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List of publications & Communications

Articles :

1. **Makhlouf, Y.**, Bouaziz, A., Bentahar, A., Djidel, S., Barghout, N., & Khennouf, S. (2023). Ethnobotanical study of medicinal plants in "ELMERGUEB" nature reserve, M'sila province, northern Algeria. *Applied Ecology and Environmental Research, 22(1)*(1), 459-472. https://doi.org/10.15666/aeer/2201_459472.

2. **Makhlouf Y,** Bouaziz A, Benazi N, Djidel S, Bentahar A, Barghout N, Khennouf S, Dahamna S. Assessment of antioxidant and antiinflammatory activities and acute toxicity of the aqueous extract from a mixture of leaves and flowers of Anabasis articulata (Forssk.) Moq.. Arch Biol Sci. 2024. https://doi.org/10.2298/ABS240122011M.

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1. Makhlouf Yasmina, Bouaziz Amel, Benazi Nabil, Seddik Khennouf, 2022. Antioxydant, anti-inflammatory, and antiulcerogenic activities of selected plant extracts from Algerian folk medicine. "3rd Doctorial Scientific Days" organized at the Faculty of Nature and Life Sciences: 10 February 2022, Sétif, Algeria.

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ملخص

باستخدام مجموعة متنوعة من النماذج *in vitro*، *in silico* و *in vivo*، تهدف هذه الدراسة إلى تقييم النشاطية المضادة للأكسدة والمضادة للالتهاب والمسكنات للألم والمضادة للتقرح لعدة مستخلصات من نبات العجرم *Anabasis articulata*، وهو من النباتات الجزائرية المتواجدة في المناطق القاحلة وشبه القاحلة و تنتمي إلى عائلة Chenopodiaceae تم اختيارها بعد دراسة اثتنوباتية أجريت في محمية المرقب الطبيعية (عدد المشاركين = 182 مستجوب). في هذه الدراسة تم تحديد الجزيئات الحيوية، وتحديد كمية المستقلبات الثانوية وإجراء ثمانية اختبارات لدراسة النشاط المضاد للأكسدة للمستخلصات: إجمالي القدرة المضادة للأكسدة؛ اختبار أزاحة جذور ABTS ، DPPH، جذر الهيدروكسيل، بيروكسيد الهيدروجين، استخلاب المعادن، اختبار قوة الإرجاع، اختبار تبييض β-carotene. تم تقييم النشاطية المضاد للالتهابات في المختبر باستخدام تخريب ألبومين البيض وBSA للمستخلص المائي DEAA والمستخلص الايثانولي EEAA وأجزائه. كما تم إجراء Molecular docking باستخدامAutodock vina . تم إعطاء جرعات فموية مفردة لكل من DEAA وEEAA بتركيز 2000 و5000 ملغم/كغم من وزن الجسم للفئران البيضاء لتقييم السمية الحادة. تم تقييم التأثير المضاد للالتهابات باستخدام نموذج الوذمة induced-carrageen في الجرذان، وذمة الأذن باستخدام croton oil، وذمة الأذن المستحدثة بالxylene في الفئران. تم تحقيق نشاط مسكن عن طريق الحقن acetic acide داخل الصفاق. تم تقييم النشاط الوقائي للمعدة باستخدام تقرح المعدة المستحث بالإيثانول في الجرذان بجرعات 50 و100 و200 ملغم/كغم من DEAA و200 و400 و600 ملغم/كغم من وEEAA. كما تضمنت الدراسة الميكانيكية المعالجة المسبقة بمضادات/مثبطات مناسبة مثل: glibenclamide, indomethacin and L-NAME. تم تحديد 46 نوعًا من النباتات التي تنتمي إلى 23 عائلة نباتية لعلاج أمراض مختلفة. تم تحديد سبعة مستقلبات موجودة في أجزاء n-butanol وethyl acetate وchloroform بشكل نوعي بواسطة LC-MS-MS وهي: الأنثرون، وبيتاكاروتين، وبوتيل هيدروكسي أنيسول، وبوتيل هيدروكسي تولوين البوتيلات، وحمض الغاليك، والميريسيتين، والروتين. أظهرت النتائج أن ChFA يمثل أكبر كمية من إجمالي البوليفينول والفلافونويدات. أظهر جزء n-butanol أعلى كمية من العفص الكلي. أظهر التقييم الكمي لقدرة الكسح DPPH أن المستخلص الإيثانولي كان الأكثر نشاطاً بقيم IC_{50}= 0.074±0.003مجم/ملل، وكان لجزء الكلوروفورم (ChFA) التأثير الأقوى في نشاط ازاحت جذر الهيدروكسيل وازاحت جذر الهيدروكسيل وازاحت جذر البيروكسيد الهيدروجين بقيم EC_{50}= 0. 151 ± 0.0017 ، و IC_{50}= 0.361 ± 0.0087و IC_{50}= 1.777 ± 0.0402 مجم/مل) ،كما كان لجزء n-butanol أعلى نشاط في اختبار ازاحت جذر ABTS بقيمة IC_{50}= 0.021 ± 0.0007مجم/مل. أظهرت جميع المستخلصات تثبيطاً جيداً لأكسدة linoleic acid. وقد ثبت وجود نشاط جيد مضاد للالتهابات في المختبر باستخدام تخريب ألبومين البيض وBSA في كل من DEAA و EEAA وأجزائها. كشف Molecular docking للجزيئات الحيوية المحددة والدواء المرجعي (indomethacin) عن تفاعل جيد مع بروتين COX_2 الالتهابي (3LN0). وأظهر اختبار السمية الحادة أن DEAA و EEAA لم يسبب الوفيات أو الآثار الضارة. وقد أدى إعطاء 100 أو 200 ملغم/كغم من DEAA و EEBAعن طريق الفم للجرذان والفئران إلى تثبيط الوذمة في الاختبارات الثلاث، مع نسب جيدة لكلا الجرعتين من كلا المستخلصين. أظهرت نتيجة النشاط المسكن تأثيراً جيداً مقارنة بالمسكن القياسي (aspirin) لنفس الجرعات والمستخلصات. كما أن الإعطاء الفموي لـ DEAA بجرعات 100 مجم/كجم أو 200 مجم/كجم يحمي الغشاء المخاطي المعدي من الإيثانول المطلق. أدت المعالجة المسبقة بجرعةL-NAME ، وهو مثبط ل nitric oxide synthesis ، أو indomethacin ، وهو مثبط لإنتاج prostaglandin، أو glibenclamide ، وهو مثبط لقنواتKATP ، إلى عكس النشاط الوقائي للمعدة 200 DEAAملغم/كغم، عن طريق الفم. قلل DEAA بشكل فعال من إنتاج العصارة المعدية القاعدية دون أي تأثير على الحموضة الكلية. وقد اشتمل تأثير DEAA الواقي للمعدة على زيادة في الإنزيمات المضادة للأكسدة وإفراز المخاط. توفر هذه الدراسة لأول مرة الأساس العلمي للاستخدام التقليدي لهذا النبات للتأثير الوقائي للمعدة وتدعم الاستخدام التقليدي لهذا النبات لعلاج بعض الاضطرابات المرتبطة بالالتهاب والإجهاد التأكسدي. يمكن استغلال هذه النتائج في الصناعات الدوائية والغذائية ومستحضرات التجميل

