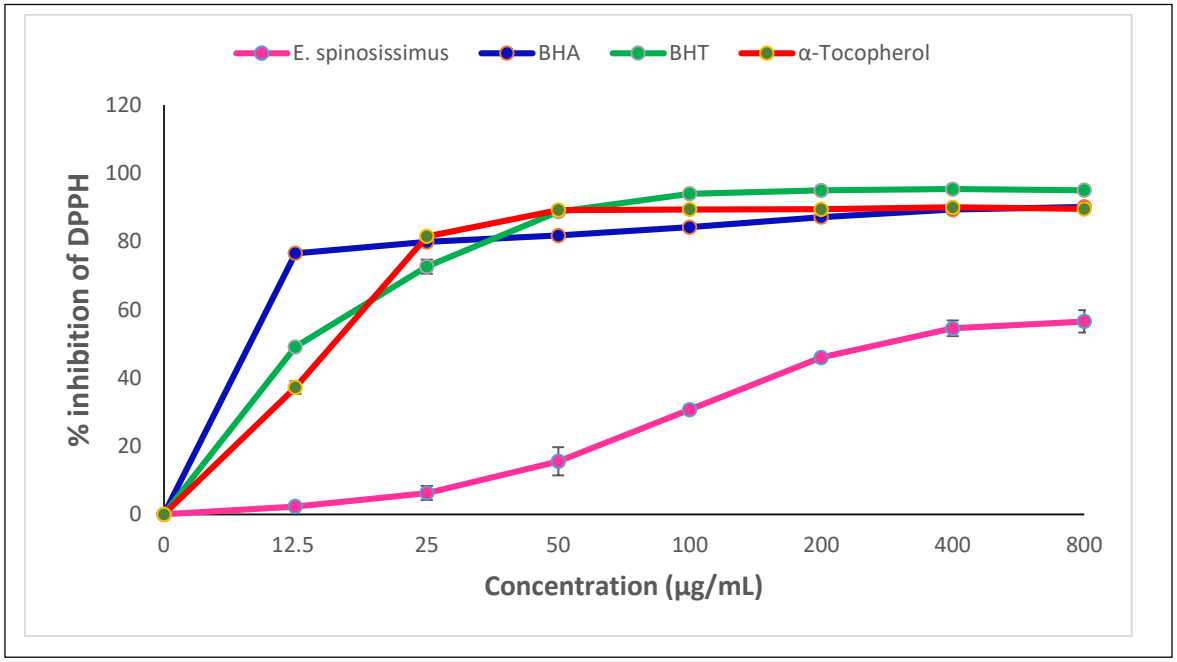

Figure 28: Percentage of inhibition of the free radical DPPH as a function of the concentration of *Echinops spinosissimus* extract.

The aqueous extract of *E. spinosissimus* demonstrated a lower percentage of DPPH radical inhibition compared to the reference antioxidants across all tested concentrations. At 200 µg/mL, the inhibition rates were 94.97% for BHT, 87.13% for BHA, and 89.45% for α-Tocopherol, whereas the plant extract achieved only 45.97%. This moderate antioxidant activity was visually confirmed by the color shift of the DPPH solution from purple to pale yellow, indicating partial radical scavenging.

The IC_{50}% value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%. The lower the IC_{50}% value, the higher is the antioxidant activity of the test sample (Bizuayehu *et al.*, 2016).

The concentration required to reduce the DPPH free radical by 50% was calculated using the linear regression equations from the graphs. These values are presented in the table below

Table 4: The antioxidant power of the DPPH radical (expressed by IC50 (in µg/mL) of the reference antioxidants and the tested extract.

	IC50 ± standard deviation (In µg /mL)
Echinops spinosissimus	337.81±12,98
α-Tocophérol	13.02±5,17
BHA	6.14±0.41
BHT	12.99±0.41

According to the data in Table 4, the extract exhibits weak antioxidant activity, with an IC_{50} of 337.81 ± 12.98 µg/mL compared to the reference compounds BHA (6.14 ± 0.41 µg/mL), BHT (12.99 ± 0.41 µg/mL), and α-Tocopherol (13.02 ± 5.17 µg/mL).

II.2. ABTS scavenging activity

The ABTS assay for evaluating antioxidant activity relies on the capacity of antioxidants to neutralize free radicals, which leads to a decrease in color intensity. ABTS itself is a nitrogen-centered radical that appears blue-green. When antioxidants interact with ABTS, they convert it to its non-radical form, resulting in a color shift from blue-green to colorless. This method is highly sensitive to light, and the antioxidant potential is quantified by measuring the reduction in absorbance at 734 nm using a spectrophotometer (Minarti *et al.*, 2024).

