الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

University Ferhat Abbas Sétif 1
Faculty of Nature and Life Sciences

جامعة فرحات عباس سطيف
كلية علوم الطبيعة والحياة

N°…./SNV/**2024**

DEPARTMENT OF BIOLOGY AND ANIMAL PHYSIOLOGY

THESIS

Presented by

Hind AMIRA

For the fulfillment of the requirements for the degree of

3RD CYCLE DOCTORATE
IN BIOLOGICAL SCIENCES

Special filed**: PHYSIOLOGY AND PHARMACOLOGY**

TOPIC

Valorization of the traditional pharmacopeia: *In vitro* and *in vivo* activities of *Achillea odorata* L. extracts

Presented publically in: **22/05/2024**

Examination Board:

President:	Saliha DAHAMNA	**Pr. UFA Setif 1**
Supervisor:	Hassiba BENABDALLAH	**Pr. UFA Setif 1**
Co-supervisor	Walid MAMACHE	**MCA. UFA Setif 1**
Examiners:	Souad AMEDDAH	**Pr. Univ Constantine 1**
	Djamila ZAMA	**Pr. Univ Constantine 1**
	Romyla BOUROUBA	**MCA. UFA Setif 1**

Laboratory of Phytotherapy Applied to Chronic Diseases

List of publications

Amira, H., Benabdallah, H., Mamache, W., Benchikh, F., Ounis R., Ejike O. Okpala., Amira S., Mehmet Emin duru. (2024) HPLC Profile and Different Pathways Involved in *Achillea odorata*-Induced Gastric Emptying and Intestinal Transit Delay. *Tropical Journal of Natural Product Research*, 8(1):5759-5764. http://www.doi.org/10.26538/tjnpr/v8i1.6

Amira, H., Benabdallah, H., Mamache, W., Benchikh, F., Ounis R., Bensouici Ch., Amira S. (2023). Evaluation of the phytochemical content and antioxidant properties of different extracts of *Achillea odorata* L. *Revista Bionatura*, 8 (3) 31. http://dx.doi.org/10.21931/RB/2023.08.03.31

Amira, H., Amira, I., Benchikh, F., Benabdallah, H., Mamache, W., Amira S. (2020). Evaluation of Antioxidant activities and total phenolic content of hydro-ethanol extract from *Phlomis bovei* De Noé areal parts. *Journal of Drug Delivery & Therapeutics*, 10(5):45-48.

Mamache, W**.,** Benabdallah, H., Hannachi, A., Boukabes, A., Bencheikh, A., Benslama, A., **Amira, H**., Benchikh, F., Amira S. (2024). Preventing ethanol-induced stomach ulcers in rats using *Senecio perralderianus* leaf extract. *Revista Científica*, 34(1):8. https://doi.org/10.52973/rcfcv-e34348

Ounis, R., Benchikh, F., Amira, S., Benabdallah, **H., Amira**, H., Mamache, W., Bensouici, C., & Hellal, K. (2023). Impact of Extraction Solvent Polarity: Antioxydant Activity of Methanolic, Hydromethanolic and Aqueous Decocted Extracts of Algerien *Thymelaea hirsuta* (L.) Endl. Areal Parts. *Turkish Journal of Agriculture - Food Science and Technology*, *11*(6), 1161–1167. https://doi.org/10.24925/turjaf.v11i6.1161-1167.5931

Mamache, W., Benslama, A., Benchikh, F., Benabdallah, H., Lassas, S., **Amira, H**., Amira S. (2022). Phytochemical Screening, Antioxidant, Antiulcer, Anti-Inflammatory and Analgesic Activity of the Aqueous Extract of *Angelica archangelica*. *Turkish Journal of Agriculture - Food Science and Technology*, 10(2): 334-340. DOI: https://doi.org/10.24925/turjaf.v10i2.334-340.5023

Benabdallah, H., Benchikh, F., Mamache, W., **Amira, H.,** Amira S. (2022). Anti-ulcer, Analgesic and Antioxidant Activities of Aqueous Extract of *Foeniculum vulgare* Mill Seeds. *Turkish Journal of Agriculture - Food Science and Technology*, 10(10): 2010-2015. DOI: https://doi.org/10.24925/turjaf.v10i10.2010-2015.5185

Benchikh, F., Benabdallah, **H., Amira**, H., Mamache, W., Amira S. (2022). Free Radical Scavenging, Reducing Power and Lipid Peroxidation Inhibition Activities of *M. communis* Berries Methanol Extract and its Fractions. *Turkish Journal of Agriculture - Food Science and Technology*, 10(4): 760-765. DOI: https://doi.org/10.24925/turjaf.v10i4.760-765.5060

Benchikh, F., Benabdallah, H., **Amira, H**., Amira I., Mamache, W., Amira S. (2022). Free Radical Scavenging, Metal chelating and Antiperoxidative Activities of *M. communis* Berries Methanol extract and its Fractions. *Turkish Journal of Agriculture - Food Science and Technology*, 10(6): 1089-1094. DOI: https://doi.org/10.24925/turjaf.v10i6.1089-1094.5062

List of communications

Amira H., Benabdallah H., Mamache W., Benchikh., Amira S., effect of aqueous and hydromethanolic extracts of *Achillea odorata* L. areal parts on gastrointestinal motility in mice. IV. International agricultural, biological & life science conference 29-31 August, 2022, Edirne, Turkey.

Amira H., Benabdallah H., Mamache W., Benchikh., Amira S. Anti-ulcer and antioxidant activities of aqueous extract from *Achillea odorata* L. areal parts. IV. International agricultural, biological &life science conference 29-31 August, 2022, Edirne, Turkey.

Amira H., Benabdallah H., Mamache W., Benchikh., Ounis R., Amira S. Scavenging and chelating activities of the aqueous extract from *Salvia Verbenaca L.* areal parts. La valorisation des ressources naturelles et de l'environnement. (VRNE 2022). University Ferhat Abbas Sétif 1.

Amira H., Benabdallah H., Mamache W., Benchikh., Ounis R., Amira S. Scavenging and redox potential activities of hydro-methanolic extract from *Achillea odorata* L. areal parts. the First national Seminar on Green Chemistry and Natural Products (GCNP'22), 2022, University of Echahid Hamma Lakhdar, El-Oued.

Amira H., Benabdallah H., Mamache W., Benchikh., Ounis R., Amira S. Scavenging and redox potential activities of decocted extract from *Achillea odorata* L. areal parts. 1er séminaire national sur l'évaluation des activités biologiques des plantes médicinales et Docking Moléculaire (SNABPMDM-1), 2022, université Mostafa Benbou laid Batna 2.

Benabdallah H., Benchikh F; Mamache W., **Amira H**., Amira S. Phenolic Content and Antioxidant Activity of the aqueous Extract of *Syzygium aromaticum* Flower Buds. 5th International Conference on Food, Agriculture and Animal Sciences. November 2023, Antalya/ Turkey.

Benabdallah H., Benchikh F; Mamache W., **Amira H**., Amira S. Phenolic contents, DPPH Radical Scavenging and β-carotene Bleaching Activities of aqueous Extract from *Magydaris tomentosa.* 5th International Conference on Food, Agriculture and Animal Sciences. November 2023, Antalya/ Turkey.

Benchikh F; Benabdallah H., Mamache W., **Amira H**., Amira S. Gastroprotective, antinociceptive and antioxidant activities of the aqueous extract of *Centaurium erythraea* L. 5th

International Conference on Food, Agriculture and Animal Sciences. November 2023, Antalya/ Turkey.

Benchikh F; Benabdallah H., Mamache W., **Amira H**., Amira S. Iron Chelating, Free Radical Scavenging and Lipid Peroxidation İnhibition Activities of *Centaurium erythraea* L. hydroethanolic Extract. 5th International Conference on Food, Agriculture and Animal Sciences. November 2023, Antalya/ Turkey.

Mamache W., Benabdallah H., Benchikh F; **Amira H**., Amira S. Chemical profile, Antiglycation, anti-inflammatory and cytotoxic effect of four Algerian Salvia species; S. aegyptiaca, S. verbenaca, S. barrelieri and S. argentea. 6th International conference on sustainable sciences and technology (ICSuSAT). July 2023, Istanbul, Turkey.

Ounis R., Benchikh F., Benabdallah H., **Amira H.**, Mamache W., Amira S. Scavenging and redox potential activities of decocted extract from *Thymeleae hisrtusa* areal parts. 1er séminaire national sur l'évaluation des activités biologiques des plantes médicinales et Docking Moléculaire (SNABPMDM-1), 2022, université Mostafa Benbou laid Batna 2.

Ounis R., Benchikh F., Benabdallah H., **Amira H.**, Mamache W., Amira S. Scavenging and redox potential activities of hydro-methanolic extract from *Thymeleae hisrtusa* areal parts. the First national Seminar on Green Chemistry and Natural Products (GCNP'22), 2022, University of Echahid Hamma Lakhdar, El-Oued.

Benchikh F; Benabdallah H., Mamache W., **Amira H**., Amira S. Effect of Capparis spinosa aqueous extract against methotrexate-induced damage on sperm parameters, liver and kidney in male mice. International conference on basic science and technology (ICBAST). November 2022. Antalya, Turkey.

Amira S., Benchikh F; Benabdallah H., **Amira H**., Mamache W. Antioxidant, analgesic and gastroprotective effects of the aqueous extract from *Origanum majorana* L. against ethanol-induced gastric ulcer in mice. International conference on basic science and technology (ICBAST). November 2022. Antalya, Turkey.

Benabdallah H., Benchikh F; Mamache W., **Amira H**., Amira S. Evaluation of the analgesic, gastroprotective and antioxidant activities of the aqueous extract of *Myrtus communis* L. fruits. National seminar on phytotherapy and pharmacognosy (NSPP-2023). Setif.

Acknowledgments

First and foremost, I would like to express my gratitude to Allah, who has granted me good health and the courage to complete this work.

I extend my deep appreciation and sincere thanks to **Pr. Hassiba Benabdallah** and **Dr. Walid Mamache**, who generously shared their knowledge and insightful guidance to help me refine this work.

My heartfelt thanks go to the members of the jury: **Pr. Saliha Dahamna, Pr. Souad Ameddah, Djamila Zama, Dr. Romyla Bourouba** for accepting the responsibility of evaluating my work.

I want to thank the director of the Laboratory of Applied Phytotherapy for Chronic Diseases (LPAMC) for providing the necessary resources and support for my research topic.

I wish to express my profound gratitude and appreciation **to Dr. Fatima Benchikh** and **Pr. Smain Amira**, who provided guidance, invaluable advice, and unwavering support throughout this research. I also thank them for their availability and encouragement.

I would like to extend my heartfelt gratitude and recognition to **Dr. Chawki Bensouici** for his assistance and valuable guidance within the Biochemistry laboratory at CRBT Constantine.

My sincere thanks to **Professor Mehmet Emin Duru**, PhD Professor in Organic Chemistry and Natural Products Chemistry, University of Muğla Sıtkı Koçman, as well as Dr. **Selçuk Küçükaydın** and **Dr. Meltem Küçükaydın.**

I appreciate the assistance provided by my lab colleagues: **Roumaissa Ounis, Karima Loucif, Soulef Mehlous, Ismahan Dherafa and Chahrazed Kaoudoune** in the LPAMC laboratory.

I thank **Dr. Amina Safsaf** for her support in the pathology laboratory, University Hospital of Setif, for providing all the facilities to conduct histological sections.

I am also grateful to the Ministry of Higher Education and Scientific Research for their financial support of the research project

الملخص

تهدف الدراسة الحالية إلى تثمين الموارد الطبيعية ذات الاستعمال الواسع في الطب الشعبي, كما تهدف الى التحليل الكيميائي و خصائص السمية الخلوية والمضادة للأكسدة خارج الكائن الحي وكذلك تقييم نشاط مضادات إنزيمات الزهايمر، مثبطات إنزيمات مرض السكري، ومضاد التيروزيناز لمستخلصات المغلي المائي (ADE)، الهيدرو-ميثانولي (AME)، الهيدرو-إيثانولي (AEE)، الهيدرو-إيثيل أسيتات (AEAE) والهيدرو-كلوروفورمي (ACHE) من نبتة ذات استعمال طبي معروفة بالاسم.*Achillea odorata* L ، علاوة على ذلك، تم القيام بدراسات في الكائن الحي بخصوص تفريغ المعدة وحركة الأمعاء وتقييم النشاط المضاد للقرحة المعدية للمستخلصات ADE وAME، بالإضافة إلى تقدير السمية الحادة. أظهر التحليل الكيميائي وجود تسع مركبات مشتركة داخل مستخلصات النبتة المدروسة: حمض البروتوكاتيكوي، حمض الكلوروجينيك، حمض بارا-هيدروكسي بنزوي، الكومارين، الروتين، حمض الإيلاغيك، حمض الروزمارينيك، الميريسيتين، واللوتيولين. أظهرت مستخلصات .*A. odorata* L مستويات نشاط متنوعة ضد الخلايا السرطانية في الرئة H1299، مما أدى إلى تثبيط تكاثر الخلايا من فعالية متوسطة إلى بارزة. لم تُلاحظ اي آثار سامة عند 2000 مغ/كغ و5000 مغ/كغ في الفئران، مما يؤكد سلامة هذه النبتة. تم تحديد الخصائص المضادة للأكسدة لمستخلصات نبات .*A. odorata* L باستخدام اختباراتDPPH، ABTSلإزالة الجذر، تبيض بيتا-كاروتين، القدرة الإرجاعية والنشاط المخلبي للمعادن. أظهرت جميع المستخلصات قدرة عالية على مكافحة الأكسدة، مع أفضل أداء ل AMEفي معظم الاختبارات. أظهرت معظم المستخلصات المستعملة في هذه الدراسة نشاطًا معتدلاً في تثبيط الإنزيمات. سُجِّل أفضل نشاط مع AEAE في جميع الاختبارات. أدى ADE و AME إلى تأخير ملحوظ في العبور المعوي لدى الفئران بنسبة 28.80% و 42.47% على التوالي، وقد يكون تأثير كلا المستخلصين مرتبطًا بمسارات أكسيد النيتريك (NO)و إنزيمات الأكسدة الحلقية. علاوة على ذلك، وجد أن كلا المستخلصين ADE و AME سببا تأخيرًا كبيرًا في إفراغ المعدة إلى نسبة 42.81% و 48.89% على التوالي. يرجع هذا التأثير الى تدخل مسارات NO وإنزيمات الأكسدة الحلقية في عمل ADE على إفراغ المعدة، ومسارات إنزيمات الأكسدة الحلقية فقط فيما يخص تأثيرAME . أدى علاج الجرذان بجرعات مختلفة من ADE و AMEإلى حماية ملحوظة للغشاء المخاطي للمعدة ضد تقرح الإيثانول بنسبة 100%، وقد تكون هذه الحماية مرتبطة بزيادة مستويات المخاط المعدي وتقليل الضرر التأكسدي على الغشاء المخاطي للمعدة. وعلاوة على ذلك، قد يشمل التأثير الحامي لـ ADE تنشيط مسار أكسيد النيتريك. تسلط نتائج هذه الدراسة الضوء على الفوائد الصحية الواعدة ل.*A. odorata* L على اضطرابات الجهاز الهضمي.

كلمات مفتاحية: .*A. odorata* L، الأنشطة المضادة للأكسدة، الأنشطة المضادة للإنزيمات، إفراغ المعدة، عبور الأمعاء، تقرح المعدة الناتج عن الإيثانول.

Abstract

The present study aims to perform the valorization of natural resources widely used in folk medicine and also aimed to chemical analysis and assess the *in vitro* cytotoxic and antioxidant properties, as well as evaluate the anti-acetylcholinesterase and anti-butyrylcholinesterase, anti-tyrosinase, anti-α-amylase, and anti-α-glucosidase activities of decocted (ADE), hydro-methanolic (AME); hydro-ethanolic (AEE); hydro-ethylacetate (AEAE) and hydro-chloroformic (ACHE) extracts from an important plant of the Algerian flora named *Achillea odorata* L. moreover, studies *in vivo* were realized on gastric emptying, intestinal motility and gastro-protective effects of the ADE and AME, in addition to the evaluation of the acute toxicity. The phytochemical analysis identified nine common compounds within the plant extracts: protocatechuic acid, chlorogenic acid, p-hydroxy benzoic acid, coumarin, rutin, ellagic acid, rosmarinic acid, myricetin, and luteolin. *A. odorata* L. extracts showed diverse activity levels against the lung cancer cell line H1299, resulting in the inhibition of cellular proliferation ranging from moderate to notably effective. No toxic effects at 2000 mg/kg and 5000 mg/kg were observed in mice, confirming the safety of this plant. The antioxidant properties of *A. odorata* L. extracts were determined using DPPH, ABTS scavenging, β-carotene bleaching, reducing power, and metal chelating activity assays. All extracts showed high antioxidant capacity with the best performance in AME in the majority of tests. most extracts examined in this study displayed moderate enzymatic inhibitory activity. The best activity was recorded with AEAE in all tests. ADE and AME induced significative intestinal transit delay in mice to 28.80 % and 42.47 % respectively, the effect of both extracts may be related to the nitric oxide (NO) and the cyclooxygenase pathways. Moreover, it was found that both extracts ADE and AME induced a significant delay in gastric emptying to 42.81% and 48.89%, respectively. This effect may involve NO and the cyclooxygenase pathways in ADE effect and only cyclooxygenase pathways in AME gastric emptying effect. Treatment of rats with different doses of ADE and AME induced a significant protection of gastric mucosa against ethanol-induced ulceration 100%, This protection may be related to the augmentation of levels of gastric mucus and reduction in gastric mucosal stress. Moreover, the gastroprotective effect of ADE may potentially involve the activation of the NO pathway. The findings of this study highlight promising health benefits of *A. odorata* L. for the gastrointestinal tract disorders.

Key words: *A. odorata* L., antioxidant activities, anti-enzymatic activities, gastric emptying, intestinal transit, ethanol induced gastric ulcer.

Résumé

La présente étude vise à valoriser les ressources naturelles largement utilisées en médecine populaire, ainsi à l'analyse chimique et à évaluer les propriétés cytotoxiques et antioxydantes *in vitro*, ainsi qu'à évaluer les activités anti-acétylcholinestérase et anti-butyrylcholinestérase, anti-tyrosinase, anti-α-amylase et anti-α-glucosidase des extraits décocté (ADE), hydro-méthanolique (AME), hydro-éthanolique (AEE), hydro-éthylacétate (AEAE) et hydro-chloroformique (ACHE) d'une plante importante de la flore algérienne nommée *A. odorata* L. De plus, des études *in vivo* ont été réalisées sur la vidange gastrique, la motilité intestinale et les effets gastro-protecteurs de l'ADE et de l'AME ainsi que l'évaluation de la toxicité aiguë. L'analyse phytochimique a permis d'identifier neuf composés communs dans les extraits de la plante : l'acide protocatéchuique, l'acide chlorogénique, l'acide p-hydroxy benzoïque, la coumarine, la rutine, l'acide ellagique, l'acide rosmarinique, la myricétine et la lutéoline. Les extraits d'*A. odorata* L. ont montré divers niveaux d'activité contre la lignée cellulaire de cancer du poumon H1299, entraînant une inhibition de la prolifération cellulaire allant d'une efficacité modérée à une efficacité notable. Aucun effet toxique n'a été observé chez la souris à 2000 mg/kg et 5000 mg/kg, ce qui confirme l'innocuité de cette plante. Les propriétés antioxydantes des extraits d'*A. odorata* L. ont été déterminées à l'aide d'essais de piégeage DPPH, ABTS, de blanchiment du β-carotène, de pouvoir réducteur et d'activité de chélation des métaux. Tous les extraits ont montré une capacité antioxydante élevée, AME étant le plus performant dans la majorité des tests. La plupart des extraits examinés dans cette étude ont montré une activité inhibitrice enzymatique modérée. La meilleure activité a été notée avec l'AEAE dans tous les tests. L'ADE et l'AME ont induit un ralentissement significatif du transit intestinal chez les souris de 28,80 % et 42,47 % respectivement, l'effet des deux extraits pouvant être lié aux voies de l'oxyde nitrique (NO) et de la cyclo-oxygénase. En outre, il a été constaté que les deux extraits ADE et AME induisaient un retardement significatif de la vidange gastrique de 42,81% et 48,89%, respectivement. Cet effet peut impliquer le NO et les voies de la cyclo-oxygénase dans l'effet de l'ADE et seulement la voie de la cyclo-oxygénase dans l'effet de vidange gastrique de l'AME. Le traitement de rats avec différentes doses d'ADE et d'AME a induit une protection significative de la muqueuse gastrique contre l'ulcération induite par l'éthanol 100%. Cette protection peut être liée à l'augmentation des niveaux de mucus gastrique et à la réduction du stress de la muqueuse gastrique. En outre, l'effet gastro-protecteur de l'ADE peut potentiellement impliquer l'activation de la voie du NO. Les résultats de cette étude mettent en évidence des bénéfices potentiels de *A. odorata* L. pour la santé concernant les troubles du tractus gastro-intestinal.

Mots clés : *A. odorata* L., activités antioxydantes, activités anti-enzymatiques, vidange gastrique, transit intestinal, ulcère gastrique induit par l'éthanol.

List of abbreviations

5-HT: 5-hydroxyryptamine or Serotonin

A. Odorata* L.: Achillea odorata* L.

ABTS:2.2´-Azino-bis-(3-ethylbenzenothiazoline-6-sulfonic acid)

Ach: Acetylcholine.

AChE: Acetylcholinesterase

AChE: Hydro-Chloroform extract

AChEI: Acetylcholinesterase inhibitors

AD: Alzheimer's disease

ADE: Decocted extract

AEAE: Hydro-ethyl acetate extract

AEE: Hydro-ethanol extract

AlCl3: Aluminium tri-chloride

ALT: Alanine aminotransferase

AME: Hydro-methanol extract

AST: Aspartate aminotransferase

ATP: Adenosine triphosphate

Aβ: Amyloid beta

BChE: Butyrylcholinesterase

BChEI: Butyrylcholinesterase inhibitors

BER: Fundamental electrical rhythm

BHA: Butylated hydroxyanisole

BHT: Butyl hydroxyl toluene

CAT: Catalase

CCK: Cholecystokinin

CMC: Carboxymethyl cellulose

COX: Cyclooxygenase

CUPRAC: Cupric ion reducing antioxidant capacity

DMSO: Dimethyl sulfoxide

DPPH: 1, 1-Diphenyl-2-picryl-hydrazyl

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

DW: Dried weight.

EDTA: Ethylenediaminetetraacetic acid

eNOS: Endothelial nitric oxide synthase

FBS: Fetal Bovine Serum

GE: Gastric emptying

GI: Gastrointestinal

GPx: Glutathione peroxidase

GR: GSH reductase

GSH: Glutathione

GSSG: Glutathione disulphide

H. pylori*: Helicobacter pylori*

H_2O_2: Hydrogen peroxide

HNO_2: Nitrous acid

HOCl: Hypochlorous acid

HPLC-DAD: High-performance liquid chromatography with photodiode-array detection

IL: Interleukin

iNOS: Inducible nitric oxide synthase

LD_{50}: Lethal dose, 50%

L-NNA: L-nitro-n-arginine

M: Muscarinic receptor

MDA: Malondialdehyde

MeOH: Methanol

MMC: Migranting motor complexes

MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

NADPH: Nicotinamide adenine dinucleotide phosphate

NANC: Non-adrenergic non-cholinergic

NFTs: Neurofibrillary tangles

nNOS: Neuronal nitric oxide

NO: Nitric oxide

NO•: Nitrogen free radical

NOS: Nitric oxide synthase

Nox: Nicotinamide adenine dinucleotide phosphate oxidase

NSAIDs: Non-steroidal anti-inflammatory drugs

O2•: Superoxide radical

OECD: Organization for Economic Cooperation and Development's guideline 425

OH•: Hydroxyl radical

PDA: Photodiode array detector

PGs: Prostaglandins

R: Bleaching rate

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

RT: Retention time

SOD: Superoxide dismutase

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

TFC: Total flavonoid content

TK: Tachynin

TNB: 2-Nitro-5-thiobenzoic acid

TNF: Tumor necrosis factor

TPC: Total phenolics content

TTC: Total tannins content

VIP: Vasoactive intestinal peptide

WHO: World Health Organization

XO: Xanthine oxidase

List of figures

List of tables

List of content

II. Materials and methods

III.Results

IV.Discussion

Introduction

Medicinal plants play a significant role in the healthcare system, embodying a rich history of human interaction with the natural world. Traditional medicine has relied on plants that embrace a diverse array of substances capable of addressing both chronic and infectious ailments. Many of today's modern pharmaceuticals have their origins in these medicinal plants. Despite the profound therapeutic benefits possessed by certain medicinal plants, it has been discovered that some of their constituents may potentially be toxic. Therefore, evaluating the toxicity of extracts from any medicinal plants intended for use in humans and animals is of utmost importance.

Oxidative stress, often defined as the excessive generation and inadequate removal of highly reactive molecules like reactive oxygen species (ROS), can lead to the overproduction of these free radicals. This, in turn, can result in oxidative damage to vital biomolecules such as lipids, proteins, and DNA. Such damage stands as a central contributor to numerous chronic diseases affecting the human population, including aging-related conditions, metabolic disorders such as diabetes and neuro-degenerative disorders like Alzheimer (Halliwell, 1994; Poulsen *et al*., 1998). Medicinal plants and plant-derived products can encompass a diverse array of molecules that excel in scavenging free radicals. These molecules include phenolic compounds like phenolic acids, flavonoids and tannins. These phenolics exhibit potent antioxidant properties and are known for their ability to scavenge radicals, chelate metals, act as reducing agents and quench singlet oxygen (Vona *et al*., 2021). According to Atta-ur-Rahman *et al*., (2002), regular antioxidants have the potential to stop or slow down the deterioration of neurons, which could potentially hinder the progression of Alzheimer's disease. This disease is a severe form of dementia, and the use of acetylcholinesterase (AChEI) and butyrylcholinesterase inhibitors (BChEI) is the preferred and highly desirable modern therapeutic approach. However, most of these medications can cause cardiac arrhythmia (bradycardia) and toxic effects in the liver, stomach, and intestines. Therefore, there is a desire to improve and utilize safe natural anticholinesterases and antioxidants. Moreover, the treatment of diabetes focus on molecules that inhibit the body's ability to assimilate glucose either by acting on sugar-degrading enzymes, or by regulating the absorption and transport of the simple sugars formed. Controlling the activity of these diabetic enzymes (alpha-amylase and alpha-glucosidase is a crucial aspect of diabetes treatment (Mechchate *et al*., 2021). However, the treatment of diabetes with drugs like acarbose is no longer considered safe and free from side effects. On the other hand, secondary metabolites from medicinal plants are recognized as inhibitors and modulators of the activity of these enzymes (Wink, 2015).

The digestive system plays a vital role in meeting the body's nutritional requirements, including sugars, proteins, vitamins, water, and electrolytes, through the performance of several crucial functions. Any disruption or imbalance in these functions can give rise to a range of diseases. Among these disorders: gastric ulcer and gastrointestinal (GI) motility disorders. Gastric ulcer is one of the most prevalent conditions impacting the digestive system resulting from the erosion of the protective mucous layer of stomach that can cause persistent pain and discomfort. These ulcers often result from a combination of factors, including the presence of *Helicobacter pylori* (*H. pylori*) bacteria, excessive stomach acid production, and lifestyle choices like alcohol consumption or excessive use of nonsteroidal anti-inflammatory drugs (NSAIDs). Ulcers represent an ailment that can be easily acquired yet proves to be challenging to cure. Their impact on human health is substantial, with potential consequences even including fatality. Its therapy might be complicated at times, contingent on the specific causative factors and the unique ulcer conditions. Moreover, modern medical treatments often come with a hefty price tag, placing a financial burden on patients and are often associated with undesirable side effects, Therefore, in Algeria and other countries, people have made diligent efforts to explore alternative solutions that are safer, more cost-effective, efficient, and reliable. Medicinal plants are among the potential options due to their content, which offers an abundant source of substances with a wide range of biological and pharmacological activities (Amira *et al*., 2012; Benchikh *et al*., 2016).

Achillea Genus (yarrow) is one of the youngest evolutionary genera of the Asteraceae family, which is present throughout the world (Farajpour *et al*., 2012). More than 100 species have been recognized in this genus (Goli *et al*., 2008). Yarrow is a perennial herb and well-known medicinal plant, widely used in folk medicine (Bimbiraitė *et al*., 2008). These plants are native to Europe, North Africa and western Asia but are also found in Australia, New Zealand, and North America (Gharibi *et al*., 2013). Whereas Achillea *odorata* L. (*A. odorata* L.) belongs to the genus Achillea. Limited research has been dedicated to exploring these plants and their valuable contributions to the healthcare system. The key aims of this study were to establish the scientific foundation for assessing the potential of these plant extracts in the treatment of various diseases by:

1. Analyzing the phytochemical constituents.
2. Evaluating *in vitro* antioxidant properties using various methods.
3. Evaluating their inhibitory effects on key enzymes associated with Alzheimer's, hyperpigmentation and diabetes mellitus.

4. Conducting acute oral toxicity tests *in vivo* and assessment the cytotoxicity of these extracts on cancer cell lines.
5. Investigating their impact on GI motility and the underlying mechanisms.
6. Assessing their ability to protect the gastric mucosa against ethanol-induced ulceration and understanding the mechanisms involved.

Literature review

1. Oxidative stress

Oxidative stress is an imbalanced state between free radicals (ROS) and antioxidants due to excessive generation of ROS or insufficient antioxidant defense. Free radicals are unstable molecules, with one or more unpaired electrons in the backbone cycle, and can be oxygen, nitrogen, or other reactive species. In mitochondria, ROS Generation is facilitated primarily by electron transport chains and oxidative phosphorylation. Other enzymes contribute to ROS construction, where xanthine Oxidase, lipoxygenase, irrigated nitric oxide (nos), and nicotinamide adenine dinucleotide phosphate oxidase (Nox) (Gordan *et al*., 2020; Martemucci *et al*., 2022; Zhang *et al*., 2022) (Figure 1).

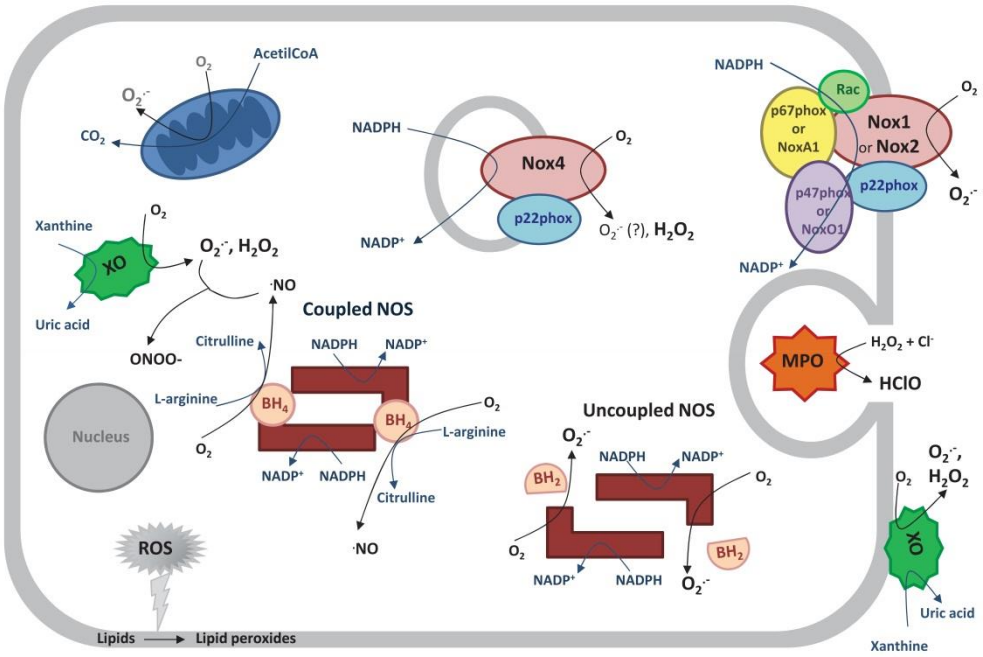


Figure 1. Sources of ROS and/or RNS generation (Teresa *et al*., 2012).

Environmental variables such as smoking, environmental toxins, UV radiation, xenobiotics, and alcohol can also contribute to ROS production. These factors interact with endogenous factors or enzymes to increase ROS formation (Aranda-Rivera *et al*., 2022).

ROS/RNS damage DNA, causing oxidative stress. These highly reactive molecules can modify and break the DNA strands, contributing to genetic mutations and potential disruptions in cellular function. (Kelainy *et al*., 2019). Oxidative stress can disrupt the membrane's lipid bilayer, leading to lipid peroxidation and increased tissue permeability. This can inactivate receptors and enzymes, making cellular proteins inactive (Bumrungpert *et al*., (2018). Lipid peroxidation products, including isoprostanes and thiobarbituric acid, are detectable in the

exhaled breath condensate, bronchoalveolar lavage fluid, and lungs of individuals who smoke or patients with chronic obstructive pulmonary disease (Ito *et al*., 2019).

Proteins are susceptible to oxidation due to the fragmentation of peptide chains, changes in electrical charge, cross-linking, and oxidation of specific amino acids. Metal-catalyzed oxidation affects enzymes with metals on or near their active sites, inhibiting their activity (Jamdade & Bodare, 2022).

Antioxidants, such as superoxide dismutase (SOD), catalase (CAT), GSH peroxidase (GPx), and GSH reductase (GR), play a crucial role in combating oxidative stress. SOD catalyzes the quick dismutation of O_2•, lowering the risk of •OH production in metal-catalyzed processes. Catalase degrades H_2O_2 by switching between two conformations, converting millions of hydrogen peroxide molecules into water and oxygen per second. GPx detoxifies H_2O_2 and other H_2O_2 produced by oxidation of cholesterol or polyunsaturated fatty acids. GSH reductase, an essential cellular antioxidant, converts $NADP^+$ to NADPH, providing oxidative stress (Ighodaro & Akinloye, 2018; Dumanović *et al*., 2021; Aranda-Rivera *et al*., 2022; Sadiq, 2023) (Figure 2).

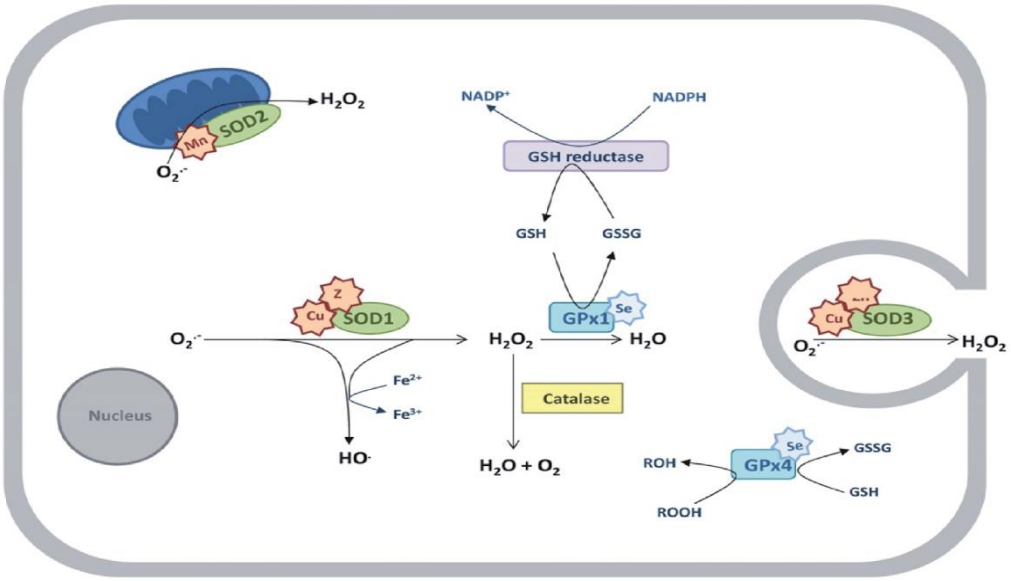


Figure 2. Major antioxidant enzyme defenses (Teresa *et al*., 2012).

Nonenzymatic antioxidants are low molecular weight compounds found in plants, including vitamins C, E, carotenoids, uric acid and polyphenols. Water-soluble vitamin C exerts antioxidant activity by scavenging oxygen free radicals. Vitamin E, a fat-soluble form, is the main protector against oxidant-induced tissue damage, carotenoids in water-insoluble cell

membranes, a compound found in plants, are powerful anti-inflammatory at low oxygen partial pressures and can regulate transcription factors and prevent oxidant-induced activation (Ribeiro *et al*., 2018; Irato & Santovito, 2021; Jurado-Campos *et al*., 2021).

A significant portion of plant secondary metabolites includes polyphenols, flavonoids, phenolic acids, tannins (water soluble), stilbenes and lignans. Phenolic acids are hydroxy derivatives of carboxylic acid formation fragrance, whereas flavonoids are low molecular weight molecules with a C6-C3-C6 structure. Due to their high-redox- capacity, they are important antioxidants because of their ability to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They are associated with diseases such as prostate and breast cancer that decreases with regular consumption relationship (Robbins, 2003; Rudrapal *et al*., 2022) (Figure 3). Tannins, with a medium to high molecular weight of up to 30,000 Da, exert inhibitory effects on the solubility and digestion of GI peptides and proteins. Additionally, they contribute to the reduction of lipid peroxidation. Furthermore, tannins exhibit toxicity towards yeasts, fungi, and bacteria. Condensed tannins and hydrolyzable tannins are the two main subgroups of plant tannins (Figure 3)(Tong *et al*., 2022).

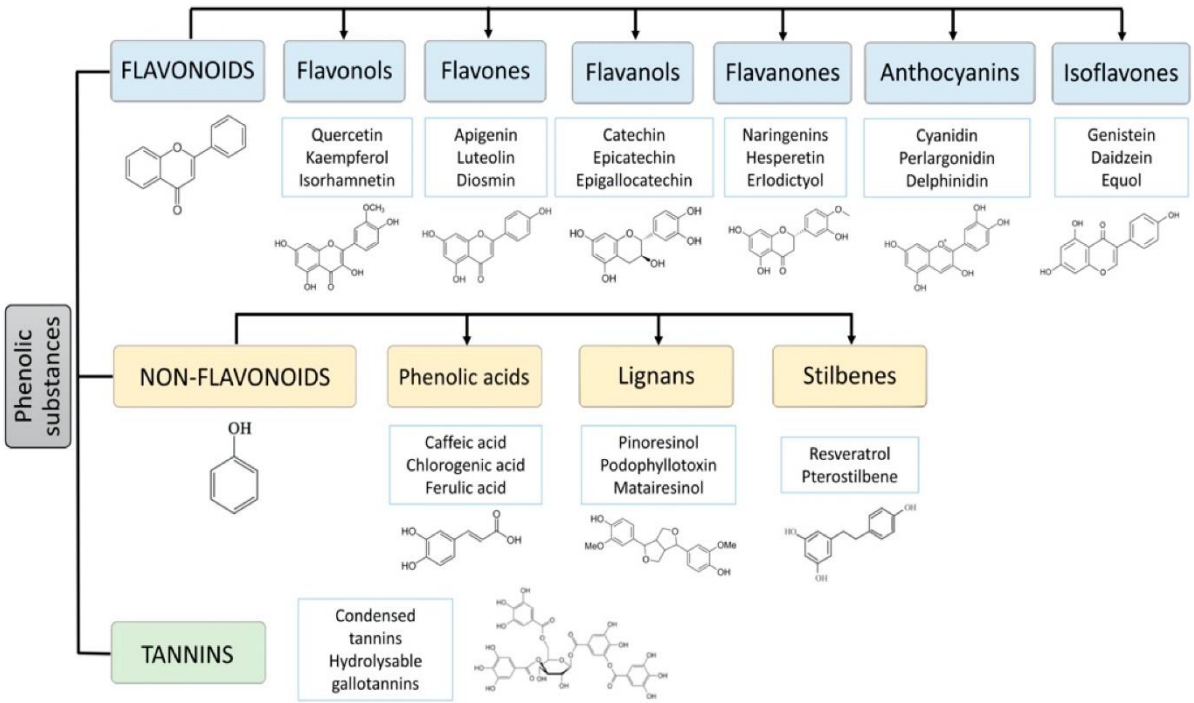


Figure 3. Main classes of polyphenols: flavonoids, non-flavonoids (phenolic acids, lignans, stilbenes) and tannins (Serra *et al*., 2021).

1.1. **Antioxidant and Alzheimer**

Alzheimer's disease (AD) is characterized by the presence of neurofibrillary tangles (NFTs) and amyloid-β peptides (Aβ), which contribute significantly to the progressive cognitive decline observed in affected individuals (Ahsan, 2022). In the early stages of AD, there is a notable reduction in hippocampal and basal forebrain neurons, including cholinergic neurons. This alteration in neurotransmitter systems, particularly the cholinergic system, plays a pivotal role and can lead to psychosis when cholinergic neurons are lost (Kumar *et al*., 2018).

Therapeutic approaches for Alzheimer's disease primarily target cholinergic factors, reflecting the central involvement of the cholinergic system in the disease pathology. The prevalence of AD is influenced by various factors, including age, genetic, medical conditions, environmental, and lifestyle factors (Schliebs & Arendt, 2011). While a definitive cure remains elusive, ongoing research aims to improve early detection methods and develop effective treatments (Winek *et al*., 2021).

The pathophysiology of AD involves oxidative stress, contributing to increased protein oxidation, glycoxidation, lipid peroxidation, and toxin production. Despite the absence of a known cure, combination therapies have shown promise in halting disease progression (Collins *et al*., 2022). Continuous efforts in research and development are critical for advancing our understanding and enhancing the management of Alzheimer's disease (Schliebs & Arendt, 2011, Winek *et al*., 2021).

Recent studies have explored the potential benefits of plant polyphenols in managing neurodegenerative diseases, including Alzheimer's disease (AD). These compounds are believed to have a positive impact on AD development through various biological mechanisms, including effects on cellular signaling networks, gene expression, connectivity, and intelligence (Taqui *et al*., 2022).

1.2. **Antioxidant and Diabetes**

Food consumption increases sugar levels, resulting in more insulin secretion, which increases glucose uptake, bio-turnover and storage in muscle and fat cells. Insulin inhibits glucagon secretion, decreases blood cholesterol levels, conserving glucose and reducing the amount produced by the liver. Insulin deficiency or insulin resistance can lead to intracellular hypoglycemia and extracellular hyperglycemia, resulting in hyperglycemic coma and osmotic diaresis (Ozougwu *et al*., 2013; Cecilia *et al*., 2019; Tomic *et al*., 2022).

There is evidence to suggest that oxidative stress has a role in the etiology of both type 1 and type 2 diabetes. Free radical generation in diabetes causes oxidative stress, which damages

enzymes, mitochondrial impairment and cellular functions and also increases insulin resistance. (Bhatti *et al*., 2022; Vassalle & Gaggini, 2022).

Antidiabetic drugs such as acarbose, miglitol, and voglibose work by inhibiting carbohydrate digestion and absorption. Polyphenols, which inhibit carbohydrate hydrolases, have been suggested as potential alternatives to commercial hypoglycemic agents. They can modulate postprandial blood glucose levels, inhibit starch digestion and reduce symptoms of diabetes (Singh, 2014; Agarwal & Gupta, 2016).

Plant extracts such as grape extract and green tea extract have been shown to be useful in treating diabetes. Grape extract has been shown to reduce postprandial hyperglycemia and inhibit mammalian pancreatic α-glucosidase activity in streptozotocin-treated rats. The combination of green tea extracts and acarbose has shown synergy (Gu *et al*., 2015; Sun *et al*., 2020).

2. Digestive system

The digestive tract, a muscular tube crafted for the absorption and digestion of food and liquids, commences in the oral cavity and traverses different anatomical regions. Including the esophagus, stomach, duodenum, small and large intestines, rectum, and anal canal. Additionally, the digestive system features auxiliary glands that release enzymes and fluids essential for the process of digestion and nutrient transport (Johnson, 2006; Olsson, 2011).

The digestive tract structure involves four components; the mucosa, the submucosa, the muscularis externa, and the serosa. The mucosa is made up of an epithelial layer that is in direct touch with the contents of the digestive tract lumen. The epithelium of the digestive tract at various stages either digests food enzymatically or absorbs the nutrients that are produced (Ganong *et al*., 2010). the submucosa, a thick layer of connective tissue, contains nerve tissue in addition to arteries, veins, and lymphatics. The muscularis externa, which encircles the submucosa, is made up of two layers of muscle: an inner layer in which the smooth muscle cells are positioned longitudinally along the GI tract and an outer layer in which they are positioned circumferentially around the digestive tract (Patton & Thibodeau, 2018). The adventitia, the digestive tracts outermost layer, is made of connective tissue and contains fat, blood vessels, and nerves (Yang *et al*., 2022) (Figure 4).

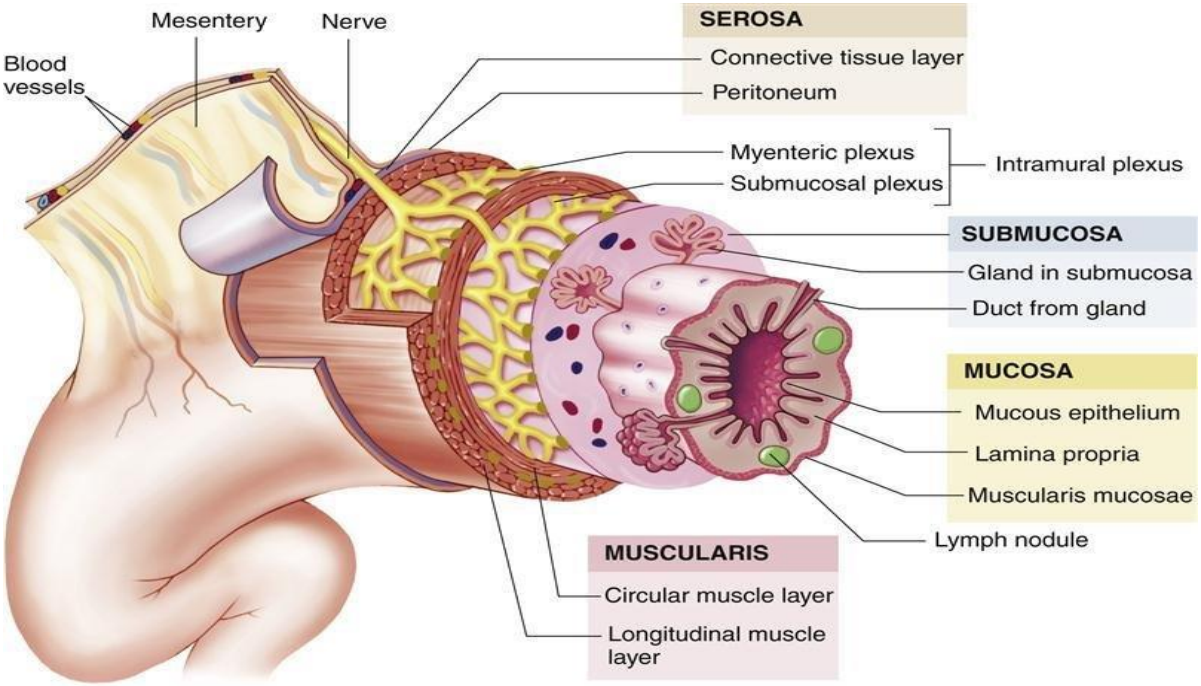


Figure 4. Wall of the gastrointestinal tract (Patton & Thibodeau, 2018).