الكلمات الرئيسية :العجرم *Anabasis articulata*، الإجهاد التأكسدي، Molecular docking، السمية الحادة، الالتهاب، مسكن الالم، قرحة المعدة.

Abstract

Using a variety of *in-vitro*, *in-silico* and *in vivo* models, the aim of the present study was to investigate the antioxidant, anti-inflammatory, analgesic and anti-ulcerogenic activities of several extracts of Anabasis articulata, a plant from the Algerian flora of arid and semi-arid regions belonging to the Chenopodiaceae family selected on the basis of an ethnobotanical survey (n=182 respondents) conducted at the El-Mergueb nature reserve. Identification of biomolecules, quantification of secondary metabolites and performance of eight tests to study the antioxidant activity of the extracts: Total antioxidant capacity (TAC), DPPH - 2,2-diphenyl-1picrylhydrazyl; ABTS - 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid, hydroxyl radical scavenging, metal-chelating hydrogen peroxide scavenging, reducing power test and β-carotene bleaching assay. In vitro anti-inflammatory activity was attested using the ovalbumin denaturation and BSA assay for DEAA, EEAA and their fractions. Molecular docking was performed using Autodock vina. Single oral doses of 2000 and 5000 mg/kg body weight were administered to albino mice to assess acute toxicity. The anti-inflammatory effect was assessed using the carrageenan-induced paw edema model in rats, the croton oil-induced ear edema model and the xylene-induced ear edema model in mice. Analgesic activity was achieved by intraperitoneal injection of acetic acid. Gastroprotective activity was assessed using ethanol-induced gastric ulceration in rats at doses of 50, 100 and 200 mg/kg p.o. for DEAA and 200, 400 and 600 mg/kg p.o. for EEAA. The mechanistic study involved pretreatment with appropriate antagonists/inhibitors such as glibenclamide, indomethacin and L-NAME. 46 plant species belonging to 23 botanical families were identified for the treatment of various ailments. Seven metabolites present in the n-butanol, ethyl acetate and chloroform fractions were qualitatively identified by LC-MS-MS: anthrone, beta-carotene, butylhydroxyanisole, butylhydroxytoluene, gallic acid, myricetin and rutin. The results showed that ChFA represents the largest quantity of total polyphenols and flavonoids. The n-butanol fraction showed the highest amount of total tannins. Quantitative evaluation of DPPH scavenging capacity showed that the ethanolic extract was the most active with IC50 values of 0.074±0.003 mg/mL, the chloroform fraction (ChFA) had the strongest effect in TAC, hydroxyl radical scavenging and hydrogen peroxide scavenging activity with EC50= 0.151±0.0017, IC50= 0.361±0.0087 and IC50= 1.777±0.0402 mg/mL), however, the nbutanol fraction had the highest activity in the ABTS radical scavenging test with an IC50 of 0.021±0.0007 mg/mL. All extracts showed good inhibition of linoleic acid oxidation. Good in vitro anti-inflammatory activity was attested using the ovalbumin denaturation and BSA assays for DEAA, EEAA and their fractions. Molecular docking of the identified biomolecules and the reference drug (indomethacin) revealed good interaction with the inflammatory protein COX2 (3LN0). Acute toxicity testing showed that DEAA and EEAA did not cause mortality or adverse effects. Oral administration of 100 or 200 mg/kg of DEAA and EEAA to rats and mice inhibited edema in three tests, with good percentages for both doses of both extracts. The analgesic activity result showed a good effect compared with the standard analgesic (aspirin) for the same doses and extracts. Oral administration of DEAA at doses of 100 mg/kg or 200 mg/kg protected the gastric mucosa against absolute ethanol. Pretreatment with L-NAME, an inhibitor of nitric oxide synthesis, indomethacin, an inhibitor of prostaglandin production, or glibenclamide, a KATP channel blocker, reversed the gastroprotective activity of (DEAA200 mg/kg, p.o.). DEAA effectively reduced basal gastric juice production without any effect on total acidity. The gastroprotective action of DEAA involved an increase in antioxidant enzymes and mucus secretion. This study provides for the first time the scientific basis for the traditional use of this plant for gastro-preventive effect and supports the traditional use of this plant to treat certain disorders linked to inflammation and oxidative stress. These results can be exploited in the pharmaceutical, food and cosmetics industries.