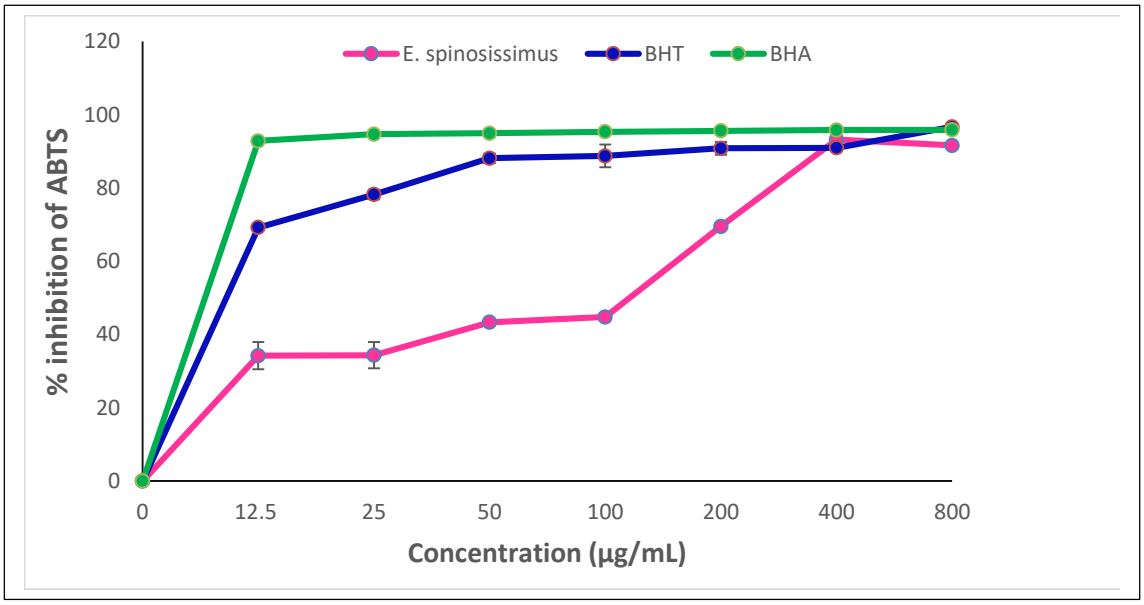

Figure 29: Percentage of inhibition of the free radical ABTS as a function of the concentration of *Echinops spinosissimus* extract.

The results presented in Figure 29 demonstrate an increase in the ABTS•+ radical inhibition rate in correlation with the increase in concentration.

At a concentration of 400 µg/mL, the aqueous extract of *Echinops spinosissimus* showed an ABTS•+ radical inhibition percentage of 93.21%, which is comparable to the values observed for the reference standards: 95.83% for BHA and 93.95% for BHT.

Table 5: The antioxidant power of the ABTS•+ radical (expressed by IC50 (in µg/mL) of the reference antioxidants and the tested extract.

	IC50 ± standard deviation (In µg /mL)
Echinops spinosissimus	102.85±2,48
BHA	1,81±0,10
BHT	1.29±0,30

According to the results presented in Table 5, the extract exhibits moderate antioxidant activity, with an IC_{50} of 102.85 ± 2.48 µg/mL, which is considerably higher than those of the reference standards BHA (1.81 ± 0.10 µg/mL) and BHT (1.29 ± 0.30 µg/mL), indicating a weaker antioxidant potential.

II.3. Reducing power assay

The antioxidant activity of the *Echinops spinosissimus* extract was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, which relies on measuring the extract's capacity to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) (Bhalodia *et al.*, 2013).

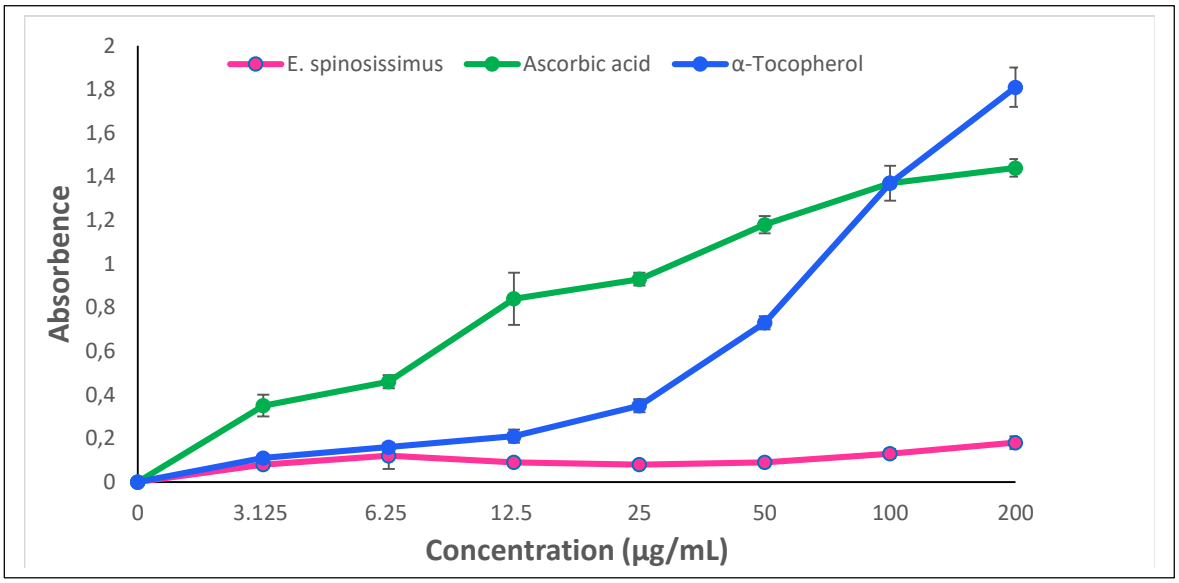

Figure 30: Iron reduction test by *Echinops spinosissimus* extract and standards (α-Tocopherol, ascorbic acid).