The gastrointestinal (GI) tract's innervation is responsible for many aspects of digestion, including motility, secretion, absorption, and blood flow. There are two types of innervation: intrinsic (enteric) primarily within the digestive wall and extrinsic with neurons cell bodies located outside the digestive wall (Bohórquez *et al*., 2015).

Intrinsic innervation including sensory neurons, responsible for detecting chemical stimuli, located between two membranes. And motor nervous system can be divided into two regions; the menteric plexus and the Meissner plexus. These plexuses receive axons that transmit signals to the nervous system while also receiving inputs from the extrinsic nervous system (sympathetic and parasympathetic) as mentioned by Boesmans *et al*., (2015) (Figure 5).

Interneurons exist between motor neurons and the extrinsic nervous system, helping keep motor activity coordinated.

There are two types of extrinsic innervation: sympathetic and parasympathetic. The extrinsic nervous system indirectly controls the smooth muscle by acting on the neurons of the enteric nervous system (Furness & Sanger, 2002; Schemann *et al*., 2008).

The digestive parasympathetic neural system consists of two contingents: one bulbar and one sacral. The enteric nervous system's plexuses, excitatory and inhibitory motor neurons, form synapses where parasympathetic fibers form synapses. The parasympathetic system primarily has an excitatory role at both ends of the digestive tract, particularly in vomiting and

defecation (Niu *et al*., 2020). Postganglionic sympathetic fibers act as inhibitors and are implicated in various reflexes, both digestive and non-digestive. They work by inhibiting synaptic transmission and are mediated by noradrenaline. The sympathetic nervous system has an excitatory function at the sphincter level, with postganglionic sympathetic neurons, having serotonin (5-HT) receptors that affect how neuromediators are released by the postganglionic fibers (Brookes & Costa, 2003) (Figure 5).

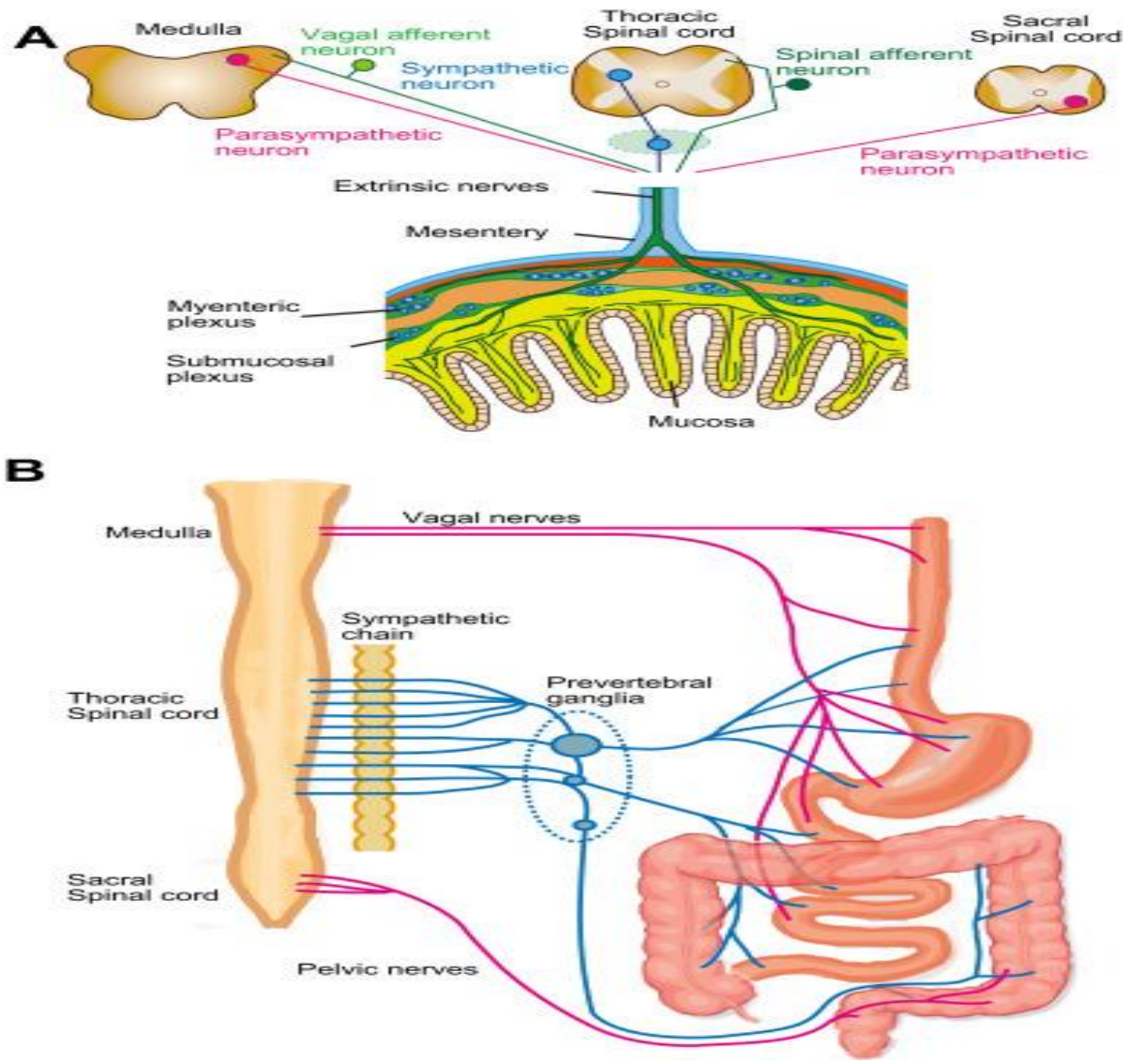


Figure 5. Intrinsic and extrinsic nerve innervation of gastrointestinal tract *(*Uesaka *et al., 2016).*

(A)Schematic diagram showing different types of extrinsic neurons projecting to the gut. Postganglionic sympathetic neurons (blue) project to the gut to regulate secretion, gut motility, and blood flow. Parasympathetic extrinsic neurons (pink) in the brainstem and sacral spinal cord run through the vagus and pelvic nerves, respectively, to innervate the gut. Vagal afferent neurons (green) in the nodose and inferior jugular ganglia and spinal afferent neurons (green) in the dorsal root ganglia receive sensory information from the gut. The gastrointestinal tract is composed of two major ganglionated plexuses, myenteric and submucosal plexuses. (B) Sympathetic (blue) and parasympathetic (pink) innervation of the gastrointestinal tract through vagal, sympathetic pelvic pathways.

2.1. **Gastro-intestinal motility**

For living vertebrates, eating, digesting food, and absorbing nutrients through the GI tract wall are basic physiological processes. The GI system serves as the entrance way for food, and

it is widely recognized that GI motility impacts eating behavior favorably and helps to maintain energy balance. In general, smooth muscle contractions mediated by extrinsic parasympathetic and sympathetic neurons, intrinsic enteric sensory and motor neurons, and certain GI hormones modulate GI motility in vertebrates. As a result, three different centers of control were implicated (Svorc *et al*., 2001):

- Myogenic control: The intrinsic rhythm of the GI musculature. The interstitial cells of Cajal (ICC), which operate as a pacemaker, set the rate at which this rhythm is largely produced by slow waves, a characteristic of GI smooth muscle.
- Hormonal regulation: Cholecystokinin, gastrin, and secretin, among many others, are used for a variety of purposes.
- Neural control, which includes the autonomic nervous system and the GI's intrinsic enteric nerve system (Furness *et al*., 2014).

Motility, secretion, digestion, and absorption are the four main functions of the GI tract that must all be accomplished for it to operate properly. The primary emphasis of this activity will be neural regulation, especially the physiological operation of the enteric nervous system and the autonomic nervous system and any disease that may be present (Furness *et al*., 2014).

2.1.1 **Gastric emptying**

The movement of the stomach's contents into the duodenum is known as gastric emptying. Peristaltic waves, antrum systolic contractions, and stomach shrinkage are the three processes that make this possible (Figure 6). Smaller items usually empty more quickly than bigger ones, and liquids usually do so faster than solids. The stomach emptying rate is a meticulously controlled procedure with several stages. A balanced emptying of the stomach's contents into the duodenum for small intestine absorption is made possible by metabolic load, brain regulation systems, and hormonal impact (Maisiyiti & Chen, 2019).

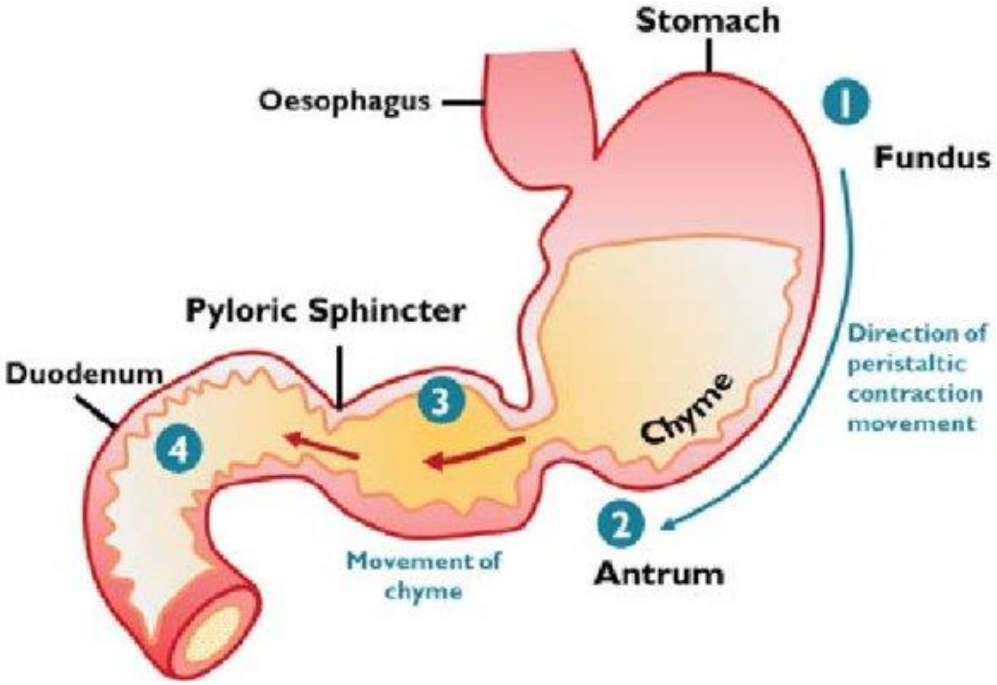


Figure 6. Gastric emptying process (Sherwood, 1993).

2.1.1.1 Gastric emptying during the digestive period

Fundic compliance rises when the meal is digested and the stomach fills, allowing for the accommodation of a significant amount of food without an increase in pressure. The peristaltic and pressure pumps do not contract during this filling phase and remain inhibited. A pumping phase follows the filling phase and is characterized by increased peristaltic contractions in the peristaltic stomach and a gradual tonic contraction of the fundus. This enables the transport of ingested food to the pylorus after combining with stomach acid and pepsin. For recycling, the peristaltic pump also takes food that is not well pulverized. Before food starts to reach the duodenum, the antrum must fill to a particular point. This is reflected as the lag phase on the whole stomach-emptying curve (Pal *et al.*, 2007).

Together with the stomach's reduced curvature, the stomach creates the "Magenstrasse," a functional tunnel that diverts liquids away from the main stomach and into the duodenum (Pal *et al.*, 2007).

Contractions that intensify in the antrum transport the tenderized food into the pyloric grinder. To take food from the proximal antrum, the pylorus relaxes (Yuan *et al.*, 2001). Pyloric contractions cause an anterograde jet of chyme into the duodenum and a strong retrograde jet of food that avoids pulverization. These occurrences are consistent with the antropyloric pressure waves on intraluminal manometry, which are closely related to a pulsatile flow into the duodenum and "sieving function" (Ramkumar & Schulze, 2005).

Isolated pyloric pressure waves on intraluminal manometry in humans are a result of the pyloric sphincter closing completely, which shuts down gastroduodenal communication. Slow or quick stomach emptying is caused by improved or compromised pressure pump relaxation, respectively. Poor mixing and sluggish stomach emptying result from weak or disorganized peristaltic pump contractions, whereas quick gastric emptying of the digestible solids results from stronger peristaltic contractions (Bharucha *et al*., 2011).To control stomach emptying, the pyloric sphincter and the duodenum collaborate well. The pyloric complex can help or hinder stomach emptying during the digesting phase because it functions as both a grinder and a flexible filter. Antral contractions cause the duodenum to relax, a process known as "antroduodenal coordination." The duodenal bulb contracts to discharge the chyme in a constant flow into the second part of the duodenum after absorbing chyme injections (Pal *et al*., 2007).

2.1.1.2 Gastric emptying in the inter- digestive period

Gastric motility during the inter-digestive (fasting) interval is intended to rid the stomach of leftovers that can't be digested. The migrating motor complex (MMC), a cyclical motor activity, is what gives it its distinctive characteristics. The MMC consists of four segments. Phase I lasts for around 45–60 minutes and is characterized by electrical slow waves that are not connected to muscle contractions in the peristaltic pump. Tonic suppression of the motor activity is what causes motor quiescence. Phase II is characterized by slow waves and regular phasic contractions. Phase III (also known as the "activity front") is characterized by a front of long-lasting, high-amplitude contractions that advance toward the pyloric sphincter over the course of 5–15 minutes. Phase III of the MMC is independent of the slow waves and is neurally mediated. The pylorus and duodenum remain untense and open during the migration front to let phase III activity to remove meal remnants from the stomach (Deloose *et al*., 2012). According to Mazzuoli *et al*., (2008), vagal stimulation completely eliminates stomach motor and neurohormonal activity during the digestive and inter-digestive phases.

2.1.1.3 Neurol and hormonal regulation

The preparation of ingested food into chyme and controlled distribution into the small intestine, known as gastric emptying, are the two main jobs of the stomach. Two notable properties of gastric emptying were previously found by studies: (a) the capacity to control the time and rate of emptying of ingested food with varying physical compositions; and (b) the capacity to control emptying based on the caloric density of food (Kar *et al*., 2015). The activity of various anatomical components of the stomach was merged to construct functional "peristaltic" and "pressure" pumps as well as a grinder-filter that played clearly defined roles in

gastric emptying, according to studies on the biomechanics of this process. In stomach emptying of solids, the peristaltic pump plays a major role (Murthy, 2006; Hinshaw *et al*., 2014).

Multiple neuronal and hormonal mechanisms control gastric emptying (Phillips *et al*., 2015). The vagus nerve, which receives extrinsic inputs from the central nervous system, and enteric nerves, which receive intrinsic modulation, are both involved in the neural control of stomach emptying. In the upper GI system, the vagus nerve's extrinsic or parasympathetic input is particularly significant. By releasing excitatory neurotransmitters like acetylcholine (ACh) and inhibitory neurotransmitters like nitric oxide (NO), the vagus nerve transmits information through a complex circuitry made up of both afferent and efferent components (Travagli & Anselmi, 2016).

The intrinsic route modulates responses by releasing both excitatory and inhibitory neurotransmitters, as well as ascending and descending enteric neurons. There may be more durable endocrine-mediated processes than these neuronally mediated effects. Amylin, cholecystokinin, glucagon-like peptide-1, and peptide YY are examples of hormones that slow gastric emptying, whereas ghrelin and motilin are examples of hormones that speed up gastric emptying (Santacruz *et al*., 2017; Summers *et al*., 2014).

2.1.2 **Intestine motility**

Two different forms of contractions are made possible by intestinal motility: intestinal segmentation (chyme churning in the gut) and peristalsis. Intestinal transit, digestion, and nutritional absorption are all directly impacted by the timing and pattern of these contractions (Sanderson *et al*., 2017).

As a result of peristaltic and other contractions regulated by the fundamental electrical rhythm (BER), MMCs that normally move down the gut at regular intervals during a fast are replaced (Hasler, 2009; Barrett *et al*., 2018). The average number of BER cycles per minute in the small intestine is 12 in the proximal jejunum and 8 in the distal ileum. Peristaltic waves, segmentation contractions, and tonic contractions are the three different forms of smooth muscle contractions. It moves the chyme (intestinal contents) towards the direction of the big intestines. By moving the chyme and increasing its exposure to the mucosal surface, segmentation contraction. The focal increases in Ca^{2+} inflow that trigger these contractions cause waves of elevated Ca^{2+} concentration to radiate out from each point. Tonic contractions, which are relatively long-lasting contractions, effectively divide the gut into separate segments. These latter two types of contractions cause the small intestine to transit more slowly, resulting

in a transit time that is longer in the fed condition than in the fasting state. This promotes absorption by enabling prolonged interaction between the chyme and the enterocytes (Barrett *et al*., 2018).

2.1.3 Neurotransmitter involved in gastrointestinal motility

2.1.3.1 Nitric oxide

Nitric oxide (NO) is a Non-adrenergic non-cholinergic (NANC) neurotransmitter of the central, peripheral, and enteric neural systems, according to Li *et al*., (2022). It is produced by the enzyme NOS, which is found in enteric neurons. When soluble guanylate cyclase is activated by NO, it penetrates the smooth muscle cell membrane and relaxes the cell. Inhibiting NOS with L-nitro-n-arginine (L-NNA) delays transit in the small intestine and colon of mice and dogs, respectively. Pharmacological NOS inhibitors in humans shorten the time it takes for the stomach to empty, increase the frequency of gastric contractions, and decrease the volume of the fundus before and after meals (Chiba *et al*., 2002).

2.1.3.2 Serotonin

It has long been assumed that 5-HT (5-hydroxytryptamine), plays a significant role in intestinal health. There have been more than 50 years of studies on the function of 5-HT in GI motility. The gut wall contains the majority of the body's 5-HT, which is found in descending interneurons and subsets of mucosal cells (enterochromaffin cells). Studies showed that 5-HT may start the peristaltic reflex and propel motion, and that it is released in response to elevated intraluminal pressure (Kendig & Grider, 2015).

2.1.3.3 Achetylcholine

ACh is the neurotransmitter that causes GI smooth muscle contractions most frequently in the enteric nervous system. The muscarinic ACh receptors on the surface of smooth muscle cells mediate cholinergic signaling (Lecci *et al*., 2002). All of the known and defined muscarinic receptor subtypes (M (1)-M (5)) are members of the G-protein-coupled receptor superfamily. The main muscarinic receptor subtype expressed by smooth muscle tissues in the GI tract is the muscarinic M(2) ACh receptor, where it is co-expressed with a lesser population of M(3) receptor (Sanders *et al*., 2012).

2.1.3.4 Vasoactive intestinal polypeptide

A neuropeptide called vasoactive intestinal polypeptide (VIP) serves as a neuromodulator and neurotransmitter. It is a strong vasodilator that controls blood flow, smooth muscle activity, and epithelial cell secretion in the digestive system (Iwasaki *et al*., 2019).

2.1.3.5 Tachykinins

In neuronal circuits that control intestinal motility, secretion, and vascular activities, tachykinins are peptide neurotransmitters. These include substance P, neurokinin A, and neurokinin B, which, according to Shimizu *et al*., (2008) activate NK1, NK2, and NK3 receptors, respectively. Tachykinins play a part in the GI tract's inflammatory reactions and are transmitted by spinal afferents that innervate it. Tachykinins function as a co-neurotransmitter of excitatory neurons and coexist with ACh, the principal transmitter of excitatory neurons innervating the muscle (Olsson & Holmgren, 2001).

2.2. **Polyphenols effect on gastrointestinal motility**

The contraction of smooth muscle cells in the GI tract is inhibited by certain polyphenols. Flavonoids can effectively lessen the contractions of the ileum brought on by PGE_2, LTD4, ACh, and $BaCl_2$. Chrysin, kaempferol, quercetin, and apigenin are all inhibitors of PGE_2-induced contractions. Apigenin, quercetin, and kaempferol all actively block the LTD4-induced contractions (Capasso *et al*., 1991).

Apigenin and quercetin have been shown to relax the mice's gastric smooth muscles in the stomach in a concentration-dependent manner (1 M to 100 M); apigenin effect is more potent than quercetin. In calcium-free medium or in the presence of nifedipine, a voltage-gated L-type calcium channel blocker, apigenin and quercetin's muscle-relaxing effects are significantly diminished, indicating that this effect is primarily brought on by their ability to prevent calcium from entering cells through these channels (Rotondo *et al*., 2009). Polyphenols also have antidiarrheal effects that impact intestinal transit. The intraperitoneal administration of 12.5–50 mg/kg of a few flavonoids (apigenin, flavone, kaempferol, morine, myricetin, naringin, and rutin) to mice slowed intestinal transit by 28–69%. The addition of flavonoids (naringenin, silibinin, silymarin, and taxifolin, (100-200 mg/kg) reduces intestinal transit by 23-41% (Di Carlo *et al*., 1993).

2.3. **Ulcer**

2.3.1 **Physiology of stomach**

The stomach contains a variety of cell types. The glands of the cardia predominantly release mucus and bicarbonate. The fundus and corpus are where the majority of the parietal, chief, enterochromaffin-like, and D cells are found. The majority of the cells in the antrum are G and D cells, also known as enteroendocrine cells (Schubert & Peura, 2008).

The bulk of parietal cells are highly differentiated epithelial cells that secrete hydrochloric acid (HCl), which is responsible for the high acidity (pH 1.5 - 3.5) of the stomach. Pepsin, an

enzyme required for protein digestion, must also be activated by HCl. Pepsinogen, the inactive version of pepsin, is secreted by chief cells. Histamine is produced and secreted by cells that resemble enterochromaffins. In addition to somatostatin, which is generated by D cells, enteroendocrine cells also emit gastrin, which is mostly secreted by G cells (Soybel, 2005).

Parietal cells secrete HCl under the influence of hormones and the neurological system. Gastrin, which mediates the so-called gastric phase of secretion, is the primary stimulant of acid secretion. Gastrin likely does not directly activate the parietal cells; instead, it works to release histamine from the mucosal enterochromaffin-like cells. The parietal cells are then prompted by histamine to release HCl. Additionally, protein consumption, stomach distension, and cholinergic activation from the vagal nerve can all cause parietal cells to create HCl (O'Connor & O'Moráin, 2014).

The H^+/K^+_ATPase, commonly known as the proton pump, is a major transporter that contributes to the stomach's acidity. It is found on the cell's surface. In return for a potassium ion (K^+), parietal cells leak a proton (H^+) into the lumen of the stomach. Somatostatin is involved in the negative control of secretion as well. Somatostatin is released in response to a low pH in the antrum, and this inhibits the production of gastrin from the G cells. The stomach's acidic environment serves as a barrier against germs (Schubert & Peura, 2008).

High turnover, tight connections, mucus, and bicarbonate secretion shield the GI mucosa from acid. Ulcer development and associated pathologies may arise if the mucosa's defenses are compromised by the harmful effects of gastric acid and pepsin (Beiranvand, 2022).

2.3.2 **Peptic ulcer**

Peptic ulcer is the most common upper GI acid-related disorder of the digestive system, affecting millions of individuals worldwide. It develops in the stomach or upper section of the small intestine. In the lower end of the esophagus where stomach fluids commonly reflux, or less frequently along the smaller curve of the antral end of the stomach, peptic ulcers are more common (Kansara & Sakhreliya, 2013). According to Moses *et al*., (2015), a gastric ulcer is primarily defined by damage to the gastric mucosa in the stomach lining, which causes symptoms such as nausea, vomiting, exhaustion, and weight loss in addition to abdominal discomfort, potential bleeding, chest pain, and other GI symptoms.

2.3.3 **Physiopathology of peptic ulcer**

Anatomo-pathology describes a peptic ulcer, whether it be gastric or duodenal, as a loss of mucosal material that extends to the mucosal muscle layer. A cicatrice is left behind when it

heals. It differs from exulcerations, abrasions, and superficial erosions that do not penetrate the muscle layer and heal without cicatrizing (Xie *et al*., 2022). According to Bonnet & Sultan, (2022), the ulcer is the result of an imbalance between the mucosa's defense mechanisms (mucus, blood flow, endogenous prostaglandins (PGs) secretion, NO, antioxidant and bicarbonate, etc.) and the attack factors (acid, pepsin, *H. pylori*, NSAIDs).

The injury determines the pathophysiology of stomach ulcer formation. Since NSAID usage and/or *H. pylori* infection cause 80 to 90% of stomach ulcers.

The first is *H. pylori*, which colonizes between 45 and 50 percent of the stomach mucosa globally. People get immunized against these bacteria at a young age, particularly in developing nations with a poor socioeconomic position and crowded houses. Gastritis, which is caused by these bacteria, is an inflammatory reaction in the host that results in epithelial response, degeneration, and damage (Bereda, 2022). Pan-gastritis is a typical complication of this illness; it harms antral somatostatin release and causes an increase in gastrin secretion, which in turn drives higher acid production. Depending on the aggressiveness of the bacteria and other host risk factors, not all patients with this infection exhibit symptoms (Annunziata *et al*., 2021).

The second most frequent cause generating stomach ulcers is NSAID medicines. When compared to persons who don't take these drugs, patients who do have a relative chance of getting stomach ulcers. NSAIDs can cause ulceration through a variety of different methods. When exposed to stomach acid, the medications themselves are weak acids. They persist in the epithelial cells and enhance cellular permeability, which causes actual damage to the cells (Sverdén *et al*., 2019). The reduction in prostaglandin production is the main cause of NSAID-induced ulceration. NSAIDs prevent the cyclooxygenase-1 (COX-1) enzyme from increasing prostaglandin synthesis, which in turn promotes the secretion of gastric bicarbonate, the formation of mucus barriers, an increase in mucosal blood flow, and an expedited rate of epithelial cell restitution and repair following damage or cell death (Skogar & Sundbom, 2022). NSAID make the stomach mucosa more susceptible to injury from pepsin and gastric acid. Overall, the gastric blood flow drop and the mild ischemia it induces in the stomach mucosa are what cause the most detrimental physiological damage (Skogar & Sundbom, 2022).

Both drinking alcohol and smoking pose risks. Chronic alcohol use alters stomach mucosal defense mechanisms by inhibiting COX-1 receptor enzymes, which reduces the release of cytoprotective prostaglandin (Razvodovsky, 2006). Alcohol has also been demonstrated to influence the mucosal barrier and histology. Epidermal growth factor levels in the blood

decrease and free radical release increases in the stomach mucosa as a result of cigarette smoking (Chiu *et al*., 2021a).

An unbalanced stomach is brought on by stress. It has an impact on histamine generation, acid production, mucosal integrity, gastric motility, emptying pace, and vascular changes in the stomach wall. Additionally, it lessens the mucosal barrier's resilience and boosts the production of glucocorticoids from pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor (TNF) (Chiu *et al*., 2021b). Coffee and caffeine, which are frequently thought to cause or exacerbate ailments, appear to have fewer negative effects. By skipping meals, gastric acid is allowed to directly interact with the surface of the stomach lining, creating irritation that eventually contributes to gastric ulcers. Abdominal discomfort that worsens with meals is a symptom of gastric ulcers (Alves-Santos *et al*., 2020).

2.3.4 **Models of gastric ulcer**

Different models are employed to assess possible antiulcer medication candidates. However, it has been demonstrated that choosing a suitable model may be challenging because each model has both significant good and bad elements. The study objectives, the hypothesis being tested, or the research issues being addressed by the researcher can all have an effect on the model that is chosen. The model's suitability for the specific kind of peptic ulcer illness under study may also have an impact on the decision to use it (Karunakaran *et al*., 2017).

Peptic ulcers can develop in a variety of animal species as a result of physiological, pharmacological, or surgical procedures. However, the bulk of peptic ulcer research studies employ rats. In experimental testing or assessment of a drug's or agent's antipeptic ulcer action, the primary models used are: (Adinortey *et al*., 2013).

1. NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers.

2. Water-immersion stress or cold-water-restraint or cold-restraint stress

3.. Acetic acid-induced gastric ulcers.

4. Histamine-induced gastric ulcers.

5. Reserpine-induced gastric ulcers.

6. Serotonin-induced gastric ulcers.

7. Pylorus-ligated-induced peptic ulcers

8. Ethanol-induced gastric ulcers rodent model is widely used to study acute gastritis (Cho & Ogle, 1992). Numerous investigations have shown that the morphological and physiological changes in the hemorrhagic and necrotic foci, mucus, and stomach acid of the experimental animals are comparable to those seen in humans (Fu *et al*., 2018; Aziz *et al*., 2019).

Since ethanol stimulates macrophages and increases inflammatory cytokine release (TNF and IL-6) and NF-kB signaling, it can result in ulcerative gastritis (Laloo *et al*., 2013). Ethanol administration caused an accumulation of ROS/RNS, which causes oxidative stress and inflammation in the gastric mucosa and has been linked to the development of gastric lesions (Aziz *et al*., 2019). According to Albaayit *et al*., (2016), ethanol-induced ulcerative gastritis is characterized by inflammation, mucosal ulceration, bleeding, and perforation. Apoptosis is induced by ethanol as well as the hypersecretion of gastric acid, proinflammatory cytokines, and ROS/RNS, which together suppress NO and prostaglandin E2 production and have anti-inflammatory and gastric healing effects (Antonisamy *et al*., 2014).

2.3.5 **Medicinal plants and gastric ulcer**

A frequent digestive system ailment is gastric ulcer. The majority of current treatment plans use herbal medication. However, multiple studies have shown that via diverse methods, herbal medications may successfully heal stomach ulcers in both people and other animal models (Saurabh *et al*., 2021). In people and animal models, studies have shown that the efficacy of herbal medicines is equal to or greater than that of medications like omeprazole or cimetidine, also, herbal medicines have fewer side effects (Gupta *et al*., 2021). Inhibiting stomach acid secretion, H^+/K^+-ATPase activity, increasing mucus formation, and stimulating mucous cell proliferation are only a few of the mechanisms by which herbal medicines treat gastric ulcers. Some herbal medications have antibacterial qualities as well (Gupta *et al*., 2021). Utilizing herbal remedies might be a beneficial solution to heal human stomach ulcers successfully and with few side effects. According to a prior study, taking *Centella asiatica* orally for just three days sped up the healing of ulcers in a dose-dependent manner (Cheng *et al*., 2004). In a study using an acetic acid-induced gastric ulcer model, Dharmani *et al*., (2004) found that oral treatment of *Ocimum sanctum* Linn at a dosage of 100 mg/kg per day for 10 days was equally effective as omeprazole in promoting ulcer healing. Herbal treatments can sometimes be more effective than prescription ones. For instance, a greater curative rate than cimetidine was attained using oral *Alchornea glandulosa* extract at a dosage of 250 mg/kg per day for 14 days (Calvo *et al*., 2007). Additionally, administering a herbal mixture's extract sped up ulcer recovery and decreased recurrence rates (Ling *et al*., 2006). Polyphenols, which are naturally

occurring dietary components essential for a balanced diet and are widely prevalent in vegetables, fruits, and medicinal plants. A growing body of research suggests that dietary polyphenols, which have many biological modes of action, are crucial in the treatment of duodenal and stomach ulcers. The ideal prevention and treatment of peptic ulcers can be achieved by combining the administration of dietary polyphenols in appropriate amounts with conventional therapy (Bi *et al*., 2014).

3. Asteraceae family

The Greek term Aster, which refers to a star in reference to the form of the flower, is where the name Asteraceae originates. The biggest family of flowering plants, often known as the Compositae, is a worldwide family. According to (Singh *et al*., 2016), it is mostly found in temperate, subtropical, or tropical climates, frequently in hilly terrain.

One of the plant kingdom's most widely dispersed families is the Asteraceae. According to Herman & Swelankomo, (2011), it has more than 13 tribes, 1000 genera, and 23,000 different species. The Asteraceae family alone accounts for between 8% and 10% of species compared to the projected total number of plant species in the globe, which is around 298,000 species (Mora *et al*., 2011). There are 408 species and 109 genera in Algeria (Quézel *et al*., 1962). The best illustration of the economic importance of this large family is the sunflower, which is grown for its oil seeds, along with the Jerusalem artichoke, lettuce, chicory, chamomile, and gaillards, among other ornamental plants like dahlias, asters, rudbeck, chrysanthemums, gerberas, and zinnias (Singh *et al*., 2016).

3.1. Achillea genus

One of the most recent evolutionary genera in the widespread Asteraceae family is yarrow (Achillea sp.) (Farajpour *et al*., 2012). In this genus, more than 100 species have been identified (Goli *et al*., 2008). A well-known medicinal plant and perennial herb, yarrow is frequently utilized in folk medicine (Bimbiraitė *et al*., 2008). These plants may also be found in Australia, New Zealand, and North America, however they are native to Europe, north Africa, and western Asia (Gharibi *et al*., 2013).

3.1.1 Traditional use

Since ancient times, the genus Achillea (Compositae or Asteraceae) has been utilized as a source of medicine all across the world. Traditional indications for their usage include GI issues, disorders of the liver and gallbladder, irregular menstruation, cramping, fever, and wound healing (Saeidnia *et al*., 2005). According to Nemeth & Bernath, (2008), it has been approved for internal use to address issues such as loss of appetite and dyspeptic conditions, including

gastric catarrh and spastic pain. Externally, it serves as a compress to treat skin irritations, promote wound healing, and combat bacterial or fungal infections.

3.1.2 **Pharmacological properties**

The aerial parts of different species of the genus Achillea are widely used in folk medicine due to various purposes and biological activities, such as, anti-inflammatory (Boutennoun *et al*., 2017), antimicrobial (Benali *et al*., 2020), antispasmodic (Karamenderes & Apaydin, 2003), antiulcer (Mehlous, 2023), anti-diabetic (Idm'hand *et al*., 2020) and antiradical activities (Bali *et al*., 2015; Benali *et al*., 2020). Furthermore, according to Ghavami *et al*., (2010) and Bali *et al*., (2015), this plant is also utilized to cure malignant cells.

3.2. ***Achillea odorata* L.**

3.2.1 **The botanical aspect of *Achillea odorata* L.**

Achillea odorata L. is a perennial plant, which proliferates in the edges of paths. It has circular stems with a tortuous-knotty stock and raised stems that range in height from 12 to 30 cm. The leaves are tiny, oblong-shaped, and greyish green with 7–15 segments on each side. They are beautifully cut. This yarrow may be identified by its tiny, 3–5 mm capitula. The flowers have short ligules and are dirty-white or slightly yellowish. The flowering is from May to July.

3.2.2 **Geographic distribution**

A.odorata L. is well represented all around the West Mediterranean basin, it native range is France to Spain, Morocco, Algeria (Figure 7) (Bremer, 1993).

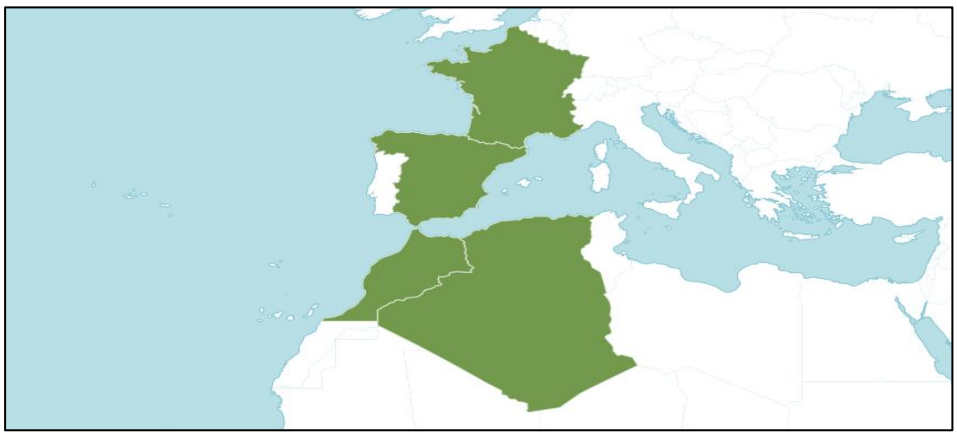


Figure *7*. Geographic distribution of *Achillea odorata* L. (Bremer, 1993).

3.2.3 **Systematics of *Achillea odorata* L. (Guo et al., 2004)**

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Super order: Euasteridae

Order: Asterales

Family: Asteraceae

Genus: Achillea

Species: *Achillea odorata* L.

3.2.4 Chemical composition

The phytochemistry of *A. odorata* L. was the subject of a few investigations that were documented. In fact, many triterpenoids (Achileol A and B) and bisabolane derivatives (different bisabolane derivatives) were isolated from solvent extracts and their structures were clarified (Barrero *et al*., 1990). The presence of 21 and 25 compounds was detected in the essential oil of *A. odorata* L. using gas chromatography-mass spectrometry (GC-MS), and the major compounds of *A. odorata* L. were camphor (45.01%), bornyl acetate (15.07%), borneol (11.33%), β-eudesmol (4.74%), camphene (3.58%), and 1.8-cineole (eucalyptol) (2.96). The chemical composition of the essential oil extracted from aerial portions of *Achillea odorata* L. from Algeria was further examined by GC (retention indices) and 13C-NMR (Bekhechi *et al*., 2011). The three main substances were camphor (22.9–26.3%), 1,8-cineole (15.7–17.8%), and α-pinene (11.3–12.5%) over the complete flowering period. Thymol (2.7-4.5%) and iso-thymol (1.3-2.7%) were also detected at considerable concentrations near the end of flowering (Benali *et al*., 2020).

Materials and methods

1. Materials

All chemicals were of analytical grade and purchased from Sigma (St Louis, MO, USA) or Fluka Chemical Co. (Buchs, Switzerland).

1.1. Plant material

From Jijel (in the northeast of Algeria), the medicinal plant *A. odorata* L. was collected in June 2020. Professor S. AMIRA, Department of Biology and Animal Physiology, University of Ferhat Abbas Setif 1, Algeria, determined the plant's taxonomic identity, and a voucher with the identification number 302 AO 16/06/20 Jij/SA was deposited at the laboratory of Phytotherapy Applied to Chronic Diseases. Debris from the plant material was removed. The plant was dried for approximately 10 days in the shade to ensure its proper preservation. Then, crushed by an electric grinder until obtaining a fine powder and preserved in clean bags.

Figure 7. *Achillea odorata* L. areal parts *(Picture photographed by Pr. Amira., 2019)*

1.2. Animals

In this investigation, albino Swiss mice weighing 25–30 g and albino Wistar rats weighing 150–200 g were employed. From the Pasteur Institute in Kouba, Algiers, animals were purchased. They were initially kept in cages for seven days under standard lab conditions and had free access to food, water for a week. The animals were individually housed in cages with wide-mesh wire bottoms before the experiment began. They were deprived of food for 18 to 20 h, but were given access to drink up until 60 min before the trial began.

2. Methods

2.1. Preparation of extracts

2.1.1 Preparation of decocted extract

The powder was heated to a boil in 500 mL of water for 10 min and allowed to cool. The homogenate was obtained and filtered through muslin after the decoction period. The filtrate obtained was subjected to further filtration through filter paper and dried in an oven at 38°C (Perera *et al*., 2008). The resulting extract was then kept at 4°C in a dark, covered bottle to prevent any molecular breakdown from light.

2.1.2 Preparation of hydro-ethanol, hydro-methanol, hydro-ethyl acetate and hydro-chloroform extracts (50%)

In order to make the extracts of *A. odorata L.*, various solvents with various polarities (methanol, ethanol, ethyl acetate, chloroform) were used (Markham, 1982). For each extract, 50 g of the plant's ground material was macerated for 72 h at room temperature using the greatest agitation in 50% of the suitable solvent (ethanol, methanol, ethyl acetate, chloroform). At a temperature of 40 to 50°C, the mixture is then filtered and evaporated using a rotary evaporator (BÜCHI). Then, this mixture was dried in a 38°C oven. The obtained extracts hydro-methanolic (AME), hydro-ethanolic (AEE), hydro-ethylacetate (AEAE) and hyro-chloroformic (AChE) were then placed in a secured bottle and kept in a cold place at 4°C.

2.2. Screening of phytochemical compounds

2.2.1 Determination of phenolic compounds

The Folin Ciocalteu reagent (FCR) method was used to assess the amount of phenolic compounds in the extract (Li *et al*., 2007). This technique was based on the generation of reduction products with a blue color and a maximum absorption at 765 nm when phosphotungstic and phosphomolybdic reagents of FCR were reduced in an alkaline medium by the oxidizable groups of phenolic compounds. To 100 µL of extract or standard (prepared in methanol or distilled water) with the serial dilution, 500 µL of FCR were added. 400 µL of a sodium carbonate solution with a 75 mg/mL concentration was added to the reaction medium after 4 min. The absorbance at 765 nm is measured after 2 h of incubation at room temperature and in the dark. The regression equation of the calibration line ($Y = 0.0266X + 0.011$, $R^2 = 0.99$) established with gallic acid was used to determine the total polyphenol content, which was reported in mg gallic acid equivalent per milligram of extract (mg GAE/mg extract).

2.2.2 Determination of flavonoids

The flavonoids in the extracts were measured using the aluminum trichloride technique (Bahorun *et al*., 1996). The procedure involves mixing 500 µL of extract or standard with 500

µL of 2% methanol-based $AlCl_3$ solution. The absorbance was measured at 430 nm after 10 min of reaction. The flavonoid concentration was calculated using a calibration line with quercetin and reported in mg of quercetin equivalent per milligram of extract (mg EQ/mg of extract).

2.2.3 Determination of total tannins content

Using FCR and tannic acid as the reference, the total tannins content (TTC) was calculated (NG & Rahate, 2013). 0.5 mL of extract, 2.5 mL of 10% FCR diluted in water, and 2.5 mL of 7.5% Na_2CO_3 were combined to create the reaction mixture. Same procedure was used to prepare a blank without a sample. The samples had been incubated for 45 min at 45°C in a thermostat. Using a spectrophotometer, the absorbance was calculated at λmax = 765 nm. For each analysis, the samples were prepared in triplicate, and the mean absorbance value was calculated. The calibration line was constructed after applying the same procedure to the tannic acid standard solution. The TTC concentration (mg/mL) was derived from the calibration line based on the measured absorbance. Each determination was made three times. In grams of dry extract, the amount of total tannins is represented as mg tannic acid equivalent (TAE).

2.2.4 HPLC-DAD analysis

The phenolic compounds were analyzed using the High-performance liquid chromatography with photodiode-array detection (HPLC-DAD) technique. The obtained extracts were split on an Intertsil ODS-3 reverse phase C18 column after being dissolved in water: methanol (80:20) and filtered through a 0.20 m disposable LC filter disk for HPLC-DAD (Chirinos *et al*., 2008; Barros *et al*., 2009). The injection volume of the sample was 20 µL, and the solvent flow rate was 1.0 mL/min. Mobile phase A was consisting of 0.5% acetic acid in water and mobile phase B was 0.5% acetic acid in methanol. The elution gradient was as follows: 0–10% B (0–0.01 min); 10–20% B (0.01–5 min); 20–30% B (5–15 min); 30–50% B (15–25 min); 50–65% B (25–30 min); 65–75% B (30–40 min); 75–90% B (40–50 min) 90–10% B (50–55 min).

A photodiode array detector (PDA) with the selected wavelength of 280 nm was used for the detection. In order to characterize the phenolic compounds, UV measurements and retention periods were compared to industry standards. The analysis was repeated three times. The calibration curve was created by injecting standard chemicals at specified concentrations (0.0, 0.00782, 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5, and 1.0 ppm) in order to identify and quantify the phenolic compounds. Totally 26 phenolic compounds were used namely, gallic protocatechuic, chlorogenic, p-hydroxy benzoic, vanillic, 3-hydroxy benzoic, syringic, p-

coumaric, ferulic, ellagic, rosmarinic, trans-cinnamic acids, catechin, pyrocatechol, 6,7-dihydroxy coumarin, vanillin, taxifolin, coumarin, rutin, myricetin, quercetin, luteolin, hesperetin, kaempferol, apigenin and chrysin. The results were given in mg per g of dry weight.

2.3. Toxicity

2.3.1 Determination of cytotoxic activity (Cell viability assay)

Lung cancer cell line H1299 was cultured in RPMI1640 medium with 10% FBS (Fetal Bovine Serum). Cultures were kept at 37°C in a humid environment with 5% CO_2. The cytotoxic activity of the extracts was evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) cytotoxicity assay (Deveci *et al*., 2019). 96-well microliter plates were filled with 100 µL of the H1299 cell suspension after it had been diluted with 5-10% FBS to yield 5×10^4 cells at 1 mL. After 24 h, the extracts were added to the cells. After 72 h, 200 µL MTT (0.5 mg/mL) was added in each well and incubated 4 h. After incubation, 100 µL DMSO was added to each well to dissolve formazan crystals. The results of the MTT assay were read at 540 nm. Cell viability (absorbance) for the control group was taken to be 100%, and cell viability for the extracts was calculated.

2.3.2 Acute oral toxicity

Rats were used to test the acute oral toxicity of *A. odorata* extracts according to the Organization for Economic Cooperation and Development's guideline 425 (OECD, 2008). Group I served as the control group, receiving only distilled water, while groups II, III, and IV were given oral doses of *A. odorata* L. decocted extract (ADE) and *A. odorata* L. hydro-methanolic extract (AME) of 2.0 and 5.0 g/kg body weight, respectively (B.W.). One animal from each group received the corresponding plant extract by gavage in a single dose. The same dose was sequentially given to four additional animals, for a total of five treated animals in each group, at intervals of 48 h. After administration, the animals were not fed for 3h. Mortality, severe behavioral and clinical symptoms (such as unusual aggression, restlessness, dullness, sedation, somnolence, twitch, tremor, agitation, catatonia, paralysis, convulsion, writhing, prostration, etc.) were noted over the course of 24 h at regular intervals. All of the experimental animals were kept under close observation for 14 days if they lived, and the number of rats that passed away during the research period was recorded. Rats were sacrificed after 14 days, their blood was drawn and tested for urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), and two organs (kidney and liver) were taken out for gross pathological analysis. If three or more rats survived, it was predicted that the LD_{50} would be higher than the tested dose (2 g/kg or 5 g/kg).