Key words: Anabasis articulata, Oxidative stress, Molecular docking, Acute toxicity, Inflammation, Analgesic, Gastric ulcer.

Résumé

En utilisant une variété de modèles *in-vitro, in-silico et in vivo,* le but de la présente étude était d'étudier les activités antioxydante, anti-inflammatoire, analgésique et anti-ulcérogène de plusieurs extraits d'*Anabasis articulata*, une plante de la flore algérienne des régions arides et semi-arides appartenant à la famille des Chenopodiaceae choisis sur la base une enquête ethnobotanique (n=182 interrogés) menée à la réserve naturelle El-Mergueb. L'identification des biomolécules, la quantification et de métabolites secondaires et la réalisation de huit tests pour l'étude de l'activité antioxydante des extraits : Capacité antioxydante totale (TAC), DPPH - 2,2-diphényl-1picrylhydrazyl; ABTS - 2,2′-azino-bis 3-éthylbenzothiazoline-6-acide sulfonique, piégeage du radical hydroxyle, piégeage du peroxyde d'hydrogène chélatrice des métaux, test du pouvoir réducteur et dosage du blanchiment du β-carotène. L'activité anti-inflammatoire *in vitro* a été attestée en utilisant la dénaturation de l'ovalbumine et le test BSA pour le DEAA, l'EEAA et leurs fractions. Le docking moléculaire a été effectuer en utilisant l'Autodock vina. Des doses orales uniques de 2000 et 5000 mg/kg de poids corporel ont été administrées à des souris albinos pour évaluer la toxicité aiguë. L'effet anti-inflammatoire a été évalué en utilisant le modèle d'œdème de la patte induit par la carragénine chez le rat, l'œdème de l'oreille induit par l'huile de croton et l'œdème de l'oreille induit par le xylène chez la souris. L'activité analgésique a été réalisée par injection intrapéritonéale d'acide acétique. L'activité gastroprotectrice a été évaluée en utilisant l'ulcération gastrique induite par l'éthanol chez les rats à des doses de 50, 100 et 200 mg/kg p.o. de DEAA et 200, 400 et 600 mg/kg p.o pour l'EEAA. L'étude mécaniste a impliqué des prétraitements avec des antagonistes/inhibiteurs appropriés tels que le glibenclamide, l'indométacine et le L-NAME. 46 espèces de plantes appartenant à 23 familles botaniques ont été recensées pour le traitement de différentes affections. Sept métabolites présents dans les fractions de n-butanol, d'acétate d'éthyle et de chloroforme ont été identifiés qualitativement par LC-MS-MS: anthrone, bêta-carotène, butylhydroxyanisole, butylhydroxytoluène, acide gallique, myricétine et rutine. Les résultats ont montré que le ChFA représente la plus grande quantité de polyphénols totaux, de flavonoïdes. La fraction n-butanol a montré la plus grande quantité de tannins totaux. L'évaluation quantitative du pouvoir de piégeage de DPPH a montré que l'extrait éthanolique était le plus actif avec des valeurs IC_{50} de 0.074±0.003 mg/mL, la fraction chloroforme (ChFA) a eu l'effet le plus fort dans le TAC, le piégeage du radical hydroxyle et l'activité de piégeage du peroxyde d'hydrogène avec EC_{50}= 0.151±0.0017, IC_{50}= 0.361±0.0087 et IC50= 1.777±0.0402 mg/mL), cependant, la fraction n-butanol a eu la plus grande activité dans le test de piégeage du radical ABTS avec un IC_{50} de 0.021±0.0007 mg/mL. Tous les extraits ont montré une bonne inhibition de l'oxydation de l'acide linoléique. Une bonne activité anti-inflammatoire *in vitro* a été attestée en utilisant la dénaturation de l'ovalbumine et le test BSA pour le DEAA, l'EEAA et leurs fractions. L'amarrage moléculaire des biomolécules identifié et le médicament de référence (l'indométacine) a révélé une bonne interaction vis à vis la protéine inflammatoire COX_2 (3LN0). Le test de toxicité aiguë a montré que DEAA et EEAA n'ont pas provoqué de mortalité ou d'effets indésirables. L'administration orale de 100 ou 200 mg/kg de DEAA et d'EEAA à des rats et à des souris a inhibé l'œdème dans trois tests avec un bon pourcentage pour les deux doses des deux extraits. Le résultat de l'activité analgésique montre un bon effet comparé à l'analgésique standard (l'aspirine) pour les mêmes doses et extraits. L'administration orale de DEAA à des doses de 100 mg/kg ou 200 mg/kg a permis de protéger la muqueuse gastrique contre l'éthanol absolu. Le prétraitement par le L-NAME, un inhibiteur de la synthèse de l'oxyde nitrique, l'indométacine, un inhibiteur de la production de prostaglandines, ou le glibenclamide, un bloqueur du canal KATP, a inversé l'activité gastroprotectrice de (DEAA200 mg/kg, p.o.). La DEAA a réduit efficacement la production basale de suc gastrique sans aucun effet sur l'acidité totale. L'action gastroprotectrice de la DEAA a impliqué une augmentation des enzymes antioxydantes et de la sécrétion de mucus. Cette étude fournit pour la première fois les bases scientifiques de l'utilisation traditionnelle de cette plante pour l'effet gastro-préventif et soutient l'utilisation traditionnelle de cette plante pour traiter certains troubles liés à l'inflammation et au stress oxydatif. Ces résultats peuvent être exploités dans le domaine des industries pharmaceutiques, alimentaires et cosmétiques.