The results shown in the figure 30 indicate a proportional relationship between the tested concentrations and the reducing power. An increase in absorbance reflects a higher reducing potential of the tested extract.

The plant extract used in this study demonstrated the ability to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), with an absorbance value of 0.18 at a concentration of 200 µg/mL. This reflects weak reducing activity compared to the reference standards, ascorbic acid (1.4) and α-Tocopherol (1.8).

Table 6: The iron reducing power (expressed by A0.5 (in µg/mL) of the reference antioxidants and the tested extract.

	$A_{0.5}$ ± standard deviation (In µg /mL)
Echinops spinosissimus	640±90.93
α-Tocophérol	34.93±2.38
Acide ascorbique	6.77±1.15

According to the results presented in Table 6, the *Echinops spinosissimus* extract exhibits weak reducing activity, with an $A_{0.5}$ value of 640 ± 90.93 µg/mL, compared to the reference compounds α-Tocopherol ($A_{0.5}$ = 34.93 ± 2.38 µg/mL) and ascorbic acid ($A_{0.5}$ = 6.77 ± 1.15 µg/mL).

III. *In vitro* sun protection factor (SPF) determination

Sun Protection Factor, or SPF, is essentially a measure of how much UV energy is required to cause a minimal erythemal dose (MED) on protected skin compared to unprotected skin. It is a useful way to assess the effectiveness of a sunscreen. One quick and reliable method to determine SPF *in vitro* is by measuring how much light the product absorbs between 290 and 320 nm (Malsawmtluangi *et al.*, 2013).

Table 7: Sun protection factor calculation for aqueous extract obtained from *Echinops spinosissimus*.

Aqueous extract of the *Echinops spinosissimus*			
λ(nm)	**EE x l (normalized)**	**Absorbance**	**SPF**
290	0.0150	1.9±0.19	0.28±0.02
295	0.0817	1.45± 0.18	1.18±0.15
300	0.2874	1.17± 0.18	3.38±0.52
305	0.2780	1.03± 0.17	3.38±0.58
310	0.1864	0.95±0.17	1.78±0.33
315	0.0837	0.90±0.17	0.75±0.14
320	0.0180	0.84±0.17	0.15±0.03
Total	**1**	**/**	**10.94 ±1.81**

The *in vitro* photoprotective potential of *Echinops spinosissimus* was assessed by determining its sun protection factor (SPF). As shown in Table 7, the extract demonstrated weak activity, with an estimated SPF of 10.94 ± 1.81.

IV. Results of the *in vivo* experimental Study

IV.1.The effect of different treatments on cytosolic oxidative status

IV.1.1.Efect on MDA level

According to Figure 31, ethanol (EtOH) administration led to a marked elevation of malondialdehyde (MDA) levels in the rat stomach, reaching 155.55% compared to the control group (100%). Pre-treatment with either omeprazole (20 mg/kg) or *Echinops spinosissimus* aqueous extract (100 mg/kg) significantly mitigated this increase, reducing MDA levels to 44.44% and 59.25%, respectively ($p < 0.001$ and $p < 0.01$). These findings highlight the protective effects of both treatments against EtOH-induced lipid peroxidation.

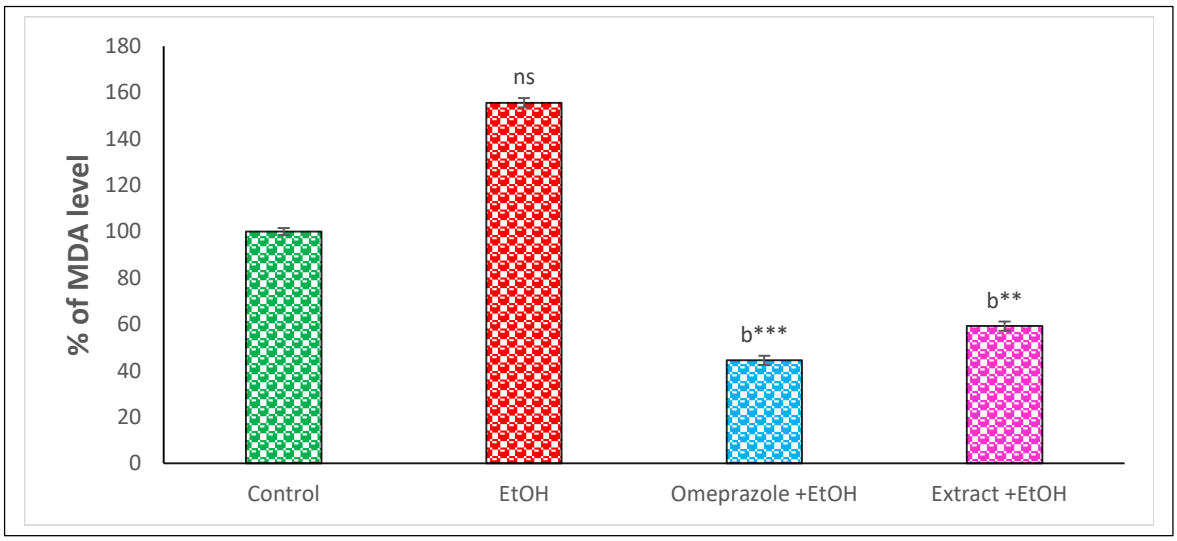

Figure 31: The preventive effect of *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) on MDA levels in the stomach of rats treated with EtOH (5mg/kg). Each value represents the mean ± SD (n= 3). **: p< 0.01, ***: p < 0.001 and ns: not significant. a: compared to the control group. b: compared to the EtOH group.