2.3.3 **Preliminary selection of tested doses for biological activities *in vivo***

To determine the most effective dose for biological activities, various doses of ADE and AME extracts from *A. odorata* L. plant were tested on mice and rats. The tested extracts had a notable impact starting at a dose of 100 mg/kg and a good impact at a dose of 400 mg/kg.

2.4. ***In vitro* biological activities**

2.4.1 **Evaluation of the antioxidant activity**

2.4.1.1 DPPH free radical-scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Blois, 1958) was used to spectrophotometrically measure the free radical scavenging activity of extracts. This test was designed to measure the antioxidants capacity to reduce the DPPH radical. Briefly, 160 µL of DPPH solution was combined with 40 µL of sample solutions at various concentrations. A 96-well microplate reader was used to measure the absorbance at 517 nm after 30 min in the dark. Methanol and distillate water were used as a standard. As a synthetic reference, butylated hydroxyanisole (BHA), a stable antioxidant, was used. The following equation was used to calculate the scavenging capacity of DPPH radical.

$$\text{DPPH scavenging effect (\%)} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100$$

Where $A_{control}$ represents the absorbance of the reaction mixture in the absence of sample, and A_{sample} represents the absorbance of the reaction mixture in the presence of extract or standard.

2.4.1.2 ABTS radical scavenging activity

Using the 2.2´-Azino-bis-(3-ethylbenzenothiazoline6-sulfonic acid (ABTS) radical, the extract's antiradical activity was assessed utilizing the Sha & Schacht (1999) method. In a brief, 2.45 mM of $K_2S_2O_8$ was added to react 2 mM of ABTS to generate ABTS cation. The mixture was then kept at room temperature for 16 h in complete darkness. Prior to use, distilled water was used to dilute the ABTS solution to an absorbance of 0.700 at 734 nm. After mixing 40 µL of each sample at various concentrations with 160 µL of the ABTS solution for 10 min, the absorbance at 734 nm was measured. The following equation was used to calculate the percentage of inhibition as previously reported. Results were contrasted with the conventional BHA.

$$\text{ABTS scavenging effect (\%)} = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100$$

2.4.1.3 β-Carotene/linoleic acid assay

Using the β-carotene/linoleic acid test system, the inhibitory activity of lipid peroxidation was evaluated (Ouknin *et al*., 2021). The following steps were used to make a stock solution of a β-carotene/linoleic acid mixture: 200 mg of Tween 40 emulsifier mixture and 25 µL of linoleic acid were added after 0.5 mg of beta-carotene had been dissolved in 1 mL of chloroform. Following the vacuum-assisted evaporation of the chloroform, 100 mL of distilled water that had been saturated with oxygen was added. 160 µL of this mixture was transferred into 40 µL of the samples at different concentrations. The emulsion was quickly added to each tube, and a 96-well microplate reader was used to measure the zero-time absorbance at 470 nm absorbance of the emulsion was read again at the same wavelength after incubation of the plate for 2 h at 50°C. Ethanol was used as a control. BHA and α-tocopherol were used as antioxidant standards for comparison of the activity. The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \ln (A_0/ A_t)/t.$$

where A_0=absorbance at time zero, A_t =absorbance at time t (120 min). Antioxidant activity was calculated in terms of percentage inhibition relative to the control, using the equation:

$$\text{Antioxidant activity (\%)} = [(R_{control} - R_{sample})/R_{control}] \times 100$$

2.4.1.4 Ferrous ion chelating activity

With a few minor modifications, the chelating activity of the extracts on Fe^{2+} was assessed as described by Çayan (2019). 40 µL (0.2 mM) of $FeCl_2$ was added to the extract solution (80 µL dissolved in ethanol at various concentrations). Then 80 µL of ferrozine at 0.5 mM was added. After giving the mixture a vigorous shake, it was allowed to stand at room temperature for 10 min. At 593 nm, the absorbance was then measured. The metal chelating activity was calculated using the following. To compare the activity, Ethylenediaminetetraacetic acid (EDTA) was used as a antioxidant standard.

$$\text{Metal chelating activity (\%)} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

where $A_{Control}$ is the absorbance of control devoid of sample and A_{Sample} is the absorbance of sample in the presence of the chelator.

2.4.1.5 Reducing power of $FeCl_3$

A. odorata's potential reducing power capacity was calculated using the protocol described by Oyaizu (1986). Following the addition of 50 µL of potassium ferricyanide (1%), a total of

40 µL of phosphate buffer (pH = 6.6; 0.2 M) was added to 10 µL of sample solution (extract or standard) in methanol. At 50°C, the mixture was incubated for 20 min. The absorbance was then measured at 700 nm after the addition of 50 µL of trichloroacetic acid solution (TCA) (10%), 40 µL of distilled water, and 10 µL of ferric chloride solution (0.1%). The results were presented as $A_{0.5}$ (µg/ml) corresponding to the concentration indicating 50% absorbance intensity of the activity of various extracts was compared with that of α-tocopherol.

2.4.1.6 Cupric-reducing antioxidant capacity

The cupric-reducing antioxidant capacity was determined according to the method of Apak *et al.* (2004). In a brief, a 96-well microplate was loaded with 50 µL of Cu (10 mM), 50 µL of neocuproine (7.5 mM), and 60 µL of NH_4 buffer (1 M, pH 7.0) solutions. The initial mixture (making the final volume 200 µL) was added to 40 µL of the extract at various concentrations. Using a 96-well microplate reader, the absorbance was measured at 450 nm after 1 h. Results were presented as $A_{0.5}$ (µg/ml) and compared with those obtained using BHA as antioxidant standard.

2.4.1.7 Phenanthroline assay

We followed the procedure outlined by Szydowska-Czerniak *et al*. (2008) to evaluate the phenanthroline antioxidant assay. A total of 50 µL of $FeCl_3$ (0.2%) was mixed with 10 µL of various dilutions of sample solutions (extract or standard) in methanol, followed by the addition of 30 µL of O-phenanthroline (0.5%) and adjusted with 110 µL of methanol. After 20 min of incubation at 30°C, the absorbance was measured at 510 nm. The obtained results were expressed as $A_{0.50}$ values and compared with the used antioxidant positive control (BHA).

2.4.2 Evaluation of the anti-enzymatic activity

2.4.2.1 Cholinesterase inhibition

The ability of extracts to inhibit BChE and AChE was assessed using Ellman's Method, with a few minor modifications (Ellman *et al*., 1961). Next, acetylthiocholine iodide (0.71 mM) and butyrylthiocholine chloride (0.2 mM) were used as the substrates for the AChE and BChE assays in 96-well microplates. 10 mL of the sample were combined with 20 µL of either the AChE or BChE enzymes in a 96-well plate that was filled with 150 µL of sodium phosphate buffer (pH = 8). Following a 15 min incubation period at 25°C, 10 µL of substrates and 10 µL of 5, 5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (0.5 mM) were added to the mixture, bringing the total volume to 200 µL. After 10 min, the absorbance was measured at 412 nm. The formula below was used to calculate the percentage inhibition of AChE or BChE:

$$\% \text{ inhibitory activity} = (A_{\text{enzyme without sample}} - A_{\text{enzyme with sample}}) \times 100 / A_{\text{enzyme without sample}}$$

2.4.2.2 Tyrosinase Inhibition

Tyrosinase enzyme inhibitory activity was measured by the spectrophotometric method as described by Masuda *et al*. (2005). Mushroom tyrosinase was used, while L-DOPA was employed as substrates of the reaction. Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 6.8), 10 µL of sample solution dissolved in ethanol at different concentrations, and 20 µL tyrosinase enzyme solution in buffer were mixed and incubated for 10 min at 37°C, and 20 µL L-DOPA was added. The sample and blank absorbances were read at 475 nm after 10 min incubation at 37°C in a 96-well microplate. The tyrosinase inhibition ability was evaluated relative to the control using the following equation:

$$\text{Tyrosinase inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control} \times 100]$$

2.4.2.3 Determination of α-amylase inhibitory activity

The method previously described by Quan *et al*. (2019) was used to test the extract's ability to inhibit the activity of the enzyme α-amylase, with a few minor adjustments to the incubation time, reagents, and sample amounts. A 96-well microplate was filled with a mixture of 25 µL sample solution and 50 µL α-amylase solution (0.1 units/mL) in phosphate buffer (20 mM pH=6.9 phosphate buffer prepared with 6 mM NaCl). The mixture underwent a 10 min pre-incubation at 37°C. After pre-incubation, 50 µL of 0.05% starch solution was added, and the mixture was then incubated at 37°C for 10 min. A volume of 100 µL of Lugol solutions and 25 µL of 0.1 M HCl were added to finish the reaction. The absorbance at 565 nm was measured using a 96-well microplate reader. The industry standard was acarbose. The activity of α-amylase inhibition was assessed in comparison to a blank that contained an enzyme-free sample.

$$\text{α-Amylase inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control} \times 100]$$

2.4.2.4 Determination of α-glucosidase inhibitory activity

The method first described by Kim *et al.* (2000) was used to determine the extracts α-glucosidase inhibitory activity, with a few minor adjustments to the incubation time, reagents, and quantities of the used reagents and samples. A 96-well microplate was filled with α-glucosidase (0.1 units/mL) in phosphate buffer (0.01 M, pH=6.0), 25 µL PNPG (4-N-nitrophenyl-a-D-glucopyranoside) in phosphate buffer (0.01 M, pH=6.9), 10 µL sample solution, and 50 µL of phosphate buffer (0.01 M, pH=6.9). At 37°C, the mixture was incubated for 20 min. To stop the reaction, 90 µL of sodium carbonate (0.1 M) was added to the microplate. On a 96-well microplate reader, absorbance at 400 nm was measured. The industry

standard was acarbose. The following equation is how the activity of α-glucosidase inhibition was determined.

$$\alpha\text{-Glucosidase inhibition activity (\%)} = [(A_{control} - A_{sample}) / A_{control} \times 100].$$

2.5. *In vivo* biological activities

2.5.1 Gastric emptying and small intestine transit in mice

Measurements of gastric emptying and small intestine transit were made using the technique outlined by Amira *et al*. (2005). The test meal was made up of 1.5% carboxymethyl cellulose (CMC), a non-nutritive material that could be easily seen and detected, and 0.1% phenol red. The test meal was given orally to the animals for 0.3 mL, and they were killed 20 min later. Under a laparatomy, the stomach and the small intestine were excised after ligation of the pylorus and the cardia. In 25 mL of 0.1 N NaOH, the stomach's contents were homogenized. In order to precipitate proteins, 8 mL of the homogenate's supernatant was added to 1 mL of 33% TCA after the mixture had been allowed to sit at room temperature for 1h. The supernatant was then treated with 2 mL of 2 N NaOH following centrifugation (1600 g for 30 min). After homogenizing the mixture, the absorbance was measured at 560 nm. 4 animals were sacrificed on the day of each experiment immediately following the administration of the test meal and were used as standards (0% emptying). The following formula was used to calculate the gastric emptying (GE) rate over the course of the 20-min interval:

$$GE\ (\%) = (A_{standard} - A_{test} / A_{standard}) \times 100$$

The entire small intestine was greatly freed from its mesenteric attachments after the stomach was removed, and its length was measured. A drop of 0.1 N NaOH was used to precisely localize the test meal after the intestine was opened at the level of the test meal's front. The ratio of the distance covered by the test meal to the length of the entire small intestine was used to express the rate of intestinal transit.

In order to investigate the possible interference of the extracts with the nervous transmission controlling GI motility, gastric emptying and intestinal transit of the meal were evaluated in animals treated with one of the following drugs: atropine (3.45×10^{-3}mmol/kg), L-Arginine (1.72 mmol/kg) or indomethacin (1.39×10^{-2} mmol/kg).

2.5.2 Ethanol-induced gastric ulceration in rats

2.5.2.1 Gastroprotective effect evaluation

The method followed is that described by Gharzouli *et al*. (1999). It involves evaluating how well ADE and AME protect against the ulcer that pure ethanol administration causes in animals.

One hour after the administration of test solutions, oral administration of ethanol is used to induce gastric lesions. One hour prior to the 0.5 mL ethanol gavage, plant extracts at doses of 100, 200, and 400 mg/kg (dissolved in NaCl 0.9%) were given orally. In addition to the oral administration of ethanol, the control group also received NaCl 0.9% by the same route, while the positive control also received ranitidine 5 mg/kg (Gupta *et al*,. 2012). Rats were killed by cervical dislocation 30 min later. Following a laparotomy, the stomach was removed, cut open along the great curvature, and spread out on a plate to determine the surface of the lesions using the program Image J 1.52o (Wayne Rasband, National Institutes of Health, USA). The percent of the ulceration was calculated for each group treated according to this formula:

$$\% \text{ Ulceration} = [U_{As}/ U_{Ac}] \times 100.$$

Where: U_{Ac}: ulcer area of the control. U_{As}: ulcer area of the Sample.

To assess the severity of the ulcers, histological analyses of the glandular gastric mucosa were carried out. In addition to determining the amount of mucus in the gastric layer, antioxidant activity was also assessed using the following biochemical indicators: estimation MDA, GSH, CAT, SOD, and total proteins.

Using the chosen dose (200 mg/kg) in combination with indomethacin (1.39×10^{-2} mmol/kg), L-Arginine (1.72 mmol/kg), L-NNA (1.36×10^{-1} mmol/kg) or yohimbine (1.28×10^{-2} mmol/kg), additional experiments were conducted to examine any potential interference between the extract and the neurotransmitters that regulate the GI protective effect. Yohimbine, L-NNA, and indomethacin were intraperitoneally injected, while arginine was given intragastrically. % ulceration, histological analyzes of the glandular gastric mucosa were determined.

2.5.2.2 Histopathological preparations

The histological sections were made at the pathology anatomy laboratory (CHU of Setif). Each experimental group had a piece of their stomach's glandular region fixed in 10% formalin. By passing these samples through three ethanol baths of 30 min each at 70, 90, and 100°C in succession, these samples are dehydrated. Then they are diluted in two toluene baths of 20 min each and added to the paraffin (two baths of 2 h each). The operation is automated using an automaton (TISSUE-TEK). After that, the final inclusion is created in metal molds. The obtained paraffin blocks are then cut with a microtome, the 5 µm thick sections are spread on slides with a 2% gelatin gel, dried in an oven at a temperature of 35-42°C, rehydrated and dried, and stained with hematoxylin-eosin. A pathologist examined the tissue samples using microscopic analysis. The slides were later captured on camera.

2.5.2.3 Determination of mucus content in gastric layer

Alcian blue was used to determine the stomach's mucus content, as described by Corne (1974). Without entering the mucosal cells, this cationic colorant binds to glycoproteins and changes the mucopolysaccharides into an insoluble complex. Each section of the stomach's glandular portion is submerged in a solution containing 0.1% alcian blue. The excess of the colorant is removed by two successive washes with sucrose for 10 min and 45 min, respectively, after 2h of incubation. The stained glandular component is then added to magnesium chloride and stirred for 2h. To make sure that no particles impacted the spectrophotometric reading at 605 nm, a volume of the blue extract was combined with an equal volume of di-ethyl ether. A calibration line made from various alcian blue concentrations was used to determine the total amount of mucus present. Concentrations are expressed as µg of alcian blue/g of tissue.

2.5.3 Evaluation of *in vivo* antioxidant activity of gastric homogenate

2.5.3.1 Preparation of homogenate

After the sacrifice, the stomach from each animal was removed, opened along the great curvature, washed with ice-cold saline, blotted with filter paper, and the glandular portion was cut, weighed, and homogenized in an ice-cold solution of 50 mM Tris HCl buffer (pH 7.4) to produce 10% (w/v) homogenate. The homogenate was then centrifuged at 4000 g for 15 min at 4 °C, and the supernatant was collected and stored at - 20 °C for the estimation of the following biochemical parameters: total proteins, CAT, GSH, MDA, SOD.

2.5.3.2 Determination of total gastric proteins

Using a Biuret reagent kit (potassium iodide, sodium potassium tartrate, copper sulfate and sodium hydroxyl), the total amount of gastric protein was measured in accordance with the procedure described by Gornall *et al*. (1949). The procedure is as follows: 1 mL of Biuret reagent is added to 25 µL of tissue homogenate or standard. The mixture is incubated for 10 min at room temperature in the dark, and then the absorbance is measured at 540 nm. The following equation is used to calculate the total protein concentration:

Total protein (mg/mL) = $(A_{assay}/A_{standard})$ x n (n :concentration of standard)

2.5.3.3 Assessment of reduced glutathione concentration

The GSH content was measured following the method of Ellman (1959), which measures the reduction of DTNB (Ellman's reagent) by sulfhydryl groups to 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color and maximum absorption at 412 nm. In a brief, 10 mL of phosphate buffer were used to dilute 50 µL of supernatant (0.1 M, pH 8). Then, 20 µL of

DTNB (0.01 M) were added to 3 mL of the dilution mixture. After 5 min, the yellow color developed was measured at 412 nm. Utilizing the molecular absorption coefficient (NBT: $13.6.10^3$ $M^{-1}.cm^{-1}$), the amount of GSH was calculated. The results are given in nmole GSH/g tissue.

2.5.3.4 Assessment of lipid peroxidation

The method of assessment of lipid peroxidation (MDA) of Ohkawa *et al.* (1979), which is based on spectrophotometric measurement of the pink color produced by the reaction of thiobarbituric acid (TBA) with MDA, was used to measure the MDA levels. A volume of 1 mL of TBA (0.67% w/v) and 0.5 mL of tissue homogenate were combined. The mixture was then heated to a boil for 15 min in a water bath. Each sample was mixed with 4 mL of n-butanol and allowed to cool to room temperature before being centrifuged for 15 min at 3000 rpm. At 532 nm, the absorbance was calculated in relation to a blank solution. The molecular absorption coefficient was used to determine the MDA concentration (ε MDA-TBA: 156 $mM^{-1}.cm^{-1}$). The results are given in nmole MDA/g tissue.

2.5.3.5 Estimation of catalase activity

With some modifications, the method outlined by Clairbone (1985) was used to measure the catalase activity. The idea behind this test is based on how hydrogen peroxide breaks down when catalase is present. In a quartz vessel, 19 mM H_2O_2 (2.9 mL) in phosphate buffer (50 mM, pH 7.4) was added, followed by 50 µL of tissue homogenate. The enzymatic activity was expressed in nmole H_2O_2 /min/ mg, and the rate of H_2O_2 decomposition in the presence of CAT is measured spectrophotometrically at 240 nm immediately and every 15 seconds for 1 min.

3. Statistical analysis

All samples were tested three times (*in vitro*), whereas for *in vivo* tests a number of 6 to 9 animals was used. Results are presented as mean ± SD or SEM for the *in vitro* or *in vivo* tests respectively and analyzed by a one-way analysis of variance (ANOVA) followed by a Tukey test using GraphPad Prism version 8.0.2 (GraphPad Software, Inc., USA). P values <0.05 or less were considered significantly different.

Results

1. Phytochemical study of the extracts

1.1. Extraction yield

Extraction yields obtained for the various extracts of *A. odorata* L. are shown in figure 9. AME extract demonstrated the most significant yield of 16.6%, then ADE (16.1%) and AEE (14.9%). While AEEA and AChE showed the lower extraction yield.

When combined, the yield using polar solvents (AME, ADE and AEE) was 45.6%, while the yield from semi-polar and nonpolar solvents (AEAE and AChE) was only 12.2%, suggesting that *A. odorata* L. extracts had more polar components.

1.2. Total polyphenols, flavonoids and tannins content

In the present study, the contents of total phenolic, flavonoids, and tannins in extracts of *A. odorata* L. were quantified. The obtained results are summarized in figure 9. The methanol extract recorded the highest total phenolic content with a value of 424.94 ± 0.58 mg GAE/g DW followed by the ADE and AEE with a value of 372.88 ± 1.91 and 349 ± 0.03 mg GAE/g DW, respectively. Whereas, the ethyl acetate and chloroform extracts gave the lowest value (113.78 ± 0.33 and 97.01 ± 0.14 mg GAE/g DE, respectively).

Furthermore, the AEE showed the highest levels of flavonoids and tannins (154.71 ± 0.35 mg QE/g DW and 72.62 ± 0.30 mg TAE/g DW, respectively) followed by the AME (98.33 ± 0.85 mg QE/g DW and 47.15 ± 0.32 mg TAE/g DW, respectively), then ADE with value close to the latter extract (86.01 ± 1.02 mg QE/g DW and 34.87 ± 0.93 mg TAE/g DW, respectively). Moreover, AEAE and AChE showed the smallest amount in flavonoids and tannins as shown in figure 9.

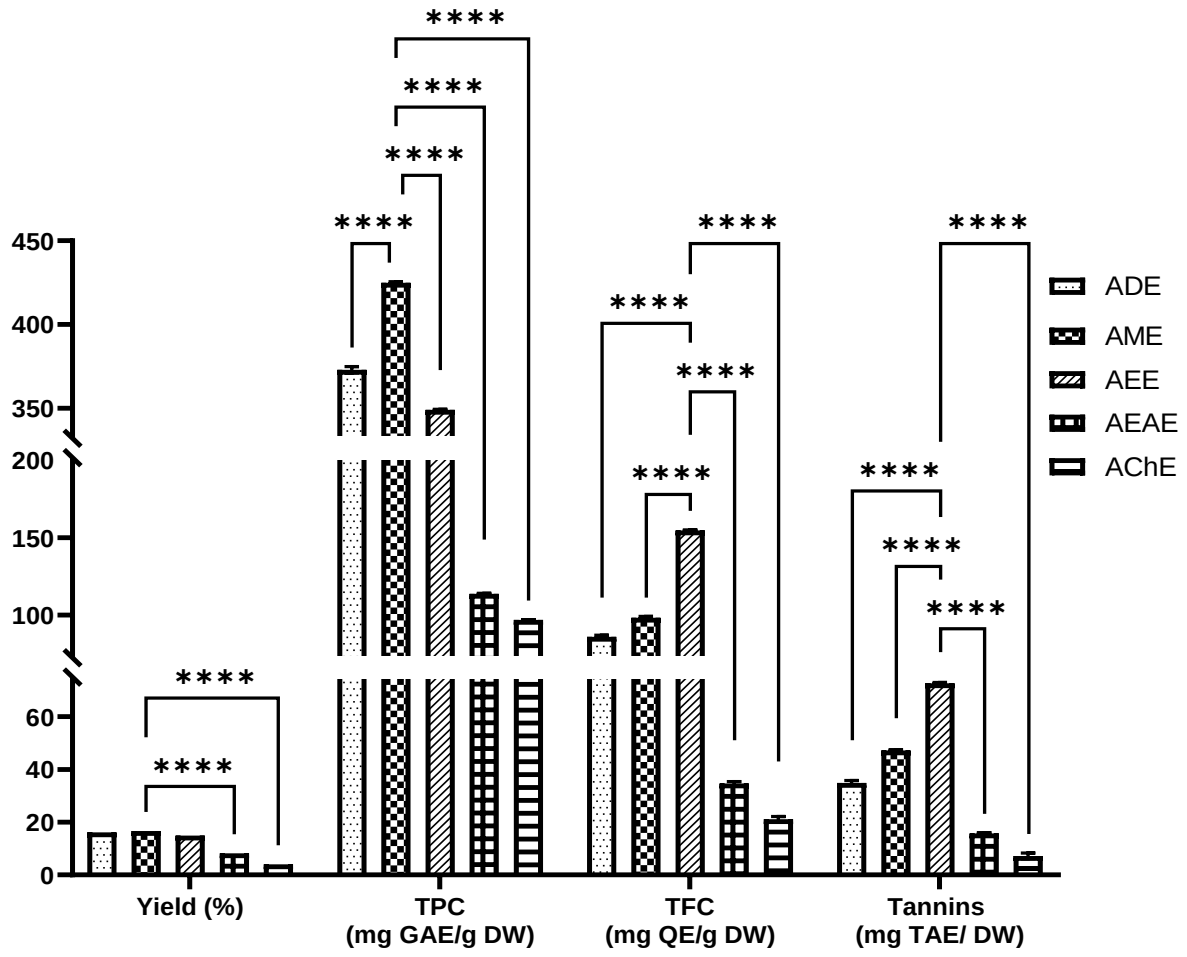


Figure 8. Yield extraction, total phenolics, flavonoids and tannins contents of *A. odorata*'s extract.

ADE: decocted extract, AME: hydro-methanol extract, AEE: hydro-ethanol extract, AEAE: hydro-ethyl acetate extract, AChE: hydro-chloroform extract, GAE: gallic acid equivalent, QE: quercetin equivalent, TAE: tannic acid equivalent, DW: dry weight. Results are expressed as means ± SD (n=3). ****; P≤0.0001 *vs* AME or AEE.

1.3. HPLC-DAD analysis

A comparative investigation was conducted on the various extracts derived from the aerial parts of *A. odorata* L. using HPLC-DAD based on retention time and absorption spectra of the available standards in the sample. As depicted in table 1 provides a comprehensive list of all the identified compounds of the extracts used along with essential data such as their retention time (Rt). 13, 12, 9, 10 and 9 phenolic compounds were characterized from AME, ADE, AEE, AEAE and AChE.

A total of 07 phenolic compounds was commonly and majorly identified in all extracts tested (Figure10), namely chlorogenic acid (Rt: 12.35 min); p-hydroxy benzoic acid (Rt: 12.77 min); rutin (Rt: 25.30 min); ellagic acid (Rt: 26.11 min); rosmarinic acid (Rt: 26.77 min); myricetin

(Rt: 27.35 min) and luteolin (Rt: 31.70 min). Chlorogenic acid was identified as the major compounds in all tested extracts. Also, ellagic acid and luteolin were identified among main compounds of ADE and AEAE while coumarin was identified as a major compound in AME. Moreover, kaempferol and apigenin were identified only in ADE and AEAE at the retention time of 33.21 and 33.77 min, respectively while coumarin was identified only in ADE, AME, AEE and AChE at the retention time of 24.49 min. Minor marks of gallic acid, pyrocatechol and p-coumaric acid were elected only with AME at time of retention of 5.7; 11.04 and 20.56 min.

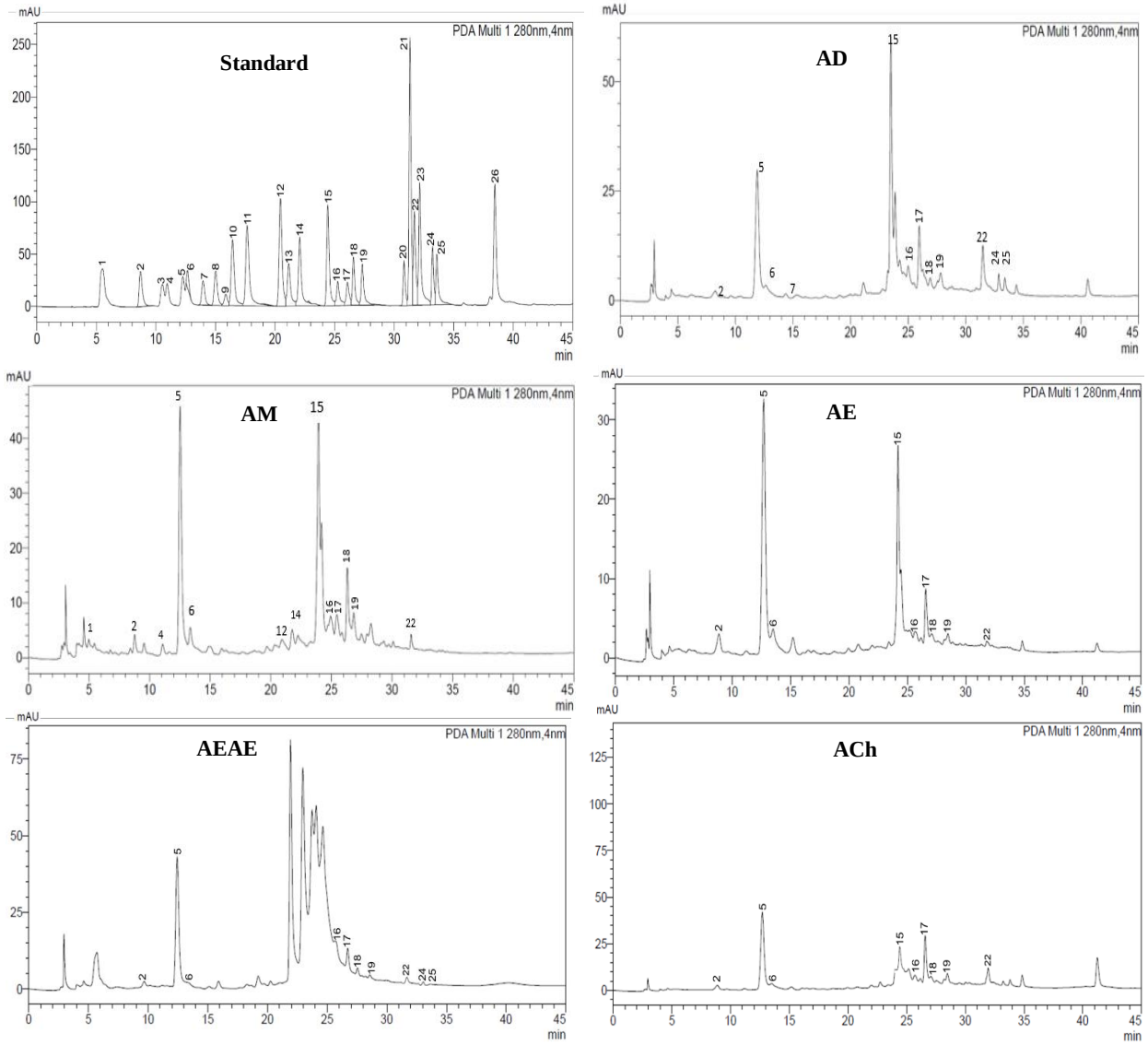


Figure 9. HPLC-DAD Chromatograms of *A. odorata* L. extracts.

ADE: decocted extract, AME: hydro-methanol extract; AEE: hydro-ethanol extract; AEAE: hydro-ethyl acetate extract and AChE: hydro-chloroform extract.

Table 1. Phenolic composition of ADE; AME; AEE; AEAE; AChE by HPLC-DAD (mg/g).

No	Phenolic compounds	RT (min)	ADE	AME	AEE	AEAE	AChE
1	Gallic acid	5.70	-	0.80±0.05	-	-	-
2	Protocatechuic acid	8.75	2.24±0.02	2.86±0.12	3.92±0.10	3.75±0.08	2.70±0.14
3	Catechin	10.68	-	-	-	-	-
4	Pyrocatechol	11.04	-	1.28±0.07	-	-	-
5	Chlorogenic acid	12.35	33.43±0.18	45.08±0.36	39.75±0.26	56.44±0.48	27.17±0.52
6	p-hydroxy benzoic acid	12.77	1.80±0.06	4.20±0.17	3.17±0.11	2.65±0.14	1.42±0.06
7	6.7-Dihydroxy coumarin	14.10	0.59±0.03	-	-	-	-
8	Caffeic acid	15.09	-	-	-	-	-
9	3- hydroxy benzoic acid	15.98	-	-	-	-	-
10	Syringic acid	16.56	-	-	-	-	-
11	Vanillin	17.78	-	-	-	-	-
12	p-coumaric acid	20.56	-	0.63±0.05	-	-	-
13	Taxifolin	21.26	-	-	-	-	-
14	Ferulic acid	22.14	-	3.45±0.13	-	-	-
15	Coumarin	24.49	7.95±0.08	12.84±0.21	5.79±0.31	-	3.96±0.27

16	Rutin	25.30	6.12±0.05	6.35±0.09	2.85±0.09	4.20±0.10	1.56±0.11
17	Ellagic acid	26.11	12.35±0.14	9.75±0.11	6.12±0.05	14.95±0.17	5.13±0.16
18	Rosmarinic acid	26.77	3.50±0.06	3.70±0.04	2.84±0.13	3.71±0.20	2.20±0.05
19	Myricetin	27.35	0.87±0.02	1.12±0.05	0.75±0.01	1.19±0.04	0.59±0.03
20	Quercetin	30.83	-	-	-	-	-
21	trans-cinnamic acid	31.33	-	-	-	-	-
22	Luteolin	31.70	16.58±0.22	2.90±0.02	0.52±0.04	4.98±0.16	0.66±0.02
23	Hesperetin	32.14	-	-	-	-	-
24	Kaempferol	33.21	4.10±0.08	-	-	0.87±0.06	-
25	Apigenin	33.77	3.95±0.03	-	-	0.69±0.03	-
26	Chrysin	38.40	-	-	-	-	-

ADE: decocted extract, AME: hydro-methanol extract; AEE: hydro-ethanol extract; AEAE: hydro-ethyl acetate extract and AChE: hydro-chloroform extract. [a]Values expressed as means ± SEM of three parallel measurements ($P<0.05$). -: not detected.

2. Toxicity

2.1. Determination of cytotoxic activity (Cell viability assay)

Cellular proliferation was assessed using the MTT assay on the lung cancer cell line H1299 for 72 hours. As illustrated in the figure 11, extracts from *A. odorata* L. exhibited varying degrees of activity against the tested cancer cell line, leading to the inhibition of cellular proliferation ranging from good to moderate. AChE displayed the most potent antiproliferative activity, followed by AME, AEAE, AQE, and AEE, in descending order of efficacy.

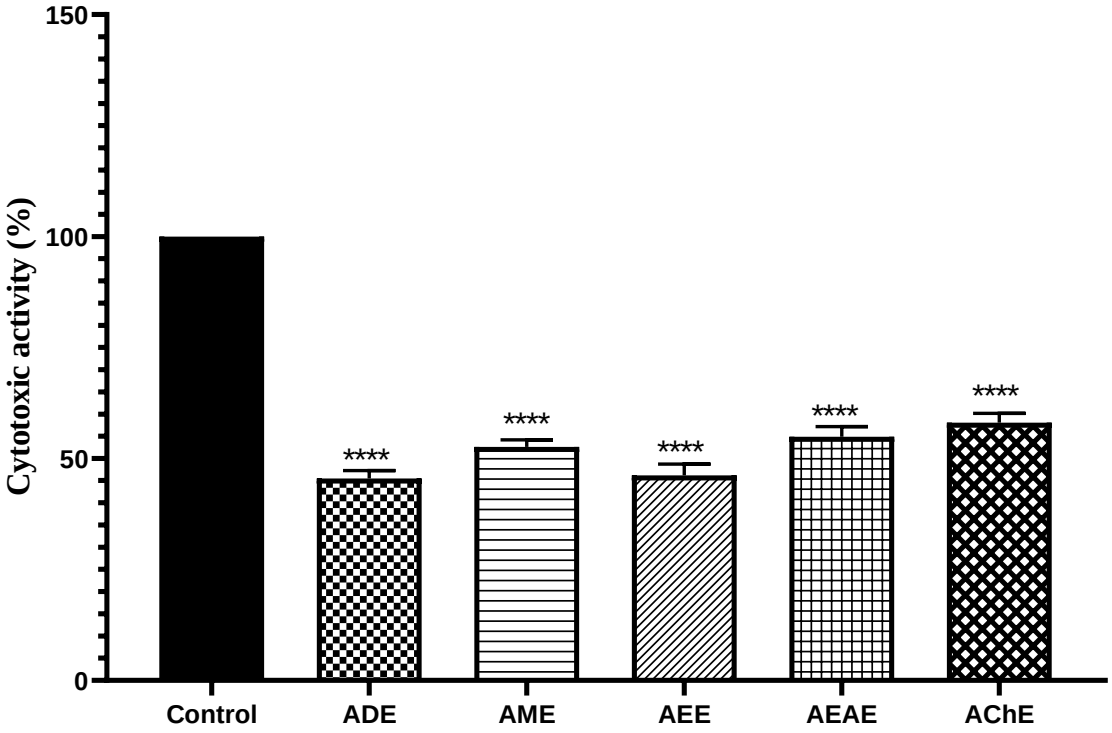


Figure 10. Cytotoxic activities of *A. odorata* L. extracts against lung cancer cell line (HT-29). ADE: decocted extract, AME: hydro-methanol extract; AEE: hydro-ethanol extract; AEAE: hydro-ethyl acetate extract and AChE: hydro-chloroform extract. ****; P≤0.0001 *vs* control.

2.2. Acute oral toxicity

2.2.1 Behavioral observations and mortality patterns

The current research, conducted in accordance with OECD guideline 425, demonstrated that both ADE and AME did not induce any alterations in the behavior of rats over the study duration. Moreover, no instances of mortality were observed during the two-week period, indicating that the LD_{50} exceeds 5000 mg/kg.

2.2.2 **Biochemical parameters**

Statistical analysis of the assessed parameters indicates that there were no significant differences between the treated males and the control group, with the exception of a significant reduction in creatinine levels (P≤0.1) observed in treated rats at a 2000 mg/kg of ADE (Figure 12).

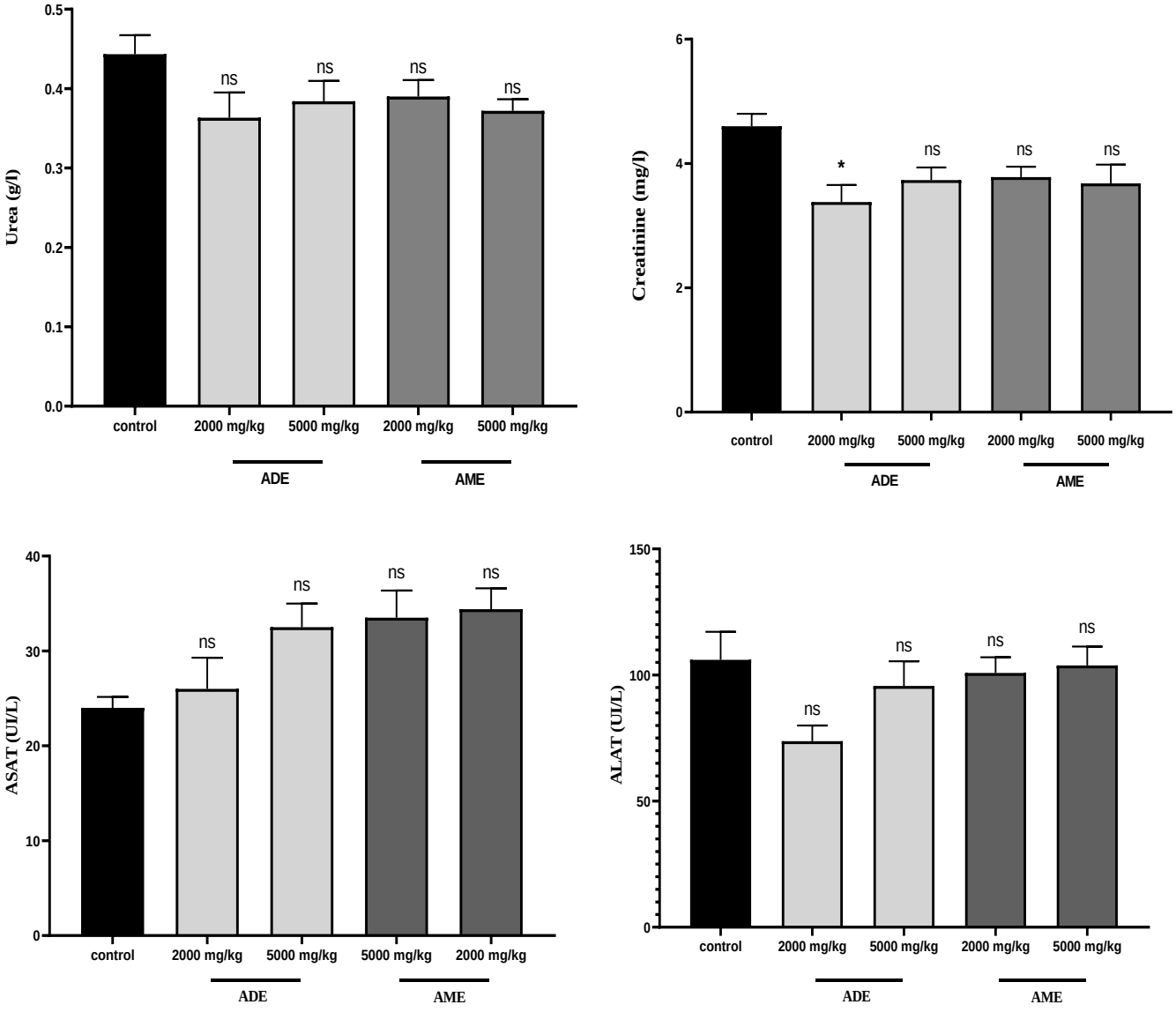
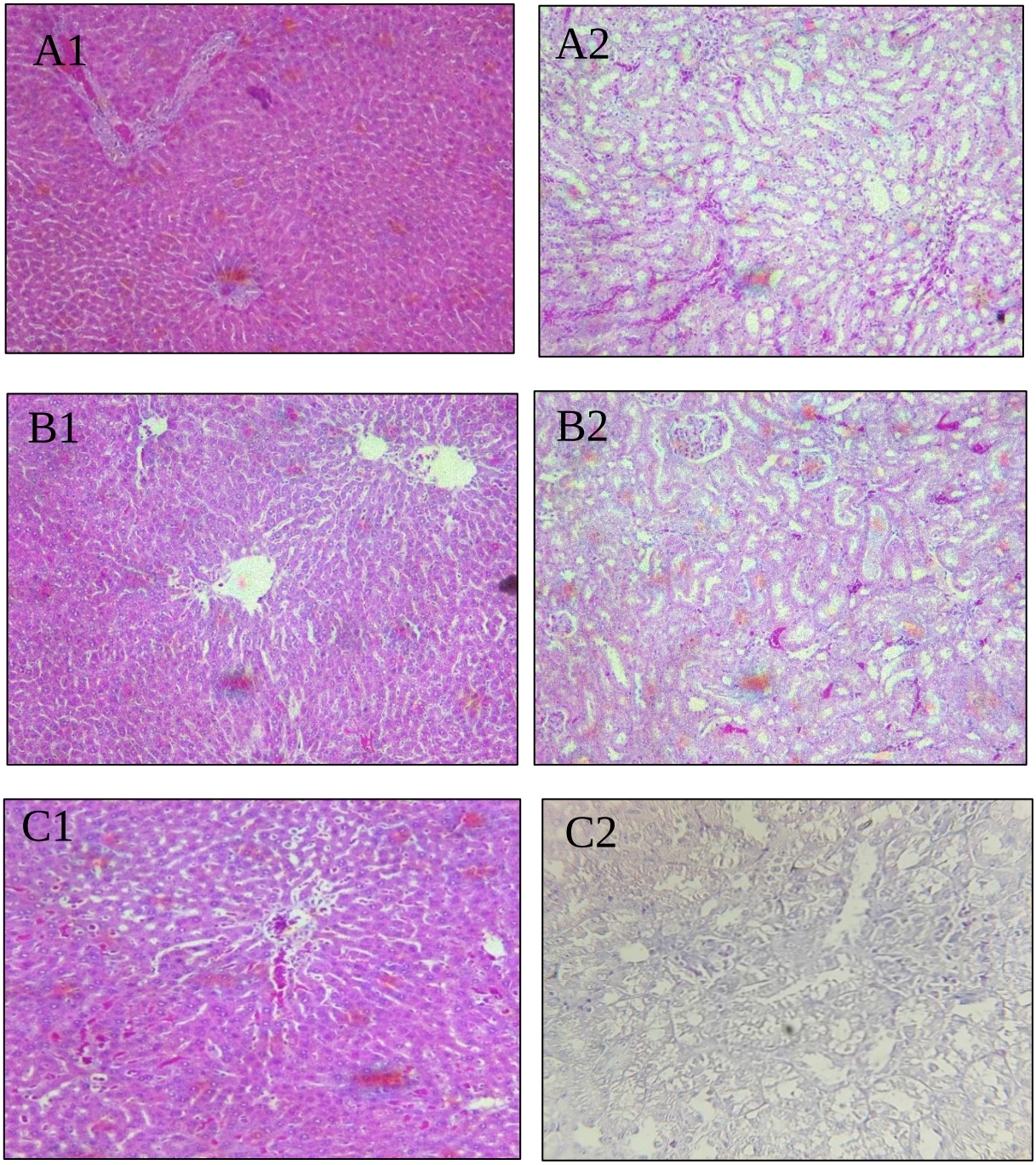


Figure 11. Biochemical parameters of control and rats treated with ADE and AME measured during the acute toxicity.

ADE: decocted extract, AME: hydro-methanol extract. Values were expressed as mean ± SEM (n = 5). *: P<0.05 *vs* control, ns; no significant difference (P>0.05).

2.2.3 **Liver and kidneys histopathology**

The histopathological assessment of the vital organs, specifically the liver and kidney (Figure 13) did not reveal any significant macroscopic or histological alterations in animals administered with ADE and AME at doses of 2000 and 5000 mg/kg. The liver exhibited a regular structural pattern with no signs of lesions or inflammation. The kidney displayed well-preserved glomeruli and normal tubules.