Mots clés: *Anabasis articulata*, Stress oxydatif, Docking moléculaire, Toxicité aiguë, Inflammation, Analgésique, Ulcère gastrique.

List of abbreviations

ABTS: 2.2′-Azino-bis-(3-ethylbenzenothiazoline-6-sulfonic acid)
Ach: Acetylcholine.
AChE: Acetylcholinesterase
AChEI: Acetylcholinesterase inhibitors
$AlCl_3$: Aluminium tri-chloride
BHT: Butyl hydroxyl toluene
CAT: Catalase
CE: Crude extract
COX: Cyclooxygenase
DPPH: 2, 2-diphenyl-1-picryl-hydrazyl
DW: Dried weight.
FRAP: Frric reducing antioxidant power assay
GAE: Gallic acid equivalents
GPx: Glutathione peroxidase
GSH: Glutathione
H_2O_2: Hydrogen peroxide.
HPLC: High-performed liquid chromatography
IC 50%: Inhibitory concentration for 50% of activity
LD_{50}: Lethal dose, 50%.
LPO: Lipid peroxidation
MDA: Malondialdehyde
NO: Nitric oxide
NO• : Nitrogen free radical
NO_2• : Nitrogen dioxide
NOS: Nitric oxide synthase
NSAIDs: Non-steroidal anti-inflammatory drugs
O_2•− : Superoxide radical
OECD: Organisation for Economic Co-operation and Development
PGs: Prostaglandins
QE: Quercetin equivalents
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SEM: Standard error of the mean
SOD: Superoxide dismutase
TAC: Total antioxidant capacity
TAE: Tannic acid equivalents
TBA: Thiobarbutiric acid
TCA: Trichloro-acetic acid
TFC: Total flavonoid content
TNF: Tumor necrosis factor
TPC: Total phenolics content
UHPLC-MS: Ultra-High performance liquid chromatography-mass spectrometry

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Introduction

The usage of medicinal plants has increased in recent years, most likely as a result of their cultural value, local abundance, and low cost of acquisition (Thomford *et al*., 2015). Medicinal plants are thought to be a promising source of bioactive chemicals. The majority of these plant derived medications were found via the study of traditional treatments and indigenous knowledge. Despite great advances in synthetic chemistry, some substances them could not be replaced (Hudaib *et al.*, 2008). Medicinal plants are important for maintaining public health since up to 80% of people in underdeveloped nations, such those in Africa, still rely on them for their primary medical requirements (Okaiyeto and Oguntibeju, 2021). Over 25% of contemporary medications contain one or more active ingredients derived from plants, according to the World Health Organization (WHO) (Benarba *et al*., 2015).

Oxidative stress has gained attention across the board in the medical community because it plays a significant role in the pathophysiologic process of numerous chronic diseases, including atherosclerosis, cancer, diabetes, rheumatoid arthritis, cardiovascular disease, aging, and other degenerative diseases in humans (El-Bahr, 2013). The imbalance between pro-oxidant and antioxidant homeostasis, which results in an excess of free radicals damaging biomolecules, is what is known as oxidative stress. Additionally, there is proof that oxidative stress is essential to the initiation and maintenance of inflammation, which in turn promotes the emergence of chronic inflammation-related diseases (Lugrin *et al*., 2014).

The reactive state of hyperemia and blood vessel exudation, followed by redness, heat, swelling, and pain that a tissue displays in response to chemical or physical harm or bacterial invasion, is called inflammation (Okoli *et al*., 2003). Unfortunately, the drugs that are currently available to treat pain and inflammation, particularly in chronic illnesses, have a number of side effects and a limited effectiveness (Abdallaha *et al*., 2014). Long-term use of nonsteroidal antiinflammatory medicines (NSAIDs) has been associated with severe side effects, including bleeding in the gastrointestinal tract (Li *et al*., 2003).

Many individuals all around the world suffer from the chronic illness as gastric ulcers. (Kangwan *et al*., 2014) *Helicobacter pylori*, NSAID usage, and stress all have an impact on (Stewart and Ackroyd, 2011). Its detrimental effects came from the disruption of the natural defensive agents such as the mucosal bicarbonate barrier, prostaglandin E2, cell regeneration, and antioxidant markers and endogenous offensive factors such as hydrochloric acid, pepsin, and reactive oxygen species (Farzaei *et al*., 2015).

Pain is a warning system that alerts the body to tissue damage. The pathophysiology of pain involves several biological events, including the activation of nociceptive receptors and the enzyme cyclooxygenase 2 (COX-2) (Van Rensburg and Reuter, 2019). Pain, regardless of aetiology, is usually caused by inflammation. With the discovery of the involvement of inflammatory cytokines, many anti-inflammatory medications may ease inflammation and pain (Bost *et al*., 2010).