IV.1.2. Effect on GSH level

According to the results presented in Figure 32, ethanol (EtOH) treatment caused a slight reduction in gastric GSH levels, reaching 88.25% compared to the control group (100%). Pre-treatment with *Echinops spinosissimus* extract and omeprazole led to an increase in GSH levels, reaching 110.57% and 91.34%, respectively, relative to the EtOH group. However, these changes were not statistically significant in any of the groups.

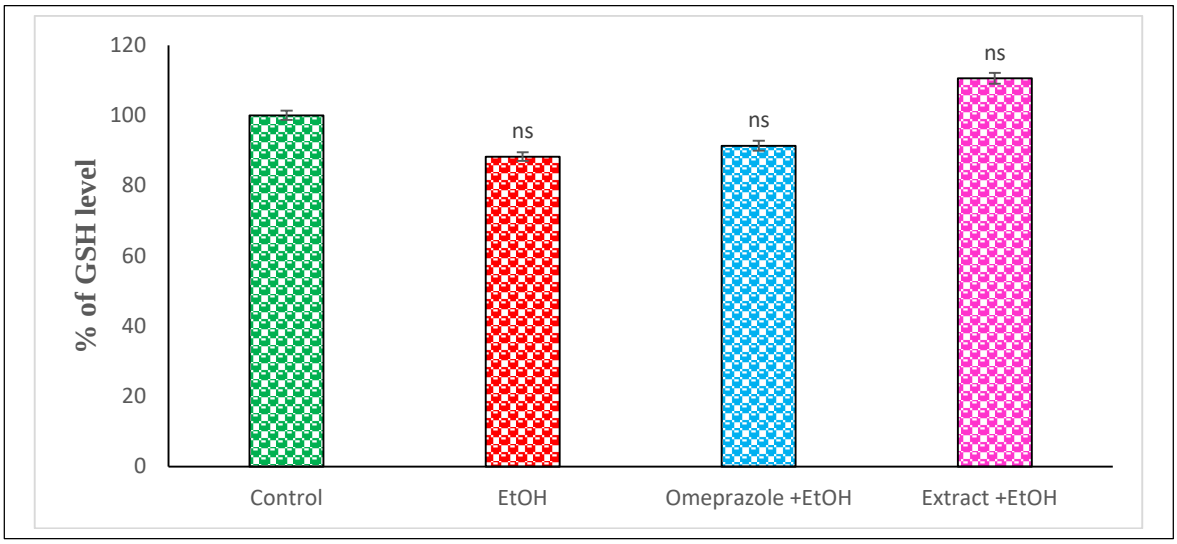

Figure 32: The preventive effect of *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) on GSH levels in the stomach of rats treated with EtOH (5mg/kg). Each value represents the mean ± SD (n= 3) ns : not significant.

IV.1.2.Effect on GPx activity

The results shown in Figure 33 revealed a highly significant reduction in glutathione peroxidase activity in the stomach of the ethanol-treated group, reaching 37.14% compared to the control (100%). In contrast, pre-treatment with *Echinops spinosissimus* extract markedly restored enzymatic activity to 85.71%, while the omeprazole group showed a slight, non-significant improvement, reaching 68.57%, relative to ethanol exposure.

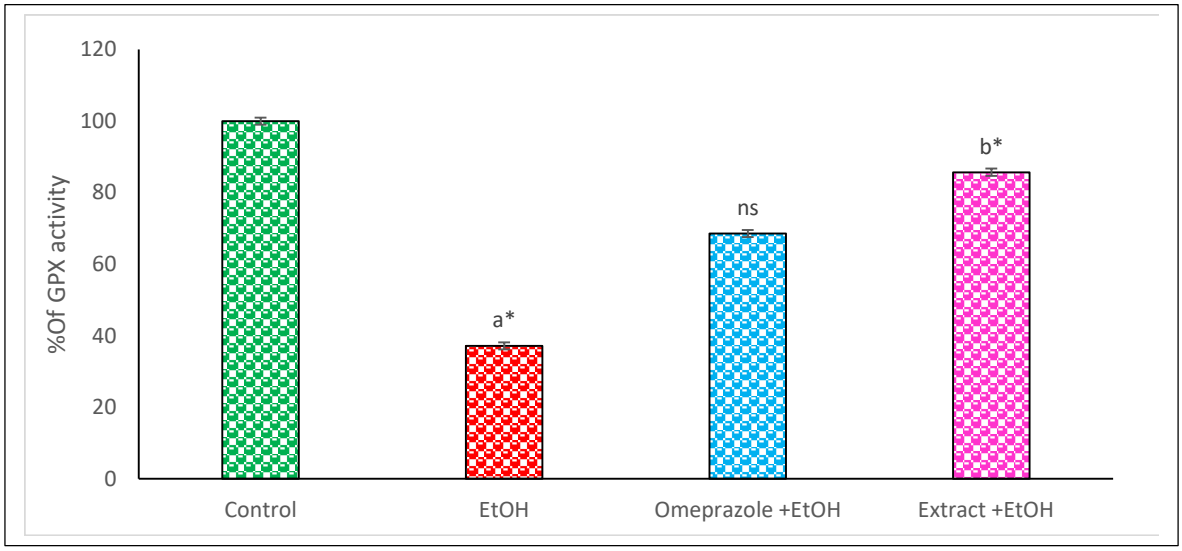

Figure 33: The preventive effect of *Echinops spinosissmus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) on GPx levels in the stomach of rats treated with EtOH (5mg/kg). Each value represents the mean ± SD (n= 3). *: p< 0.05, ***: p < 0.001 and ns : not significant. a: compared to the control group. b: compared to the EtOH group.