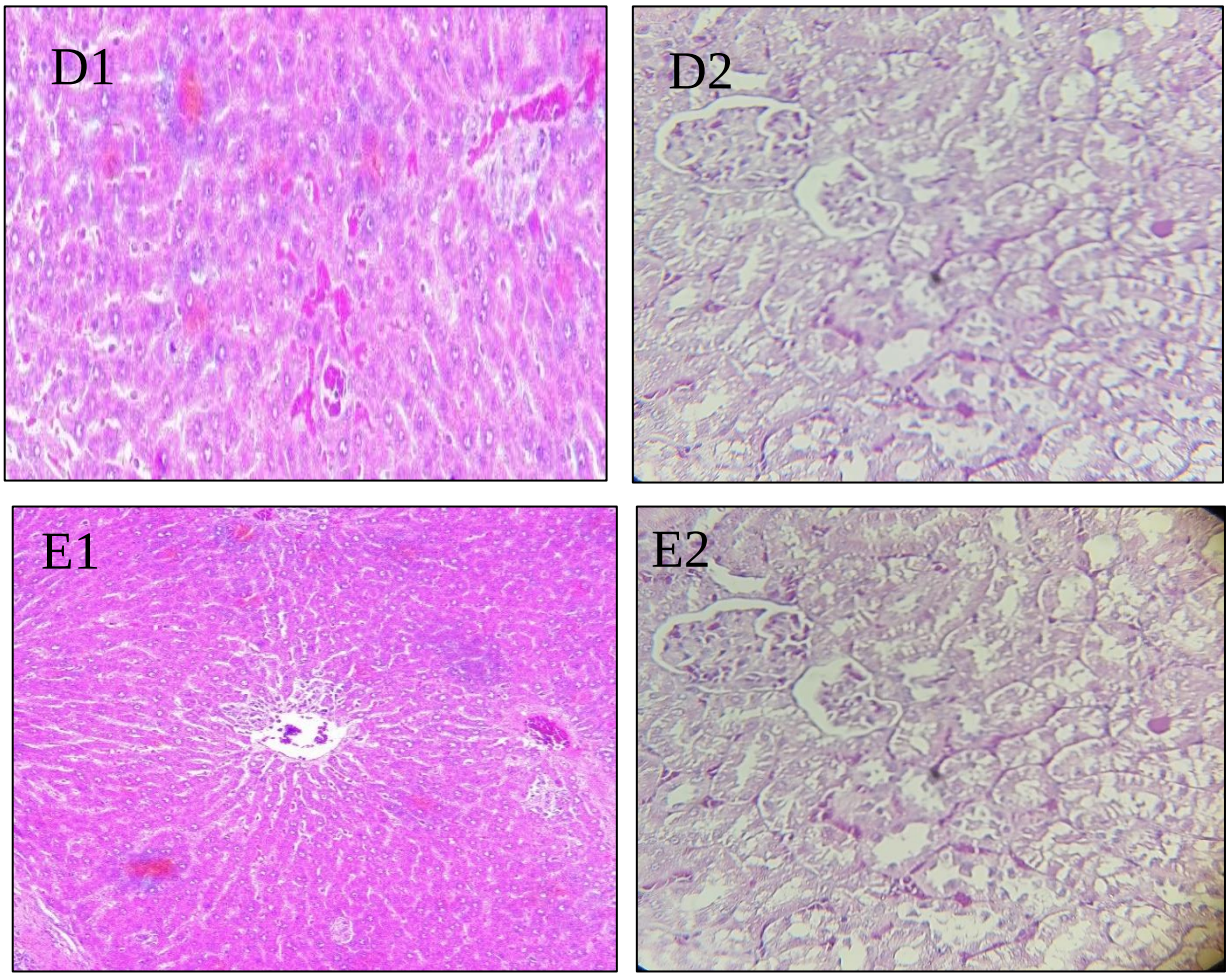


Figure 12. Histopathological analysis of liver and kidney treated with ADE and AME in acute toxicity (x 100).

ADE: decocted extract, AME: hydro-methanol extract. (A1, B1, C1, D1 and E1): liver histopathology of control group, ADE 5g/kg, ADE 2g/kg, AME 5g/kg and AME 2g/kg groups, respectively. (A2, B2, C2, and E2): kidney histopathology of control group, ADE 5g/kg, ADE 2g/kg, AME 5g/kg and AME 2g/kg groups, respectively.

3. *In vitro* biological activities

3.1. Antioxidant activities

3.1.1 DPPH free radical-scavenging assay

The anti-radical activity was assessed using the DPPH radical method, which is frequently employed due to its simplicity. This method is based on the reduction of a methanolic solution of DPPH in the presence of an antioxidant, which donates a hydrogen or an electron to convert DPPH into its non-radical form, DPPH-H. The antioxidant activity of the extracts is expressed as IC_{50} (Table 2). Most of the extracts exhibited very strong anti-radical activity against DPPH, with values ranging from 15.17 ± 0.22 to 31.88 ± 0.71 µg/mL compared to the BHA as standard (19.82 ± 0.33 µg/mL), The majority of the extracts (AME, AEAE and AEE) showed a superior scavenging activity ($P \leq 0.0001$) than BHT.

3.1.2 **ABTS radical scavenging activity**

ABTS assay, one of the well-established methods to measure the antioxidant activity, in terms of hydrogen donation and inhibition of oxidation chain reactions. This test is based on the oxidation of The ABTS radical by peroxyl radicals to form a stable radical that absorbs light between 600 and 750 nm. Absorption decreases in the presence of antioxidants, indicating radical scavenging ABTS. According to the present work, the ability of *A. odorata* L. extracts to scavenge the radical ABTS are shown in table 2. Comparing to standards (α-tocopherol and BHA), all extracts exhibited high antioxidant activity and in the following order: AME (IC_{50} = 13.46 ± 1.158 µg/mL) > AEE (IC_{50} = 15.34 ± 1.16 µg/mL) > ADE (IC_{50} = 16.38 ± 1.153 µg/mL) > AEAE (IC_{50} = 18.84 ± 1.156 µg/mL) > AChE (IC_{50} = 20.25 ± 1.167 µg/mL). The AME and AEE showed a significant activity almost similar to those of BHA (IC_{50}= 13.21 µg/mL; P >0.05).

3.1.3 **β-carotene/linoleic acid assay**

In β-carotene bleaching assay, linoleic acid produces hydrogen peroxide. The bleaching of yellow color of β-carotene is due to peroxide free radicals. The rate of β-carotene bleaching can be delayed with the presence of antioxidants. As the β-carotene bleaching assay employs a model lipid substrate (Linoleic acid), the measuring environment is closer to the real lipid system.

In this assay, all of the extracts, α-tocopherol and BHA were able to prevent degradation of β-carotene during the assay. As shown in table 2. AEAE is the best inhibitor of the oxidation of β-carotene (IC_{50}= 5.97 ± 0.60 µg/mL) followed by AME (8.51 ± 0.16 µg/mL), AEE (11.55 ± 0.95 µg/mL) and AChE (13.80 ± 0.37 µg/mL) and ADE (14.66 ± 0.30 µg/mL). None of the extracts exhibited activity comparable to that of the standard α-tocopherol (P≤0.0001).

3.1.4 **Ferrous ion chelating activity**

In the study of the chelation of metals by antioxidants in the *A. odorata* L. extracts were showed in table 2 compared with EDTA. Extracts show good to moderate metal chelating activity in the following order AME >ADE > AEAE > AEE >AChE. The methanolic extract showed the moderate antioxidant activity with an IC_{50} value of 56.12 ± 0.61 µg/mL. This value found moderate compared to the strong metal chelate standard EDTA (P≤0.0001). The chelating ability of all extracts was lower than that of EDTA (P≤0.0001).

3.1.5 **Reducing power of $FeCl_3$**

Reducing power assays are often used as an indicator of electron-donating activity, which is an important mechanism of antioxidant compounds. Therefore, FRAP and CUPRAC and phenanthroline methods were applied to evaluate reducing power potentials of *A. odorata* L. extracts. Lower $A_{0.5}$ value indicates higher reducing potential.

The ability to convert Fe^{3+} ions into Fe^{2+} ions by the various studied extracts appears to be highly important. It was found that all extracts prepared from *A. odorata* L., demonstrate very potent reducing activities and are ranked in the following ascending order AME ($A_{0.5}$= 9.918 ± 0.019 µg/mL) <ADE ($A_{0.5}$= 19.50 ± 0.83 µg/mL) < AEE ($A_{0.5}$= 20.02 ± 0.40 µg/mL) < AEAE ($A_{0.5}$= 24.38 ± 0.43 µg/mL) < AChE ($A_{0.5}$= 39.61 ± 0.05 µg/mL). AME showed the better reducing power than other extracts while AChE showed the lowest one. Only AME exhibited activity comparable to that of α-tocopherol ($A_{0.5}$= 9.063 ± 0.030 µg/mL, P>0.05) (Table 2).

3.1.6 **Cupric-reducing antioxidant capacity (CUPRAC)**

The CUPRAC test is one of the most commonly used methods to determine antioxidant capacity by reducing Cu^{2+} using bis(neocuproine) to form a stable complex that absorbs at 450 nm. Absorption increases in the presence of antioxidants. The ability of the various extracts to reduce copper ions is shown in Table 2. The enhancement of the reducing effect of various extracts is ranked as follows: AME < AEAE < AEE < ADE < BHA < AChE. Overall, all the extracts exhibit potent antioxidant activity, AME demonstrating the highest activity. Notably, AME, AEAE, AEE, and ADE show potential superiority compared to the standard.

3.1.7 **Phenanthroline assay**

The phenanthroline activity of the extracts was determined and compared to standard (BHA), the results were in the following order of activity; AME > ADE > AEE > AEAE > AChE, the highest reducing power was shown by AME ($A_{0.5=}$ 6.22±0.04) which was clearly the best and quite closer compared with standards (Table 2).

Table 2. Antioxidant activity of the extracts by β-Carotene-linoleic acid, DPPH•, ABTS•, CUPRAC and metal chelating assays.

		Antioxidant Activity						
		β-Carotene-linoleic acid assay	**DPPH• assay**	**ABTS•+ assay**	**Metal chelating assay**	**CUPRAC assay**	**Reducing power assay**	**Phenanthroline assay**
		IC_{50} (µg/mL)	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**	**$A_{0.50}$ (µg/mL)**	**$A_{0.50}$ (µg/mL)**	**$A_{0.50}$ (µg/mL)**
Extracts	**ADE**	14.66±0.30****	31.88±0.71****	16.38±1.153**	68.41±0.89****	22.48±0.93 **	19.50±0.83****	8.50±0.07**
	AME	8.51±0.16****	15.17±0.22****	13.46±1.158 ns	56.12±0.61****	10.28±0.67 ****	9.918±0.019 ns	6.22±0.04*
	AEE	11.55±0.95****	15.78±0.33****	15.34±1.16 ns	107.2±0.81****	22.39±0.76 **	20.02±0.40****	12.16±0.8****
	AEAE	5.97±0.60****	17.31±0.20****	18.84±1.156***	86.28±1.18****	12.27±0.70 ****	24.38±0.43****	24.05±0.29****
	AChE	13.80±0.37****	26.06±0.41****	20.25±1.167****	128.5±0.85****	31.14±0.95 ****	39.61±0.05****	24.75±0.06****
Standards	**α-Tocopherol**	2.25±0.07	NT	35.50±0.56	NT	NT	9.063±0.030	NT
	BHA	1.40±0.03	19.82±0.33	12.47±0.35	NT	25.50±0.43	NT	5.024±0.04
	EDTA	NT	NT	NT	5.60±0.45	NT	NT	NT

Table 2. Antioxidant activity of the extracts by β-carotene-linoleic acid, DPPH, ABTS, CUPRAC and metal chelating assay

[c] NT: not tested.

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).

ADE: decocted extract, AME: hydro-methanol extract; AEE: hydro-ethanol extract; AEAE: hydro-ethyl acetate extract and AChE: hydro-chloroform extract. *; P≤0.1, **; P≤0.01, ****; P≤0.0001 *vs* standards. ns; no significant difference (P>0.05).

3.2. Enzymatic inhibition activities

3.2.1 AChE and BChE inhibition activity

The table 3 illustrates the enzyme inhibitory activities of *A. odorata* L. extracts and standard compounds against AChE and BChE, expressed as the percentage of inhibition. ADE, AME, and AChE exhibited relatively weak anti-AChE and BChE activities compared to galantamine (85.48 ± 0.62% for AChE and 74.63 ± 0.25% for BChE; P≤0.0001). In contrast, AEE and AEAE showed good anti-BChE activity (51.23 ± 0.85% and 53.70 ± 0.49%, respectively) but remained incomparable to the standard (P≤0.0001).

3.2.2 α-amylase and α-glucosidase inhibition activity

The table 3 reveals the α-amylase inhibition activity of *A. odorata* L. extracts. The best α-amylase inhibition activity from *A. odorata* L. was obtained by AEAE (67.11 ± 0.79%), followed by AEE (27.44 ± 0.32%), ACHE (25.66 ± 0.27%), AME (22.71 ± 0.64%), and the lowest one was ADE (15.25 ± 0.33%). All extracts had a significant difference compared to the standard (P≤0.0001).

The α-glucosidase inhibitor effects of the extracts are presented in the table 3. The investigation showed that AEAE had a good α-glucosidase inhibition effect with a percentage of inhibition of 52.18 ± 0.35, followed by a moderate inhibition of AME (37.56 ± 0.76%), AEE (33.56 ± 0.17%), AChE (24.22 ± 0.43%), and ADE (23.17 ± 0.63%). The activity of all extracts was lower than acarbose as a standard (57.70 ± 0.75; P≤0.0001).

3.2.3 Tyrosinase inhibition activity

The inhibitory effects of *A. odorata* L. extracts on tyrosinase activity are presented in table 3 (% of inhibition). The extracts inhibited tyrosinase activity in the following order: AEAE (%=36.70 ± 0.62) < AEE (%= 31.54 ± 0.53) < AME (%=30.71 ± 0.43) < AChE (%=23.46 ± 0.71) < ADE (%=17.85 ± 0.36). All extracts exhibited inhibition lower than the standard, kojic acid (%= 79.50 ± 0.32).

Table 3. Antidiabetic, cholinesterase, and tyrosinase inhibitory activities of the extracts.

	Cholinesterase inhibitory activity		**Anti-diabetic activity**		
	AChE	**BChE**	**α-glucosidase**	**α-amylase**	**Tyrosinase inhibitory**
Extracts/ Standards	**Inhibition (%) (at 100 µg/mL)**	**Inhibition (%) (at 100 µg/mL)**	**Inhibition (%) (at 200 µg/mL)**	**Inhibition (%) (at 200 µg/mL)**	**Inhibition (%) (at 100 µg/mL)**
ADE	39.25±0.75****	31.44±0.17****	23.17±0.63****	15.25±0.33****	17.85±0.36****
AME	46.40±0.33****	43.67±0.54****	37.56±0.76****	22.71±0.64****	30.71±0.43****
AEE	18.27±0.50****	51.23±0.85****	33.56±0.17****	27.44±0.32****	31.54±0.53****
AEAE	28.67±0.57****	53.70±0.49****	52.18±0.35****	67.11±0.79****	36.70±0.62****
AChE	23.20±0.22****	39.21±0.25****	24.22±0.43****	25.66±0.27****	23.46±0.71****
Galantamine	85.48±0.62	74.63±0.25	*NT*	*NT*	*NT*
Acarbose	*NT*	*NT*	57.70±0.75	82.10±0.27	*NT*
Kojic acid	*NT*	*NT*	*NT*	*NT*	79.50±0.32

Values represent the means ± SEM of three parallel sample measurements ($p \leq 0.05$).

NT: not tested. ADE: decocted extract, AME: hydro-methanol extract; AEE: hydro-ethanol extract; AEAE: hydro-ethyl acetate extract and AChE: hydro-chloroform extract.

*; P≤0.1 **; P≤0.01, ****; P≤0.0001 *vs* standards. ns; no significant difference (P>0.05).

4. Gastric emptying and small intestine transit in mice

4.1. Effect of ADE and AME on intestinal transit in mice

The results about the effects of various doses of *A. odorata* L. extracts, and standard drug on intestinal motility are depicted in figure 14. Both two tested extracts decreased the transit of phenol red through the small intestine compared to the vehicle (65.17 ± 3.69%). Indeed, the ADE at 100, 200, and 400 mg/kg significantly decrease the intestinal transit (45.62 ± 2.69%; P≤0.01, 42.92 ± 4.91%; P≤0.001, 28.80 ± 3.02%; P≤0.0001, respectively) compared to the control group (CMC 1,5%) (62.31% ± 2.34; P≤0.0001). The doses (200 and 400 mg/kg) from ADE showed a dose dependent reduction of intestinal transit (P≤0.01). Moreover, as expected, antagonist effects have been observed following the use of standard drug, Atropine (decreased 29.29 ± 3.11%) on the transit motility assessment. Only 400 mg/kg dose showed no significant difference (P>0.05) compared to the atropine as standard. All doses of AME attenuated the intestinal transit 49.53 ± 2.34%; P≤0.1, 45.61 ± 1.98%; P≤0.01, 42.47 ± 2.77%; P≤0.001, respectively) compared to the vehicle. The intestinal transit effect of the tested doses except 400 mg/kg dose was not significantly different in comparison between ADE and AME (P>0.05).

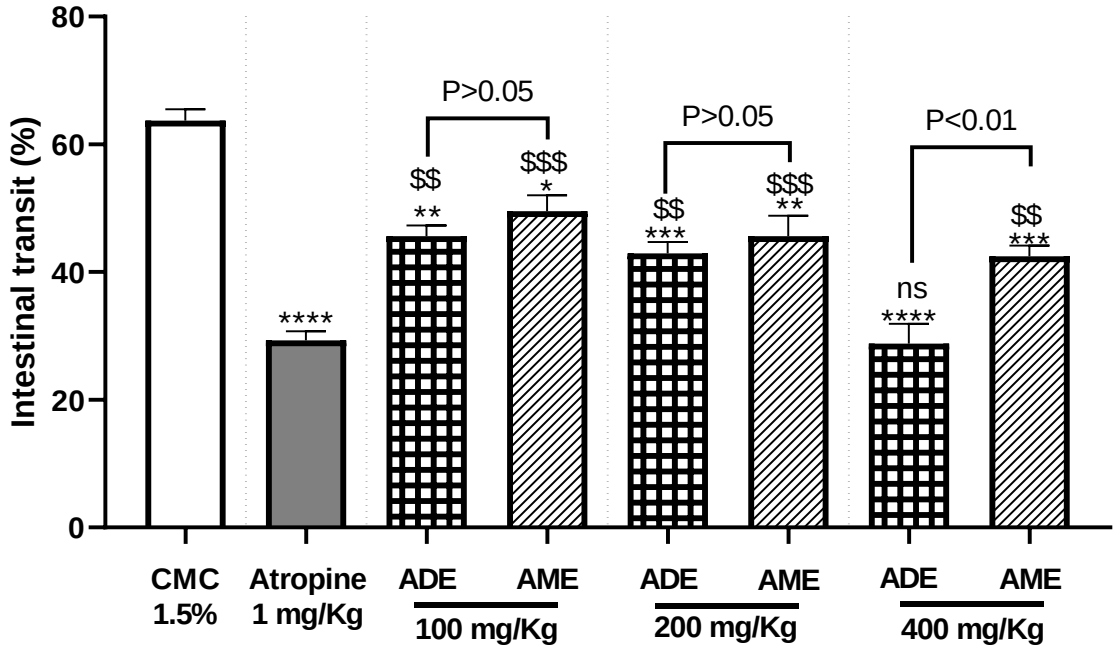


Figure 13. Effects of ADE and AME on intestinal transit in mice.

ADE: decocted extract. Bars represent means ± SEM (n=9). *; P≤0.1, **; P≤0.01, ***; P≤0.001, ****; P≤0.0001 *vs* vehicle as negative control. $$; P≤0.01, $$$; P≤0.001 *vs* atropine as positive control. ns; no significant difference (P>0.05).

4.1.1 **Mechanisms of the ADE effect on intestinal transit**

In order to determine the mechanism of action of ADE and AME on gastric emptying and intestinal transit, another series of experiments was conducted. ADE was administered at a dose of 200 mg/kg in the presence of different pharmacological substances. The treatment of mice with atropine (Atr) (3.45×10^{-3} mmoL/kg), L-arginine (L-arg) at 1.72 mmoL/kg, or indomethacin (Indo) (1.39×10^{-2} mmoL/kg) significantly reduced transit to 29.29 ± 2.01%, 39.99±1.44%, and 44.27 ± 1.87%, respectively ($P\leq0.0001$). Similarly, these pharmacological agents, which were used previously, reduced gastric emptying rates to 45.82%, 60.78%, and 43.58% ($P\leq0.0001$) for atropine, L-arginine, and indomethacin, respectively (Figure 15).

Blocking muscarinic receptors with atropine in the presence of ADE significantly reduced intestinal transit (39.09 ± 2.36%; $P\leq0.0001$) compared to the vehicle. This effect on intestinal transit observed with atropine was still non-significant ($P\leq0.05$) than that obtained with the extract alone, although it showed a significant difference ($P\leq0.01$) compared to the animals pretreated only with atropine.

Treatment of mice with L-arginine (a NO donor *via* NOS) and ADE significantly reduced intestinal transit (46.81 ± 2.87%, $P\leq 0.0001$) compared to the vehicle. There was no significant difference compared to the effect of L-arginine alone ($P>0.05$).

The intraperitoneal administration of indomethacin (a prostaglandin production inhibitor) with ADE reduced intestinal transit (48.20 ± 2.13%, $P\leq 0.0001$) compared to the vehicle but remained unchanged compared to the extract alone ($P>0.05$). The intestinal transit rate of ADE in the presence of indomethacin was comparable to that of indomethacin alone ($P>0.05$) (Figure 15).

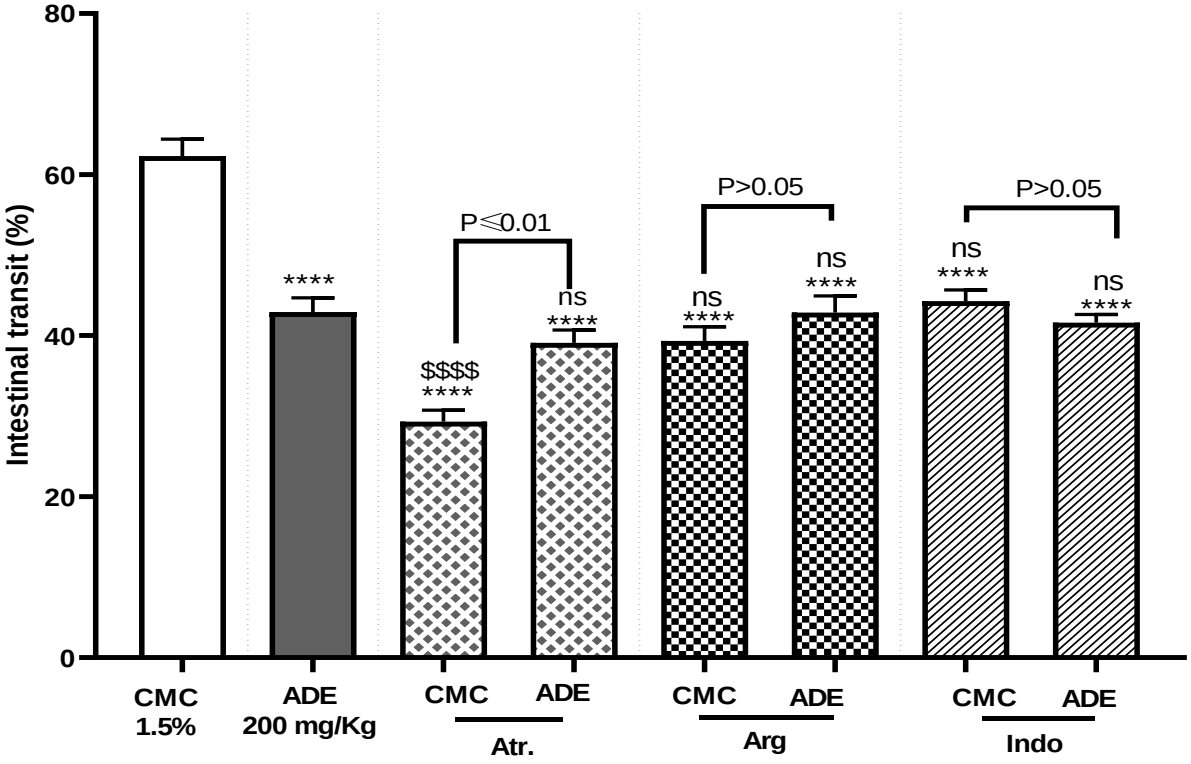


Figure 14. Effect of ADE in absence and in presence of atropine, L-arginine or indomethacin on intestinal transit.

ADE; decocted extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 *vs* vehicle as negative control. $$$$; P≤0.0001 in comparison between both effects of ADE in absence and in presence of atropine, or indomethacin. ns; no significant difference (P>0.05).

4.1.2 **Mechanisms of the AME effect on intestinal transit**

Treatment of mice with atropine and AME (200 mg/kg) simultaneously reduced intestinal transit rates to 41.95 ± 1.24 % (P≤0.0001) compared to the vehicle. Although the decrease in transit was non-significant compared to AME alone (P>0.05), the latter's rate remained non-comparable to that of atropine alone (P≤0.01). Intraperitoneal administration of indomethacin with AME induced a slight but non-significant decrease in intestinal motility to 38.61 ± 1.88 % (P>0.05), comparable to AME alone. No significant difference (P>0.05) was demonstrated compared to the group pretreated with indomethacin alone. Oral administration of L-arginine with AME induced intestinal transit rates that were statistically unchanged compared to L-arginine alone (45.13 ± 1.60 %, P>0.05) (Figure 16).

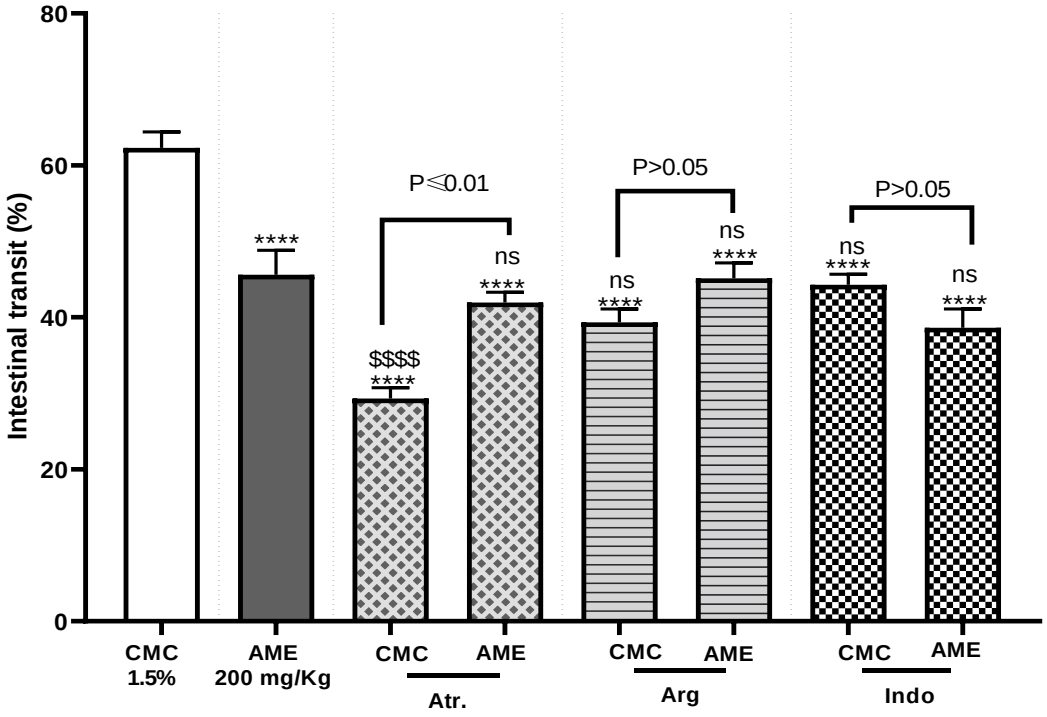


Figure 15. Effect of AME in absence and in presence of atropine, L-arginine or indomethacin on intestinal transit.

AME; hydro-methanol extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 *vs* vehicle as negative control. $$$$; P≤0.0001 in comparison between both effects of ADE in absence and in presence of atropine or indomethacin. ns; no significant difference (P>0.05).

4.2. **Effect of ADE and AME on gastric emptying in mice**

As illustrated in figure 17, the present investigation suggests that oral administration of *A. odorata* L. extracts produced relaxing actions on emptying of contents from the stomach into the duodenum and leads to a reduced effect on stomach emptying process. Indeed, the pretreatment of mice with ADE sample at three different doses (100, 200, 400 mg/kg) reduce this action significantly (57.87 ± 3.97%, 48.72 ± 2.01% and 42.81 ± 3.96%; P≤0.0001, respectively) compared to the control group (80.11 ± 3.99%). ADE at 200 and 400 mg/kg showed no significant difference effects (P>0.05) compared to atropine as positive control (44.26 ± 2.73%). Furthermore, AME at all doses significantly and not dose dependently (P≤0.0001) reduce gastric emptying compared to the vehicle, only 200 and 400 mg/kg doses show no significant difference (P>0.05) compared to the atropine. The gastric emptying effect of the tested doses was not significantly different in comparison between ADE and AME (P>0.05).

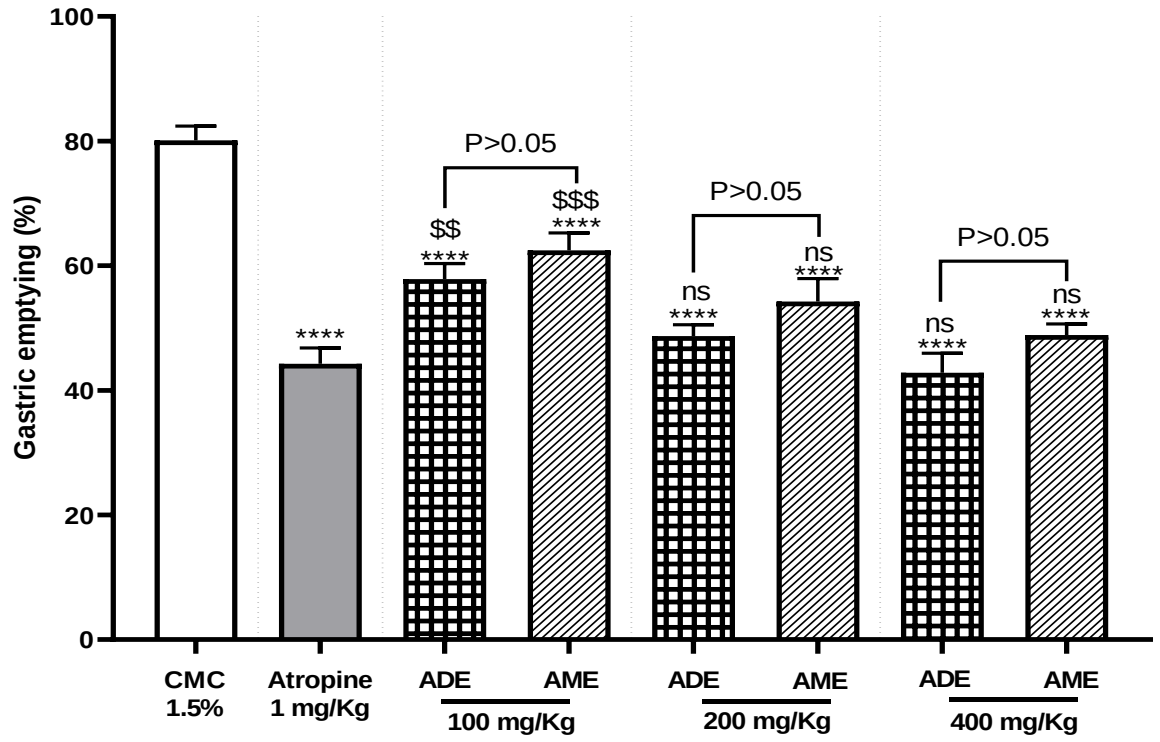


Figure 16. Effects of ADE and AME on gastric emptying in mice.

ADE; decocted extract, AME; hydro-methanol extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 vs vehicle as negative control. $$; P≤0.01, $$$; P≤0.001 *vs* atropine as positive control. ns; no significant difference (P>0.05).

4.2.1 **Mechanisms of the ADE effect on gastric emptying**

A significant reduction in gastric emptying was observed using atropine in the presence of the extract at a dose of 200 mg/kg compared to the vehicle (P≤0.0001) and to the extract alone (P≤0.01). The gastric emptying rate in this case was 32.66 ± 2.45%. Moreover, a significant difference was recorded between atropine + ADE (45.88 ± 2.14%) and atropine alone (P>0.01). Under the influence of NOS donation, oral administration of the ADE had a significant effect on gastric emptying delay (46.81 ± 2.45%; P≤0.0001) compared to the vehicle. There was no significant difference between the effects of ADE in the absence and presence of L-arginine (P>0.05). No significant difference (P>0.05) was recorded between the effects of ADE and the vehicle in animals pretreated only with L-arginine. Moreover, the combination of the extract with indomethacin remained non-significant when compared to the group of indomethacin alone (48.20 ± 1.13%; P>0.05) (Figure 18).

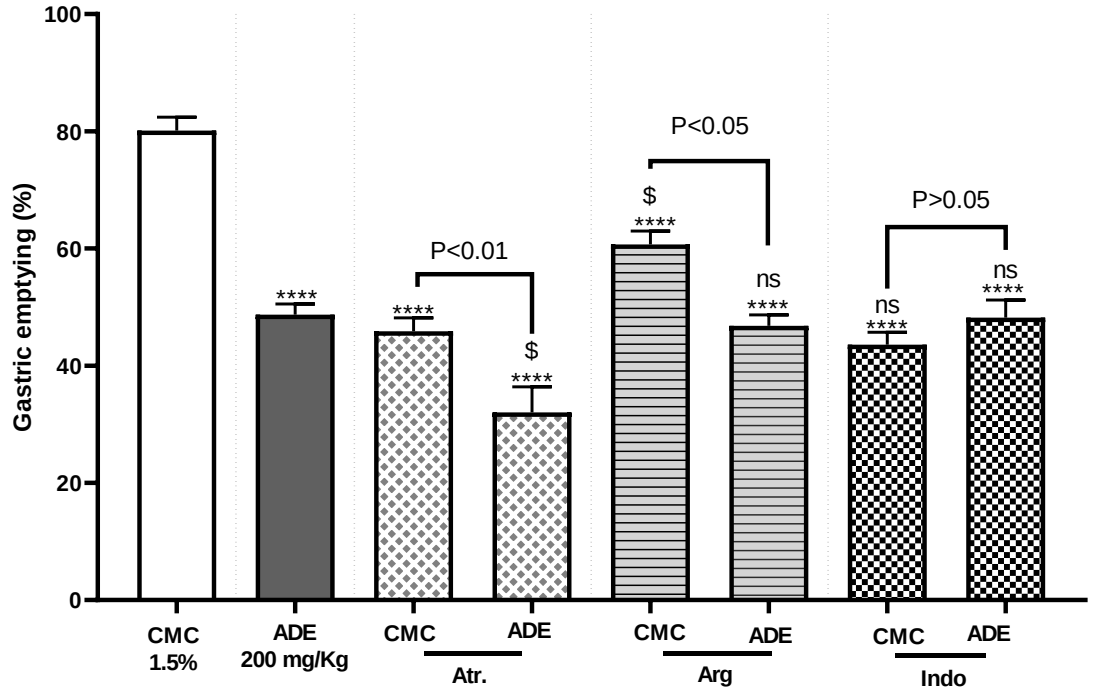


Figure 17. Effect of ADE in absence and in presence of atropine, L-arginine or indomethacin on gastric emptying.

ADE; decocted extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 *vs* vehicle as negative control. $$; P≤0.01 in comparison between both effects of ADE in absence and in presence of atropine, L-arginine or indomethacin. ns; no significant difference (P>0.05).

4.2.2 **Mechanisms of the AME effect on gastric emptying**

The blockade of muscarinic receptors by atropine in the presence of AME induced a significant reduction (51.56 ± 2.34%; P≤0.0001) in gastric emptying compared to the vehicle. This gastric emptying effect observed with atropine remained lower (P>0.05) but not significantly different than that obtained with the extract alone, while it had a significant difference (P≤0.0001) compared to the animals pretreated only with atropine (Figure 19).

Treatment of mice with L-arginine (NO donor by NOS) and AME significantly reduced gastric emptying (45.04 ± 2.69%, P≤ 0.0001) compared to the vehicle, and a significant difference compared to the effect of L-arginine alone (P>0.001) was recorded. The intraperitoneal administration of indomethacin (prostaglandin production inhibitor) with AME reduced gastric emptying (50.60 ± 2.86%, P≤ 0.0001) compared to the vehicle and remained unchanged compared to the extract alone (P≤0.05). Gastric emptying rate of AME in the presence of indomethacin is comparable to that of indomethacin alone (P≤0.05) (Figure 19).

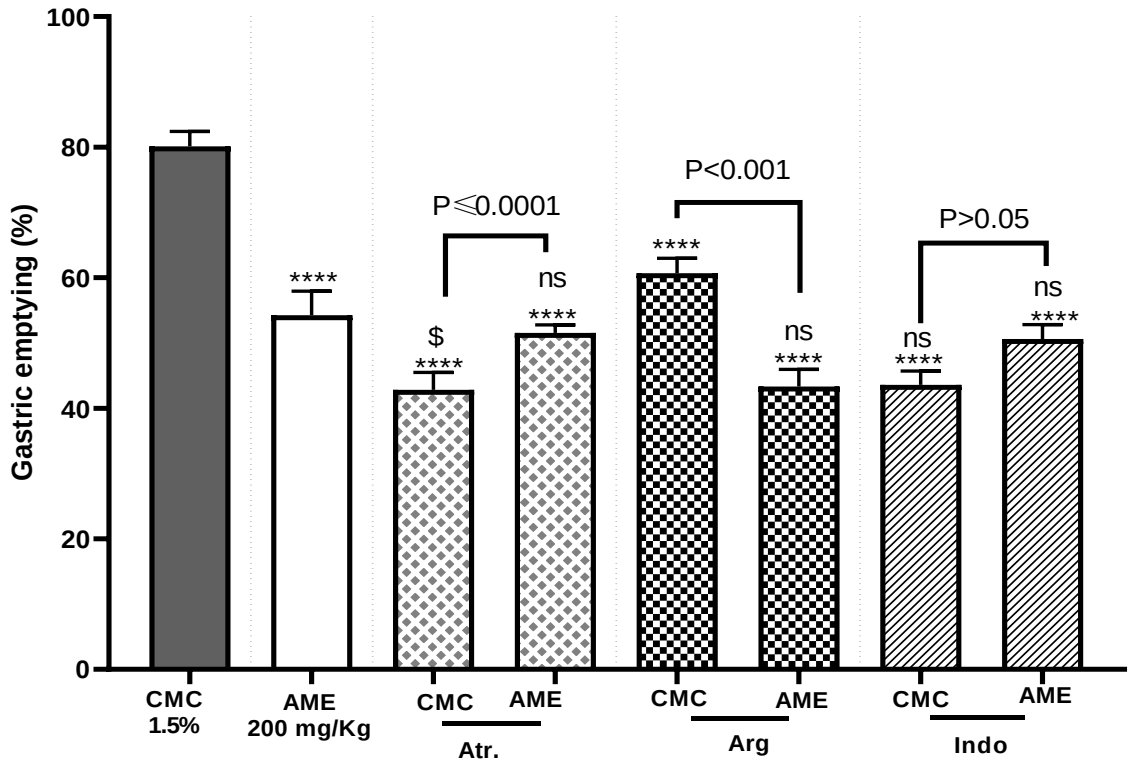


Figure 18. Effect of AME in absence and in presence of atropine, L-arginine or indomethacin on gastric emptying.

AME; hydro-methanol extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 *vs* vehicle as negative control. $; P≤0.1 in comparison between both effects of AME in absence and in presence of atropine, L-arginine or indomethacin. ns; no significant difference (P>0.05).

5. Ethanol-induced gastric ulceration in rats

5.1. **Gastroprotective effect of ADE and AME**

Intragastric administration of ethanol (100%) to the control group resulted in substantial, band-like hemorrhagic erosions in the glandular stomach. Pre- treatment with ADE and AME at the tested doses (100, 200 and 400 mg/kg, *p.o.*) and ranitidine (5 mg/kg) offered different degrees of protection to the mucosa against all such damages caused by ethanol (Figure 20). Compared with the ethanol treated animals (31.11 ± 1.89%), all tested doses of ADE and AME extracts significantly (P ≤ 0.0001) decreased the ulcer gastric. Oral administration of 100, 200 and 400 mg/kg ADE induced a significant reduction of ulceration percentage ranging from 2.01 to 0.56 % respectively and comparable to that of ranitidine 5 mg/kg (0.80 ± 2.01%, P>0.05). However, significant ulceration diminution (P≤0.05) of 4.44 ± 2.45 and 1.72 ± 2.34% was observed following the use of 200 and 400 mg/kg AME respectively compared to positive

control. No significant difference (P>0.05) between the different types of extracts except 100 mg/kg dose (P<0.1).

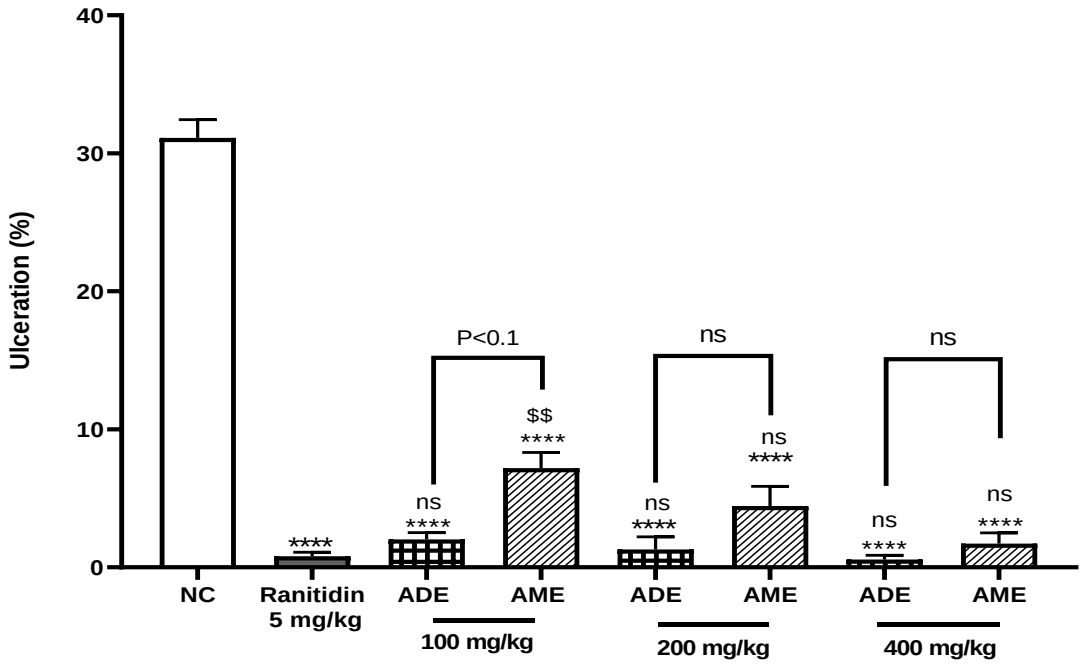


Figure 19. Effects of ADE and AME on gastric ulcer in rats.

ADE; decocted extract, AME; hydro-methanol extract. Bars represent means ± SEM (n=8). ****; P≤0.0001 *vs* vehicle as negative control., $$; P≤0.01 *vs* ranitidine as positive control. ns; no significant difference (P>0.05).

5.1.1 **Mechanisms of the ADE gastroprotective effect**

In a series of experiments, the mechanisms of the ADE gastroprotective effect was evaluated, the extract was administered at a dose of 200 mg/kg in the presence of different pharmacological substances: indomethacin (1.39×10^{-2} mmoL/kg), L-arginine (1.72 mmoL/kg), L-NNA (1.36×10^{-1} mmoL/kg) or yohimbine (1.28×10-^{2} mmoL/kg) to investigate the potential pathways involved in the gastroprotective effect of the extract.

L-arginine significantly reduced gastric ulcer formation to 2.60±0.23% (P≤0.0001) compared to the vehicle groupe (31.11 ± 1.89%). When ADE was administered in the presence of L-arginine, there was no significant difference in its effect (4.71 ± 0.11%; P>0.05) compared to ADE alone. Additionally, it showed no significant difference compared to the group treated with L-arginine alone (P>0.05) (Figure 21).

No significant difference was observed between the rate of ulceration in ADE pretreated with L-NNA and the group treated with L-NNA alone; in fact, it caused an increase in ulceration (33.16 ± 3.56%; P>0.05) (Figure 21).

On the other hand, when ADE was administered in conjunction with indomethacin and yohimbine, it significantly protected against ethanol-induced ulcers (3.19 ± 1.27%; 3.20 ± 2.09%; P≤0.0001) compared to indomethacin and yohimbine treatments alone (20.90 ± 3.21%; 14.55 ± 3.10%). A significant difference was recorded (P≤0.0001) (Figure 21).

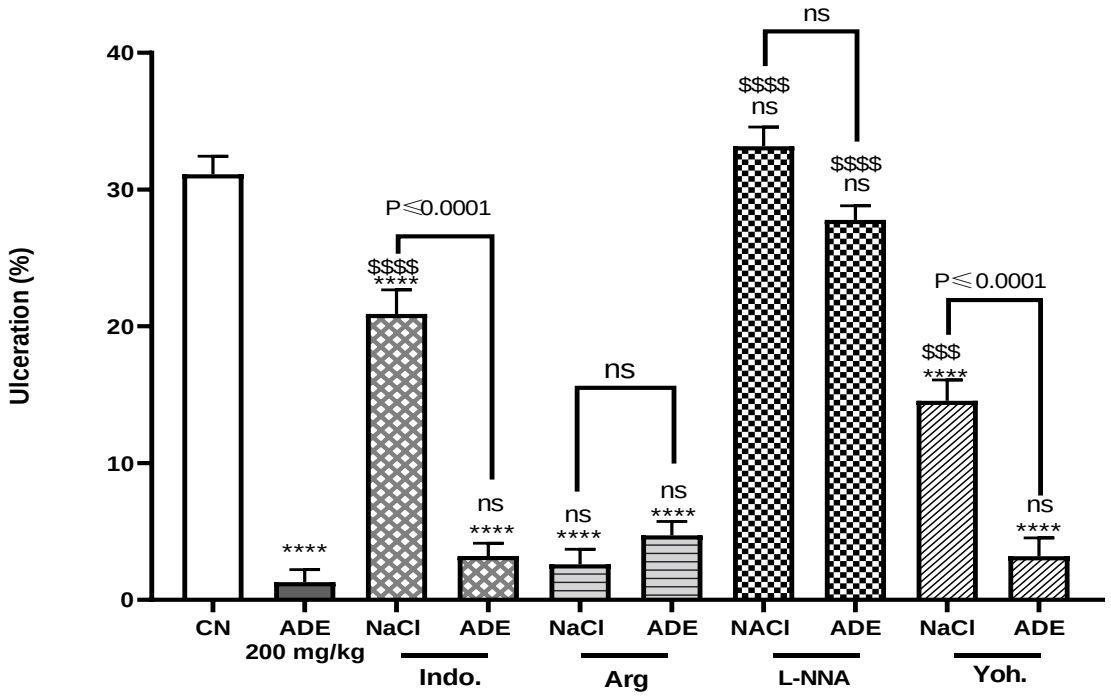


Figure 20. Effect of ADE in absence and in presence of indomethacin, L-arginine, L-NNA or yohimbine on gastric ulcer in ethanol-induced gastric mucosal lesions in rats.