Molecular docking is a crucial bioinformatic method that forecasts the probable direction of experimentation and the binding affinity necessary to create a stable complex structure between a ligand and a target. (Kumar and Kumar, 2019). The results of molecular docking can be used to evaluate and supplement biological experimentation findings. The impact of a molecule on the functioning of a system is evaluated experimentally in biological experiments. Binding assays and cell or organism activity tests are examples of these tests (Mathammal *et al*., 2013).

In the present study, the following objectives are formulated:

After choosing our plant *Anabasis articulata* (Forssk.) Moq. or El-Ajrem, as it is known locally and, belongs to the Chenopodiaceae family using an ethnobotanical survey we investigated,

- The extraction of phenolic compounds from medicinal plants using organic solvents.
- The identification qualitatively of polyphenols in fractions of *A. articulata* using ultra-High performance liquid chromatography (UPLC) coupled with triple quadrupole mass spectrometry (MS/ MS) based on multiple reactions monitoring (MRM).
- The determination of polyphenols, flavonoids and tannins contents in different plant extracts.
- The evaluation of the *in vitro* antioxidant activity of plant extracts using different methods (DPPH, ABTS radical scavenging activities, metal chelating activity, reducing power test, Hydroxyl radical scavenging, scavenging effect on hydrogen peroxide and β-carotene bleaching assay).
- The molecular docking study of the organic molecules that have been detected from the plant was performed.
- The studying of *in vitro* and *in vivo* anti-inflammatory activity of plant extracts using different methods as protein denaturation method, Carrageenan induced paw edema and Croton-oil/Xylene-induced ear edema.

- The exploring of the analgesic properties of extracts.
- The studying of the anti-ulcerogenic activity of plant extracts (decocted and ethanolic extracts) using ethanol-induced gastric ulceration in rats.
- The evaluation of the *in vivo* antioxidant activity of decocted extract by assessing the MDA and SOD levels, and catalase activity.

Literature review

I. Oxidative stress

I.1. Definition

Oxygen is essential to our survival, our life, our development and our ability to adapt, but it is also a source of toxicity, acidity, deterioration and degeneration. In fact, when oxygen metabolism is deregulated, it can lead to what is known as "oxidative stress" (Gibson *et al*., 2007). Oxidative stress can form partially reduced, highly toxic species known as free radicals or reactive oxygen species (ROS) and reactive nitrogen species (RNS). Radical or non-radical, they are permanently produced by several cell types in aerobic organisms (Phaniendra *et al*., 2015). Oxidative stress is the inability to defend against the aggression of ROS, due to an imbalance between the production of these substances and the defense capacity of antioxidants (Vona *et al*., 2021).

I.2. Reactive oxygen species

Reactive species are oxygenated chemical species such as O_2•-derived free radicals (ROS), while reactive nitrogen species (RNS) are considered a subclass of ROS, and are generated from the reaction of oxygen with nitrogen (Jha *et al*., 2017). These reactive oxygen and nitrogen species include not only free radicals but also non-radical derivatives. The latter can be highly reactive, being precursors of free radicals (Jomova *et al*., 2023). ROS represent the most important class of reactive species generated in living organisms, due to the importance of aerobic metabolism (Arnhold, 2020). In the body, the physiological production of reactive species is continuous.

Reactive oxygen species (ROS) include free radicals with at least one free electron on the outer layer (hydroxyl radical OH•, superoxide radical, peroxyl radical ROO•) and non-radical derivatives with very high reactivity, such as hydrogen peroxide H_2O_2, singlet oxygen $1O_2$ and hypochlorous acid HOCl (Chu *et al*., 2010).

I.2.1. Free radical species

I.2.1.1. Superoxide anion $O_2•^-$

The $O_2•^-$ radical spontaneously dismutes at physiological pH to produce hydrogen peroxide and is generated by the monovalent reduction of molecular oxygen. This reaction seems to be catalyzed mainly by membrane NADPH oxidases (Pisoschi and Pop, 2015).

I.2.1.2. Hydroxyl radical HO•

The pathways leading to the formation of this radical: Fenton reaction (the one involving transition metals) and Haber-Weiss reaction (H_2O_2 can also react with the superoxide radical, resulting in the production of HO) (Collin, 2019).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\bullet + HO^-$$

I.2.1.3. Nitric oxide NO

Synthesized in endothelial cells from arginine and O_2 by NO synthase enzymes as illustrated in Figure 1. It can react with most oxygen species to form nitrogen dioxide (NO_2), which in turn can form nitrogen trioxide (N_2O_3), and finally a stable nitrate ion (NO_2^-); in addition, nitric oxide forms peroxynitrite with O_2^- (Maron and Michel, 2012; Umbrello *et al*., 2013).

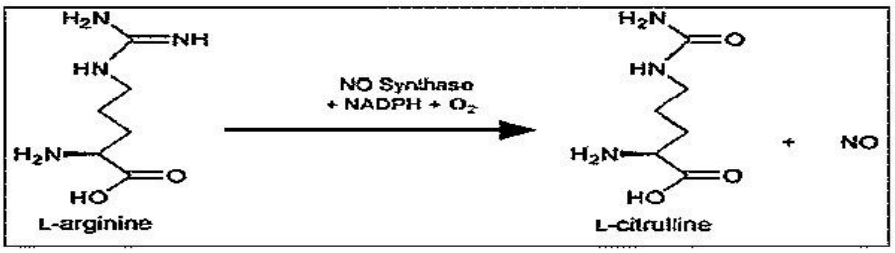

Figure 1: Summary of biological NO- synthesis (Pacher *et al*., 2007).