V. Histopathological study

Histopathological examination of gastric sections stained with hematoxylin and eosin from rats in the control group revealed a normal histological architecture of the gastric mucosa. In contrast, sections from the ethanol-treated group exhibited marked infiltration of inflammatory cells, indicative of tissue damage. Pretreatment with the aqueous extract of *Echinops spinosissimus* (100 mg/kg) exerted a significant protective effect by preserving the integrity of the gastric tissue structure. Conversely, no significant protection was observed in the group pretreated with omeprazole (20 mg/kg) (Figure 34).

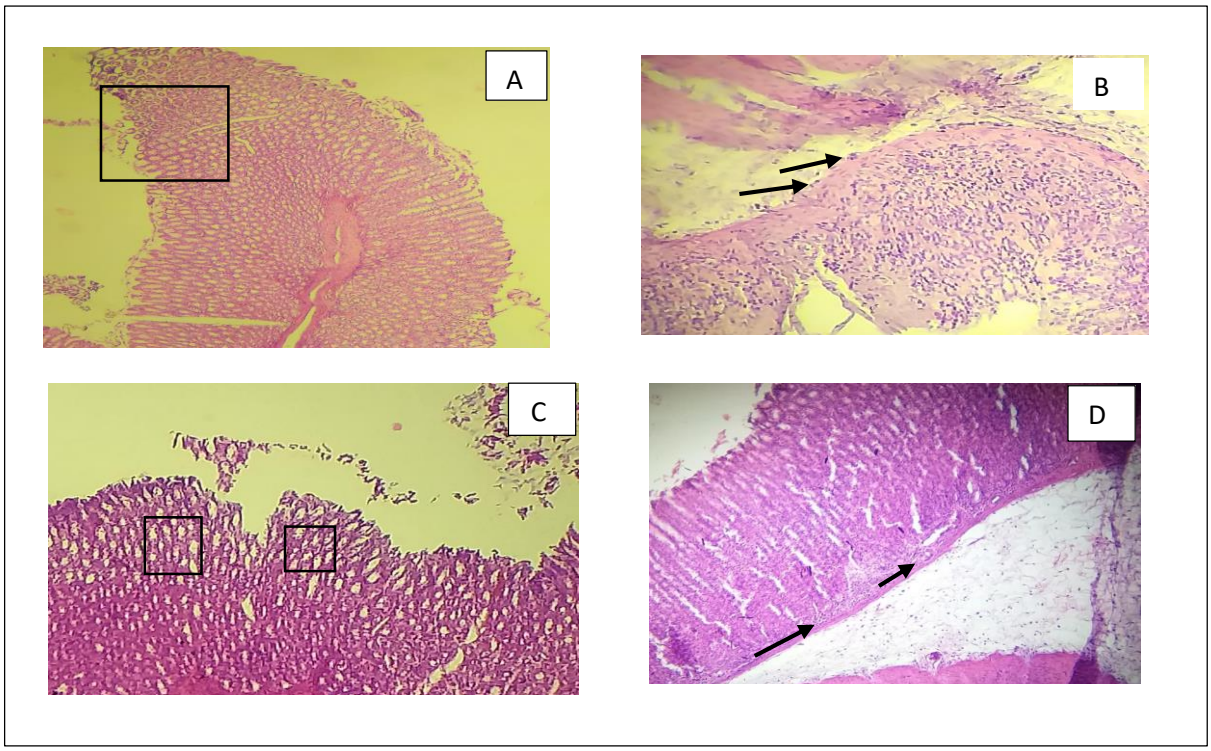

Figure 34: Histopathological examination of gastric tissue. (A) Normal control group showing normal histoarchitecture ; (B) Ethanol-treated group showing significant inflammatory infiltration cells (polymorphonuclear leukocytes, neutrophils) (arrows) ; (C) *E. spinosissimus* + ethanol group showing no disturbance in the gastric mucosa; (D) Omeprazole + ethanol group showing infiltration and signs of inflammation (arrows). Magnification, ×100 and ×400.

Discussion

Discussion

Every year, millions of people suffer from stomach ulcers, which represent a significant health challenge worldwide. If left untreated, these ulcers can lead to serious complications such as gastrointestinal bleeding, perforation, and even cancer, due to the erosion of the stomach lining. Several factors have contributed to changes in the prevalence of stomach ulcers over the years, including the widespread use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, the prevalence of *Helicobacter pylori* infections, and dietary changes. Although the overall incidence has decreased in some regions, certain populations particularly the elderly and those with chronic health conditions continue to experience high rates. Because of their widespread impact, gastric ulcers have been the focus of extensive research into their causes, consequences, and effective treatments (Badr *et al.,* 2019 ; Sawant *et al.,* 2025). Therefore, the use of medicinal plants and their active ingredients have been used in traditional medicine for stomach ulcer remedies for a long time. Numerous studies by several peptic ulcer researchers have reported on using medicinal plants and their active compounds for anti-ulcer effects in rats (Shareef *et al.,* 2022).