ADE; *A. odorata* L. decocted extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 *vs* vehicle as negative control. $$$$; P≤0.0001, $$$; P≤0.001 in comparison between both effects of ADE in absence and in presence of indo., L-agr., L-NNA or yoh. ns; no significant difference (P>0.05).

5.1.2 **Mechanisms of the AME gastroprotective effect**

The mechanisms of AME's gastroprotective effect are illustrated in the figure 22. Pre-treatment with AME in the presence of L-arginine offered highly significant gastric protection against ulcers caused by ethanol (9.23 ± 0.12%; P≤0.0001) compared to the vehicle groupe (31.11 ± 1.89%). There was a significant difference between the gastroprotective effects of AME in the presence of L-arginine and the group treated with L-arginine alone (P≤0.1). A significant difference was observed in the rate of ulceration between the presence of AME and L-NNA or the group treated with L-NNA alone (8.18 ± 2.56%; P≤0.0001).

On the other hand, AME under the influence of indomethacin and yohimbine provided significant gastric protection against ulcers caused by ethanol (5.08 ± 0.27%; 1.05 ± 1.61%;

P≤0.0001). However, there was no significant difference (P≤0.05) between the effects of AME in the presence and absence of indomethacin or yohimbine.

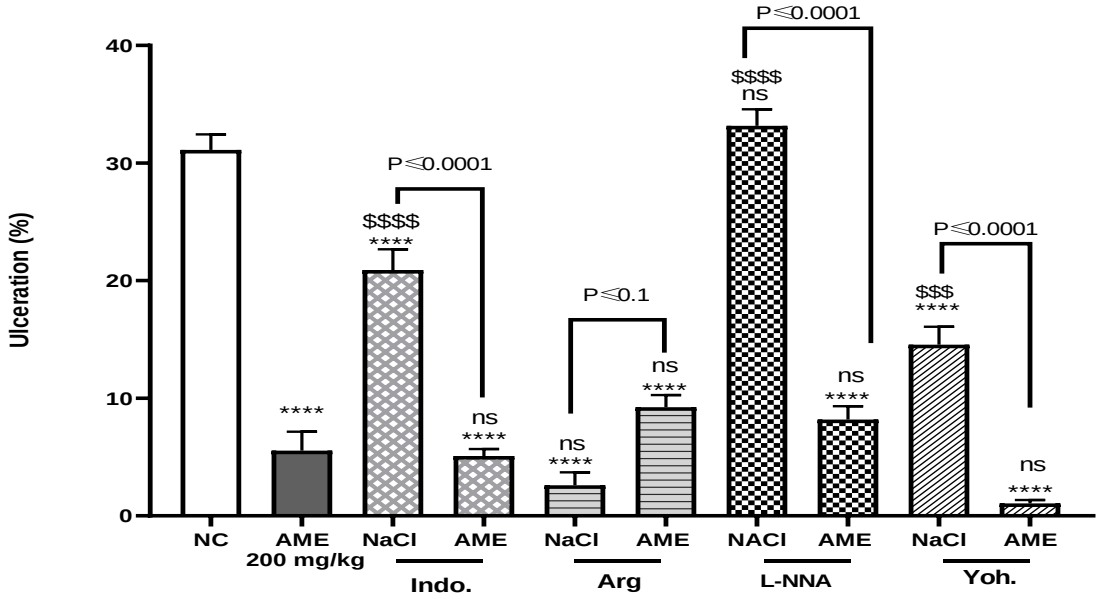


Figure 21. Effect of AME in absence and in presence of indomethacin, L-arginine, L-NNA or yohimbine on gastric ulcer in ethanol-induced gastric mucosal lesions in rats.

AME; hydro-methanol extract. Bars represent means ± SEM (n=9). ****; P≤0.0001 *vs* vehicle as negative control. $$$$; P≤0.0001, $$$; P≤0.001 in comparison between both effects of AME in absence and in presence of indo., L-agr., L-NNA or yoh., ns; no significant difference (P>0.05).

5.2. Macroscopic and histopathological examination of ADE and AME effect on ethanol-induced gastric mucosa damage in rats

a) Macroscopic examination

Acute exposure of the gastric mucosa of rats to ethanol can result in gastric lesions similar to those occurring in gastric ulcer; hence, ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity (Boligon *et al*., 2014). Accordingly, it was observed that animals received 100% ethanol showed macroscopic lesions in gastric tissue, such as loss of normal color and mucus along with presence of petechiae, hemorrhage and edema (Figure 23, B). It is attenuated by the administration of ranitidine (5 mg/kg) (Figure 23, C) and *A. odorata* L. extracts (100, 200,400 mg/kg) with a few fields of hyperemia (Figure 23).

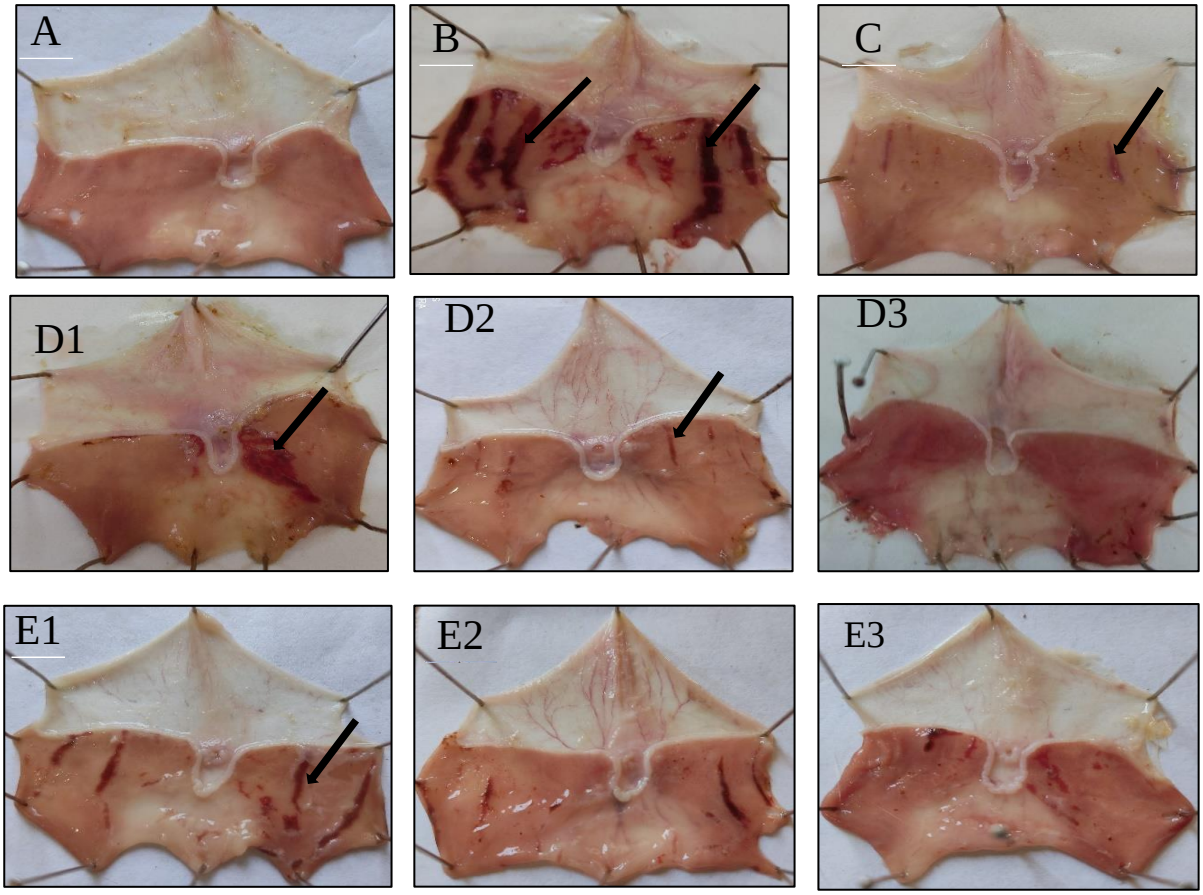


Figure 22. Effects of ADE and AME on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(A) Normal control group. (B) The group pre-treated with NaCl vehicle as negative control. (C) The group pretreated with ranitidine (5 mg/kg) as positive control. (D1, D2 and D3): The group pre-treated with ADE (100, 200 and 400 mg/kg, respectively). ADE: decocted extract. (E1, E2 and E3): The group pre-treated with AME (100,200 and 400 mg/kg, respectively). AME: hydro-methanol extract. Black arrow: elongated bands of hemorrhagic lesions.

b) Histopathological examination

In the present study, ethanol administration to rats induced macroscopic lesions of gastric tissue, such as petechiae, hemorrhages and oedemas. These lesions are most likely linked to depletion of mucus from the veins and arteries of the gastric mucosa, producing constriction of hemorrhage, inflammation and tissue damage. In order to confirm the results of antiulcer experiment, the stomachs were also evaluated by histopathological examination (Figure 24). In histological observation, the stomach of the healthy animals showed no damage with a normal histological structure in the mucosa, muscularis mucosa and submucosa (Figure 24, A). However, rats, 30 min after of exposure to ethanol presented damage to gastric tissue at a microscopic level. Histopathological injury caused by ethanol administration is characterized

by severe detachment of the surface epithelium, edema, formation of hemorrhagic and gastric lesions, and an inflammatory process characterized by neutrophil infiltration (Figure 24, B). In contrast, the stomach of a rat in the positive control group, pretreated with ranitidine plus ethanol (Figure 24, C), maintains an intact histological mucosa structure, with mild edema, infiltration of inflammatory cells (blue arrow), and mildly dilated blood vessels (green arrow) in the submucosa compared to the ulcer control rats. Rats pre-treated with ADE (Figure 24, $D_{1,2,3}$) and AME (Figure 24, $E_{1,2,3}$) exhibit progressively better protection, characterized by an intact gastric epithelium and reduced or complete absence of edema and leukocyte infiltration (blue arrow).

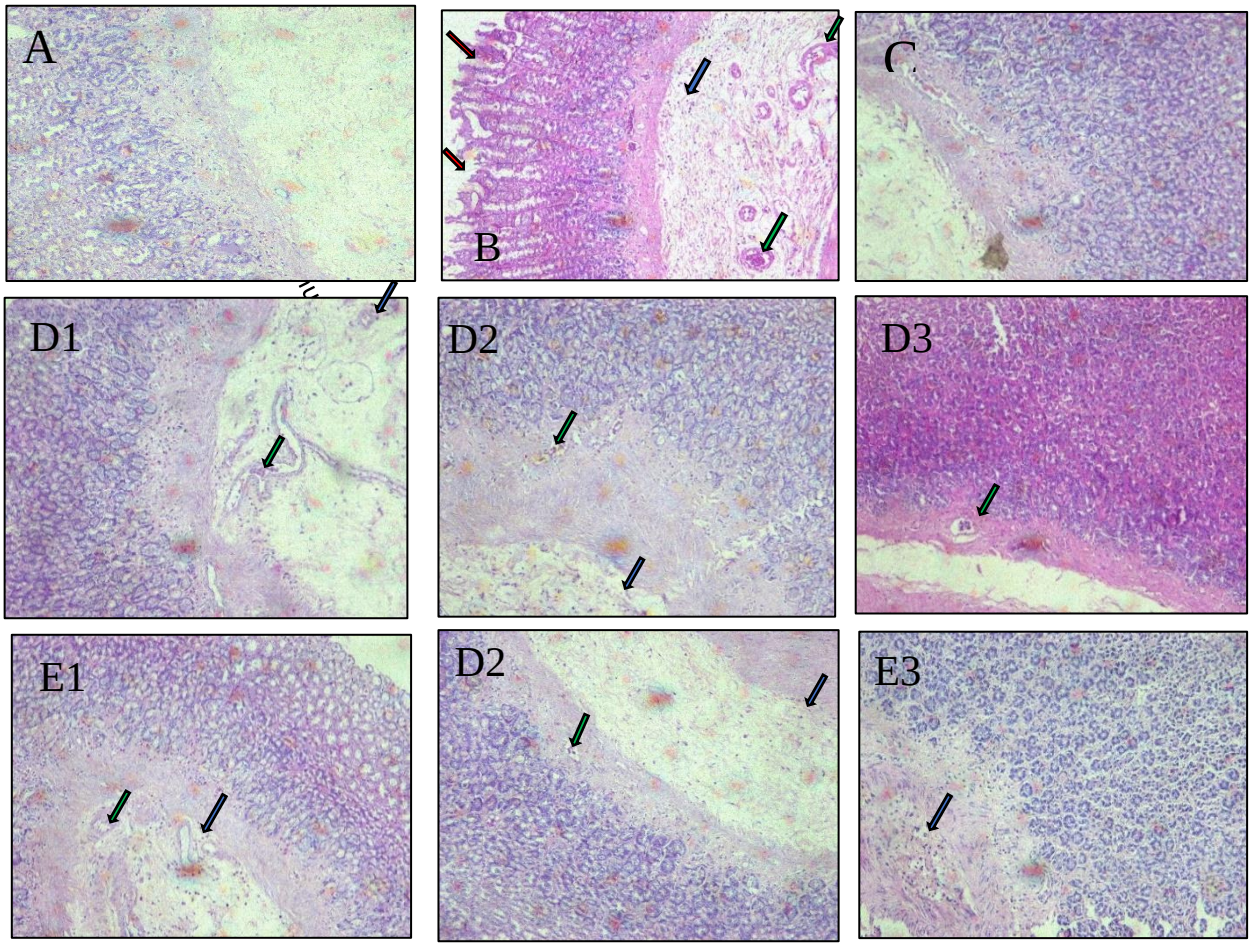


Figure 23. Histological examination for the protective effect of ADE and AME against ethanol-induced gastric damage in rat stomach tissue (magnification 100x).

(A): Normal control group. (B): The group pre-treated with NaCl as negative control. (C): The group pretreated with ranitidine (5 mg/kg) as positive control. (D1, D2 and D3): The groups pre-treated with ADE (100, 200 and 400 mg/kg, respectively). ADE: A. *odorata* L. decocted extract. (E1, E2 and E3): The groups pre-treated with AME (100, 200 and 400 mg/kg, respectively). AME: *A. odorata* L. hydro-methanol extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: edema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels. muc: mucosa, musc muc: muscularis mucosa, sub muc: submucosa.

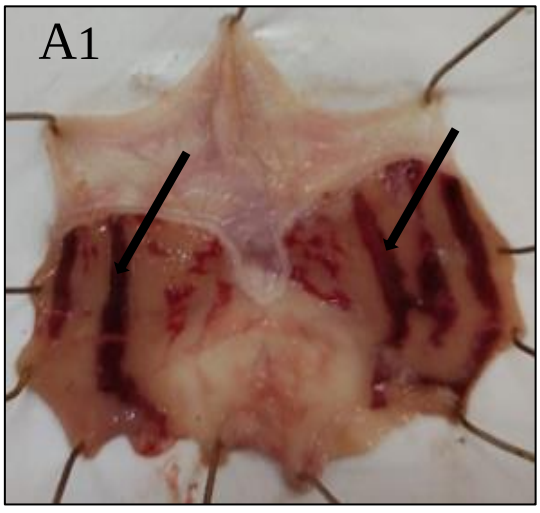
5.2.1 Macroscopic and histopathological examination of mechanisms of ADE effect on ethanol-induced gastric mucosa damage in rats

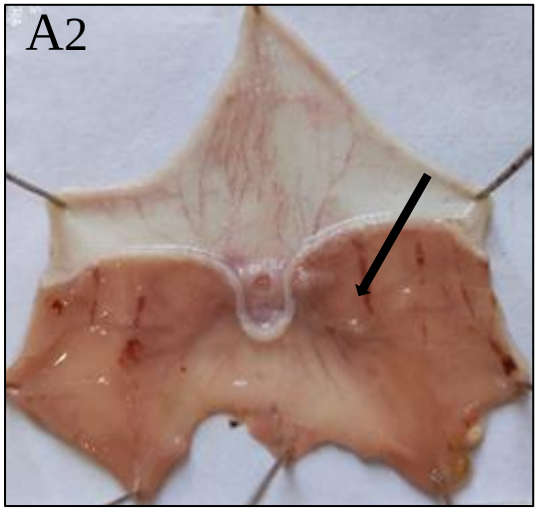
a) Macroscopic examination

The figure 25 illustrates the impact of ADE on ethanol-induced gastric mucosa damage in rats when combined with different pharmacological substances. Moderate injuries are seen in the gastric mucosa with indomethacin either in the absence of ADE (Figure 25, B1) or in its presence (Figure 25, B2), compared to negative control (Figure 25, A1).

Rats pre-treated with L-arginine, both with and without ADE (Figure 25, C1 and C2), showed significantly reduced gastric ulcer formation compared to the negative control group. This effect was similar to using ADE at a dose of 200 mg/kg (Figure 25, A2).

In contrast, L-NNA, whether administered with or without ADE (Figure 25, D1 and D2), resulted in a higher gastric ulcer area compared to the negative control group. Rats pre-treated with yohimbine (Figure 25, E1) exhibited milder gastric mucosa injuries than the negative control group. However, when yohimbine was combined with ADE (Figure 25, E2), it reduced the formation of gastric lesions, similar to the effect of ADE at a dose of 200 mg/kg.





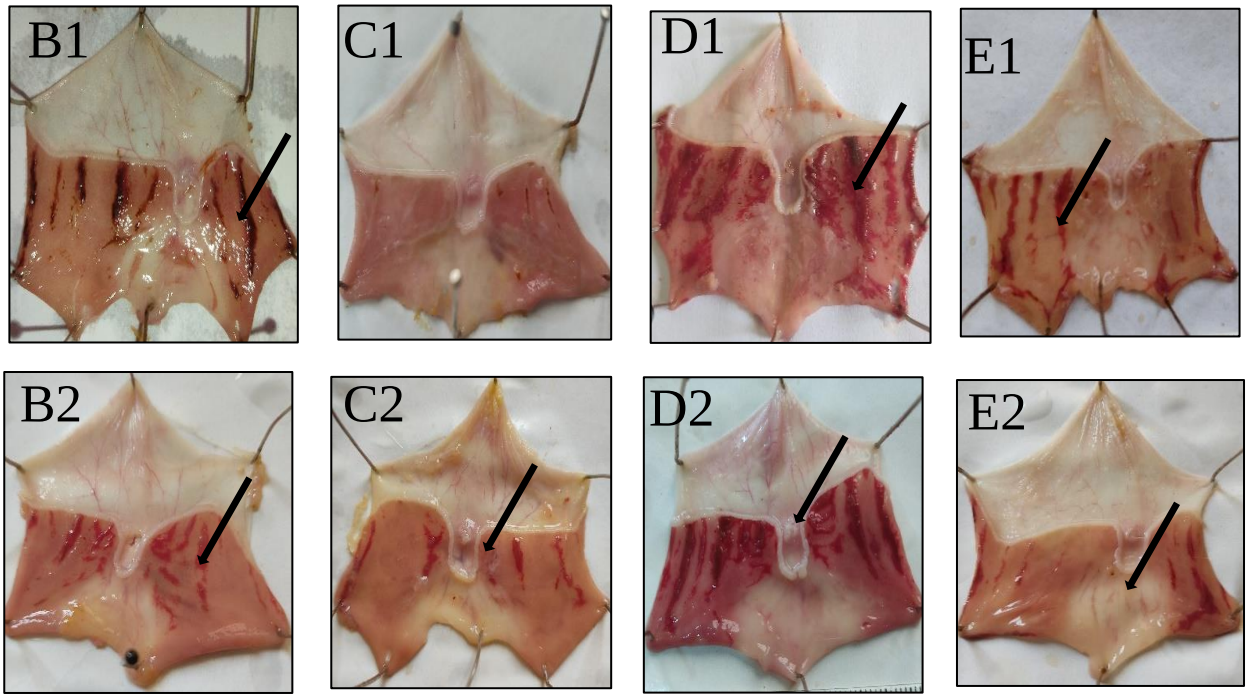


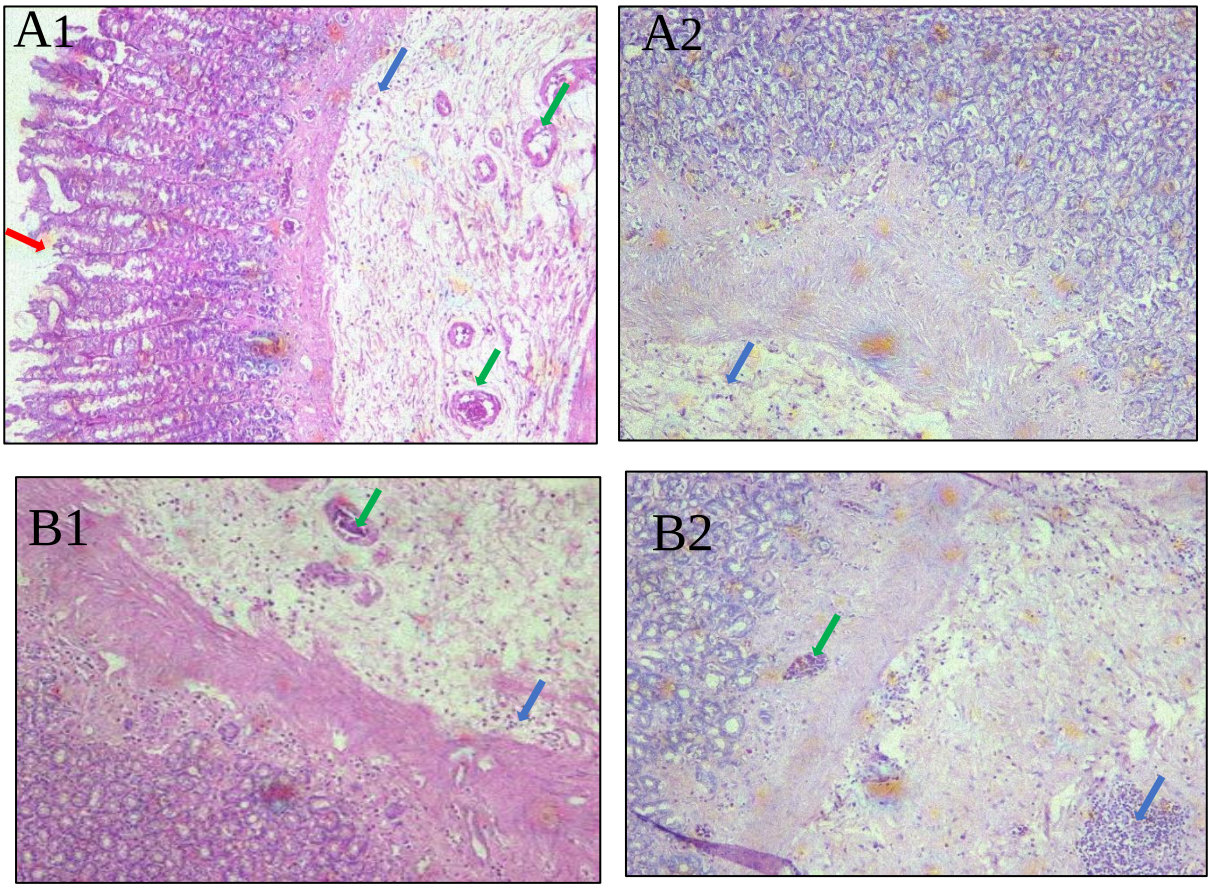
Figure 24. Effect of ADE in absence or presence of different pharmacological substances on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(A1): The group pre-treated with NaCl as negative control. (A2): The group pre-treated with ADE (200 mg/kg). (B1, C1, D1, E1): The groups pre-treated with indomethacin, L-arginine, L-NNA or yohimbine in absence of ADE. (B2, C2, D2, E2): The groups pre-treated with indomethacin, L-arginine, L-NNA or yohimbine, respectively in presence of ADE. ADE: decocted extract. Black arrow: elongated bands of hemorrhagic lesions.

b) Histopathological examination

Figure 26 showed a histopathological analysis of ADE effect in absence or presence of different pharmacological substances, on the microscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats. Histological observation confirms the ability of ADE (Figure 26, A2) at dose 200 mg/kg to reduce ethanol-induce gastric damage in the superficial layer of the gastric mucosa and further highly significantly reduced oedema and leucocytes infiltration (Blue arrow) in submucosa compared to the ulcer control group (Figure 26, A1). Indomethacin administration in the presence of ADE extract (Figure 26, B2) significantly reduced moderately leucocytes infiltration (blue arrow) and the congestion of blood vessels (Green arrow) in the submucosal compared to indomethacin effect in absence of ADE (Figure 26, B1). There is no disruption to the surface mucosal epithelium, reduced leucocyte infiltration and moderate dilated blood vessels in the gastric submucosa of rats treated with the L-arginine in the absence of ADE (Figure 26, C1) and in its presence (Figure 26, C2).

It appeared similar to the ADE alone (Figure 26, A2). There are severe submucosal oedema and leucocytes infiltration (Blue arrow) in rats pretreated with L-NNA (Figure 26, D1) alone and in rats pretreated with both L-NNA and ADE (Figure 26, D2), compared to the negative control (Figure 26, A1). In rats pretreated with yohimbine (Figure 26, E1); there is a moderate disruption to the surface epithelium and, significant leucocytes infiltration (Blue arrow) and congestion of blood vessels (Green arrow) in the submucosal layer, compared to that seen in the vehicle as negative control rats. However, the yohimbine in the presence of ADE extract (Figure 26, E2) significantly reduced the oedema, leucocytes infiltration (Blue arrow) and the congestion of blood vessels (Green arrow) in the submucosal compared to yohimbine effect in absence of ADE. ADE effect in presence of yohimbine appeared better to its effect in absence of yohimbine.



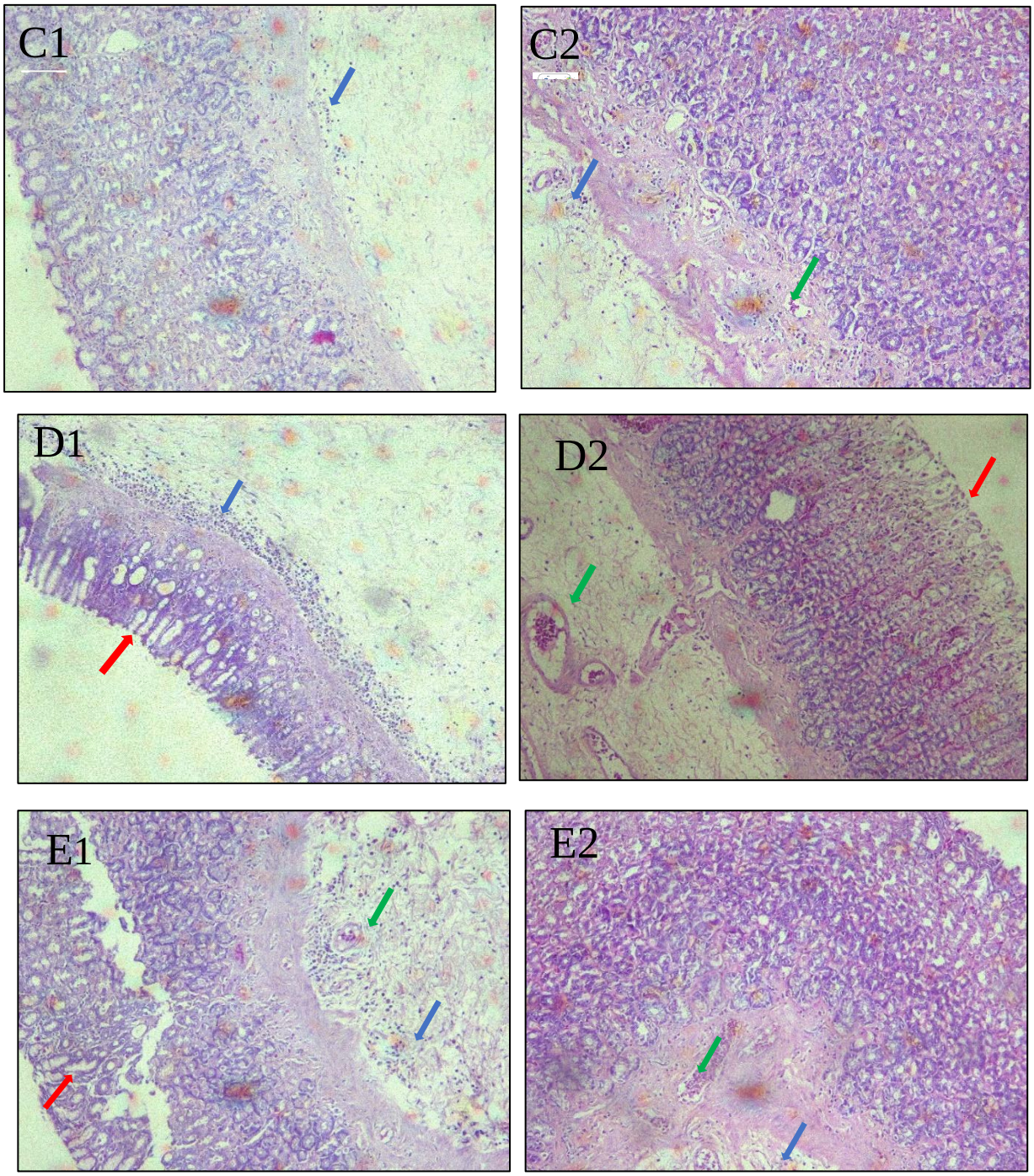


Figure 25. Histological examination for the protective effect of ADE in absence or presence of different pharmacological substances, against ethanol-induced gastric damage in rat stomach tissue (magnification 100x).

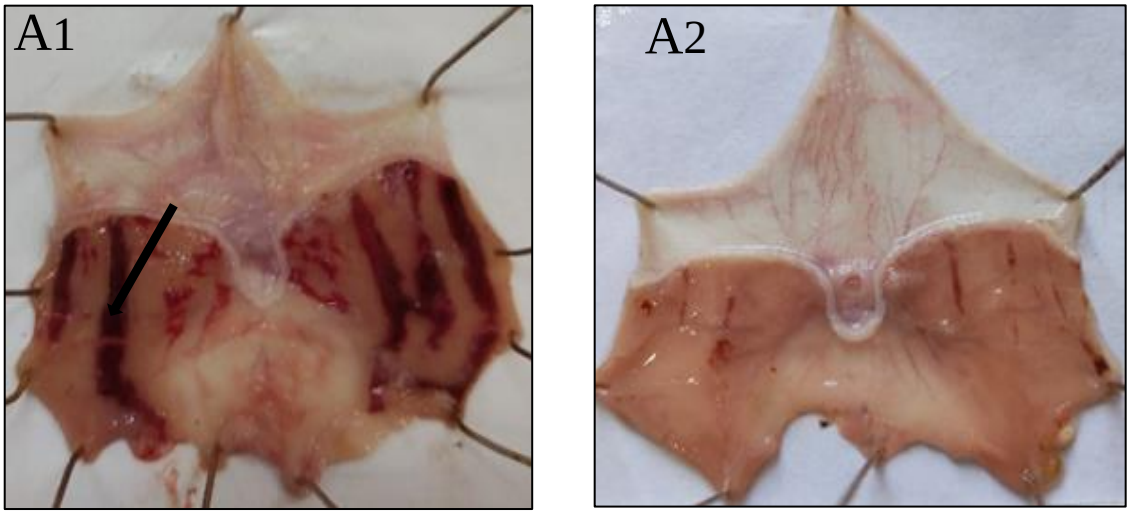
(A1): The group pre-treated with NaCl as negative control. (A2): The group pre-treated with ADE (200 mg/kg). (B1, C1, D1 and E1): The groups pre-treated with indomethacin, L-arginine, L-NNA or yohimbine, respectively in absence of ADE. (B2, C2, D2 and E2): The groups pretreated with indomethacin, L-arginine, L-NNA or yohimbine, respectively in presence of ADE. ADE: decocted extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: oedema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels.

5.2.2 Macroscopic and histopathological examination of mechanisms of AME effect on ethanol-induced gastric mucosa damage in rats

a) Macroscopic examination

The macroscopic examination of AME effect in absence or presence of different pharmacological substances, on ethanol-induced gastric mucosa damage is shown in figure 27. The administration of indomethacin provided a moderate to severe gastric injury against ulcer caused by ethanol (Figure 27, B1). Whereas, the pre-treatment with both indomethacin and AME provides a milder effect (Figure 27, B2) compared to that in presence of indomethacin alone. It was similar to the AME effect (Figure 27, A2).

Intragastric administration of L-arginine alone (Figure 27, C1) or in combination with AME (Figure 27, C2) ameliorated gastric lesions to a lesser extent than those observed in the vehicle-treated negative control (Figure 27, A1). However, a difference was observed in the effect of L-arginine in the presence and absence of AME on stomach lesions. L-NNA caused severe damage to the gastric mucosa when administered without AME (Figure 27, D1). However, in the presence of AME (Figure 27, D2), the extent of damage was reduced, resulting in moderate lesions compared to the negative control. On the other hand, yohimbine resulted a moderate gastric injury (Figure 27, E1) compared to the ethanol-induced ulcer negative group. However, pretreatment with both yohimbine and AME provided better protection (Figure 27, E2) than yohimbine alone. This effect was similar to the AME effect (Figure 27, A2).



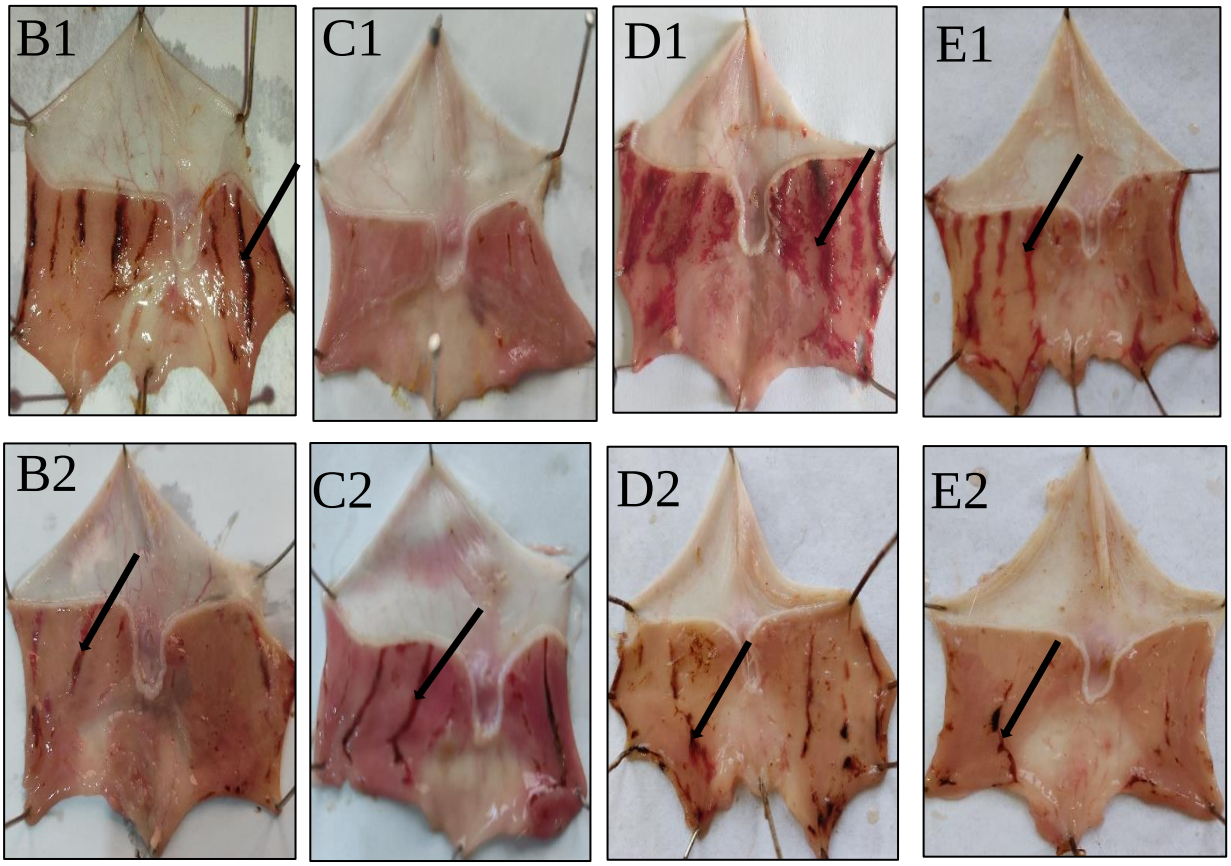


Figure 26. Effect of AME in absence or presence of different pharmacological substances on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(A1): The group pre-treated with NaCl as negative control. (A2): The group pre-treated with AME (200 mg/kg). (B1, C1, D1, E1): The groups pre-treated with indomethacin, L-arginine, L-NNA or yohimbine in absence of AME. (B2, C2, D2, E2): The groups pre-treated with indomethacin, L-arginine, L-NNA or yohimbine, respectively in presence of AME. AME: hydro-methanol extract. Black arrow: elongated bands of hemorrhagic lesions.

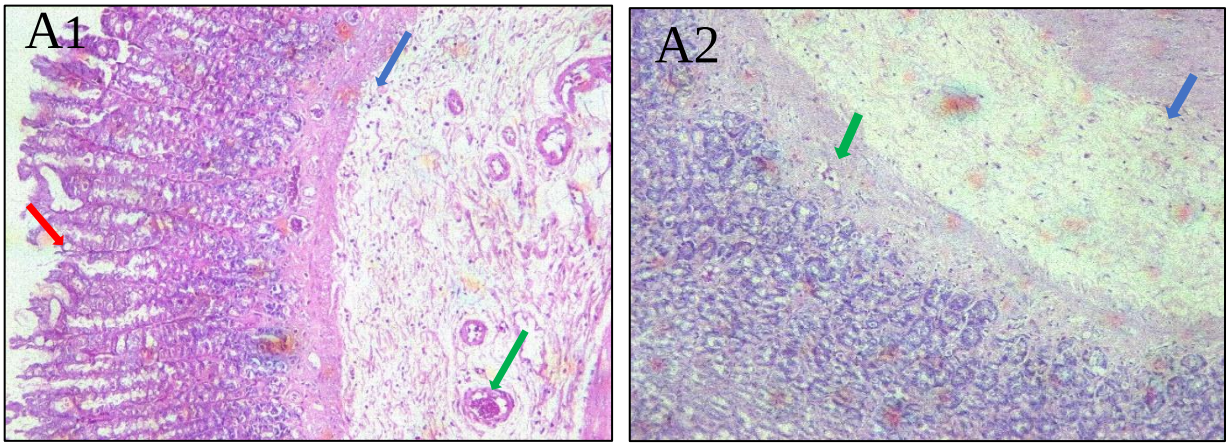
b) Histopathological examination

The microscopic analysis of the effects of AME in the presence or absence of various pharmacological substances on ethanol-induced damage to the gastric mucosa is presented in Figure 28. When indomethacin was administered along with AME extract (Figure 28, B2), there was a significant reduction in moderate leukocyte infiltration (indicated by the blue arrow) and congestion of blood vessels (indicated by the green arrow) in the submucosal area compared to the effect of indomethacin alone in the absence of AME (Figure 28, B1). This effect resembled the impact of AME treatment alone (Figure 28, A2).

In the absence of AME, animals receiving L-arginine (Figure 28, C1) were completely protected against the damaging effects of ethanol, preserving all histological aspects when compared to the control animal group (Figure 28, A1). However, in the presence of AME, there was a moderate leukocyte infiltration (blue arrow) and congestion of blood vessels (Green arrow) observed in the submucosal region due to L-arginine administration (Figure 28, C2).

L-NNA caused severe necrosis of the gastric mucosa (indicated by the red arrow) with leukocyte infiltration (Blue arrow), and congestion of blood vessels (Green arrow) in the submucosa when administered without AME (Figure 28, D1). However, in the presence of AME (Figure 28, D2), the extent of damage was reduced, resulting in moderate leukocyte inflammation compared to the negative control (Figure 28, A1).

On the other hand, yohimbine induced moderate gastric injury (Figure 28, E1) compared to ethanol-induced ulcer group. However, pretreatment with both yohimbine and AME provided better protection (Figure 28, E2) than yohimbine alone (Figure 28, E1). This combination significantly reduced the infiltration and congestion of both mucosa and submucosa. This effect was similar to the AME effect (Figure 28, A2).



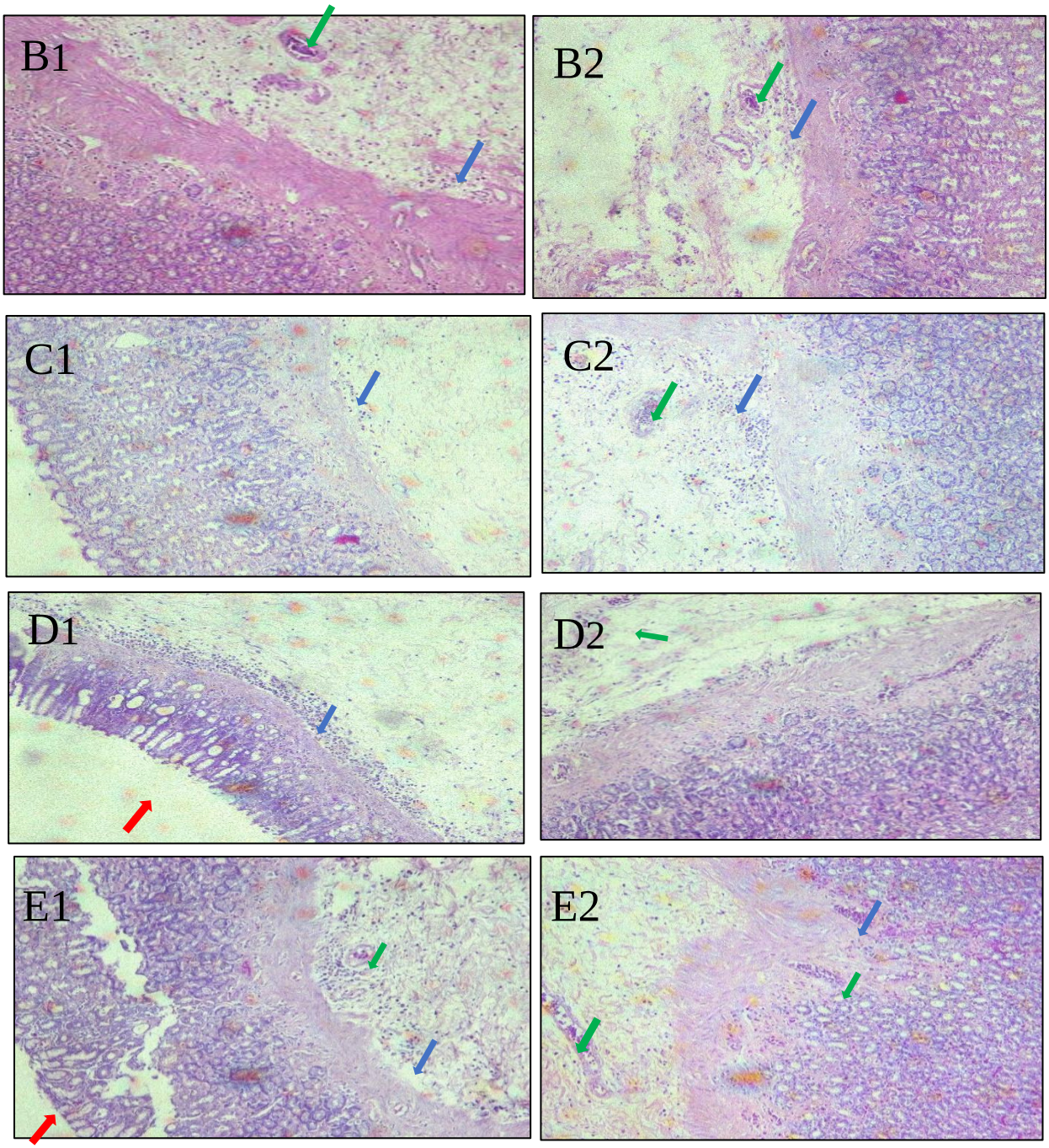


Figure 27. Histological examination for the protective effect of AME in absence or presence of different pharmacological substances, against ethanol-induced gastric damage in rat stomach tissue (magnification 100x).

(A1): The group pre-treated with NaCl as negative control. (A2): The group pre-treated with AME (200 mg/kg). (B1, C1, D1 and E1): The groups pre-treated with indomethacin, L-L-arginine, L-NNA or yohimbine, respectively in absence of AME. (B2, C2, D2 and E2): The groups pretreated with indomethacin, L-arginine, L-NNA or yohimbine, respectively in presence of AME. AME: hydro-methanol extract. Red arrow: surface epithelium damage and hemorrhagic necrosis penetrating deeply into gastric mucosa. Blue arrow: oedema of submucosa and inflammatory cell infiltration. Green arrow: congestion of blood vessels.

5.3. **Determination of mucus in gastric layer**

The effects of ADE and AME on gastric mucus content are shown in figure 29. Compared to the vehicle (64.78 µg of Alcian blue/g of wet tissue), ADE and AME extracts increased the gastric mucus content. All ADE doses (100, 200 and 400 mg/kg) had very highly significantly ulcer protective effect (124.01 ± 10.92 µg of Alcian blue/g of wet tissue, 158.08 ± 20.11 µg of Alcian blue/g of wet tissue, 185.28 ± 9.43 µg of Alcian blue/g of wet tissue, respectively; P≤0.0001). AME at 400 mg/kg exhibited a good gastric mucus content (145.91 ± 12.01 µg of Alcian blue/g of wet tissue, P≤0.0001) compared to the vehicle.