I.2.1.4. Alkyl radicals R•

Generally derived from the action of hydroxyl radicals on biological substrates by hydrogen atom abstraction or addition to double bonds (Crespi and Fagnoni, 2020).

I.2.1.5. Peroxyl radicals ROO•

Peroxyl radicals are secondary radicals formed by the addition of oxygen to carbon-centered radicals R• (Crespi and Fagnoni, 2020).

I.2.2. Non-radical species

I.2.2.1. Hydrogen peroxide H_2O_2

Hydrogen peroxide formed by the dismutation of $O_2\bullet^-$ by superoxide dismutase or produced by the bivalent reduction of oxygen by a large number of dehydrogenases. In the presence of transition metals (iron and copper), H_2O_2 gives rise to a highly reactive HO• hydroxyl radical via the Fenton reaction (Huang and Groves, 2018).

I.2.2.2. Singlet oxygen ($1O_2$)

Oxygen with antiparallel spins. It produced at high oxygen pressures by absorption of UV rays disappears too quickly to be toxic *in vivo* (Hackbarth *et al*., 2023).

I.2.2.3. Hypochloric acid HOCl

Produced in neutrophils, its toxicity is mediated by halogenation and oxidation reactions (Eryilmaz and Palabiyik, 2013).

I.2.2.4. Peroxynitrite $ONOO^-$

A highly reactive and toxic compound, responsible for the oxidation of many biomolecules (proteins, lipids and nucleic acids) (Martemucci *et al*., 2022).

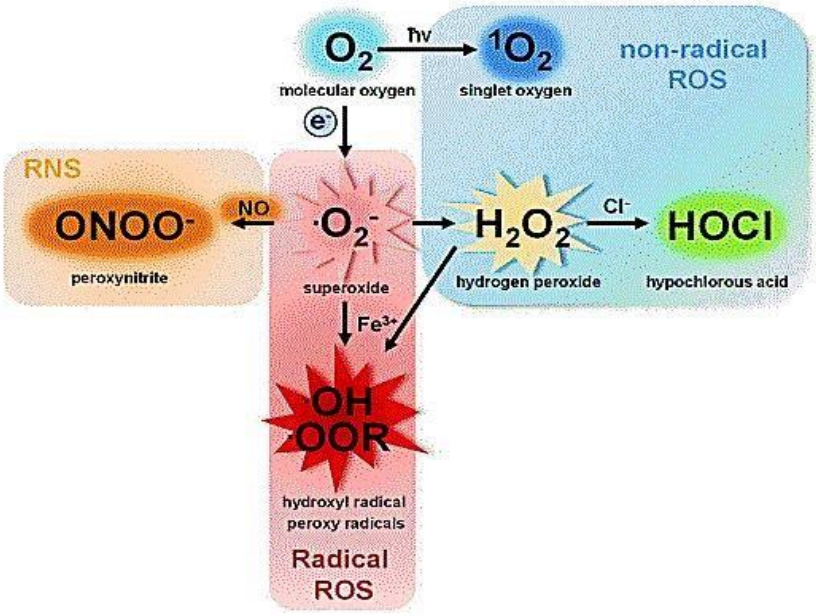

Figure 2: Reactive species: Reactive nitrogen species (RNS) and reactive oxygen species (ROS) including free radicals and non-radical derivatives (Herb and Schramm, 2021).

I.3. Sources of ROS and RNS

There are many different sources of oxidative stress as illustrated in figure 3. Excessive production may occur, resulting in heavy metal toxicity and mitochondrial malfunction. Activation of enzymes, free iron release from chelating proteins, and molecular oxidation, among other things (Ranjbar *et al*., 2014). Oxidative stress can come from a variety of environmental and endogenous factors.

The main source of ROS is the mitochondria, via its respiratory chain. In fact, it produces 90% of cellular ROS (Pisoschi and Pop, 2015). There are many other sources, including the autooxidation of small molecules, cyclooxygenases and lipo-oxygenases, xanthine oxidase, NADPH oxidase, the endoplasmic reticulum and peroxisomes (Verma *et al*., 2023).
Environment and lifestyle can play a direct or indirect role in the generation of these reactive species in biological systems. Ionizing and non-ionizing radiation, toxic gases such as ozone O_3, toxins, drugs, pesticides, tar, certain food preservatives such as sulfur dioxide, smoking, alcohol ingestion and industrial pollutants are all examples (Kardeh *et al*., 2014).

I.3.1. Environmental factors

They are mainly of physical and chemical origin and exist as a result of interactions with the surrounding environment for examples: much of the food we eat is oxidized, containing various types of oxidants such as peroxides, aldehydes, oxidized fatty acids and transition metals (Birben *et al*., 2012). Tobacco smoke directly delivers oxygen radicals to the lungs and also releases them by indirect mechanisms. In total, cigarette smoke contains more than 200 prooxidant compounds (Taylor *et al*., 2016). Toxic metals (chromium, vanadium, copper) and also free iron generate HO• radicals in the presence of H_2O_2 by the Fenton reaction (Valko *et al*., 2005). Ionizing radiation such as X-rays or gamma rays can directly generate HO• by radiolysis, whereas ultraviolet A rays generate $O_2^{\bullet -}$ and $^1O_2$ by photo-activation of molecules present in the skin (Reisz *et al*., 2014). Various pollutants, industrial solvents (Antiseptics and pesticides) and chemical reagents can generate reactive oxygen species (ROS) (Tandon and Singh, 2016). Inhalation of certain particles such as asbestos or silica, which also generate ROS, partly because they exacerbate phagocytosis, and partly because their surface is coated with iron salts (Dostert *et al*., 2008).