The present study was conducted in several stages. Firstly, it focused on the quantitative determination of polyphenols and flavonoids in the aqueous extract of *Echinops spinosissimus*. Secondly, the study aimed to assess the *in vitro* biological activities of this extract, including its antioxidant, and photoprotective properties. Thirdly, the research investigated the *in vivo* preventive effects of *Echinops spinosissimus* against ethanol-induced gastric ulcers. To support these findings, the study included both the evaluation of oxidative stress parameters and a histopathological examination of gastric tissue.

The results obtained indicate that the aqueous seed extract of *Echinops spinosissimus* contains low levels of polyphenols and flavonoids, with values of 12 µg GAE/mg extract and 2.55 µg QE/mg extract, respectively. These concentrations are significantly lower than those reported in other studies. For instance, Bouzabata *et al.* (2022) reported much higher levels in a methanolic extract from *E. spinosissimus* collected in El Tarf, located in northwestern Algeria, with total phenolic content reaching 125.16 mg GAE/g of dried residue and flavonoid content at 25.40 mg QE/g of dried residue. Similarly, Benrahou *et al.* (2022) found that the aqueous root extract of *E. spinosissimus* from Morocco had a higher total phenolic content of 34 ± 0.58 mg GAE/g extract and a total flavonoid content of 10.33 ± 4.2 mg RE/g extract.

This variability in polyphenol and flavonoid content depends on several factors, including the origin of the plant, the drying method, the harvest period, the type of extraction solvent, and the storage conditions, which may themselves be influenced by the geographical area of the harvest (Addab *et al.*, 2020).

The anti-oxidant activities of the *Echinops spinosisssimus* aqueous extract were evaluated *in vitro* using the following tests: ABTS, DPPH and FRAP.

When measuring the antioxidant properties of various greens and plants, the most commonly used spectrophotometric assays are based on radical scavenging methods, such as DPPH and ABTS. These antioxidants can directly interact with chromogenic radicals. Due to their simplicity, speed, reproducibility, and sensitivity, these assays are widely used. (Gulcin & Alwasel, 2023).

The results of our assays on the aqueous extract reveal that it has weak free radical scavenging activity in the DPPH assay, with an IC_{50} value of 337.81 ± 12.98 µg/mL. These results are significantly higher than those reported by Khedher *et al.* (2020), who found an IC_{50} of 9.23 ± 0.04 µg/mL for the ethyl acetate extract of *E. spinosissimus* roots cultivated in Tunisia.

Gheffour and his collaborators have shown that the antioxidant activity of *Echinops spinosus* extracts is likely due to the presence of phenolic compounds, such as flavonoids and tannins. These molecules, along with other known antioxidants like ascorbic acid and tocopherol, have been shown to reduce and decolorize DPPH radicals through their hydrogen-donating ability (Gheffour *et al.*, 2015).

On the other hand, the studied plant showed a moderate antioxidant activity against the ABTS radical, with an IC_{50} value of 102.85 ± 2.48 µg/mL. These results are not consistent with those reported by Jamila *et al.* (2020), who obtained an IC_{50} of 5.88 µg/mL for the ethyl acetate stem extract of *Echinops echinatus* from Pakistan.

This difference in values can be explained by the low content of total phenolic compounds in the extract, as research has shown that the main antioxidant constituents in medicinal plants, vegetables, fruits, and spices are phenolic compounds, including polyphenols and flavonoids. Furthermore, the effectiveness of these antioxidant agents is influenced by their solubility in either lipids or water (Belattar *et al.*, 2023).

The reducing power assay is a common method for evaluating how well an antioxidant can donate electrons. This test is often used to evaluate how well foods, beverages, and dietary supplements rich in polyphenols can act as antioxidants (Irshad *et al.,* 2012; Singh *et al.,* 2021).

Our FRAP assay results indicated that the aqueous extract of *Echinops spinosissimus* exhibits a weak ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), with an $A_{0.5}$value of 640 ± 90.93 µg/mL. This result is not consistent with the findings of Amira *et al.* (2022), who evaluated the reducing capacity of the hydroethanolic extract from the aerial parts of *Achillea odorata* L. (family Asteraceae) collected in Algeria and reported a significantly lower $A_{0.5}$ value of 20.02 µg/mL.

Research on the iron-reducing ability of phenolic compounds has shown that the catechol ring is the only structural feature positively associated with reducing power. The presence of this ring can increase a compound's reducing capacity by up to 36% compared to those lacking it. This activity is thought to arise from the interaction of the hydroxyl (OH) groups attached to the catechol ring (Boukada *et al.,* 2021).

Skin damage caused by UV radiation ranks among the most prevalent concerns throughout the world. Research has demonstrated that photoprotective agents, particularly sunscreens, are vital in decreasing the occurrence of skin disorders, such as pigmentation issues and premature aging caused by UV exposure. Numerous recent studies have explored natural substances as potential resources for sunscreen due to their capacity to absorb UV radiation and their antioxidant properties (Bouteche *et al.,* 2024).

In this study, the photoprotective activity of *Echinops spinosissimus* extract was evaluated *in vitro* using the SPF test. The obtained results showed that the aqueous extract has a low sun protection potential with a value of 10.94 ±1.81.