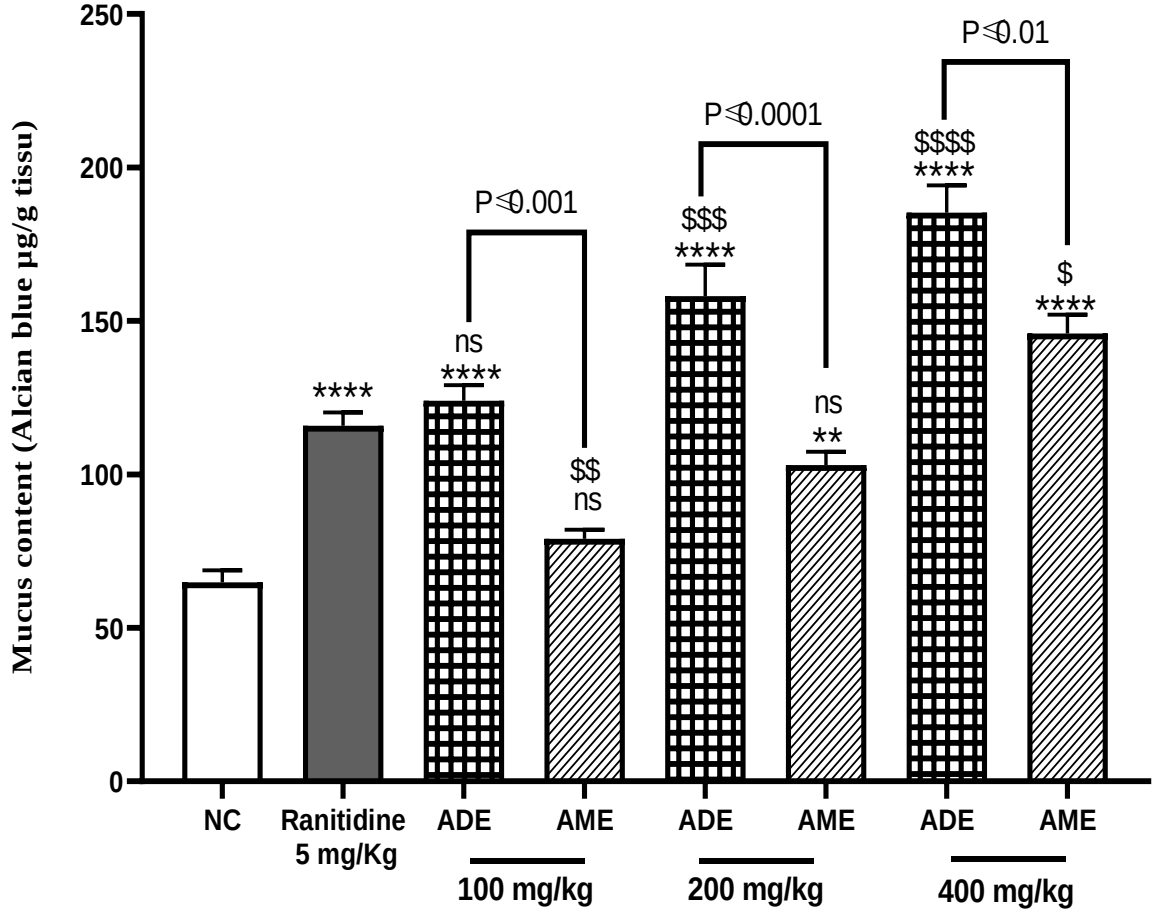


Figure 28. Effects of ADE and AME on gastric mucus content in ethanol-induced gastric ulcer.

ADE; decocted extract, AME; hydro methanol extract. Bars represent means ± SEM (n=8). **; P≤0.01, ****; P≤0.0001 vs vehicle as negative control. $$; P≤0.01, $$$; P≤0.001, $$$$; P≤0.0001 vs ranitidine as positive control. ns; no significant difference (P>0.05).

5.4. Evaluation of *in vivo* antioxidant activity of gastric homogenate

5.4.1 Estimation of gastric total proteins content

Treatment of rat's stomachs with different doses (100, 200 and 400 mg/kg) of ADE and AME result a significant augmentation (from P≤0.001 to P≤0.0001) of total gastric proteins level except groups treated with 100 mg/kg of both extracts showed any effect compared to the vehicle (P>0.05). 200 and 400 mg/kg doses from ADE (0.48 ± 0.034 proteins mg/mL; 0.53 ± 0.039 proteins mg/mL) and AME (0.41 ± 0.029 proteins mg/mL; 0.49 ± 0.034 proteins mg/mL) showed no significant difference in gastric proteins contents compared to ranitidine (P>0.05) (Figure 30).

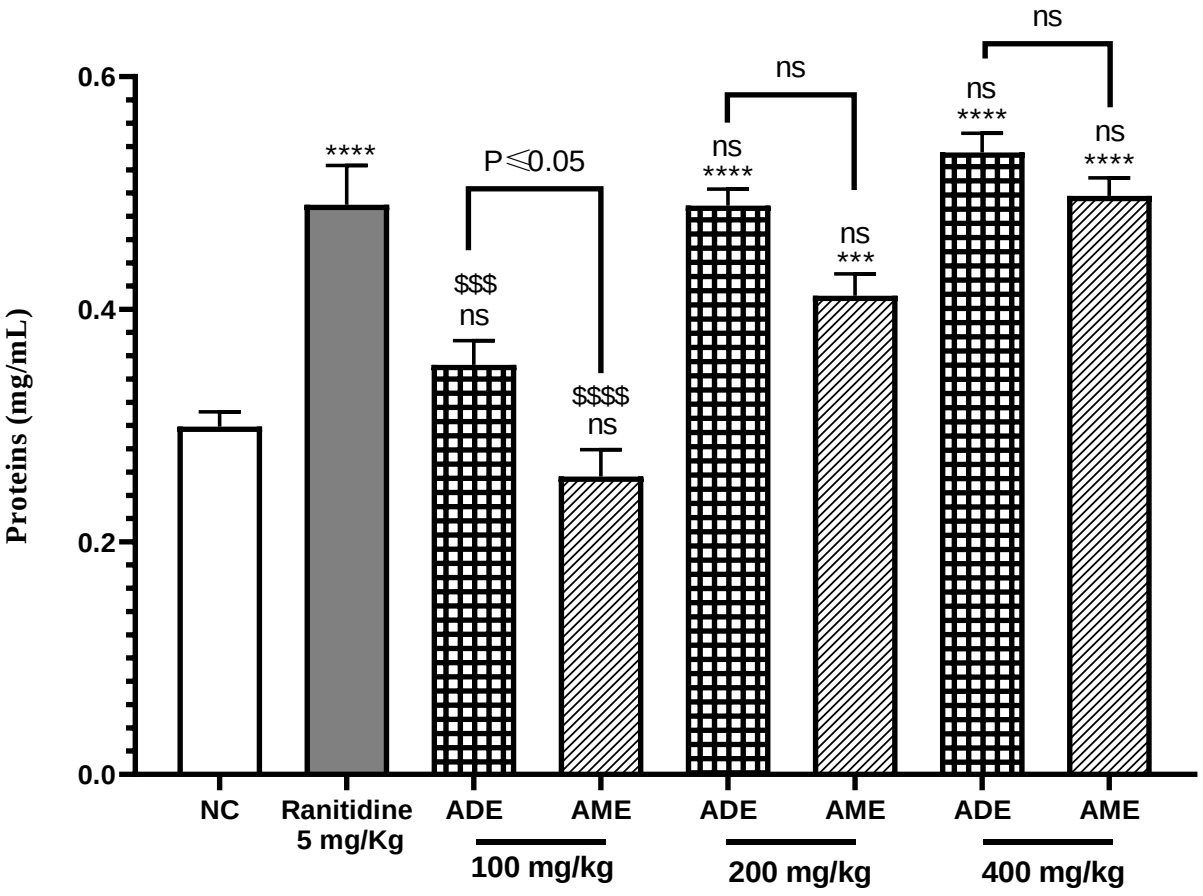


Figure 29. Effects of ADE and AME on total protein level of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

ADE; decocted extract, AME; hydro-methanol extract. Bars represent means ± SEM (n=8). ***; P≤0.001, ****; P≤0.0001 *vs* vehicle as negative control., $$$; P≤0.001, $$$$; P≤0.0001 *vs* ranitidine as positive control. ns; no significant difference (P>0.05).

5.4.2 **Estimation of catalase activity**

Statistical analysis revealed a significant increase in CAT activity in gastric tissue after treatment with ADE and AME (200 and 400 mg/kg) when compared to control group (2.90 ± 0.98 CAT nmoL/min/mg). In addition, the ranitidine at 5 mg/kg group was able to significantly reversed the decrease on CAT activity induced by ethanol (8.65 ± 0.81 nmoL/min/mg) (Figure 31).

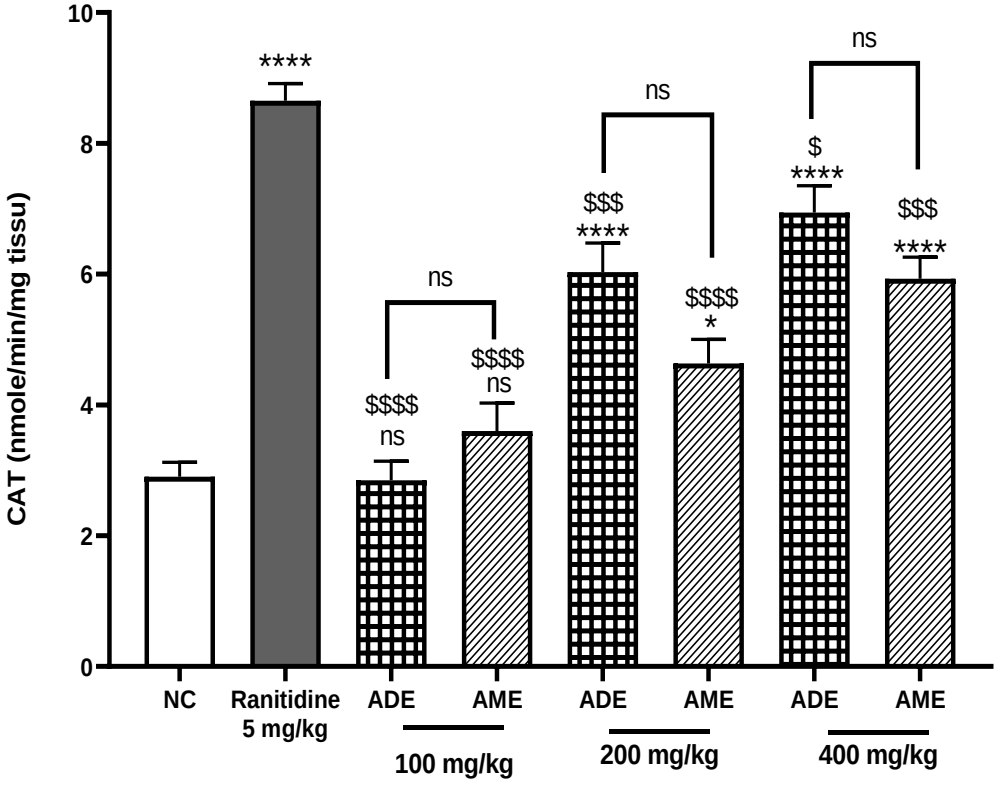


Figure 30. Effects of ADE and AME on CAT activity of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

ADE; decocted extract, AME; hydro-methanol extract. Bars represent means ± SEM (n=8). *; P≤0.1, ****; P≤0.0001 *vs* vehicle as negative control, $; P≤0.1, $$$; P≤0.001, $$$$; P≤0.0001 *vs* ranitidine as positive control. ns; no significant difference (P>0.05).

5.4.3 **Assessment of reduced glutathione**

Treatment of rats with both ADE and AME extracts showed significant increase at all doses (100, 200 and 400 mg/kg) (P≤0.1; P≤0.001; P≤0.0001) compared with the vehicle treated animal group (40.44 ± 0.69 nmoL TNB/g tissue) (Figure 32). Whereas, these values were higher than the positive control (45.48 ± 0.51 nmoL TNB/g tissue).

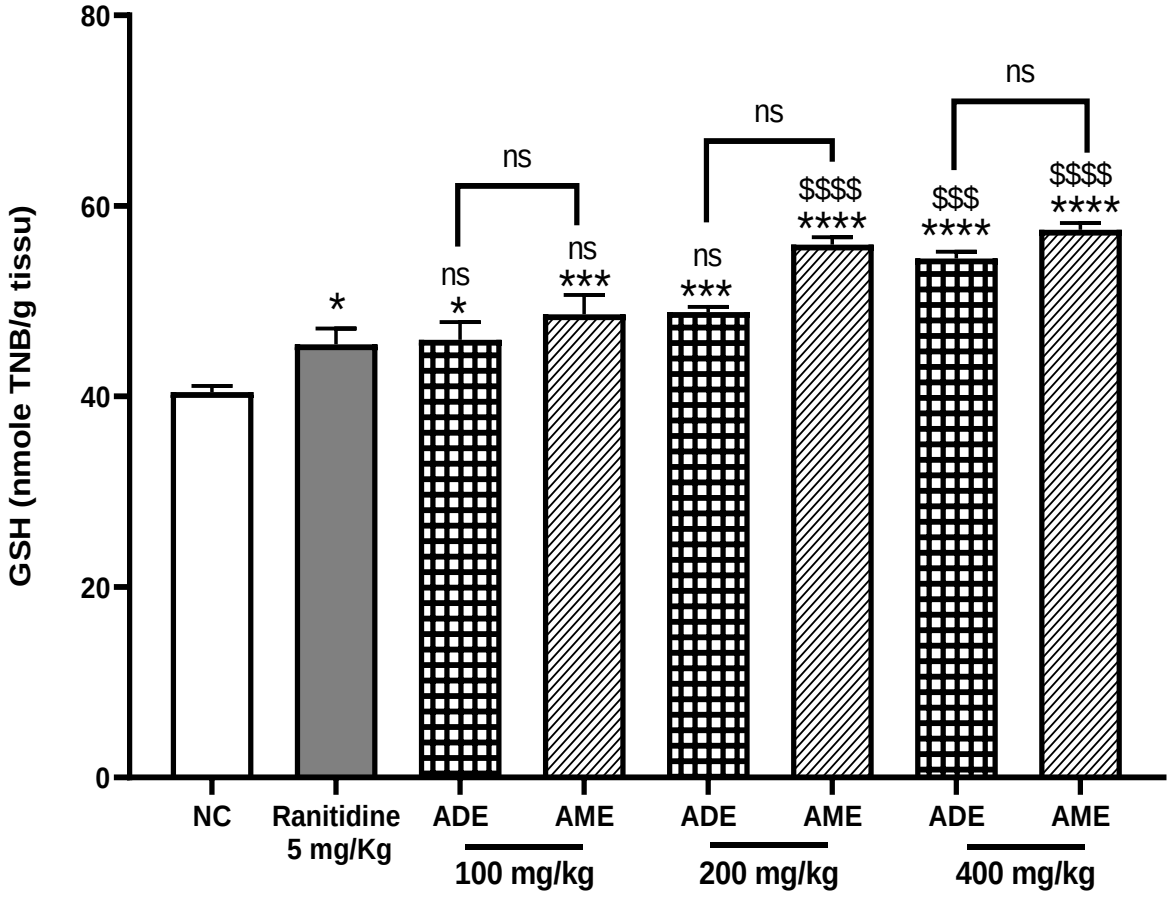


Figure 31. Effects of ADE and AME on GSH level of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

ADE; decocted extract, AME; hydro-methanol extract. Bars represent means ± SEM (n=8). *; P≤0.1, ***; P≤0.001, ****; P≤0.0001 *vs* vehicle as negative control., $$$; P≤0.001, $$$$; P≤0.0001 vs ranitidine as positive control. ns; no significant difference (P>0.05).

5.4.4 **Lipid peroxidation estimation**

The ethanol group showed a change on oxidative markers with an increase on lipid peroxidation (13.16 ± 0.64 MDA µmoL/g tissue). However, the animals which received ranitidine at 5 mg/kg, ADE or AME at 100, 200 and 400 mg/kg completely attenuated the damage induced by ethanol (P≤0.0001). Furthermore, ADE and AME, at all doses tested, were able to significantly prevent the increase on lipid peroxidation better than the positive control (5.75 ± 0.69 µmoL/g tissue) (Figure 33).

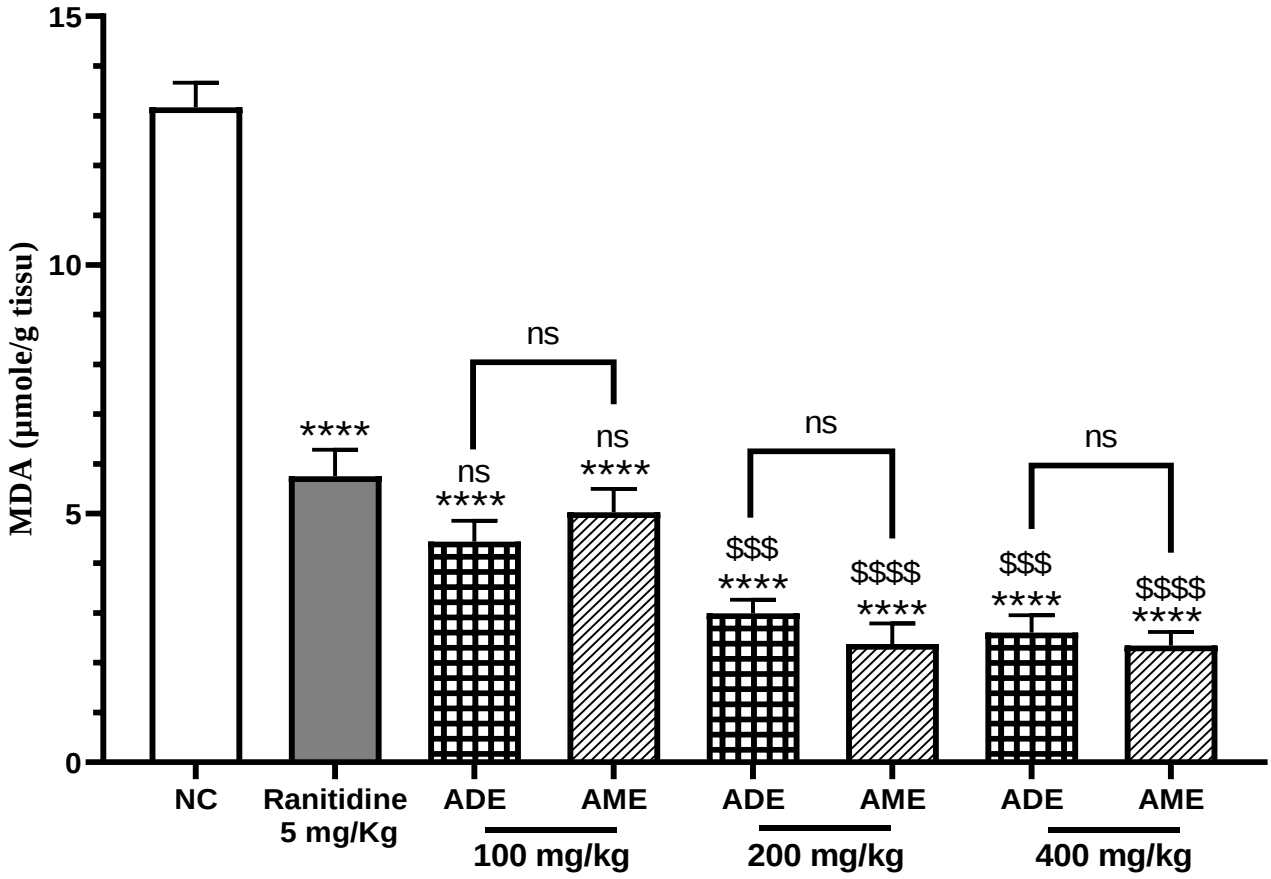


Figure 32. Effects of ADE and AME on MDA level of stomach tissue in ethanol-induced gastric mucosal lesions in rats.

ADE; decocted extract, AME; hydro-methanol extract. Bars represent means ± SEM (n=8). ****; P≤0.0001 *vs* vehicle as negative control., $$$; P≤0.001, $$$$; P≤0.0001 *vs* ranitidine as positive control. ns; no significant difference (P>0.05).

Discussion

1. Extraction yield

Achillea genus is used traditionally for treating many disorders and is viewed as a popular plant in many countries in the world and may be considered a functional food; this feature has been attributed to its high bioactive content and diversity of terpenes, flavonoids, coumarins, phenolic acids (Rahimmalek *et al*., 2009). The distinctive structures and functional groups of plant phytochemicals are known to affect their polarity and solubility in the different extraction solvents used, and thus the yields would be dependent on such characteristics. Methanol is frequently reported to generate the highest yields when used as an extracting solvent (Abrahim *et al*., 2012). Moreover, the extraction yield for most extract plants was directly proportional to the polarity of solvents. For instance, methanol and chloroform each gave the highest and lowest extraction yield, respectively. This finding is in agreement with Do *et al*., (2014) who demonstrated the superiority of polar solvents with hydroxyl groups such as methanol and water in extracting solid mass from plant samples. In the present study, the overall crude extraction yield which ranged from 4.0% to 16.6% was lower than those reported by Boutennoun *et al*., (2017) (18.2%) and higher than the yield extraction found by Benali *et al*., (2020) (0.37-1.04%) for the essential oil of the same plant. This could be explained by the type of solvents and method of extraction used in those studies.

2. Phytochemical screening

A. odorata L. extracts were examined for their total polyphenols, flavonoids and tannins, the results revealed that these extracts have high amounts of polyphenols. The highest polyphenol level has been identified in AME. These findings were lower than the previous study realized by Benali *et al*., (2020). These results are in agreement with those of Spigno *et al*., (2007), who suggested that polar solvents are the best extracting media for polyphenols and flavonoids, which may be due to an increase in polarity of flavonoids upon conjugation through glyscosides with hydroxyl groups that enhances their solubility in polar solvents. The chemical make-up of the extraction media used has a significant impact on the extraction yield of phenolics as well as their antioxidant efficiency (Dai & Mumper, 2010). These factors include plant part/type, genetic make-up of the species, agroclimatic conditions, harvest timing, and post-harvest processing. The type of extraction solvent chosen has a significant impact on the accuracy of botanical chemicals separated from plant material (Kalidindi *et al*., 2015). For the extraction of phenolic chemicals in a prior study, polar and nonpolar solvents like methanol, ethanol, acetone, propanol, ethyl acetate, and water were frequently employed (Alothman *et al*., 2009). The

solubility of these phenolic compounds in the solvent employed for the extraction procedure affects the recovery of phenolics from plant sources.

3. Phytochemical analysis (HPLC-DAD)

In this study, a fast and efficient HPLC-DAD method was developed for the determination of phenolic compounds of *A. odorata* L. extracts. The method uses a binary gradient system consists of methanol, deionized water and acetic acid. The separation was achieved in 40 min using a reversed phase C18 column and detection using a diode array detector. The method was precise, accurate, specific and highly reproducible for quantitative and qualitative purposes. The method was used to assess the phenolic composition of *A. odorata* L. areal parts extracts. A total of 9 common phenolic compounds was tentatively identified, namely Protocatechuic acid; Chlorogenic acid; p-hydroxy benzoic acid; Coumarin; Rutin; Ellagic acid; Rosmarinic acid; Myricetin; Luteolin.

Similary to our findings, Benetis *et al*., (2008) and Bobis *et al*., (2015) quantified some phenolic compounds in another specie of achillea genus named *Achellia millefolium* and the results revealed that chlorogenic acid, rutin and luteolin were the major compound in the leaves of this species. Fourteen Achillea species were extracted and analyzed using RP-HPLC-DAD gradient technique to obtain the fingerprint profiles. The presence of selected standards (chlorogenic acid, kaempferol, luteolin, naringenin, naringin, para-coumaric acid, quercetin, rutin, trans- ferulic acid, and vanillic acid) was confirmed in most of the studied plants based on their spectra and retention time values (Hawrył *et al.*, 2018).

4. Toxicity

4.1. Cytotoxic activity (Cell viability assay)

The cytotoxicity was assessed using the MTT assay on the lung cancer cell line H1299, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. *A. odorata* L. extracts exhibited varying degrees of activity against the tested cancer cell line, leading to the inhibition of cellular proliferation ranging from good to moderate. The high cytotoxic activity of the chloroform extract may be attributed to the presence of non-polar compounds. Our findings align with those of Elbatrawy *et al*., (2015) who also noted that extracts obtained using solvents of low to intermediate polarity exhibited greater cytotoxicity against cancer cells compared to highly polar solvents. In another study, cytotoxic activities of N-hexane, chloroform, aqueous-methanol and aqueous extracts of the aerial parts of the *A. millefolium* aggregate on MCF-7 cell lines were investigated by means of

MTT assays and after 48 h incubation of chloroform-soluble extract at 10 µg/mL exerted high tumor cell growth inhibitory activities on MCF-7 cells (53.95%) (Csupor-Löffler *et al*., 2009).

A. *odorata*'s cytotoxic properties have been documented in previous study on methanolic extract using three tumor cell lines: MCF-7, Hep2, and WEHI. The extract demonstrated activity against all three cancer cell lines and effectively inhibited cell proliferation in a dose-dependent manner. Specifically, at an extract concentration of 50 µg/mL, the percentage of deceased cells for MCF-7, Hep2, and WEHI was approximately 42.90%, 61.54%, and 81.13%, respectively (Boutennoun *et al*., 2017). A literature survey revealed that anticancer potential of some Achillea species and their metabolites has been investigated *in vitro* using various tumor cell lines. Among them, 80% of ethanol extracts of A. *biebersteini* and A. *millefolium* exhibited cytotoxic effect against MCF-7 cells (IC_{50} = 47.468 µg/mL and 64.058 µg/mL, respectively) after 24 h of incubation (Ghavami *et al*., 2010). Furthermore, Bali *et al*., (2015) reported that the methanol extract of *Achillea teretifolia* exhibited moderate cytotoxic effects on prostate cancer cells, with calculated IC_{50} values of 0.40 ± 0.05 mg/mL and 0.14 ± 0.04 mg/mL for 24 and 48 hours, respectively.

There is substantial evidence supporting a synergistic potential between antioxidant and anticancer properties in a wide range of medicinal plants. In this regard, significant attention has been directed towards plant polyphenols. All classes of phenolic compounds possess the necessary structural attributes to act as scavengers of free radicals and hold promise as antioxidants in the realms of chemistry, food, and medicine (Olivero-Verbel *et al*., 2010). Empirical investigations have shown that numerous naturally occurring substances and plant extracts exhibit anticancer potential across various bioassay systems and animal models, bearing relevance to human health (Abdel-Aziz *et al*., 2014). Among these compounds, phenolic substances, recognized for their potent anti-cancer capabilities (Surakka *et al*., 2005).

Numerous plant species, particularly those abundant in polyphenols like flavonoids, are known for their disease-preventive and therapeutic properties. This observation carries significant weight, given that flavonoids are constituents found in many fruits and vegetables, and the consumption of these foods has been linked to a reduced risk of cancer (Kanadaswami *et al*., 2005; Granado-Serrano *et al*., 2007). Furthermore, *in vitro* data corroborate these findings, demonstrating that a mixture containing these sterol compounds exhibits cytotoxic effects on human lung and breast cancer cells (Sundarraj *et al*., 2012). Certain phenolic compounds may also influence hormone production and inhibit aromatase, potentially thwarting cancer development (Zhao *et al*., 2007). The cytotoxic activity observed in our

current study aligns with these findings, as the phytochemical analysis points to the presence of promising flavonoids. It is evident from our study that some extracts from *A. odorata* L. exhibit strong, dose-dependent *in vitro* cytotoxic activity against the tested cell lines.

4.2. **Acute oral toxicity**

The lack of scientific data about the effectiveness and safety of medicinal plant is one of the major problems in the use of traditional medicinal preparations. This is probably due to the non-evaluation of mechanism action and toxicological profile of these plants. Acute or lethal toxicity refers to a chemical compound's ability to lead to death relatively soon after being orally ingested, or after being exposed. The period has conventionally been expressed in minutes, hours, and weeks (up to two weeks) (Walum, 1998).

Achellia odorata L. is used in traditional medicine, but to date, there is no documented evidence corroborating their safety in an acute toxicity study. A one-time oral administration of the ADE and AME at dosages of 2000 mg/kg and 5000 mg/kg did not result in any treatment-related alterations in behavior or fatalities among male and female mice over a 14-day observation period. Consequently, this implies that the LD_{50} exceeds 5000 mg/kg, in accordance with the OECD guideline. Therefore, the extract is classified as a Category 5 substance under the Global Harmonisation System of Chemical Substances, signifying it is reasonably low in toxicity. This study represents the initial investigation into the acute toxicity of *A. odorata* L., which explains the absence of prior literature findings on the subject. Jafari *et al*., 2019 reported similar findings regarding the acute toxicity of *A. wilhelmsii* hydroalcoholic extract. Their study suggests that the safe dose was ≤5000 mg/kg. Another study that assessed the acute toxicity of *A. biebersteinii* essential oil on mice at various doses did not reveal any instances of mortality or pathological symptoms, even at the highest dose (0.5 mL/kg) (Al-Said *et al*., 2016).

Certain enzymes (ALAT, ASAT) have been identified as sensitive indicators of hepatocellular effects (Rawi *et al*., 2012). In the present study, these enzymes (ALAT and ASAT) did not exhibit any noteworthy changes, whether in the ADE or AME groups at doses of 2000 mg/kg and 5000 mg/kg, when compared to their corresponding control groups. The liver histopathology results revealed the absence of a significant vascular congestion, infiltration or necroses in the groups treated with 2000 and 5000 mg/kg of both extracts. The absence of these pathological changes can confirm the safety effects of ADE and AME on the livers of rats.

Creatinine and urea are recognized as biomarkers for assessing renal (Wasung *et al*., 2015). Notably, a significant ($P < 0.1$) decrease in creatinine levels was observed at the 2000 mg/kg dose of ADE, possibly suggesting muscle wastage. This decrease could be influenced by extrarenal factors like a high-protein diet or increased protein catabolism (Perrone *et al*., 1992). However, renal histological analysis indicated a normal and non-pathological state for both extracts.

5. *In vitro* biological activities

5.1. Antioxidant activities

The antioxidant activity of natural substances *in vitro* can be determined by several analytical methods. However, the vast diversity in phytochemical composition and oxidative processes necessitates the assessment of the antioxidant activity of plant extracts using a variety of methods (Inchuen *et al*., 2010). Various mechanisms can be responsible for antioxidant activity, such as the elimination of free radicals, the decomposition of peroxide, the prevention of the initiation of the oxidation chain, the hydrogen atom abstraction, the reduction of capacity, and the binding of transition metal ionic catalysts (Mao *et al*., 2006). The antioxidant activity observed in the extracts results from the neutralization of free radicals (DPPH) through either electron or hydrogen atom transfer (Benchikh *et al*., 2018).

DPPH is a compound known for its proton free radical properties, characterized by a distinct absorption spectrum that significantly decreases in the presence of proton radical scavengers (Moreira *et al*., 2023). It is widely accepted that antioxidants scavenge the DPPH free radical through their hydrogen-donating ability. DPPH has been commonly used to assess the free radical-scavenging capabilities of various substances. Antioxidants interact with DPPH, reducing the number of DPPH molecules by donating hydroxyl groups.

Previous studies have highlighted the potent antiradical activity of BHA (Gülçin *et al*., 2004). In our study, the crude extracts of *A. odorata* L. demonstrated notable DPPH radical scavenging activity. Among all the extracts investigated, the AME exhibited the highest radical scavenging activity, surpassing that of alpha-tocopherol. Furthermore, the total phenolic content in the AME extract was found to be exceptionally high compared to the other extracts, supporting our conclusion that phenolic compounds play a significant role in this activity.

Recent reports have highlighted a strong positive correlation between polyphenols, flavonoids, and antioxidant activities in various plant species (Kim *et al*., 2007; Kumaran & Joel Karunakaran, 2007; Ozsoy *et al*., 2008). Antioxidant activity is closely associated with the

chemical composition of the plant, with all constituents interacting synergistically or antagonistically. The excellent antioxidant activity of the plant extract can be attributed to the presence of phenolic compounds like apigenin and chlorogenic acid, which are major phenolic constituents in Achillea species (Vitalini *et al*., 2011). These phytochemicals possess the ability to scavenge free radicals, act as reducing agents, donate hydrogen atoms, or quench singlet oxygen against reactive species involved in oxidative stress-induced cell damage (Carocho & Ferreira, 2013). Our findings suggest that phenolic acids and flavonoids are likely the primary contributors to the radical scavenging activity of Achillea extracts.

Our results closely align with those reported by Boutennoun *et al*., (2017) demonstrating a high DPPH radical scavenging effect (88.34%) for the methanol extract of *A. odorata* collected from Jijel, Algeria. In contrast, a separate study indicated weak scavenging activity for the essential oil of *A. odorata* collected from Morocco (IC_{50} = 189.8 ± 1.09 µg/mL) (Benali *et al*., 2020). Furthermore, prior research has confirmed the potent DPPH radical scavenging properties of other Achillea species. For instance, Candan *et al*., (2003) reported that A. *millefolium* exhibited DPPH radical scavenging with IC_{50} values of 45.60 ± 1.30 µg/mL. In addition, Dias *et al*., (2013) found that IC_{50} values for methanol extracts of wild and commercial *A. millefolium* were 0.50 ± 0.01 mg/mL and 0.37 ± 0.01 mg/mL, respectively. Additionally, the methanolic extract of *A. biebersteinii* reduced the stability of DPPH radicals with an IC_{50} value of 89.9 µg/mL. Furthermore, Mehlous, (2023) indicated that the methanolic extract of *A. santolinoides* L. exhibited the highest activity compared to other extracts, with an IC_{50} value of 24.20 ± 0.93 µg/mL.

ABTS reagent is a stable radical that is directly produced from the oxidation of ABTS by potassium persulfate (Dudonne *et al*., 2009). The ABTS blue/green chromophore decolorizes when it is scavenged by antioxidant molecules and this can be monitored at 734 nm. The decrease in ABTS absorbance in the presence of the tested extract reflects the percentage of ABTS inhibition. The scavenging properties of antioxidant compounds are often associated with their ability to form stable radicals (Wootton-Beard *et al*., 2011). DPPH and ABTS methods have the same mechanism of reaction based on the electron transfer (Christodouleas *et al*., 2015).

Commonly, extracts obtained with high-polarity solvents show better radical scavenging activity. The polar phase of the extract contributes to the inhibition of ABTS radicals through simple electron transfer (Prior *et al*., 2005). There are no published studies that have evaluated the ABTS radical scavenging activity of *A. odorata* L. In previous literature, a study of three

Achillea species (*A. biebersteinii, A. millefolium, and A. teretifolia*) reported that the methanol extract exhibited stronger antioxidant abilities, which correlated with higher levels of phenolic compounds when compared to the non-polar extracts (Zengin *et al*., 2017). Moreover, a study by Gevrenova *et al*., (2021) revealed that the antioxidant properties assessment of the methanol extract of *A. santolinoides* roots actively scavenged ABTS radicals (112.53 mg trolox equivalent/g). These high ABTS radical scavenging potential results could be attributed to the polyphenols and flavonoids content in the plant extracts. Phenolic compounds possess strong radical scavenging potential (Miliauskas *et al*., 2004), more specifically, the presence of a carbonyl group, such as in esters like chlorogenic acid, enhances this activity (Göçer & Gülçin, 2011).

The β-carotene bleaching assay belongs to the oldest and continuously commonly applied methods of estimating the antioxidant activity of compounds and mixtures. The basis of this assay is discoloration of β-carotene in reaction with linoleic acid free radical formed upon removal of hydrogen atom located between two double bonds of linoleic acid. The consequence is the loss of conjugation and accordingly, a decrease in absorbance at 490 nm. Antioxidants can reduce the extent of β-carotene destruction by reacting with the linoleate free radical or any other free radical formed within the system. Thus, by simulation of the oxidation of the membrane lipid components in the presence of antioxidants, this test gives an insight of the inhibitory effect of extracts on the lipid peroxidation (Petlevski *et al*., 2013).

The same results were reported by Haliloglu *et al., (2017)*, who demonstrated that the ethyl acetate extract and methanol extract had the highest estimated inhibition values for linoleic acid oxidation, at 53.9% and 55.8%, respectively. In contrast, the essential oil and methanol extract from *A. biebersteinii* Afan. were not effectively able to inhibit linoleic acid oxidation, exhibiting only 16% and 22.7%, respectively (Bariş *et al*., 2006).

Moreover, these results indicated that all the extracts can significantly inhibit oxidation of linoleic acid, but the semi-polar extract is more effective (AEAE). In conclusion, the activity of AEAE was more active than AME, the richest one by phenolic contents. It should be noted that the chemical characteristics of the solvents used during extraction have a remarkable effect on the organic type of the agent's antioxidants, resulting from the extraction process (Wijesinghe & Jeon, 2012). The potent lipid bleaching activity of AEAE may be due to its high quantity of chlorogenic acid. This is confirmed by a study that indicates that the presence of chlorogenic acid bleached β-carotene most rapidly (Terpinc & Abramovič, 2010).

The chelating capacity is very important because it reduces the concentration of transition metal catalysts of the lipid peroxidation. Indeed, iron can stimulate lipid peroxidation by the Fenton reaction, and accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Sen *et al*., 2013). The observed results demonstrate that all extracts interfered with the formation of ferrous and ferrozine complex suggesting that they have chelating activity through capturing the ferrous ion before it reacts to form a complex with ferrozine.

Iron and flavonoids are the most studied transition metal and polyphenol family, respectively. From those studies, there is a consensus on the presence of ‘‘iron chelation sites” or ‘‘iron binding motifs” in an important number of flavonoids (Kazazić *et al*., 2006) and demonstrated that flavonoids like kaempferol, chlorogenic acid and apigenin, can trap free electrons and chelate metals on the functional carbonyl groups that cause oxidative reactions (KoNO *et al*., 1998; Lue *et al*., 2010; Spiegel & Sroka, 2023). This kind of flavonoids were present in most extract of this study. Nevertheless, as far as we know, this is the first report of metal chelation of *A. odorata* L.

In line with our results, Bariş *et al*., (2006) used an EDTA solution as the standard chelator in their study with Achillea species. They found that the extracts in this system did not demonstrate superior chelating properties compared to the effective chelator EDTA solution. In a study involving three Achillea species, *A. boissieri*. *A. cretica* and *A. nobilis*, no chelating capacity was observed at all tested concentrations (12.5-125 µg/mL) (Tekin *et al*., 2021).

In the reducing power assay, the yellow color of the test solution changes to various shades of green and blue, which depend on the reducing power of each compound. The presence of reducers (i.e., antioxidants) leads to the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm allows the monitoring of Fe^{2+} concentration ((Ferreira *et al*., 2007). It was observed that all these extracts exhibited significant ferric reducing power. The reducing power capacity of the extracts can offer valuable insights into the potential antioxidant capacity of the plant. This potent activity may be attributed to the high phenolic content and their strong electron-donating abilities (Bilto & Alabdallat, 2015).

Additionally, a statistical analysis of three Achillea species (*A. pachycephalla, A. kellalensis, A. aucherii*) indicated a robust and significant correlation (R = 0.97) between the ferric reducing antioxidant power and the phenolic contents of the plant extracts (Gharibi *et al*., 2013).

In a previous study, Boutennoun *et al*., (2017) reported that the transformation of Fe^{3+} to Fe^{2+} in the presence of different concentrations of the methanolic extract of *A. odorata* may serve as a significant indicator of its potential antioxidant activity. Furthermore, Benali *et al*., (2020)'s research indicates that the reducing power of the essential oil of *A. odorata* exhibited a value of 0.85 ± 0.24 milligrams of ascorbic acid equivalence per gram of extract.

One of the most effective, recent methods of antioxidant potential estimation is the CUPRAC assay. This method possesses multiple advantages, including the fact that measurements are done at a neutral pH (about 7), which is more representative of living systems (Apak *et al*., 2007). The CUPRAC test is based on the reduction of Cu^{2+} to Cu^{+} by utilizing bis(neocuproine) Cu^{2+} as a chromogenic oxidizing agent in the presence of antioxidants. An increase in absorbance signifies an augmented reduction capacity resulting from increased complex formation. The ability of various prepared extracts to reduce copper ions was investigated for the first time in this study.

In prior findings, it was revealed that the *A. coarctata* extract exhibited strong CUPRAC activity, with values ranging from 0.15 to 2.90 at concentrations of 0.8–3 mg/mL ($P<0.05$), as reported by Albayrak & Silahtarlıoğlu, (2020). Additionally, a study conducted by Mehlous *et al*., (2022) confirmed the potential reducing power of *A. santolinoides* L., with an $A_{0.50}$ value of 76.56±2.35 µg/mL.

The obtained results demonstrated that the tested extracts possess a good ability to reduce cupric ions (Cu^{2+}). This substantial reducing potential can be attributed to the presence of natural antioxidants like chlorogenic acid and apigenin, as indicated by studies conducted by Lue *et al*., (2010).

5.2. **Enzymatic inhibitory activities**

Nowadays, inhibiting key enzymes associated with the pathology of various diseases is considered one of the most effective strategies for their treatment and management (Vecchio *et al*., 2021). For instance, targeting AChE and BChE, enzymes involved in Ach breakdown, are widely accepted as a strategy for treating Alzheimer's disease. While many synthetic compounds (e.g., galantamine and tacrine) have been developed by the pharmaceutical industry, they often come with adverse effects, including GI issues and hepatotoxicity (Mechchate *et al*., 2021). In this context, the enzyme inhibitory properties of medicinal plants and plant-derived products hold great promise in drug development. There is a growing trend

to replace synthetic inhibitors with natural ones due to their potential as natural enzyme inhibitors (AShah *et al*., 2017).

The majority of extracts used in this study exhibited a moderate AChE and BChE activities compared to the galantamine, in previous literature, no scientific research has been conducted on *A. odorata* L. effect on AChE and BChE inhibition, contrary of our results, studies reported in the literature for other Achillea species on enzyme inhibitory activities , such as *A. cucullata* (Eruygur *et al*., 2019) (0.90 mg/mL for AChE and 0.50 mg/mL for BChE) and *A. millefolium* (Barut *et al*., 2017) (0.217 mg/mL for AChE) highlighted the strong AChE and BChE inhibitory activities of these species. Another study has shown that a group of flavonoids, such as quercetin, chrysin, morin, isorhamnetin, rhamnetin, and kaempferide, exhibited significant inhibitory effects on AChE and BChE activity (Khan *et al*., 2018). It is possible that the moderate AChE and BChE inhibitory activity observed in *A. odorata* L. extracts may be due to the absence of these flavonoids.

Numerous studies have explored the potential of plant constituents for treating diabetes mellitus, with a particular focus on their ability to inhibit enzymes related to carbohydrate metabolism, especially alpha-amylase and alpha-glucosidase. (Dirir *et al*., 2022). However, there is currently no scientific data available regarding the anti-diabetic activity of the tested plant extracts. Consequently, we conducted an investigation into the impact of these extracts on the activity of alpha-amylase and alpha-glucosidase, crucial enzymes involved in carbohydrate breakdown and intestinal absorption.

The results, except AEAE, indicate a moderate level of inhibition against both enzymes, implying that the various extracts derived from the aerial parts of *A. odorata* L. exhibit limited anti-diabetic properties. And this is in agreement with a previous study that demonstrated a moderate to moderate anti-diabetic activity in *A. sieheana* (Dİkpinar & BİtİŞ, 2022). The strong activity of AEAE could be due to the high concentration of some phenolic compounds as chlorogenic acid.

Tyrosinase is a copper-containing enzyme and involved in melanin synthesis. From this point, tyrosinase inhibitors have become increasingly important in medical and cosmetics industry (Obaid *et al*., 2021). It is considered to be a key therapeutic strategy for the management of skin hyperpigmentation conditions (Pillaiyar *et al*., 2017). As can be seen from results, the tyrosinase inhibitory capacity (% of inhibition) of the five extracts exhibited a moderate activity compared to the standard. However, all these values had a significant

difference compared to the control. there are no published studies that have evaluated the tyrosinase inhibitory effect of *A. odorata* L. In previous literature and similarly to our results, the most active extracts of *A. sivasica* were found to be ethyl acetate and ethanol extracts (5.5 and 6.2 mg kojic acid equivalents/g extract, respectively) (Haliloglu *et al*., 2017).

6. *In vivo* biological activities

6.1. ADE and AME effects on gastric emptying and intestinal transit in mice and the possible mechanism (s) involved

Constipation and diarrhea are two distinct antagonistic disorders characterized by an imbalance in intestinal absorption/secretion/motility leading to unexplained abdominal pain, discomfort and bloating in association with altered bowel habits (Birru *et al*., 2016). Additionally, the perturbation of gastric emptying process and the motility changes are part of a spectrum of responses which includes nausea and vomiting as its more severe manifestations (Thompson *et al*., 1982). Thus, in this study, the aim was to investigate the dual effectiveness of *A. odorata* L. extracts on intestinal motility and gastric emptying.

Achillea genus is used traditionally for treating GI disorders and is viewed as a popular plant in many countries in the world and may be considered a functional food; this feature has been attributed to its high bioactive content and diversity of terpenes, flavonoids, coumarins, phenolic acids, lignans and essential oil some of which are responsible for the antioxidant, antiulcerogenic, antimicrobial, antispasmodic, immunosuppressive, antitumor and antidiabetic activities (Si *et al*., 2006; Fathi *et al*., 2011). Various species of the genus are traditionally used in the world for wound healing, against diarrhea and flatulence, as a diuretic, as emmenagogue agents, and for abdominal pain (Baytop, 1984). The level and concentration of chemical constituents have been of interest because of their important influence in GI tract.

The gastric emptying and intestinal motility process results from mechanism activities that inhibit or stimulate the motor activity of the stomach, pylorus and intestine (Wilson & Schey, 2015). In our experiment, results showed that the gastric emptying and intestinal motility process significantly influenced by ADE and AME pretreated mice, while causing an opposite action, compared to the controls. Gastric emptying of a phenol red meal was studied as an index of fundus functionality through its tonic contractions which increase intragastric pressure leading to a gradient of peptic pressure. Indeed, stomach emptying is a complex mechanism affected by several factors, including the size of the meal, the caloric density, water intake,

nutrient composition, pharmaceutical administration, the particle size, the state health and stress (Goggin *et al*., 1999).

The delaying effects of the extract on gastric emptying may result from the relaxation of the stomach musculature and/or from the constriction of the pyloric sphincter, while the delay of intestinal transit may involve the inhibition of muscle contraction and/or consolidation of the inhibitory component of the intestinal muscle (Nwinyi *et al*., 2004). Our results are in agreement with the study realized by Niazmand & Khoshnood, (2011) on the same genus showed an inhibitory effect on gastric emptying in both basal and vagal stimulated condition. This inhibitory effect may be exerted by an antagonistic effect on Ach dependent calcium influx or release of calcium from intracellular storage in gastric smooth muscle. Also, Karamenderes & Apaydin, (2003) showed that the *A. nobilis* extract had an inhibitory effect on Ach induced contraction in rat's duodenal smooth muscle by an antagonistic effect on Ach. The effect of *A. santolinoides* L. aqueous extract on gastric emptying and intestinal transit were studied in mice (Mehlous, 2023). It was found that the extract induced a significant delay in gastric emptying and in intestinal transit.