I.3.2. Endogenous sources

As ROS are produced continuously throughout the life of every cell in the body intracellular systems are more important. Under normal physiological conditions, the mitochondrion is the major source of cellular $O_2^{\bullet -}$ production, through the partial reduction of NADH dehydrogenase and ubiquinone/ ubisemiquinone /ubiquinol by complex I and III respectively (Tirichen *et al*., 2021). In addition, other electron transport chains (peroxisomes and microsomes) appear to be the major endogenous sources of ROS (mainly H_2O_2) via the β-oxidation of fatty acids in

aerobic cells (Chaudhary *et al*., 2023). During inflammatory processes, activated neutrophils produce $O_2^{\bullet -}$ via the action of membrane-bound NADPH oxidase on molecular oxygen. Neutrophils also produce the NO radical, which is the source of a more reactive molecule, $ONOO^-$, a powerful oxidant that can break down to form HO•. This mechanism, when controlled, is crucial in the fight against infection, as it enables the digestion of phagocytosed material (Belambri *et al*., 2018). Several enzymatic systems produce ROS during biochemical reactions. Cytochrome P450 in the electron transport chain of microsomes can produce ROS when they interrupt the normal redox cycle and divert the flow of electrons to O_2. Cytochrome P450 can directly reduce O_2 to $O_2^{\bullet -}$ causing oxidative stress (Ozcan and Ogun, 2015). During purine catabolism, xanthine oxidoreductase catalyzes the oxidative hydroxylation of hypoxanthine to xanthine and subsequently xanthine to uric acid, producing ROS (particularly $O_2^{\bullet -}$ and H_2O_2) (Kelley *et al*., 2010).

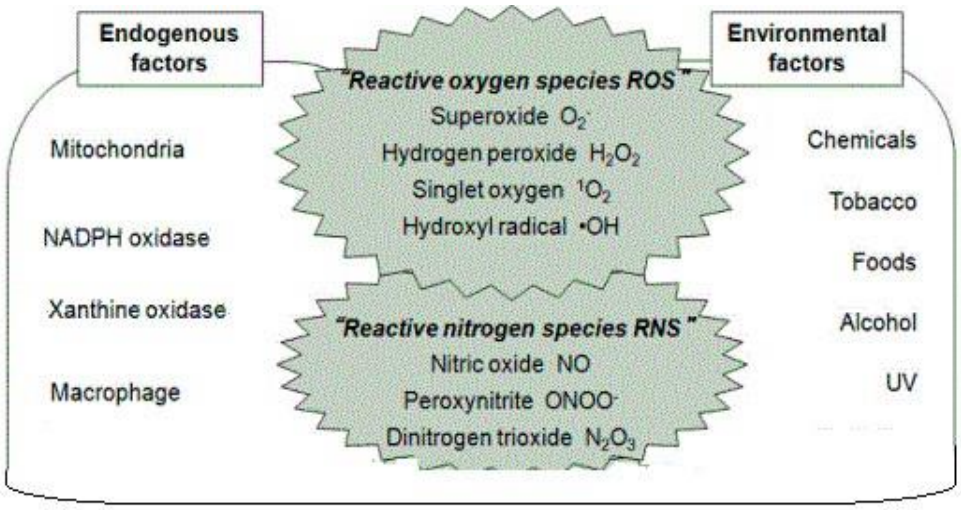

Figure 3: Reactive oxygen species and reactive nitrogen species (ROS and RNS) and their sources from endogenous and environmental factors (Thanan *et al*., 2014).

I.4. Harmful effect of ROS and RNS on biomolecules

Free radicals have important role in human body, it is product normally with low amount by various physiological mechanisms. Excess formation of free radical can initiate series of chemical reaction and cause damage to cellular components such as damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivate specific enzymes by oxidation of co-factors (Lobo *et al*., 2010).

I.4.1. Effects of oxidative stress on lipids

Polyunsaturated fatty acids are the major lipid target of ROS, the more unsaturated the fatty acid, the more likely it is to be peroxidized. Polyunsaturated fatty acids are particularly susceptible to attack by HO• (Catalá, 2013). This reaction is known as lipid peroxidation, and leads to the formation of numerous toxic derivatives, including MDA (malondialdehyde). It also leads to an increase in cell membrane permeability, resulting in irreversible alteration of the cell's functional properties, up to and including complete lysis (Ayala *et al*., 2014).

I.4.2. Effects of oxidative stress on DNA

Nucleic bases are susceptible to direct oxidation by ROS. Hydrogen abstraction from DNA by OH- leads to hydroxylation of purine and pyrimidine bases, and these actions represent the most common alterations of nuclear and mitochondrial DNA attacked by ROS. Radical attack can lead to base oxidation, producing a large number of modified bases. These modifications will subsequently disrupt transcription and translation, resulting in the formation of a truncated and/or non-functional protein. These alterations are often at the root of mutagenesis (Cadet *et al*., 2015). One frequent DNA damage is the oxidation of guanine by the hydroxyl radical to form 8-hydroxy-guanine (8-OHG). The latter is considered a marker of nucleic damage and carcinogenesis. ROS also cause DNA damage, leading to cell death and aging (Dabrowska and Wiczkowski, 2017).