Our results were not in agreement with those of Bouteche *et al.* (2024)., who tested the ethyl acetate extract of the aerial parts of *Achillea ligustica* All., a plant from the Asteraceae family collected in Mila, Northeast Algeria. Their study reported a high sun protection factor (SPF) value of 48.08 ± 0.01.

These incompatible results can be explained by the fact that polarity is a very important factor in increasing phenolic solubility and polyphenols are generally more soluble in organic solvent with a lower polarity than water's (Haminiuk *et al.,* 2014).

After establishing the *in vitro* antioxidant properties of the *Echinops spinosissimus* extract, we then investigated its preventive effects against ethanol-induced oxidative stress *in vivo* by evaluating oxidative stress biomarkers in gastric tissue.

Malondialdehyde (MDA) is a major end-product of the oxidation of polyunsaturated fatty acids, and elevated MDA levels serve as a key biomarker of lipid peroxidation (Mete *et al.*, 2016).

In the present study, MDA levels in gastric tissues were significantly elevated in the ethanol group, indicating enhanced lipid peroxidation. These findings align with previous studies reporting ethanol-induced oxidative damage Gugliandolo *et al*.(2021) and Omer *et al.* (2023). This effect is primarily attributed to the generation of reactive oxygen species (ROS), which promote lipid peroxidation in gastric epithelial cells. As a result, membrane integrity is compromised, cellular permeability increases, and the development of gastric ulcers is accelerated (Beiranvand & Bahramikia, 2020 ; Omer et *al.*, 2023).

On the other hand, pretreatment with *E. spinosissimus* extract (100 mg/kg) ($P < 0.01$)or omeprazole (20 mg/kg) ($P < 0.001$) prior to ethanol administration significantly reduced MDA levels in gastric tissues. This suggests that both treatments exert protective effects against ethanol-induced lipid peroxidation. These results are consistent with several studies demonstrating that medicinal plants can attenuate ethanol induced gastrotoxicity (Sistani Karampour *et al.*, 2019 ; Beiranvand & Bahramikia, 2020).

Furthermore, Hegazy and colleagues demonstrated that *Echinops spinosus* extract attenuates paracetamol-induced nephrotoxicity by reducing MDA levels. This protective effect may be attributed to its ability to preserve biomembrane integrity by inhibiting lipid peroxidation and enhancing cellular antioxidant defenses (Hegazy *et al.*, 2019) .

Glutathione (GSH) inhibits lipid peroxidation and scavenges radicals; it is among the most abundant cellular antioxidants and acts to detoxify hydrogen peroxide by various glutathione peroxidases (Park *et al.*, 2021). Moreover, GSH serves as a cofactor for glutathione peroxidase (GPx), further enhancing its ability to neutralize H_2O_2 (Beiranvand & Bahramikia, 2020)

Our study revealed a significant reduction in glutathione (GSH) levels and glutathione peroxidase (GPx) activity in the ethanol-treated group compared to the normal group. These

findings are consistent with those reported by Omer *et al.* (2023) and Mousa *et al.* (2019), who also observed impaired antioxidant defenses following ethanol exposure.

Glutathione (GSH) is one of the most abundant intracellular antioxidants. It plays a crucial role in cellular defense by inhibiting lipid peroxidation, scavenging free radicals, and detoxifying hydrogen peroxide (H_2O_2) through the action of glutathione peroxidases, including GPx (Park *et al.*, 2021). However, excessive production of reactive oxygen species (ROS) can lead to a depletion of GSH and a reduction in the activity of antioxidant enzymes such as GPx, thereby compromising the antioxidant defense system and promoting oxidative stress (Ahmed & Kadhim, 2024).

On the other hand, pretreatment with *Echinops spinosissimus* extract (100 mg/kg) or omeprazole led to a significant increase in GSH levels and GPx activity compared to the ethanol group. This antioxidant-enhancing effect is in line with findings by Zheleva-dimitrova and his collaborators demonstrated that *Echinops ritro* extract elevated GSH and GPx levels in hepatic tissue, highlighting the protective role of *Echinops* species against oxidative stress (Zheleva- dimitrova *et al.,* 2023).

Histopathological examination revealed that ethanol administration led to marked structural alterations in the gastric tissue. In the ethanol-treated group, the development of gastric ulcers was confirmed by a significant infiltration of inflammatory cells, predominantly lymphocytes and polymorphonuclear cells. In contrast, the control group exhibited a normal histoarchitecture of the gastric mucosa, with no signs of inflammatory infiltration or tissue degeneration. These findings are consistent with those reported by Raish and his collaborators (Raish *et al.,* 2021).

Our results also align with those of Sweilam *et al.* (2023), who observed a well-preserved gastric mucosal structure in the control group, while the ethanol-treated group showed a pronounced infiltration of inflammatory cells. Ethanol appears to trigger a strong inflammatory response, likely mediated by increased pepsin secretion and enhanced synthesis of prostaglandin E2 (PGE2). This response promotes the recruitment of leukocytes through elevated levels of pro-inflammatory cytokines and the upregulation of cyclooxygenase-2 (COX-2) and nuclear factor κB (NF-κB) (Mamache *et al.*, 2024).

Furthermore, the link between ethanol, gastric ulceration, and inflammation can be attributed to its disruptive effect on the gut's natural defenses. Ethanol compromises the

integrity of the intestinal barrier and alters the composition of the gut microbiota, leading to systemic inflammation that may contribute to mucosal damage (Wang *et al.*, 2010).