Polyphenols show relaxing effects on the contractile activity of various smooth muscles, such as vascular smooth muscles (Ajay *et al*., 2003; Mendes *et al*., 2003), muscles of the bladder (Dambros *et al*., 2005) and the muscles of the uterus (Revuelta *et al*., 1997). In the GI tract, polyphenols show an inhibitory action against the contractions of smooth muscle cells. Indeed, this strong inhibitory effect of ADE and AME against contraction of GI muscles may due to the presence of a good quantity of chlorogenic acid in the extracts and this is in agreement with study realized by Posłuszny *et al*., (2022) which confirm the antispasmodic effect of chlorogenic acid against contraction. Also Capasso *et al*., (1991) reported that apigenin present in extracts is also active inhibitor of the contractions induced by LTD4. The authors have proposed that the effect of this flavonoid involves calcium. In the stomach it is shown that apigenin (1 µM – 100 µM) induce relaxation of the gastric smooth muscles of mice in a concentration-dependent manner, suggesting that this effect is mainly due to their ability to inhibit the entry of calcium via voltage-gated calcium channels (Rotondo *et al*., 2009). On the other hand, the delaying effect of gastric emptying is probably due to the blocking of muscarinic receptors by rosmarinic acid, which is able to inhibit these receptors in the mice ileum (Demirezer *et al*., 2015). Polyphenols also act on the intestinal transit thus exerting antidiarrheal effects, the relaxation of stomach muscles in mice induced by several types of flavonoids such as apigenin, genistein, quercetin, rutin, naringenin and catechin depends essentially on the

structure of these flavonoids and the position of the hydroxyl groups (Amira *et al*., 2008). Di Carlo *et al*., (1993) showed that intraperitoneal administration of some flavonoids: apigenin, myricetin and rutin present in ADE and AME reduced intestinal transit by 28-69% in mice. Finally, it has also been demonstrated that the pathological state plays a key role in the effect of extracts on intestinal transit. The ethanolic extract of *S. divinorum* does not affect intestinal transit in normal mice, but does reduce it in the case of induced inflammation (Capasso *et al*., 2006).

Atropine, a competitive antagonist of Ach at muscarinic receptors, can reduce gastric emptying and small intestinal motility (Kimura & Sumiyoshi, 2012). The role of ways is likely that blocks M1 receptors on gastric parietal cells and helps in reduction of gastric secretions (Bahekar & Kale, 2015). Furthermore, it blocks M3 receptors on visceral smooth muscles of stomach and intestine leading to relaxation of these muscles and decrease the tone and amplitude of these organs (Sharma & Sharma, 2011). Atropine has a maximal inhibition on gastric emptying and intestinal transit, in the same time, the inhibitory effect of the extracts pretreated with atropine was significantly different from that of atropine as a control which indicate the involvement of other pathways causing this effect and is totally not due to the blockade of muscarinic receptors. Thus, might deny the involvement of muscarinic receptors in the inhibitory action of ADE and AME on gastric emptying and intestinal motility and suggest another mechanism. Oppositely to our finding and according to previous study the delayed gastric emptying effect is probably due to muscarinic receptor blockade by rosmarinic acid which is able to inhibit these receptors in the rat ileum (Demirezer *et al*., 2015). Other study on *Aquilariae Lignum* Resinatum suggest that this plant played a spasmolytic role in GI motility, which is probably mediated through inhibition of muscarinic receptors, blockade of Ca^{2+} influx and NO release (Li *et al*., 2018).

NO is generated from the terminal guanidinonitrogen atom(s) of the amino acid L-arginine by the enzyme NO synthase (NOS) (Moncada *et al*., 1991). At least two types of NOS have been so far identified. One is constitutive NOS, Ca^{2+}/calmodulin-dependent and releases NO for short periods in response to receptor stimulation. The other enzyme is inducible NOS, Ca^{2+}-independent and, once expressed, generates NO for long periods. Both enzymes are inhibited by certain L-arginine analogues (Matsuda *et al*., 1999). NO is a nonadrenergic, noncholinergic neurotransmitter. It serves as a mediator of the effects of some substances on GI motilities. Pretreatment with L-arginine delay the intestinal motility and gastric emptying (Martínez-Cuesta *et al*., 1997). In the present study, pretreatment with ADE and L-arginine delay the

intestinal transit and gastric emptying comparably to the control group, these results indicate that NO, is a mediator implicated in the intestinal motility and gastric emptying inhibitions by ADE and may imply that this effect depends on the NO pathway. Oral administration of AME with L-arginine had the same effect on intestinal transit as group of L-arginine alone but not on gastric emptying which suggest that AME follows the NO pathway only with intestinal transit effect. previous study on *Helichrysum stoechas* L. showed antispasmodic activity exerting through activation of the NO/cGMP pathway (Valero *et al*., 2022).

Endogenous prostaglandins (PGs) are generated from arachidonic acid by the enzyme cycle-oxygenase (COX). PGs have complex pharmacological activities in the modulation of GI motilities and they are well known for their ability to stimulate contraction in GI smooth muscle. It appears that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increase the rate of the emptying of liquids from the human stomach; PGE series contract the longitudinal smooth muscle layer and relax the circular layer. In contrast, PGF series contract both small muscle layers (Sanders & Ross, 1978). Both PGE_2 and $PGF_{2\alpha}$, but not PGI_2, stimulate intestinal transit and cause diarrhea (Pierce *et al*., 1971). Indomethacin is an inhibitor of COX, preventing production of PGs from arachidonic acid and has an anti-spasmodic effect. In the present study, similarly to the indomethacin effect, both intestinal motility inhibitions and gastric emptying delaying by ADE and AME were attenuated by pretreatment with indomethacin and no significant difference with group of indomethacin alone was recorded for both extracts. These results indicate that the inhibition of endogenous PGs was important in the effects of ADE and AME on intestinal motility and gastric emptying. and might indicate the participation of the cyclooxygenase pathway in these effects.

6.2. ADE and AME effects on ethanol-induced gastric ulceration in rats and possible mechanism (s) of action

Alcohol consumption stands as the primary factor contributing to the development of gastric ulcers, as noted by Shen *et al*., (2019). Excessive alcohol intake typically increases the likelihood of damage to the gastric mucosa. Consequently, the rat model of ethanol-induced gastric mucosal damage is frequently utilized in research to evaluate compounds with potential anti-ulcer properties, given that it serves as the predominant cause of gastric ulcers observed in humans, as outlined by Hobani *et al*., (2022).

Ethanol-induced damage to the gastric tissue is associated with the generation of oxygen free radicals, as reported by Li *et al*., (2013). Furthermore, the production of inflammatory mediators

represents a crucial factor in the underlying mechanisms of these lesions. Additionally, gastric blood flow and disruption of microvasculature contribute to hemorrhaging and necrotic tissue injury (Liu *et al*., 2012). Neutrophil infiltration is a pivotal process in the induction of injury and inflammation, involving the aggregation and release of tissue-disrupting substances within various tissues, including the gastric mucosa (Krawisz *et al*., 1984). A prior study has demonstrated that the infiltration of neutrophils into gastric mucosal tissues is implicated in the development of acute gastric mucosal lesions (Nishida *et al*., 1998a).

Multiple investigations have linked the protection against gastric ulcers to the presence of phenolic acids and flavonoids found in plant extracts (Cadirci *et al*., 2007; Mota *et al*., 2010; Amaral *et al*., 2013). Hussain *et al*., (2009) detailed the substantial gastroprotective effects of rutin, attributed to its anti-inflammatory properties, vasoactive attributes, and its ability to scavenge reactive oxygen species (ROS) produced during ethanol-induced lesions. Additionally, kaempferol has exhibited protective effects in ethanol-induced gastric ulcers by reducing oxidative stress and enhancing the activity of antioxidant enzymes (de Lira Mota *et al*., 2009).

Polyphenols, encompassing efficient scavengers of superoxide anion, hydroxyl, peroxyl, and alcoxyl radicals, have been shown in previous studies (Amaral *et al*., 2013; Shimoyama *et al*., 2013) to possess antioxidant, anti-obesity, and gastroprotective properties. Chlorogenic acids, in particular, have been noted for their antioxidant activity and their ability to mitigate gastric injury induced by acidified ethanol or NSAIDs. This protective effect can be attributed to the inhibition of leukocyte migration, increased antioxidant enzyme activity, including the restoration of CAT, SOD, GPx, and GSH levels, along with the suppression of TNF-α and leukotriene B4 production (Krawisz *et al*., 1984).

The presence of these beneficial compounds elucidates the protective effects of *A. odorata* L. against ethanol-induced gastric injury. Furthermore, various extracts or fractions derived from *A. millefolium, A. biebersteinii,* and *A. wilhelmsii* have been shown in other studies (Niazmandi *et al*., 2012; Abd-Alla *et al*., 2016; Alomair *et al*., 2022) to significantly reduce lesions induced by ethanol in rat models.

Mucus is widely recognized as an effective barrier against acid and pepsin exposure (Cai *et al*., 2021). It is believed that the visible mucus adhering to the stomach wall, as opposed to the mucus dissolved in gastric secretions, plays a more crucial role in safeguarding the gastric mucosa from self-digestion (Liu *et al*., 2021). In our current investigation, the extracts from *A.*

odorata L. have demonstrated the capacity to stimulate the synthesis of gastric mucus. This action likely contributes to its effectiveness in preventing lesion formation induced by ethanol in gastric ulcers. Our findings align with previous studies indicating that increased gastric mucus secretion, induced by *A. wilhelmsii* extracts, plays a vital role in the prevention of lesions caused by substances like indomethacin (Niazmandi *et al*., 2012).

Earlier research conducted in chronic experiments involving dogs has shown that an extract of *A. asiatica* Serg. induces a reduction in the aggressive properties of gastric juice (including proteolytic enzyme activity and acidity) and an enhancement in mucus synthesis and the stomach's excretory function (Vymiatnina & Gridneva, 1997).

Ethanol induces oxidative stress by increasing ROS production and depleting cellular antioxidants. This ROS-driven process leads to lipid peroxidation and protein oxidation, contributing to both acute and chronic ethanol-induced gastric ulcers, primarily caused by harmful agents like superoxide anions, hydroxyl radicals, and lipid peroxides (Kwiecien *et al*., 2002).

Scavenging free radicals is a key mechanism in gastric ulcer healing (Sowndhararajan & Kang, 2013). While gastric cells have various antioxidants, including CAT, SOD, GPx, GSH, and sulfhydryl groups (NPSH), excessive ROS production depletes these defenses (Cadirci *et al*., 2007; Liu *et al*., 2012).

The abnormal increase in reactive species is a primary aggressive mechanism of ethanol, which can result in damage and cell death in gastric cells (Ineu *et al*., 2008). In this study, ethanol induced a depletion of non-enzymatic defenses and inhibited the antioxidant enzyme CAT. Indeed, the depletion of GSH (the major non-protein thiol) and the inhibition of CAT following ethanol exposure have been previously documented (Xie *et al*., 2020), and these factors directly contribute to the observed increase in lipid peroxidation in ethanol-treated rats. Furthermore, lipid peroxidation in gastric tissue significantly contributes to the pathogenesis of ethanol-induced gastric lesions (Mohan *et al*., 2020). A previous report confirmed that ethanol increases the production of superoxide anions and hydroxyl radicals by neutrophils, which react with cellular lipids, leading to the formation of lipid peroxides that are further metabolized to form malondialdehyde (MDA) (Rahman *et al*., 2020). Lipid peroxidation is a crucial mechanism of cellular injury, and MDA is one of its end products. Therefore, determining MDA levels can be used to assess lipid peroxidation (Mohan *et al*., 2020).

It has been demonstrated that ethanol-induced damage to the gastric mucosa is associated with a significant decrease in levels of sulfhydryl compounds, particularly GSH, in experimental animals (Serafim *et al*., 2020). Depletion of GSH results in enhanced lipid peroxidation, and excessive lipid peroxidation can lead to increased GSH consumption. Conversely, an increase in gastric non-protein sulfhydryl content helps limit the production of oxygen-derived free radicals and may be related to gastric protection in various experimental models, such as the ethanol-induced ulcer model (Ugan & Un, 2020).

A. odorata L. extracts effectively restored the gastric mucosal damage and oxidative stress induced by ethanol. The broad antioxidant properties of ADE and AME were demonstrated by the reduction in MDA levels and the increase in antioxidant defenses (GSH and CAT). These protective effects attributed to the crude extracts of *A. odorata* L. can be associated with the presence of phenolic acids, primarily chlorogenic (Chen *et al*., 2018), ellagic (Qin *et al*., 2022), and rosmarinic acids (Gupta *et al*., 2021), in addition to flavonoids such as rutin and luteolin (Iova *et al*., 2021).

A previous study reported that the administration of *A. millefolium* increased proliferative activity and enhanced antioxidant mechanisms in the gastric mucosa injured by necrotizing agents (Alomair *et al*., 2022). In another study, *A. biebersteinii* reduced oxidative stress and acute gastric mucosal damage by preventing a reduction in the activity of GSH and SOD levels, which are known to scavenge reactive oxygen metabolites (Abd-Alla *et al*., 2016).

Several studies have demonstrated the positive impact of various classes of polyphenols on the activities of antioxidant enzymes such as SOD, CAT, and GPx in both animal models and human research (Wu *et al*., 2015; Rudrapal *et al*., 2022). The precise mechanisms underlying how polyphenols activate these antioxidant enzymes are not yet fully understood. However, numerous studies have suggested a link between polyphenols and the expression of antioxidant enzymes, possibly through the activation of nuclear factor-erythroid-2–related factor 2 (Nrf 2) *in vivo* and in various cultured cell lines (Oliveras-López *et al*., 2014; Nakayama *et al*., 2015).

To further clarify, other gastroprotective mechanism pathways of ADE and AME, several pharmacology substances L-arginine, L-NNA or indomethacin and yohimbine were tested.

Therefore, we conducted an investigation into the mechanisms responsible for the gastroprotective effects of *A. odorata* L. extracts against acute gastric mucosal lesions induced by ethanol. Ethanol is a potent mucosal damaging agent that causes injury through direct contact with the gastric mucosa. We observed that ethanol, as a topical irritant that directly interacts

with the gastric mucosa, resulted in extensive hemorrhagic lesions in the gastric mucosa of rats. Similar hemorrhagic lesions induced by ethanol have been documented in the human stomach (Jung *et al*., 2011).

The maintenance of gastric mucosal integrity when exposed to various damaging agents relies on a delicate balance between the epithelial and subepithelial components, which are regulated by the proliferative zone of gastric glands and mucosal defense mechanisms. These mechanisms include maintaining uninterrupted mucosal blood flow, mucus and alkaline secretion, and the restoration and proliferation of mucosal cells (Silva & de Sousa, 2011). Endogenous prostaglandins, as well as the activation of endothelial and mucosal production of NO, hydrogen sulfide, and carbon monoxide, contribute to mucosal integrity and gastroprotection (Jasnos *et al*., 2014).

L-arginine is an amino acid that acts as a substrate for the constitutive enzyme NO-synthase (cNOS). This enzyme produces NO, a potent vasodilator initially known as endothelium-derived relaxing factor. NO plays a crucial role in modulating the integrity of the gastric mucosa, and its significance in various physiological processes, including those in the GI tract, has been well established (Sharma *et al*., 2007).

Previous research has indicated that an enhanced ulcerogenic response is mediated by endogenous NO, primarily produced by iNOS (Nagai *et al*., 2009). Both NO and substances known as NO-donors, such as glyceryl trinitrate, isoamyl nitrate, and nitroprusside, have demonstrated the ability to protect the gastric mucosa from damage induced by various ulcerogenic agents, including absolute ethanol, endothelin-1, acidified aspirin, and stress (Khattab *et al*., 2001). However, it's worth noting that NO-donors like S-nitroso N-acetyl-penicilamine or sodium nitroprusside, when administered at higher doses, can exacerbate lesions caused by topical irritants. This suggests that an increased synthesis of NO, possibly via activation of an inducible form of NO-synthase, may render the gastric mucosa more susceptible to damage from potent irritants (Szlachcic *et al*., 2013). In animal models of gastric ulcer, systemic administration of L-arginine, the substrate for NOS, has been shown to reverse the adverse effects caused by NOS inhibitors, which worsen mucosal integrity and gastric blood flow. These inhibitors include substances like L-NNA or NG-nitro-L-arginine methyl ester (L-NAME). Additionally, L-arginine has been implicated in the regulation of gastric acid secretion, modulation of gastric mucosal integrity, and cooperation with endogenous prostaglandins to reduce leukocyte-endothelial cell rolling and adherence (Calatayud *et al*., 1999; Khalifa *et al*., 2002). However, there has been limited research on whether L-arginine

can protect the gastric mucosa from damage caused by potent irritants or expedite the healing of chronic gastric ulcers.

Multiple studies have emphasized the significance of endogenous NO in safeguarding the gastric mucosa (Kim & Kim, 1998; Korbut *et al*., 2020; Zhao *et al*., 2020). Specifically, NO generated by NOS plays a pivotal role in regulating gastric mucosal integrity by interacting with sensory neuropeptides and endogenous prostaglandins (Tepperman & Whittle, 1992).

A previous investigation has demonstrated that NO derived from transdermal nitroglycerin effectively shields against ethanol-induced gastric ulceration. This protection is achieved by maintaining mucosal blood flow and reducing the rolling and adherence of leukocytes to the endothelial cells (Calatayud *et al*., 1999). Moreover, NO has been reported to stimulate the synthesis and secretion of gastric mucus (Engevik *et al*., 2019; Wallace, 2019). Conversely, a decline in gastric mucosal NOS activity in ulcer-treated rats is associated with impaired gastric mucus secretion (Nishida *et al*., 1998b).

This study provides evidence that topical administration of L-arginine is highly effective in safeguarding the gastric mucosa from damage caused by acid-independent factors, such as ethanol. The co-administration of ADE and L-arginine significantly reduces gastric lesions induced by ethanol. However, when ADE and L-NNA are concurrently administered, this protective effect is significantly diminished. In contrast, AME reduces lesions in combination with L-NNA but exacerbates them when combined with L-arginine. These findings suggest that ADE, rather than AME, is responsible for the gastroprotective action observed in ADE and that the NO pathway is implicated in its mechanism. Similar study on *Croton campestris* A. St. (CCRE) have proposed that CCRE's antiulcer activity is reliant on both NO and prostaglandin pathways, likely due to its ability to stimulate NO synthesis and activate endogenous prostaglandin production (Júnior *et al*., 2014). On other hand Mehlous, (2023) reported that treatment of animals with *A. santolinoides* L. aqueous extract in the presence of L-NNA decreased the gastric ulcer induced by ethanol, suggesting that the NO pathway is not involved in the effect of this extract in the gastric protection.

PGE_2 plays a pivotal role in safeguarding the gastric mucosa due to its involvement in regulating gastric acid secretion, stabilizing mast cell membranes, and promoting repair processes. It significantly contributes to both the prevention and healing of ulcers (Kaur *et al*., 2007). Prostaglandins, particularly PGE_2, as well as NO, indirectly impact mucus production by enhancing gastric microcirculation and sulfhydryl compound levels (Pajdo *et al*., 2011).

Prostaglandin analogs, acting through EP_3 receptors, contribute to gastroprotection by increasing mucus and bicarbonate secretion while reducing cAMP formation, ultimately leading to decreased acid secretion (Zayachkivska *et al*., 2004). Notably, pretreatment with indomethacin in *A. odorata* L. groups did not affect their protective effects. This observation rules out the involvement of prostaglandins in the mechanism of action, and this outcome was consistent for both *A. odorata* L. extracts.

Noradrenergic α_2-receptors within the enteric nervous system play critical roles in various functions, including the regulation of GI tract movement patterns and the control of gastric acid secretion (Gyires *et al*., 2000). When administered in small doses, noradrenergic agonists inhibit acid secretion by blocking the vagus nerve and the actions of substances like pentagastrin and clonidine. On the other hand, yohimbine, a noradrenergic antagonist, counteracts the effects of α_2-receptor agonists by increasing gastric acid secretion (Kaur *et al*., 2007).

However, pretreatment with yohimbine in both ADE and AME groups did not impact their gastroprotective action, ruling out the involvement of α_2-noradrenergic receptors in the mechanism of action for ADE and AME.

The results of this study suggest that ADE has a gastroprotective role against ethanol-induced gastric mucosal damage. The gastroprotective mechanism may possibly involve the activation of the NO pathway, as NO could mediate the centrally-induced effect. Importantly, neither indomethacin nor yohimbine blocked the gastroprotective effect of ADE.

In the case of AME, pretreatments with indomethacin, L-NNA, or yohimbine did not result in the blockade of gastroprotection afforded by AME. This suggests that under these conditions, AME may act through a different pathway.

Conclusion

The present study demonstrated that *A. odorata* L extracts are a rich source of phenolic compounds, with significantly higher levels found in hydro-methanolic extract compared to other types of extracts. Phytochemical analysis identified nine common compounds within the plant extracts: protocatechuic acid, chlorogenic acid, p-hydroxy benzoic acid, coumarin, rutin, ellagic acid, rosmarinic acid, myricetin, and luteolin.

This study offers significant insights into the acute oral toxicity profiles of *A. odorata L.*, which can greatly benefit any forthcoming *in vivo* and clinical investigations involving this plant medicine. Additionally, *A. odorata* L. extracts showcased diverse activity levels against the tested cancer cell line, resulting in the inhibition of cellular proliferation ranging from moderate to notably effective.

The findings also highlight the potential of *A. odorata L.* as a natural antioxidant, suggesting its possible utility as functional food ingredients and in pharmaceutical applications. Notably, most extracts examined in this study displayed moderate enzymatic inhibitory activity.

Both ADE and AME extracts exhibited the ability to reduce gastric emptying and intestinal transit. The anti-intestinal motility effect of these extracts was associated with the involvement of NO and cyclooxygenase pathways.

In the case of ADE-induced delay in gastric emptying, both NO and the cyclooxygenase pathways were implicated. On the other hand, the gastric emptying delay caused by AME was exclusively associated with the cyclooxygenase pathway.

Both the decocted and methanolic extracts of *A. odorata* L. offered protection against ethanol-induced gastric ulcers. This protective effect was demonstrated by an increase in gastric mucus content and a reduction in gastric mucosal stress. Additionally, the gastroprotective mechanism of ADE may potentially involve the activation of the NO pathway.

The limitations of many current medicines, such as cost and side effects, are prompting a shift towards using natural substances for healing. The findings of this study highlight promising health benefits for the GI tract disorders.

Despite the comprehensive evaluation of numerous biological activities associated with the selected medicinal plant, there remain several unexplored avenues for future research:

-Thorough examination of the chemical components present in the plant is highly advisable.

-Conducting extensive cytotoxicity assays across diverse cell lines, particularly focusing on various cancer cell lines, is strongly recommended.

-Further exploration into the mechanisms governing the plant's effects on gastric emptying, intestinal transit, and gastroprotective functions is of great importance.

-Isolating and characterizing the active molecules responsible for the observed biological activities represents an essential progression.

-Utilizing the advantageous properties of these plant constituents in the food and pharmaceutical industries for their beneficial effects offers promising potential.

References

A Shah, A., A Dar, T., A Dar, P., A Ganie, S., & A Kamal, M. (2017). A current perspective on the inhibition of cholinesterase by natural and synthetic inhibitors. *Current drug metabolism*, 18(2), 96-111.

Abd-Alla, H. I., Shalaby, N. M., Hamed, M. A., El-Rigal, N. S., Al-Ghamdi, S. N., & Bouajila, J. (2016). Phytochemical composition, protective and therapeutic effect on gastric ulcer and α-amylase inhibitory activity of *Achillea biebersteinii* Afan. *Archives of pharmacal research*, 39, 10-20.

Abdel-Aziz, A. K., Shouman, S., El-Demerdash, E., Elgendy, M., & Abdel-Naim, A. B. (2014). Chloroquine synergizes sunitinib cytotoxicity via modulating autophagic, apoptotic and angiogenic machineries. *Chemico-Biological Interactions*, 217, 28-40.

Abrahim, N. N., Kanthimathi, M., & Abdul-Aziz, A. (2012). Piper betle shows antioxidant activities, inhibits MCF-7 cell proliferation and increases activities of catalase and superoxide dismutase. *BMC complementary and alternative medicine*, 12(1), 1-11.

Adinortey, M. B., Ansah, C., Galyuon, I., & Nyarko, A. (2013). *In vivo* models used for evaluation of potential antigastroduodenal ulcer agents. *Ulcers*, 2013.

Agarwal, P., & Gupta, R. (2016). Alpha-amylase inhibition can treat diabetes mellitus. *Res. Rev. J. Med. Health Sci*, 5(4), 1-8.

Ahsan, M. (2022). Evaluation of *in-vitro* Antioxidant and Acetylcholinesterase Inhibitory Potential of Night Blooming Jasmine (*Cestrum nocturnum*).

Ajay, M., Gilani, A.-u. H., & Mustafa, M. R. (2003). Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. *Life sciences*, 74(5), 603-612.

Albaayit, S. F. A., Abba, Y., Abdullah, R., & Abdullah, N. (2016). Prophylactic effects of *Clausena excavata Burum*. f. leaf extract in ethanol-induced gastric ulcers. *Drug design, development and therapy*, 1973-1986.

Albayrak, S., & Silahtarlıoğlu, N. (2020). Determination of biological activities of essential oil and extract obtained from *Achillea coarctata* Poir. *Advances in Traditional Medicine*, 20(1), 77-88. https://doi.org/10.1007/s13596-019-00378-w

Alomair, M. K., Alabduladheem, L. S., Almajed, M. A., Alobaid, A. A., Alkhalifah, E. A., Younis, N. S., & Mohamed, M. E. (2022). *Achillea millefolium* essential oil mitigates peptic ulcer in rats through Nrf2/HO-1 pathway. *Molecules*, 27(22), 7908.

Alothman, M., Bhat, R., & Karim, A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food chemistry*, 115(3), 785-788.

Al-Said, M. S., Mothana, R. A., Al-Yahya, M. M., Rafatullah, S., Al-Sohaibani, M. O., Khaled, J. M., Alatar, A., Alharbi, N. S., Kurkcuoglu, M., & Baser, H. C. (2016). GC-MS Analysis: *In Vivo* Hepatoprotective and Antioxidant Activities of the Essential Oil of *Achillea biebersteinii* Afan. Growing in Saudi Arabia. *Evidence-Based Complementary and Alternative Medicine*, 2016. https://doi.org/10.1155/2016/1867048

Alves-Santos, A. M., Sugizaki, C. S. A., Lima, G. C., & Naves, M. M. V. (2020). Prebiotic effect of dietary polyphenols: A systematic review. *Journal of Functional Foods*, 74, 104169. https://doi.org/https://doi.org/10.1016/j.jff.2020.104169

Amaral, G. P., de Carvalho, N. R., Barcelos, R. P., Dobrachinski, F., Portella, R. d. L., da Silva, M. H., Lugokenski, T. H., Dias, G. R. M., da Luz, S. C. A., Boligon, A. A., Athayde, M. L., Villetti, M. A., Antunes Soares, F. A., & Fachinetto, R. (2013). Protective action of ethanolic extract of *Rosmarinus officinalis* L. in gastric ulcer prevention induced by ethanol in rats. *Food and Chemical Toxicology*, 55, 48-55. https://doi.org/https://doi.org/10.1016/j.fct.2012.12.038

Amira, S., Dade, M., Schinella, G., & Ríos, J.-L. (2012). Anti-inflammatory, anti-oxidant, and apoptotic activities of four plant species used in folk medicine in the Mediterranean basin. Pak. *J. Pharm. Sci*, 25(1), 65-72.

Amira, S., Rotondo, A., & Mulè, F. (2008). Relaxant effects of flavonoids on the mouse isolated stomach: structure-activity relationships. *European journal of pharmacology*, 599(1-3), 126-130.

Amira, S., Soufane, S. and Gharzouli, K. (2005). Effect of sodium fluoride on gastric emptying and intestinal transit in mice. Experimental and Toxicologic Pathology, 57(1), 59-64.

Annunziata, G., Sureda, A., Orhan, I. E., Battino, M., Arnone, A., Jiménez-García, M., Capó, X., Cabot, J., Sanadgol, N., Giampieri, F., Tenore, G. C., Kashani, H. R. K., Silva, A. S., Habtemariam, S., Nabavi, S. F., & Nabavi, S. M. (2021). The neuroprotective effects

of polyphenols, their role in innate immunity and the interplay with the microbiota. *Neuroscience & Biobehavioral Reviews*, 128, 437-453. https://doi.org/https://doi.org/10.1016/j.neubiorev.2021.07.004

Antonisamy, P., Dhanasekaran, M., Ignacimuthu, S., Duraipandiyan, V., Balthazar, J. D., Agastian, P., & Kim, J.-H. (2014). Gastroprotective effect of epoxy clerodane diterpene isolated from *Tinospora cordifolia Miers* (Guduchi) on indomethacin-induced gastric ulcer in rats. *Phytomedicine*, 21(7), 966-969.

Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., Bektaşoğlu, B., Berker, K. I., & Özyurt, D. (2007). Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. *Molecules*, 12(7), 1496-1547. https://www.mdpi.com/1420-3049/12/7/1496

Aranda-Rivera, A. K., Cruz-Gregorio, A., Arancibia-Hernández, Y. L., Hernández-Cruz, E. Y., & Pedraza-Chaverri, J. (2022). RONS and oxidative stress: An overview of basic concepts. *Oxygen*, 2(4), 437-478.

Ashley, E. A., Ashley, E., & Niebauer, J. (2004). Cardiology explained.

Atta-ur-Rahman, Z.-u.-H., Khalid, A., Anjum, S., Khan, M. R., & Choudhary, M. I. (2002). Pregnane-type steroidal alkaloids of *Sarcococca saligna*: a new class of cholinesterases inhibitors. *Helv Chim Acta*, 85, 678-688.

Aziz, R. S., Siddiqua, A., Shahzad, M., Shabbir, A., & Naseem, N. (2019). Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-α, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. *Biomedicine & Pharmacotherapy*, 110, 554-560.

Bahekar, S. E., & Kale, R. S. (2015). Antidiarrheal activity of ethanolic extract of *Manihot esculenta Crantz* leaves in Wistar rats. *Journal of Ayurveda and integrative medicine*, 6(1), 35.

Bali, E. B., Açık, L., Elçi, P., Sarper, M., Avcu, F., & Vural, M. (2015). In vitro anti-oxidant, cytotoxic and pro-apoptotic effects of *Achillea teretifolia* Willd extracts on human prostate cancer cell lines. *Pharmacogn Mag*, 11(Suppl 2), S308-315. https://doi.org/10.4103/0973-1296.166060

Bariş, Ö., Güllüce, M., Şahin, F., Özer, H., Kiliç, H., Özkan, H., Sökmen, M., & Özbek, T. (2006). Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan. (Asteraceae). *Turkish Journal of Biology*, 30(2), 65-73.

Barrero, A. F., Arseniyadis, S., & Guittet, E. (1990). Achilleol B: a new tricyclic triterpene skeleton from *Achillea odorata* L. *Tetrahedron*, 46(24), 8161-8168.

Barrett, K. E., Barman, S. M., Boitano, S., & Brooks, H. L. (2018). Gastrointestinal Motility. In Ganong's Review of Medical Physiology. *McGraw-Hill Education*, 25e

Barut, E. N., Barut, B., Engin, S., Yıldırım, S., Yaşar, A., Türkiş, S., Özel, A., & Sezen, F. S. (2017). Antioxidant capacity, anti-acetylcholinesterase activity and inhibitory effect on lipid peroxidation in mice brain homogenate of *Achillea millefolium*. *Turkish Journal of Biochemistry*, 42(4), 493-502.

Baytop, T. (1984). Therapy with Medicinal Plants in Turkey (Past and Present) No: 3255. Istanbul: *Publications of the Istanbul University*, 359.

Bedard, K., & Krause, K.-H. (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiological reviews*, 87(1), 245-313.

Bekhechi, C., Bekkara, F. A., Casanova, J., & Tomi, F. (2011). Composition and antimicrobial activity of the essential oil of *Achillea odorata* L. subsp. pectinata (Lamk) var. microphylla (Willd.) Willk. from Northwestern Algeria. *Journal of Essential Oil Research*, 23(3), 42-46.

Benali, T., Habbadi, K., Khabbach, A., Marmouzi, I., Zengin, G., Bouyahya, A., Chamkhi, I., Chtibi, H., Aanniz, T., & Achbani, E. H. (2020). GC–MS analysis, antioxidant and antimicrobial activities of *Achillea odorata* subsp. pectinata and *Ruta montana* essential oils and their potential use as food preservatives. *Foods*, 9(5), 668.

Benchikh, F., Amira, S., & Benabdallah, H. (2018). The evaluation of antioxidant capacity of different fractions of *Myrtus communis* L. leaves. *Annual Research & Review in Biology*, 1-14.

Benchikh, F., Benabdallah, H., Dahamna, S., Khennouf, S., Flamini, G., & Amira, S. (2016). Antimotility and andidiarrhoel activity of *Myrtus communis* L. leaves essential oil in mice. *International Journal of Pharmacognosy and Phytochemical Research*, 8(7), 1238-1244.

Benetis, R., Radusiene, J., Jakstas, V., Janulis, V., Puodziuniene, G., & Milasius, A. (2008). Quantitative HPLC determination of phenolic compounds in yarrow. *Pharmaceutical chemistry journal*, 42, 153-156.

Bereda, G. (2022). Peptic Ulcer disease: definition, pathophysiology, and treatment. *Journal of Biomedical and Biological Sciences*, 1(2), 1-10.

Bharucha, A. E., Manduca, A., Lake, D. S., Fidler, J., Edwards, P., Grimm, R. C., Zinsmeister, A. R., & Riederer, S. J. (2011). Gastric motor disturbances in patients with idiopathic rapid gastric emptying. *Neurogastroenterology & Motility*, 23(7), 617-e252.

Bhatti, J. S., Sehrawat, A., Mishra, J., Sidhu, I. S., Navik, U., Khullar, N., Kumar, S., Bhatti, G. K., & Reddy, P. H. (2022). Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radical Biology and Medicine*, 184, 114-134.

Bi, W. P., Man, H. B., & Man, M. Q. (2014). Efficacy and safety of herbal medicines in treating gastric ulcer: a review. *World J Gastroenterol*, 20(45), 17020-17028. https://doi.org/10.3748/wjg.v20.i45.17020

Bilto, Y. Y., & Alabdallat, N. G. (2015). *In vitro* and in vivo antioxidant related effects of rosemary (*Rosmarinus officinalis* L.) extracts in humans. *American Journal of Clinical and Experimental Medicine*, 3(5), 213-221.

Bimbiraitė, K., Ragažinskienė, O., Maruška, A., & Kornyšova, O. (2008). Comparison of the chemical composition of four yarrow (*Achilea millefolium* L.) morphotypes. *biologija*, 54(3).

Birru, E. M., Asrie, A. B., Adinew, G. M., & Tsegaw, A. (2016). Antidiarrheal activity of crude methanolic root extract of *Idigofera spicata Forssk*.(Fabaceae). *BMC Complementary and Alternative Medicine*, 16, 1-7.

Bobis, O., Dezmirean, D., Tomos, L., Chirila, F., & Al Marghitas, L. (2015). Influence of phytochemical profile on antibacterial activity of different medicinal plants against gram-positive and gram-negative bacteria. *Applied biochemistry and microbiology*, 51, 113-118.

Boesmans, W., Lasrado, R., Vanden Berghe, P., & Pachnis, V. (2015). Heterogeneity and phenotypic plasticity of glial cells in the mammalian enteric nervous system. *Glia*, 63(2), 229-241.

Bohórquez, D. V., Shahid, R. A., Erdmann, A., Kreger, A. M., Wang, Y., Calakos, N., Wang, F., & Liddle, R. A. (2015). Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *The Journal of clinical investigation*, 125(2), 782-786.

Boligon, A. A., De Freitas, R. B., de Brum, T. F., Waczuk, E. P., Klimaczewski, C. V., de Ávila, D. S., Athayde, M. L., & de Freitas Bauermann, L. (2014). Antiulcerogenic activity of *Scutia buxifolia* on gastric ulcers induced by ethanol in rats. *Acta Pharmaceutica Sinica B*, 4(5), 358-367.

Bonnet, J.-B., & Sultan, A. (2022). Narrative review of the relationship between ckd and diabetic foot ulcer. *Kidney International Reports*, 7(3), 381-388.

Boutennoun, H., Boussouf, L., Rawashdeh, A., Al-Qaoud, K., Abdelhafez, S., Kebieche, M., & Madani, K. (2017). *In vitro* cytotoxic and antioxidant activities of phenolic components of Algerian *Achillea odorata* leaves. *Arabian Journal of Chemistry*, 10(3), 403-409.

Bremer, K. (1993). Generic monograph of the Asteraceae-Anthemideae. *Bull. Nat. Hist. Mus*. London (Bot.), 23, 71-177.

Brookes, S. J., & Costa, M. (2003). Innervation of the gastrointestinal tract. *CRC Press*.

Bumrungpert, A., Lilitchan, S., Tuntipopipat, S., Tirawanchai, N., & Komindr, S. (2018). Ferulic acid supplementation improves lipid profiles, oxidative stress, and inflammatory status in hyperlipidemic subjects: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients*, 10(6), 713.

Cadirci, E., Suleyman, H., Aksoy, H., Halici, Z., Ozgen, U., Koc, A., & Ozturk, N. (2007). Effects of *Onosma armeniacum* root extract on ethanol-induced oxidative stress in stomach tissue of rats. *Chemico-Biological Interactions*, 170(1), 40-48. https://doi.org/https://doi.org/10.1016/j.cbi.2007.06.040

Cai, X., Xu, Y., Zhao, L., Xu, J., Li, S., Wen, C., Xia, X., Dong, Q., Hu, X., & Wang, X. (2021). In situ pepsin-assisted needle assembly of magnetic-graphitic-nanocapsules for enhanced gastric retention and mucus penetration. *Nano Today*, 36, 101032.

Calatayud, S., Sanz, M. J., Canet, A., Bello, R., De Rojas, F. D., & Esplugues, J. V. (1999). Mechanisms of gastroprotection by transdermal nitroglycerin in the rat. *British journal of pharmacology*, 127(5), 1111-1118.

Calvo, T. R., Lima, Z. P., Silva, J. S., Ballesteros, K. V. n. R., Pellizzon, C. H., Hiruma-Lima, C. A., Tamashiro, J., Brito, A. R. M. S., Takahira, R. K., & Vilegas, W. (2007). Constituents and antiulcer effect of *Alchornea glandulosa*: activation of cell proliferation in gastric mucosa during the healing process. *Biological and Pharmaceutical Bulletin*, 30(3), 451-459.

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., & Akpulat, H. A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium Afan.*(Asteraceae). *Journal of ethnopharmacology*, 87(2-3), 215-220.

Capasso, A., Pinto, A., Mascolo, N., Autore, G., & Capasso, F. (1991). Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. *Phytotherapy Research*, 5(2), 85-87.

Capasso, A., Pinto, A., Sorrentino, R., & Capasso, F. (1991). Inhibitory effects of quercetin and other flavonoids on electrically-induced contractions of guinea pig isolated ileum. *Journal of ethnopharmacology*, 34(2-3), 279-281.

Capasso, R., Borrelli, F., Capasso, F., Siebert, D., Stewart, D., Zjawiony, J., & Izzo, A. A. (2006). The hallucinogenic herb *Salvia divinorum* and its active ingredient salvinorin A inhibit enteric cholinergic transmission in the guinea-pig ileum. *Neurogastroenterology & Motility*, 18(1), 69-75.

Carocho, M., & Ferreira, I. C. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, 51, 15-25.

Cecilia, O.-M., José Alberto, C.-G., José, N.-P., Ernesto Germán, C.-M., Ana Karen, L.-C., Luis Miguel, R.-P., Ricardo Raúl, R.-R., & Adolfo Daniel, R.-C. (2019). Oxidative stress as the main target in diabetic retinopathy pathophysiology. *Journal of diabetes research*, 2019.

Chen, J., Yu, B., Chen, D., Huang, Z., Mao, X., Zheng, P., Yu, J., Luo, J., & He, J. (2018). Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs. *The Journal of Nutritional Biochemistry*, 59, 84-92.

Cheng, C. L., Guo, J. S., Luk, J., & Koo, M. W. L. (2004). The healing effects of *Centella* extract and asiaticoside on acetic acid induced gastric ulcers in rats. *Life sciences*, 74(18), 2237-2249.

Chiba, T., Bharucha, A., Thomforde, G., Kost, L., & Phillips, S. (2002). Model of rapid gastrointestinal transit in dogs: effects of muscarinic antagonists and a nitric oxide synthase inhibitor. *Neurogastroenterology & Motility*, 14(5), 535-541.

Chiu, H.-F., Venkatakrishnan, K., Golovinskaia, O., & Wang, C.-K. (2021b). Gastroprotective Effects of Polyphenols against Various Gastro-Intestinal Disorders: A Mini-Review with Special Focus on Clinical Evidence. *Molecules*, 26(7), 2090. https://www.mdpi.com/1420-3049/26/7/2090

Cho, C. H., & Ogle, C. W. (1992). The pharmacological differences and similarities between stress-and ethanol-induced gastric mucosal damage. *Life sciences*, 51(24), 1833-1842.

Christodouleas, D. C., Fotakis, C., Nikokavoura, A., Papadopoulos, K., & Calokerinos, A. C. (2015). Modified DPPH and ABTS assays to assess the antioxidant profile of untreated oils. *Food Analytical Methods*, 8, 1294-1302.

Collins, A., Saleh, T., & Kalisch, B. (2022). Naturally occurring antioxidant therapy in Alzheimer's disease: Antioxidants in Age-Related Diseases and Anti-Aging Strategies, Antioxidants, 11, 213-215.

Csupor-Löffler, B., Hajdú, Z., Zupkó, I., Réthy, B., Falkay, G., Forgo, P., & Hohmann, J. (2009). Antiproliferative effect of flavonoids and sesquiterpenoids from *Achillea millefolium* sl on cultured human tumour cell lines. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 23(5), 672-676.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313-7352.

Dambros, M., de Jongh, R., van Koeveringe, G. A., van Deutekom, M., De Mey, J. G., Palma, P. C., & van Kerrebroeck, P. E. (2005). Multiple-signaling pathways are involved in the inhibitory effects of galangin on urinary bladder contractility. *Neurourology and Urodynamics: Official Journal of the International Continence Society*, 24(4), 369-373.

de Lira Mota, K. S., Dias, G. E. N., Pinto, M. E. F., Luiz-Ferreira, Â., Monteiro Souza-Brito, A. R., Hiruma-Lima, C. A., Barbosa-Filho, J. M., & Batista, L. M. (2009). Flavonoids with gastroprotective activity. *Molecules*, 14(3), 979-1012.

Deloose, E., Janssen, P., Depoortere, I., & Tack, J. (2012). The migrating motor complex: control mechanisms and its role in health and disease. *Nature reviews Gastroenterology & hepatology*, 9(5), 271-285.

Demirezer, L. Ö., Gürbüz, P., Uğur, E. P. K., Bodur, M., Özenver, N., UZ, A., & Güvenalp, Z. (2015). Molecular docking and ex vivo and in vitro anticholinesterase activity studies of Salvia sp. and highlighted rosmarinic acid. *Turkish journal of medical sciences*, 45(5), 1141-1148.

Dharmani, P., Kuchibhotla, V. K., Maurya, R., Srivastava, S., Sharma, S., & Palit, G. (2004). Evaluation of anti-ulcerogenic and ulcer-healing properties of *Ocimum sanctum* Linn. *Journal of ethnopharmacology*, 93(2-3), 197-206.

Di Carlo, G., Autore, G., Izzo, A., Maiolino, P., Mascolo, N., Viola, P., Diurno, M., & Capasso, F. (1993). Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships. *Journal of Pharmacy and Pharmacology*, 45(12), 1054-1059.

Dias, M. I., Barros, L., Dueñas, M., Pereira, E., Carvalho, A. M., Alves, R. C., Oliveira, M. B. P., Santos-Buelga, C., & Ferreira, I. C. (2013). Chemical composition of wild and commercial *Achillea millefolium* L. and bioactivity of the methanolic extract, infusion and decoction. *Food chemistry*, 141(4), 4152-4160.

Dİkpinar, T., & BİtİŞ, L. (2022). LC-MS/MS analysis and biological activities of endemic *Achillea sieheana Stapf* from Türkiye. *J Res Phar*m, 26(6).

Dirir, A. M., Daou, M., Yousef, A. F., & Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Review*s, 21(4), 1049-1079.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y.-H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of food and drug analysis*, 22(3), 296-302.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., & Mérillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, 57(5), 1768-1774.

Dumanović, J., Nepovimova, E., Natić, M., Kuča, K., & Jaćević, V. (2021). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Frontiers in plant science*, 11, 552969.

Elbatrawy, E. N., Ghonimy, E. A., Alassar, M. M., & Wu, F.-S. (2015). Medicinal mushroom extracts possess differential antioxidant activity and cytotoxicity to cancer cells. *International journal of medicinal mushrooms*, 17(5).

Engevik, M. A., Luk, B., Chang-Graham, A. L., Hall, A., Herrmann, B., Ruan, W., Endres, B. T., Shi, Z., Garey, K. W., & Hyser, J. M. (2019). Bifidobacterium dentium fortifies the intestinal mucus layer via autophagy and calcium signaling pathways. *MBio*, 10(3), e01087-01019.

Eruygur, N., Koçyiğit, U., Taslimi, P., Ataş, M., Tekin, M., & Gülçin, İ. (2019). Screening the in vitro antioxidant, antimicrobial, anticholinesterase, antidiabetic activities of endemic *Achillea cucullata* (Asteraceae) ethanol extract. *South African Journal of Botany*, 120, 141-145.

Farajpour, M., Ebrahimi, M., Amiri, R., Golzari, R., & Sanjari, S. (2012). Assessment of genetic diversity in *Achillea millefolium* accessions from Iran using ISSR marker. *Biochemical Systematics and Ecology*, 43, 73-79.

Fathi, H., Lashtoo Aghaee, B., & Ebrahimzadeh, M. (2011). Antioxidant activity and phenolic contents of *Achillea wilhemsii*. *Pharmacologyonline*, 2, 942-949.

Ferreira, I. C., Baptista, P., Vilas-Boas, M., & Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food chemistry*, 100(4), 1511-1516.

Fu, Y., Wu, H. q., Cui, H. l., Li, Y. y., & Li, C. z. (2018). Gastroprotective and anti-ulcer effects of oxymatrine against several gastric ulcer models in rats: Possible roles of antioxidant, anti-inflammatory, and prosurvival mechanisms. *Phytotherapy Research*, 32(10), 2047-2058.

Furness, J. B., & Sanger, G. J. (2002). Intrinsic nerve circuits of the gastrointestinal tract: identification of drug targets. *Current opinion in pharmacology*, 2(6), 612-622.

Furness, J. B., Callaghan, B. P., Rivera, L. R., & Cho, H.-J. (2014). The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Microbial endocrinology: The microbiota-gut-brain axis in health and disease*, 39-71.

Ganong, W. F., Barrett, K. E., Barman, S. M., Boitano, S., & Brooks, H. L. (2010). Ganong. In: *McGraw-Hill Interamericana Editores*.

Gevrenova, R., Zengin, G., Sinan, K. I., Yıldıztugay, E., Zheleva-Dimitrova, D., Picot-Allain, C., Mahomoodally, M. F., Imran, M., & Dall'Acqua, S. (2021). UHPLC-MS Characterization and biological insights of different solvent extracts of two Achillea species (*A. aleppica* and *A. santolinoides*) from Turkey. *Antioxidants*, 10(8), 1180.

Gharibi, S., Tabatabaei, B. E. S., Saeidi, G., Goli, S. A. H., & Talebi, M. (2013). Total phenolic content and antioxidant activity of three Iranian endemic Achillea species. *Industrial Crops and Products*, 50, 154-158. https://doi.org/https://doi.org/10.1016/j.indcrop.2013.07.038

Gharzouli, K., Gharzouli, A., Amira, S. and Khennouf, S. (1999a). Prevention of ethanol-induced gastric lesions in rats by natural honey and glucose–fructose–sucrose–maltose mixture. Pharmacological Research, 39(2), 151-156.

Ghavami, G., Sardari, S., & Shokrgozar, M. A. (2010). Anticancerous potentials of Achillea species against selected cell lines. *J Med Plants Res*, 4(22), 2411-2417.

Göçer, H., & Gülçin, İ. (2011). Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. *International journal of food sciences and nutrition*, 62(8), 821-825.

Goggin, J. M., Hoskinson, J. J., Kirk, C. A., Jewell, D., & Butine, M. D. (1999). Comparison of gastric emptying times in healthy cats simultaneously evaluated with radiopaque markers and nuclear scintigraphy. *Veterinary Radiology & Ultrasound*, 40(1), 89-95.

Goli, S. A. H., Rahimmalek, M., & Tabatabaei, B. E. S. (2008). Physicochemical characteristics and fatty acid profile of yarrow (*Achillea tenuifolia*) seed oil. *Int J Agric Biol*, 10(3), 355-357.

Gordan, R., Fefelova, N., Gwathmey, J. K., & Xie, L.-H. (2020). Iron overload, oxidative stress and calcium mishandling in cardiomyocytes: role of the mitochondrial permeability transition pore. *Antioxidants*, 9(8), 758.

Granado-Serrano, A. B., Angeles Martín, M., Izquierdo-Pulido, M., Goya, L., Bravo, L., & Ramos, S. (2007). Molecular mechanisms of epicatechin and chlorogenic acid on the regulation of the apoptotic and survival/proliferation pathways in a human hepatoma cell line. *Journal of Agricultural and Food Chemistry*, 55(5), 2020-2027.

Gu, C., Zhang, H., Putri, C. Y., & Ng, K. (2015). Evaluation of α-amylase and α-glucosidase inhibitory activity of flavonoids. *Int. J. Food Nutr*. Sci, 2, 1-6.

Gülçin, I., Küfrevioğlu, Ö. İ., Oktay, M., & Büyükokuroğlu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of ethnopharmacology*, 90(2-3), 205-215.

Guo, Y. P., Ehrendorfer, F., & Samuel, R. (2004). Phylogeny and systematics of Achillea (AsteraceaeAnthemideae) inferred from nrITS and plastid trnLF DNA sequences. *Taxon*, *53*(3), 657-672A.

Gupta, J., Kumar, D., & Gupta, A. (2012). Evaluation of gastric anti–ulcer activity of methanolic extract of Cayratia trifolia in experimental animals. *Asian Pacific Journal of Tropical Disease*, *2*(2), 99-102.

Gupta, M., Kapoor, B., Gupta, R., & Singh, N. (2021). Plants and phytochemicals for treatment of peptic ulcer: An overview. *South African Journal of Botany*, 138, 105-114.

Gyires, K., Müllner, K., Fürst, S., & Rónai, A. Z. (2000). Alpha-2 adrenergic and opioid receptor-mediated gastroprotection. *Journal of Physiology-Paris*, 94(2), 117-121.

Haliloglu, Y., Ozek, T., Tekin, M., Goger, F., Baser, K. H. C., & Ozek, G. (2017). Phytochemicals, antioxidant, and antityrosinase activities of *Achillea sivasica Çelik* and *Akpulat*. *International Journal of Food Properties*, 20(sup1), S693-S706. https://doi.org/10.1080/10942912.2017.1308954

Halliwell, B. (1994). Free radicals and antioxidants: a personal view. *Nutrition reviews*, 52(8), 253-265.

Hasler, W. L. (2009). Motility of the small intestine and colon. *Textbook of gastroenterology*, 1, 231-263.

Hawrył, A., Hawrył, M., Świeboda, R., & Waksmundzka-Hajnos, M. (2018). Chemometric analysis based on high-performance liquid chromatographic data in the fingerprint construction

of selected Achillea species. *Journal of Liquid Chromatography & Related Technologies*, 41(1), 1-5.

Herman, P., & Swelankomo, N. (2011). Asteraceae. *Bothalia*, 41(1), 176-178.

Hinshaw, L., Schiavon, M., Mallad, A., Man, C. D., Basu, R., Bharucha, A. E., Cobelli, C., Carter, R. E., Basu, A., & Kudva, Y. C. (2014). Effects of delayed gastric emptying on postprandial glucose kinetics, insulin sensitivity, and β-cell function. *American Journal of Physiology-Endocrinology and Metabolism*, 307(6), E494-E502.

Hobani, Y. H., Mohan, S., Shaheen, E., Abdelhaleem, A., Ahmad, M. F., Bhatia, S., & Abou-Elhamd, A. S. (2022). Gastroprotective effect of low dose Eugenol in experimental rats against ethanol induced toxicity: Involvement of anti-inflammatory and antioxidant mechanism. *Journal of ethnopharmacology*, 289, 115055.

Hussain, M. T., Verma, A. R., Vijayakumar, M., Sharma, A., Mathela, C., & Rao, C. V. (2009). Rutin, a natural flavonoid, protects against gastric mucosal damage in experimental animals. *Asian journal traditional medicines*, 4, 188-197.

Idm'hand, E., Msanda, F., & Cherifi, K. (2020). Ethnopharmacological review of medicinal plants used to manage diabetes in Morocco. *Clinical Phytoscience*, 6(1), 18. https://doi.org/10.1186/s40816-020-00166-z

Ighodaro, O., & Akinloye, O. (2018). First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, 54(4), 287-293.

Inchuen, S., Narkrugsa, W., & Pornchaloempong, P. (2010). Effect of drying methods on chemical composition, color and antioxidant properties of Thai red curry powder. *Agriculture and Natural Resources*, 44(1), 142-151.

Ineu, R., Pereira, M., Aschner, M., Nogueira, C., Zeni, G., & Rocha, J. (2008). Diphenyl diselenide reverses gastric lesions in rats: involvement of oxidative stress. *Food and Chemical Toxicology*, 46(9), 3023-3029.

Iova, G. M., Calniceanu, H., Popa, A., Szuhanek, C. A., Marcu, O., Ciavoi, G., & Scrobota, I. (2021). The antioxidant effect of curcumin and rutin on oxidative stress biomarkers in experimentally induced periodontitis in hyperglycemic Wistar rats. *Molecules*, 26(5), 1332.

Irato, P., & Santovito, G. (2021). Enzymatic and non-enzymatic molecules with antioxidant function. In *MDPI* (Vol. 10, pp. 579).

Ito, F., Sono, Y., & Ito, T. (2019). Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*, 8(3), 72.

Iwasaki, M., Akiba, Y., & Kaunitz, J. D. (2019). Recent advances in vasoactive intestinal peptide physiology and pathophysiology: focus on the gastrointestinal system. *F1000Research*, 8.

Jafari, M., Naeini, K. M., Lorigooini, Z., & Namjoo, R. (2019). Oral acute and sub-acute toxic effects of hydroalcoholic *Terminalia chebula* Retz and *Achillea wilhelmsii* extracts in BALB/c mice. *Biomedicine* (Taipei), 9(4), 25. https://doi.org/10.1051/bmdcn/2019090425

Jamdade, C., & Bodare, R. (2022). A mini review on free radicals-generated in biological system. *World Journal of Pharmaceutical Research,12(3), 661-668.*

Jasnos, K., Magierowski, M., Kwiecień, S., & Brzozowski, T. (2014). Carbon monoxide in human physiology–its role in the gastrointestinal tract. *Advances in Hygiene and Experimental Medicine*, 68, 101-109.

Johnson, L. R. (2006). Physiology of the gastrointestinal tract. *Elsevier*.

Jung, M. K., Callaci, J. J., Lauing, K. L., Otis, J. S., Radek, K. A., Jones, M. K., & Kovacs, E. J. (2011). Alcohol exposure and mechanisms of tissue injury and repair. Alcoholism: *Clinical and Experimental Research*, 35(3), 392-399.

Júnior, F. E. B., de Oliveira, D. R., Boligon, A. A., Athayde, M. L., Kamdem, J. P., Macedo, G. E., da Silva, G. F., de Menezes, I. R. A., Costa, J. G. M., Coutinho, H. D. M., Kerntopf, M. R., & Posser, T. (2014). Protective effects of *Croton campestris* A. St-Hill in different ulcer models in rodents: Evidence for the involvement of nitric oxide and prostaglandins. *Journal of ethnopharmacology*, 153(2), 469-477. https://doi.org/https://doi.org/10.1016/j.jep.2014.03.005

Jurado-Campos, A., Soria-Meneses, P. J., Sánchez-Rubio, F., Niza, E., Bravo, I., Alonso-Moreno, C., Arenas-Moreira, M., García-Álvarez, O., Soler, A. J., & Garde, J. J. (2021). Vitamin E delivery systems increase resistance to oxidative stress in red deer sperm cells: hydrogel and nanoemulsion carriers. *Antioxidants*, 10(11), 1780.

Kalidindi, N., Thimmaiah, N. V., Jagadeesh, N. V., Nandeep, R., Swetha, S., & Kalidindi, B. (2015). Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosa* Linn. leaves. *Journal of food and drug analysis*, 23(4), 795-802.

Kanadaswami, C., Lee, L.-T., Lee, P.-P. H., Hwang, J.-J., Ke, F.-C., Huang, Y.-T., & Lee, M.-T. (2005). The antitumor activities of flavonoids. *In vivo*, 19(5), 895-909.

Kansara, S., & Sakhreliya, B. (2013). Peptic ulcer-Its Pathogenesis and Recent Approaches for the Treatment. *J. Pharm. Sci. Bio. Res*, 3, 136-144.

Kar, P., Jones, K. L., Horowitz, M., Chapman, M. J., & Deane, A. M. (2015). Measurement of gastric emptying in the critically ill. *Clinical nutrition*, 34(4), 557-564.

Karamenderes, C., & Apaydin, S. (2003). Antispasmodic effect of *Achillea nobilis* L. subsp. sipylea (O. Schwarz) Bässler on the rat isolated duodenum. *Journal of ethnopharmacology*, 84(2-3), 175-179.

Karunakaran, R., Ira Thabrew, M., Thammitiyagodage, G. M., Galhena, B. P., & Menuka Arawwawala, L. (2017). The gastroprotective effect of ethyl acetate fraction of hot water extract of *Trichosanthes cucumerina* Linn and its underlying mechanisms. *BMC Complementary and Alternative Medicine*, 17(1), 1-8.

Kashtoh, H., & Baek, K.-H. (2023). New Insights into the Latest Advancement in α-Amylase Inhibitors of Plant Origin with Anti-Diabetic Effects. *Plants*, 12(16), 2944.

Kaur, R., Chopra, K., & Singh, D. (2007). Role of α2 receptors in quercetin-induced behavioral despair in mice. *Journal of medicinal food*, 10(1), 165-168.

Kazazić, S. P., Butković, V., Srzić, D., & Klasinc, L. (2006). Gas-phase ligation of Fe^{+} and Cu^{+} ions with some flavonoids. *Journal of Agricultural and Food Chemistry*, 54(22), 8391-8396.

Kelainy, E. G., Ibrahim Laila, I. M., & Ibrahim, S. R. (2019). The effect of ferulic acid against lead-induced oxidative stress and DNA damage in kidney and testes of rats. *Environmental Science and Pollution Research*, 26, 31675-31684.

Kendig, D. M., & Grider, J. R. (2015). Serotonin and colonic motility. *Neurogastroenterology & Motility*, 27(7), 899-905.

Khalifa, M., Hassan, M. K., Ashour, O. M., & Heeba, G. (2002). Evaluation of the anti-ulcer activity of pibutidine hydrochloride (IT-066); the new histamine H2-receptor antagonist, in cold-restraint stress-and ethanol-induced ulcer models in rats. *Al Azhar Med*. J, 31, 33-47.

Khan, H., Marya, Amin, S., Kamal, M. A., & Patel, S. (2018). Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. *Biomedicine & Pharmacotherapy*, 101, 860-870. https://doi.org/https://doi.org/10.1016/j.biopha.2018.03.007

Khattab, M. M., Gad, M. Z., & Abdallah, D. (2001). Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent of gastric acid secretion. *Pharmacological research*, 43(5), 463-467.

Kim, H., & Kim, K. H. (1998). Effect of nitric oxide on hydrogen peroxide-induced damage in isolated rabbit gastric glands. *Pharmacology*, 57(6), 323-330.

Kim, M.-K., Lee, H.-S., Kim, E.-J., Won, N.-H., Chi, Y.-M., Kim, B.-C., & Lee, K.-W. (2007). Protective effect of aqueous extract of *Perilla frutescens* on tert-butyl hydroperoxide-induced oxidative hepatotoxicity in rats. Food and Chemical Toxicology, 45(9), 1738-1744. https://doi.org/https://doi.org/10.1016/j.fct.2007.03.009

Kimura, Y., & Sumiyoshi, M. (2012). Effects of an *Atractylodes lancea* rhizome extract and a volatile component β-eudesmol on gastrointestinal motility in mice. *Journal of ethnopharmacology*, 141(1), 530-536.

Kono, Y., Kashine, S., Yoneyama, T., SAKAMOTO, Y., MATSUI, Y., & SHIBATA, H. (1998). Iron chelation by chlorogenic acid as a natural antioxidant. *Bioscience, Biotechnology, and Biochemistry*, 62(1), 22-27.

Korbut, E., Brzozowski, T., & Magierowski, M. (2020). Carbon monoxide being hydrogen sulfide and nitric oxide molecular sibling, as endogenous and exogenous modulator of oxidative stress and antioxidative mechanisms in the digestive system. *Oxidative Medicine and Cellular Longevity*, 3, 14.

Krawisz, J. E., Sharon, P., & Stenson, W. F. (1984). Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity: Assessment of inflammation in rat and hamster models. *Gastroenterology*, 87(6), 1344-1350. https://doi.org/https://doi.org/10.1016/0016-5085(84)90202-6

Kumar, A., Sidhu, J., Goyal, A., & Tsao, J. W. (2018). Alzheimer disease.

Kumaran, A., & Joel Karunakaran, R. (2007). *In vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - *Food Science and Technology*, 40(2), 344-352. https://doi.org/https://doi.org/10.1016/j.lwt.2005.09.011

Kwiecien, S., Brzozowski, T., & Konturek, S. (2002). Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. *Journal of physiology and pharmacology*, 53(1), 39-50.

Laloo, D., Prasad, S. K., Krishnamurthy, S., & Hemalatha, S. (2013). Gastroprotective activity of ethanolic root extract of *Potentilla fulgens* Wall. ex Hook. *Journal of ethnopharmacology*, 146(2), 505-514.

Lecci, A., Santicioli, P., & Maggi, C. A. (2002). Pharmacology of transmission to gastrointestinal muscle. *Current opinion in pharmacology*, 2(6), 630-641.

Li, H., Qu, Y., Zhang, J., Zhang, J., & Gao, W. (2018). Spasmolytic activity of *Aquilariae Lignum Resinatum* extract on gastrointestinal motility involves muscarinic receptors, calcium channels and NO release. *Pharmaceutical biology*, 56(1), 559-566.

Li, W., Huang, H., Niu, X., Fan, T., Mu, Q., & Li, H. (2013). Protective effect of tetrahydrocoptisine against ethanol-induced gastric ulcer in mice. *Toxicology and applied pharmacology*, 272(1), 21-29.

Li, Y.-R., Li, Y., Jin, Y., Xu, M., Fan, H.-W., Zhang, Q., Tan, G.-H., Chen, J., & Li, Y.-Q. (2022). Involvement of nitrergic neurons in colonic motility in a rat model of ulcerative colitis. *World journal of gastroenterology*, 28(29), 3854.

Ling, J.-H., Li, J.-B., Shen, D.-Z., & Zhou, B. (2006). Nuclear factor-kappa B mRNA and protein expression in stomach tissue of rats with gastric ulcer recurrence and effect of *jianwei yuyang* granule on its expression. *Chinese Journal of Integrated Traditional and Western Medicine*, 26(3), 228-231.

Liu, L., Tian, C., Dong, B., Xia, M., Cai, Y., Hu, R., & Chu, X. (2021). Models to evaluate the barrier properties of mucus during drug diffusion. *International Journal of Pharmaceutics*, 599, 120415.

Liu, Y., Tian, X., Gou, L., Fu, X., Li, S., Lan, N., & Yin, X. (2012). Protective effect of l-citrulline against ethanol-induced gastric ulcer in rats. *Environmental Toxicology and Pharmacology*, 34(2), 280-287. https://doi.org/https://doi.org/10.1016/j.etap.2012.04.009

Lue, B.-M., Nielsen, N. S., Jacobsen, C., Hellgren, L., Guo, Z., & Xu, X. (2010). Antioxidant properties of modified rutin esters by DPPH, reducing power, iron chelation and human low density lipoprotein assays. *Food chemistry*, 123(2), 221-230.

Mao, L.-C., Pan, X., Que, F., & Fang, X.-H. (2006). Antioxidant properties of water and ethanol extracts from hot air-dried and freeze-dried daylily flowers. *European Food Research and Technology*, 222, 236-241.

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, 2(2), 48-78.

Martínez-Cuesta, M., Barrachina, M., Beltran, B., Calatayud, S., & Esplugues, J. (1997). Nitric oxide modulates the acute increase of gastrointestinal transit induced by endotoxin in rats: a possible role for tachykinins. *Journal of Pharmacy and Pharmacology*, 49(10), 988-990.

Matsuda, H., Li, Y., & Yoshikawa, M. (1999). Roles of endogenous prostaglandins and nitric oxide in inhibitions of gastric emptying and accelerations of gastrointestinal transit by escins Ia, Ib, IIa, and IIb in mice. *Life sciences*, 66(3), 41-46.

Mazzuoli, G., Lucherini, M., Russo, D., Clavenzani, P., & Chiocchetti, R. (2008). Intrinsic neuronal control of the pyloric sphincter of the lamb. *Journal of Chemical Neuroanatomy*, 36(2), 98-106.

Mechchate, H., Es-Safi, I., Louba, A., Alqahtani, A. S., Nasr, F. A., Noman, O. M., Farooq, M., Alharbi, M. S., Alqahtani, A., & Bari, A. (2021). *In vitro* alpha-amylase and alpha-glucosidase inhibitory activity and *in vivo* antidiabetic activity of *Withania frutescens* L. Foliar extract. *Molecules*, 26(2), 293.

Mehlous, S. (2023). Chemical profile, gastric ulcer and gastrointestinal effects of *Saccocalyx satureioides Coss* and *Dur* and *Achillea santolinoides* L. extracts

Mehlous, S., Amira, S., Benchikh, F., Benabdallah, H., Mamache, W., Bensouici, C., Laouer, H., & Loucif, K. (2022). Alkaline DMSO superoxide and radical scavenging, cupric reducing antioxidant capacity (CUPRAC) and polyphenol contents of aqueous and methanol

extract from *Achilllea santolinoides* L. aerial parts. *Turkish Journal of Agriculture - Food Science and Technology*, 10(10), 2045-2050. https://doi.org/10.24925/turjaf.v10i10.2045-2050.5249

Mendes, W. B., Reis, H. T., Seery, M. D., & Blascovich, J. (2003). Cardiovascular correlates of emotional expression and suppression: Do content and gender context matter. *Journal of Personality and Social Psychology*, 84(4), 771.

Miliauskas, G., Venskutonis, P., & Van Beek, T. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, 85(2), 231-237.

Mohan, S., Hobani, Y. H., Shaheen, E., Abou-Elhamd, A. S., Alhazmi, H. A., & Abdelwahab, S. I. (2020). Ameliorative effect of Boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (Fingerroot) on oxidative stress and inflammation in ethanol-induced gastric ulcer in vivo. *Journal of ethnopharmacology*, 261, 113104.

Moncada, S., Palmer, R., & Higgs, E. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological reviews*, 43(2), 109-142.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G., & Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS biology*, 9(8), e1001127.

Moreira, K. C. C. S. R., da Silva, M. M. V., Jadjeski, M. F. P., Nogueira, C. M., & da Silveira, V. C. (2023). Antioxidative capacity evaluation of imine compounds as metal ions chelators and free radical scavengers in biodiesel. *Biofuels*, 1-10.

Moses, J. F., Hughes, C. D., Patel, P. B., Chao, T. E., Konneh, S. A., Jallabah, T. Y., Kikubaire, M. K., Meara, J. G., & Hagander, L. E. (2015). Surgical outcomes for perforated peptic ulcer: A prospective case series at an academic hospital in Monrovia, Liberia. *African Journal of Emergency Medicine*, 5(2), 60-65.

Mota, C. S., Freitas, R. B., Athayde, M. L., Boligon, A. A., Augusti, P. R., Somacal, S., Rocha, M. P., & Bauermann, L. F. (2010). Effect of *Vernonia cognata* on oxidative damage induced by ethanol in rats. *Human & Experimental Toxicology*, 30(7), 675-684. https://doi.org/10.1177/0960327110377646

Murthy, K. S. (2006). Signaling for contraction and relaxation in smooth muscle of the gut. *Annu. Rev. Physiol.*, 68, 345-374.

Nagai, N., Fukuhata, T., Ito, Y., Usui, S., & Hirano, K. (2009). Involvement of interleukin 18 in indomethacin-induced lesions of the gastric mucosa in adjuvant-induced arthritis rat. *Toxicology*, 255(3), 124-130.

Nakayama, A., Morita, H., Nakao, T., Yamaguchi, T., Sumida, T., Ikeda, Y., Kumagai, H., Motozawa, Y., Takahashi, T., & Imaizumi, A. (2015). A food-derived flavonoid luteolin protects against angiotensin II-induced cardiac remodeling. *PloS one*, 10(9), e0137106.

Nemeth, E., & Bernath, J. (2008). Biological activities of yarrow species (Achillea spp.). *Current pharmaceutical design*, 14(29), 3151-3167.

Niazmand, S., & Khoshnood, E. (2011). The effects of *Achillea wilhelmsii* extract on Rat's gastric motility at basal and vagal stimulated conditions. *Iranian Journal of Basic Medical Sciences*, 12(2), 151-157.

Niazmandi, S., Kooshaki, M., Sookhtanloo, M., Nemati, M., Kianoosh, T., Sadeghnia, H. R., & Akhlaghi, S. (2012). The preventive effects of aqous-ethanolic extract of *Achillea wilhelmsii* on indomethacine-induced ulcer and related biochemical factors in rats. *URMIAMJ*, 23(2), 209-217. http://umj.umsu.ac.ir/article-1-1333

Nishida, K., Ohta, Y., & Ishiguro, I. (1998a). Contribution of NO synthases to neutrophil infiltration in the gastric mucosal lesions in rats with water immersion restraint stress. *FEBS letters*, 425(2), 243-248.

Nishida, K., Ohta, Y., & Ishiguro, I. (1998b). Relationship between constitutive nitric oxide synthase activity and mucus level in the gastric mucosa of rats with stress. *Pharmacological research*, 38(5), 393-397.

Niu, X., Liu, L., Wang, T., Chuan, X., Yu, Q., Du, M., Gu, Y., & Wang, L. (2020). Mapping of extrinsic innervation of the gastrointestinal tract in the mouse embryo. *Journal of Neuroscience*, 40(35), 6691-6708.

Nwinyi, F., Binda, L., Ajoku, G., Aniagu, S., Enwerem, N., Orisandipe, A., Kubmarawa, D., & Gamaniel, K. (2004). Evaluation of the aqueous extract of *Boswellia dalzielii* stem bark for antimicrobial activities and gastrointestinal effects. *african Journal of Biotechnology*, 3(5), 284-288.

Obaid, R. J., Mughal, E. U., Naeem, N., Sadiq, A., Alsantali, R. I., Jassas, R. S., Moussa, Z., & Ahmed, S. A. (2021). Natural and synthetic flavonoid derivatives as new potential tyrosinase inhibitors: A systematic review. *RSC advances*, 11(36), 22159-22198.

Oliveras-López, M.-J., Berná, G., Jurado-Ruiz, E., de la Serrana, H. L.-G., & Martín, F. (2014). Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. *Journal of Functional Foods*, 10, 475-484.

Olivero-Verbel, J., González-Cervera, T., Güette-Fernandez, J., Jaramillo-Colorado, B., & Stashenko, E. (2010). Chemical composition and antioxidant activity of essential oils isolated from Colombian plants. *Revista Brasileira de Farmacognosia*, 20, 568-574.

Olsson, C. (2010). The enteric nervous system. *Elsevier*.30, 319-349.

Olsson, C. (2011). Gut anatomy and morphology. *Gut anatomy*.

Olsson, C., & Holmgren, S. (2001). The control of gut motility. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 128(3), 479-501.

Ozougwu, J., Obimba, K., Belonwu, C., & Unakalamba, C. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *J Physiol Pathophysiol*, 4(4), 46-57.

Ozsoy, N., Can, A., Yanardag, R., & Akev, N. (2008). Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food chemistry*, 110(3), 571-583. https://doi.org/https://doi.org/10.1016/j.foodchem.2008.02.037

Pajdo, R., Brzozowski, T., Szlachcic, A., C Konturek, P., Ptak-Belowska, A., Drozdowicz, D., Targosz, A., J Konturek, S., & W Pawlik, W. (2011). Lipoxins, the novel mediators of gastroprotection and gastric adaptation to ulcerogenic action of aspirin. *Current pharmaceutical design*, 17(16), 1541-1551.

Pal, A., Brasseur, J. G., & Abrahamsson, B. (2007). A stomach road or "Magenstrasse" for gastric emptying. *Journal of biomechanics*, 40(6), 1202-1210.

Patton, K. T., & Thibodeau, G. A. (2018). Anthony's Textbook of Anatomy & Physiology-E-Book. *Elsevier Health Sciences*.

Perrone, R. D., Madias, N. E., & Levey, A. S. (1992). Serum creatinine as an index of renal function: new insights into old concepts. *Clinical chemistry*, 38(10), 1933-1953.

Petlevski, R., Flajs, D., Kalođera, Z., & Končić, M. Z. (2013). Composition and antioxidant activity of aqueous and ethanolic *Pelargonium radula* extracts. *South African Journal of Botany*, 85, 17-22.

Phillips, L. K., Deane, A. M., Jones, K. L., Rayner, C. K., & Horowitz, M. (2015). Gastric emptying and glycaemia in health and diabetes mellitus. *Nature Reviews Endocrinology*, 11(2), 112-128.

Pierce, N. F., Carpenter Jr, C. C., Elliott, H. L., & Greenough III, W. B. (1971). Effects of prostaglandins, theophylline, and cholera exotoxin upon transmucosal water and electrolyte movement in the canine jejunum. *Gastroenterology*, 60(1), 22-32.

Pillaiyar, T., Manickam, M., & Namasivayam, V. (2017). Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *Journal of enzyme inhibition and medicinal chemistry*, 32(1), 403-425.

Posłuszny, M., Chłopecka, M., Suor-Cherer, S., el Amine Benarbia, M., & Mendel, M. (2022). Short Lecture "The effect of Melissa officinalis extract and chlorogenic acid on intestine motility of broiler chicken–*ex vivo* study". *Planta Medica*, 88(15), SL-F07.

Poulsen, H., Prieme, H., & Loft, S. (1998). Role of oxidative DNA damage in cancer initiation and promotion. *European journal of cancer prevention*, 9-16.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290-4302.

Qin, W., Xu, B., Chen, Y., Yang, W., Xu, Y., Huang, J., Duo, T., Mao, Y., Zhou, G., & Yan, X. (2022). Dietary ellagic acid supplementation attenuates intestinal damage and oxidative stress by regulating gut microbiota in weanling piglets. *Animal nutrition*, 11, 322-333.

Quézel, P., Santa, S., Schotter, O., & Emberger, L. (1962). Nouvelle flore de l'Algérie et des régions désertiques méridionales.

Rahimmalek, M., Tabatabaei, B. E. S., Etemadi, N., Goli, S. A. H., Arzani, A., & Zeinali, H. (2009). Essential oil variation among and within six Achillea species transferred from different ecological regions in Iran to the field conditions. *Industrial Crops and Products*, 29(2), 348-355. https://doi.org/https://doi.org/10.1016/j.indcrop.2008.07.001

Rahman, Z., Dwivedi, D., & Jena, G. (2020). Ethanol-induced gastric ulcer in rats and intervention of tert-butylhydroquinone: Involvement of Nrf2/HO-1 signaling pathway. *Human & Experimental Toxicology*, 39(4), 547-562.

Ramkumar, D., & Schulze, K. (2005). The pylorus. *Neurogastroenterology & Motility*, 17, 22-30.

Rawi, S. M., Marie, M.-A. S., Fahmy, S. R., & El-Abied, S. A. (2012). Hazardous effects of acrylamide on immature male and female rats. *Afr J Pharm Pharmacol*, 6(18), 1367-1386.

Razvodovsky, Y. E. (2006). Aggregate level association between alcohol and the peptic ulcer mortality rate. *Alcoholism*, 42(2), 61-68.

Revuelta, M., Cantabrana, B., & Hidalgo, A. (1997). Depolarization-dependent effect of flavonoids in rat uterine smooth muscle contraction elicited by CaCl2. *General Pharmacology: The Vascular System*, 29(5), 847-857.

Ribeiro, D., Freitas, M., Silva, A. M., Carvalho, F., & Fernandes, E. (2018). Antioxidant and pro-oxidant activities of carotenoids and their oxidation products. *Food and Chemical Toxicology*, 120, 681-699.

Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry*, 51(10), 2866-2887.

Rotondo, A., Serio, R., & Mulè, F. (2009). Gastric relaxation induced by apigenin and quercetin: analysis of the mechanism of action. *Life sciences*, 85(1-2), 85-90.

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., & Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism (s) of action. *Frontiers in pharmacology*, 13, 283.

Sadiq, I. Z. (2023). Free radicals and oxidative stress: Signaling mechanisms, redox basis for human diseases, and cell cycle regulation. *Current Molecular Medicine*, 23(1), 13-35.

Saeidnia, S., Gohari, A. R., Yassa, N., & Shafiee, A. (2005). Composition of the volatile oil of *Achillea conferta* DC. from Iran. *DARU Journal of Pharmaceutical Sciences*, 13(1), 34-36.

Sanders, K. M., & Ross, G. (1978). Effects of endogenous prostaglandin E on intestinal motility. *American Journal of Physiology-Endocrinology and Metabolism*, 234(2), E204.

Sanders, K. M., Koh, S. D., Ro, S., & Ward, S. M. (2012). Regulation of gastrointestinal motility-insights from smooth muscle biology. *Nature reviews Gastroenterology & hepatology*, 9(11), 633-645.

Sanderson, J. J., Boysen, S. R., McMurray, J. M., Lee, A., & Stillion, J. R. (2017). The effect of fasting on gastrointestinal motility in healthy dogs as assessed by sonography. *Journal of veterinary emergency and critical care*, 27(6), 645-650.

Santacruz, C. A., Quintairos, A., Righy, C., Crippa, I. A., Couto, L., Imbault, V., Wasineeart, M., De Ryckere, M., & Preiser, J.-C. (2017). Is there a role for enterohormones in the gastroparesis of critically ill patients? *Critical care medicine*, 45(10), 1696-1701.

Saurabh, A., Mishra, A. S., & Gupta, S. (2021). A review on medicinal plant which may effective in the treatment of ulcer or which show anti-ulcer activities. *International Journal of Pharma Professional's Research* (IJPPR), 12(1), 1-10.

Schemann, M., Rohn, M., & Michel, K. (2008). Motor control of the stomach. *European Review for Medical & Pharmacological Sciences*, 12.

Schliebs, R., & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behavioural brain research*, 221(2), 555-563.

Sen, S., De, B., Devanna, N., & Chakraborty, R. (2013). Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa Roxb*., an Indian medicinal plant. *Chinese journal of natural medicines*, 11(2), 149-157.

Serafim, C., Araruna, M. E., Júnior, E. A., Diniz, M., Hiruma-Lima, C., & Batista, L. (2020). A review of the role of flavonoids in peptic ulcer (2010–2020). *Molecules*, 25(22), 5431.

Serra, V., Salvatori, G., & Pastorelli, G. (2021). Dietary Polyphenol Supplementation in Food Producing Animals: Effects on the Quality of Derived Products. *Animals*, 11(2), 401. https://www.mdpi.com/2076-2615/11/2/401

Sharma, H., & Sharma, K. (2011). Opioid analgesics and opioid antagonists. *Principles of Pharmacology. 2nd ed. Hyderabad: Paras Medical Publishers*.

Sharma, J., Al-Omran, A., & Parvathy, S. (2007). Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, 15, 252-259.

Shen, F., Zhao, C.-S., Shen, M.-F., Wang, Z., & Chen, G. (2019). The role of hydrogen sulfide in gastric mucosal damage. *Medical Gas Research*, 9(2), 88.

Sherwood, L. (1993). Human Physiology: From cells to systems. *Minneapolis/St. Paul. In: West Publishing.*

Shimizu, Y., Matsuyama, H., Shiina, T., Takewaki, T., & Furness, J. (2008). Tachykinins and their functions in the gastrointestinal tract. *Cellular and Molecular Life Sciences*, 65, 295-311.

Shimoyama, A. T., Santin, J. R., Machado, I. D., de Oliveira e Silva, A. M., de Melo, I. L. P., Mancini-Filho, J., & Farsky, S. H. (2013). Antiulcerogenic activity of chlorogenic acid in different models of gastric ulcer. *Naunyn-Schmiedeberg's archives of pharmacology*, 386, 5-14.

Si, X. T., Zhang, M. L., Shi, Q. W., & Kiyota, H. (2006). Chemical constituents of the plants in the genus Achillea. *Chemistry & biodiversity*, 3(11), 1163-1180.

Silva, M. I. G., & de Sousa, F. F. (2011). Gastric ulcer etiology. *Peptic ulcer disease*, 1.

Singh, R., Ahluwalia, V., Singh, P., Kumar, N., Prakash Sati, O., & Sati, N. (2016). Antifungal and phytotoxic activity of essential oil from root of *Senecio amplexicaulis Kunth*. (Asteraceae) growing wild in high altitude-Himalayan region. *Natural product research*, 30(16), 1875-1879.

Singh, S. (2014). Aqueous two-phases extraction of fungal amylase and its use for desizing of cotton fabrics. *International Journal of Plant, Animal and Environmental Sciences*, 4(4), 118-123.

Skogar, M. L., & Sundbom, M. (2022). Nonsteroid anti-inflammatory drugs and the risk of peptic ulcers after gastric bypass and sleeve gastrectomy. *Surgery for Obesity and Related Diseases*, 18(7), 888-893.

Sowndhararajan, K., & Kang, S. C. (2013). Protective effect of ethyl acetate fraction of *Acacia ferruginea* DC. against ethanol-induced gastric ulcer in rats. *Journal of ethnopharmacology*, 148(1), 175-181. https://doi.org/https://doi.org/10.1016/j.jep.2013.04.007

Spiegel, M., & Sroka, Z. (2023). Quantum-mechanical characteristics of apigenin: Antiradical, metal chelation and inhibitory properties in physiologically relevant media. *Fitoterapia*, 164, 105352.

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of food engineering*, 81(1), 200-208.

Summers, M. J., Di Bartolomeo, A. E., Zaknic, A. V., Chapman, M. J., Nguyen, N. Q., Zacharakis, B., Rayner, C. K., Horowitz, M., & Deane, A. M. (2014). Endogenous amylin and glucagon-like peptide-1 concentration is not associated with gastric emptying in critical illness. *Acta Anaesthesiologica Scandinavica*, 58(2), 235-242.

Sun, C., Zhao, C., Guven, E. C., Paoli, P., Simal-Gandara, J., Ramkumar, K. M., Wang, S., Buleu, F., Pah, A., & Turi, V. (2020). Dietary polyphenols as antidiabetic agents: Advances and opportunities. *Food Frontiers*, 1(1), 18-44.

Sundarraj, S., Thangam, R., Sreevani, V., Kaveri, K., Gunasekaran, P., Achiraman, S., & Kannan, S. (2012). γ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. *Journal of ethnopharmacology*, 141(3), 803-809.

Surakka, A., Sihvonen, L. M., Lehtimäki, J. M., Wahlsten, M., Vuorela, P., & Sivonen, K. (2005). Benthic cyanobacteria from the Baltic Sea contain cytotoxic Anabaena, Nodularia, and Nostoc strains and an apoptosis-inducing Phormidium strain. *Environmental Toxicology: An International Journal*, 20(3), 285-292.

Sverdén, E., Agréus, L., Dunn, J. M., & Lagergren, J. (2019). Peptic ulcer disease. *Bmj*, 367.

Svorc, P., Bracoková, I., & Dorko, E. (2001). An overview of the regulation of basic functions of the digestive system. *Ceskoslovenská fysiologie*, 50(3), 115-118.

Szlachcic, A., Krzysiek-Maczka, G., Pajdo, R., Targosz, A., Magierowski, M., Jasnos, K., Drozdowicz, D., Kwiecien, S., & Brzozowski, T. (2013). The impact of asymmetric dimethylarginine (ADAMA), the endogenous nitric oxide (NO) synthase inhibitor, to the pathogenesis of gastric mucosal damage. *Current pharmaceutical design*, 19(1), 90-97.

Taqui, R., Debnath, M., Ahmed, S., & Ghosh, A. (2022). Advances on plant extracts and phytocompounds with acetylcholinesterase inhibition activity for possible treatment of Alzheimer's disease. *Phytomedicine Plus*, 2(1), 100184.

Tekin, Z., Küçükbay, F. Z., & Dikme, A. (2021). *In Vitro* Antioxidant Activities of Methanol Extracts of Three Achillea Species from Turkey. *Journal of the Turkish Chemical Society Section A: Chemistry*, 8(2), 483-490.

Tepperman, B., & Whittle, B. (1992). Endogenous nitric oxide and sensory neuropeptides interact in the modulation of the rat gastric microcirculation. *British journal of pharmacology*, 105(1), 171.

Teresa, S., Joana, A., António, A.-T., & Félix, C. (2012). Lipid Peroxidation and Antioxidants in Arterial Hypertension. In C. Angel (Ed.), Lipid Peroxidation. *IntechOpen*, 17. https://doi.org/10.5772/50346

Terpinc, P., & Abramovič, H. (2010). A kinetic approach for evaluation of the antioxidant activity of selected phenolic acids. *Food chemistry*, 121(2), 366-371.

Thompson, D., Richelson, E., & Malagelada, J.-R. (1982). Perturbation of gastric emptying and duodenal motility through the central nervous system. *Gastroenterology*, 83(6), 1200-1206.

Tomic, D., Shaw, J. E., & Magliano, D. J. (2022). The burden and risks of emerging complications of diabetes mellitus. *Nature Reviews Endocrinology*, 18(9), 525-539.

Tong, Z., He, W., Fan, X., & Guo, A. (2022). Biological function of plant tannin and its application in animal health. *Frontiers in Veterinary Science*, 8, 803657.

Travagli, R. A., & Anselmi, L. (2016). Vagal neurocircuitry and its influence on gastric motility. *Nature reviews Gastroenterology & hepatology*, 13(7), 389-401.

Uesaka, T., Young, H. M., Pachnis, V., & Enomoto, H. (2016). Development of the intrinsic and extrinsic innervation of the gut. *Developmental biology*, 417(2), 158-167.

Ugan, R. A., & Un, H. (2020). The protective roles of butein on indomethacin induced gastric ulcer in mice. *The Eurasian Journal of Medicine*, 52(3), 265.

Valero, M. S., López, V., Castro, M., Gómez-Rincón, C., Arruebo, M. P., Les, F., & Plaza, M. Á. (2022). Involvement of NO/cGMP Signaling Pathway, $Ca2^{+}$ and K^{+} Channels on Spasmolytic Effect of Everlasting Flower Polyphenolic Extract (*Helichrysum stoechas* (L.) Moench). *International journal of molecular sciences*, 23(22), 14422.

Vassalle, C., & Gaggini, M. (2022). Type 2 diabetes and oxidative stress and inflammation: Pathophysiological mechanisms and possible therapeutic options. *In MDPI*, 11, 953

Vecchio, I., Sorrentino, L., Paoletti, A., Marra, R., & Arbitrio, M. (2021). The state of the art on acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. *Journal of Central nervous system disease*, 13, 11795735211029113.

Vitalini, S., Beretta, G., Iriti, M., Orsenigo, S., Basilico, N., Dall'Acqua, S., Iorizzi, M., & Fico, G. (2011). Phenolic compounds from *Achillea millefolium* L. and their bioactivity. *Acta Biochimica Polonica*, 58(2).

Vona, R., Pallotta, L., Cappelletti, M., Severi, C., & Matarrese, P. (2021). The Impact of Oxidative Stress in Human Pathology: Focus on Gastrointestinal Disorders. Antioxidants, 10, 201.

Vymiatnina, Z. K., & Gridneva, V. I. (1997). Effect of *Achillea asiatica* Serg. on gastric secretory and excretory activity. *Eksperimental'naia i klinicheskaia farmakologiia,* 60(5), 54-57. http://europepmc.org/abstract/MED/9483408

Wallace, J. L. (2019). Nitric oxide in the gastrointestinal tract: opportunities for drug development. *British journal of pharmacology*, 176(2), 147-154.

Walum, E. (1998). Acute oral toxicity. *Environmental health perspectives*, 106(suppl 2), 497-503.

Wasung, M. E., Chawla, L. S., & Madero, M. (2015). Biomarkers of renal function, which and when? *Clinica chimica acta*, 438, 350-357.

Wijesinghe, W., & Jeon, Y.-J. (2012). Enzyme-assistant extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds: a review. *Fitoterapia*, 83(1), 6-12.

Wilson, N., & Schey, R. (2015). Lubiprostone in constipation: clinical evidence and place in therapy. *Therapeutic advances in chronic disease*, 6(2), 40-50.

Winek, K., Soreq, H., & Meisel, A. (2021). Regulators of cholinergic signaling in disorders of the central nervous system. *Journal of Neurochemistry*, 158(6), 1425-1438.

Wink, M. (2015). Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, 2(3), 251-286.

Wootton-Beard, P. C., Moran, A., & Ryan, L. (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and

after in vitro digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. *Food research international*, 44(1), 217-224.

Wu, F., Tian, F.-J., & Lin, Y. (2015). Oxidative stress in placenta: health and diseases. *BioMed research international*, 15, 14.

Xie, L., Guo, Y.-l., Chen, Y.-r., Zhang, L.-y., Wang, Z.-c., Zhang, T., & Wang, B. (2020). A potential drug combination of omeprazole and patchouli alcohol significantly normalizes oxidative stress and inflammatory responses against gastric ulcer in ethanol-induced rat model. *International immunopharmacology*, 85, 106660.

Xie, X., Ren, K., Zhou, Z., Dang, C., & Zhang, H. (2022). The global, regional and national burden of peptic ulcer disease from 1990 to 2019: a population-based study. *BMC gastroenterology*, 22(1), 58.

Yang, H., Yang, W. J., & Hu, B. (2022). Gastric epithelial histology and precancerous conditions. *World J Gastrointest Oncol*, 14(2), 396-412. https://doi.org/10.4251/wjgo.v14.i2.396

Yuan, S., Costa, M., & Brookes, S. (2001). Neuronal control of the pyloric sphincter of the guinea-pig. *Neurogastroenterology & Motility*, 13(3), 187-198.

Zayachkivska, O., Konturek, S., Drozdowicz, D., Brzozowski, T., & Gzhegotsky, M. (2004). Influence of plant-originated gastroproteciive and antiulcer substances on gastric mucosal repair. *Fiziol Zh*, 50(6), 118-127.

Zengin, G., Aktumsek, A., Ceylan, R., Uysal, S., Mocan, A., Guler, G. O., Mahomoodally, M. F., Glamočlija, J., Ćirić, A., & Soković, M. (2017). Shedding light on the biological and chemical fingerprints of three Achillea species (*A. biebersteinii, A. millefolium and A. teretifolia*). *Food & function*, 8(3), 1152-1165.

Zhang, B., Pan, C., Feng, C., Yan, C., Yu, Y., Chen, Z., Guo, C., & Wang, X. (2022). Role of mitochondrial reactive oxygen species in homeostasis regulation. *Redox Report*, 27(1), 45-52.

Zhao, D.-Q., Xue, H., & Sun, H.-J. (2020). Nervous mechanisms of restraint water-immersion stress-induced gastric mucosal lesion. *World journal of gastroenterology*, 26(20), 2533.

Zhao, J., Li, Q. Q., Zou, B., Wang, G., Li, X., Kim, J. E., Cuff, C. F., Huang, L., Reed, E., & Gardner, K. (2007). *In vitro* combination characterization of the new anticancer plant drug

β-elemene with taxanes against human lung carcinoma. *International journal of oncology*, 31(2), 241-2