Interestingly, pre-treatment with the aqueous extract of *Echinops spinosissimus* effectively prevented the histopathological alterations typically induced by ethanol, preserving the integrity of the gastric mucosa. These protective effects appear superior to those reported by Zitouni-Nourine *et al.* (2022), who observed mild lymphocytic infiltration in skin tissues following administration of the plant's methanolic extract.

In contrast, the omeprazole pre-treated group still exhibited signs of inflammation and cellular infiltration in the gastric tissue, differing from the findings of Raish *et al.* (2021), who reported that omeprazole significantly reduced inflammatory cell infiltration due to its gastroprotective properties.

In conclusion, Our results demonstrated that *Echinops spinosissimus* significantly attenuates histopathological damage and enhances the endogenous defense mechanisms, thereby providing protection against ethanol-induced gastric injury.

Conclusion and Perspectives

General conclusion and perspectives

Medicinal plants are widely recognized as valuable sources of bioactive compounds due to their diverse and significant therapeutic properties. In this study, the aqueous extract of *Echinops spinosissimus* was investigated for its antioxidant and gastroprotective potential using both *in vitro* and *in vivo* approaches. Quantitative analyses revealed that the extract contains low concentrations of polyphenols and flavonoids, which correlates with its limited antioxidant potency, as demonstrated by DPPH, ABTS, and FRAP assays when compared to standard reference compounds.

In addition, the photoprotective activity of *E. spinosissimus* was evaluated by measuring its sun protection factor (SPF), which indicated limited dermatoprotective potential.

Our results on ethanol-induced gastric ulcers in rats showed that pretreatment with the aqueous extract significantly reduced lipid peroxidation, as evidenced by lower levels of malondialdehyde (MDA). Furthermore, the extract enhanced the activity of the endogenous antioxidant enzyme glutathione peroxidase (GPx) in gastric tissues. Although the observed increase in glutathione (GSH) levels was not statistically significant, an overall improvement in the gastric antioxidant defense system was noted, accompanied by a reduction in both macroscopic gastric lesions and histopathological alterations.

These results suggest that *E. spinosissimus* may exert gastroprotective effects primarily by modulating oxidative stress pathways and strengthening the body's intrinsic antioxidant defenses, thereby contributing to the attenuation of ethanol-induced gastric injury.

In terms of perspectives, it would be valuable to:

- ✓ Isolate, identify, and characterize the specific active compounds responsible for the observed effects
- ✓ Evaluate the antioxidant and anti-inflammatory activities of the extract in other *in* vivo models and against other types of gastric lesions
- ✓ Explore the molecular mechanisms underlying the gastroprotective action of the extract, to better understand the correlation between its pharmacological activity and its chemical composition
- ✓ Assess the safety and toxicity of the extract in long term studies
- ✓ Investigate the potential for clinical application, including possible synergistic effects with conventional anti-ulcer therapies

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Evaluation of the antioxidant and gastroprotective effects of the aqueous extract of *Echinops spinosissimus* in an ethanol-induced gastric ulcer model

A thesis submitted to obtain a Master's Diploma in Toxicology and Health

Gastric ulcers pose a significant global health challenge, often exacerbated by factors such as alcohol consumption. This study investigates the gastroprotective potential of an aqueous extract from *Echinops spinosissimus* against ethanol-induced gastric ulcers in female *Albino Wistar* rats, along with its in *vitro* antioxidant and photoprotective properties.

Quantitative analysis revealed that the aqueous extract of *Echinops spinosissimus* contains 12 µg GAE/mg EXT of total polyphenols and 2.55 µg QE/mg EXT of total flavonoids. *In vitro* antioxidant assays demonstrated low activity: the extract exhibited DPPH and ABTS radical scavenging activity with (IC_{50} = 337.81 ± 12.98 µg/mL), (IC_{50} = 102.85 ± 2.48 µg/mL), respectively. The extract also showed the ability to reduce iron ions ($A_{0.5}$ = 640 ± 90.93 µg/mL). The *in vitro* sun protection factor (SPF) was found to be 10.94 ± 1.81, indicating weak photoprotective potential.

In the *in vivo* study, ethanol administration significantly increased malondialdehyde (MDA) levels in rat gastric tissue, a key biomarker of lipid peroxidation. Pre-treatment with *Echinops spinosissimus* aqueous extract (100 mg/kg) and omeprazole (20 mg/kg) significantly reduced MDA levels to 59.25% and 44.44%, respectively, compared to the ethanol group (155.55%), demonstrating a preventive effect against lipid peroxidation.

Furthermore, ethanol treatment caused a significant reduction in glutathione (GSH) levels and glutathione peroxidase (GPx) activity. Pre-treatment with *Echinops spinosissimus* extract and omeprazole markedly restored GPx activity to 85.71% and 68.57%, respectively, and increased GSH levels to 110.57% and 91.34%, respectively, relative to the ethanol group, indicating an enhancement of the cellular antioxidant defense system. These beneficial effects were further supported by histological examination.

In conclusion, the aqueous extract of *Echinops spinosissimus* may serve as a natural and effective alternative to conventional drugs for the prevention and protection against gastric ulcer formation in humans.

Key words: Ethanol, Gastric ulcer, *Echinops spinosissimus*, Polyphenols, Antioxidant activity, Photoprotective activity.

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