I.4.3. Effects of oxidative stress on proteins

Proteins are also targets for ROS. All amino acids are sensitive to oxidation in particular aromatic amino acids such as tryptophan, tyrosine and histidine, on which OH• is added, modifying the conformation of the protein (Domínguez *et al*., 2021). Proteins with a sulfhydrile (SH) group are the most vulnerable to radical damage (Aldini *et al*., 2018). Protein oxidation processes are classified into two types: those that cleave peptide bonds and change the peptide chain, and those that modify peptides by adding lipid peroxidation products such as 4-HNE (4hydroxynonenal) (Hu *et al*., 2017). Proteins impacted by these alterations typically lose their structural or catalytic properties, making them more vulnerable to protease activity and ultimately destroyed (Xie *et al*., 2023). Alternatively, oxidized proteins can become extremely hydrophobic by the creation of aberrant clusters inside or outside of cells, the externalization

of core hydrophobic zones, or the suppression of ionizable amine groups (Orrenius *et al*., 2007). Carbonyls are the primary byproducts of protein oxidation and are utilized as indicators of oxidative stress (Estévez *et al*., 2022).

I.4.4. Effects of oxidative stress on glucide

Specifically, ROSs tend to attack glycose and cartilage proteoglycans. Straightforward glucose oxidation produces carbonyl compounds, which can then combine with a protein to make glycosylation end products (GEPs) (Lorenzo *et al*., 2013) or by the formation of a covalent bond between an glucide and the free amino groups of a protein to give a glycated protein that can be attacked by ROS to form PFGs (Sun *et al*., 2016). In the presence of transition metals, PFGs promote O_2 release, weakening vascular walls and the retina in diabetic patients (Mengstie *et al*., 2022).

I.5. Antioxidants

An antioxidant is defined as any chemical that slows, stops, or fixes oxidative damage to a target molecule (Carocho and Ferreira, 2013). Antioxidants are chemicals that are naturally created by the body or obtained from nutrition to counteract the damaging effects of radicals during oxidative stress (Chaudhary *et al*., 2023). Antioxidants can be divided into two types based on their origin: enzymatic and non-enzymatic antioxidants.

I.5.1. Enzymatic antioxidants

Enzymatic antioxidants are molecules capable of neutralizing the harmful effects of ROS through the endogenous enzymatic defense system, such as SOD, catalase and the glutathione peroxidases and reductases found in the body (Mehta and Gowder, 2015).

I.5.1.1. Superoxide dismutase (SOD)

As the primary antioxidant enzyme, SOD serves as the first line of defense against ROS. The transformation of superoxide ions into hydrogen and oxygen peroxide is catalyzed by these metalloenzymes (Mishra and Sharma, 2019).

Three isoforms (SOD1, SOD2, and SOD3) of this family exist in mammals; they differ in terms of their cellular location and metal cofactor: Cu/Zn-SOD, which is linked with copper and zinc

ions in the cytosol; Mn-SOD, which is connected with manganese in the mitochondria; and ECSOD, which is associated with extracellular matter (Fukai and Ushio-Fukai, 2011).

I.5.1.2. Catalase (CAT)

The intracellular enzyme catalase is mostly found in peroxisomes. It is mostly present in the liver and red blood cells, where it catalyses the H_2O_2 detoxification process, which is often generated by SOD (Shin *et al*., 2018).

I.5.1.3. Glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) is responsible for reducing organic hydroperoxides (ROOH) to alcohols and hydrogen peroxide to water molecules. Two glutathione (GSH) molecules are needed for this process in order for them to be transformed into glutathione disulphide (GSSG). Glutathione peroxidase is an enzyme located in the cytosol and mitochondria. (Pei *et al*., 2023).

I.5.1.4. Glutathione reductase

An enzyme called glutathione reductase is found in the mitochondria and cytoplasm. It is a homodimeric flavoenzyme that regenerates GSH from GSSG. Each subunit of GR has FAD at its active site. NADPH, a cofactor used by glutathione reductase, first converts FAD to $FADH_2$. Then, on the -S-S-disulfide bridge of the enzyme, FADH2 serves as a source of electrons, reducing GSSG to GSH (Čėnas *et al*., 2021).

I.5.2. Non-enzymatic antioxidants

To combat harmful reactive oxygen species (ROS), our bodies have their own antioxidant defense systems, but others are provided by healthy, balanced diet rich in antioxidants. Among these exogenous antioxidant molecules are vitamins E and C, as well as plant phytoconstituents such as polyphenols, carotenoids and others (Chaudhary *et al*., 2023).

I.5.2.1. Vitamins

a. Ascorbic acid or vitamin C

It is thought to be the strongest antioxidant. It inhibits the oxidation of "light density lipids" (LDL), which are created by activated endothelial cells, myeloperoxidase, and other

mechanisms that generate reactive oxygen species. In addition to directly reacting with ROS like OH•- and O2•-, vitamin C is crucial for the regeneration of oxidised vitamin E during its oxidation to dehydroascorbic acid (Carr *et al*., 2000).

b. Vitamin E

All compounds that share the biological functions of the tocopherol family are collectively referred to as vitamin E. Four isomeric tocopherols α, β, γ, and δ are present in the natural form of vitamin E with varying levels of antioxidant activity. α-Tocopherol is the most active form of the tocopherol class and is synthesized by plants (Jiang, 2014). A pyroxyl lipid radical (LOO•) is captured by vitamin E through membrane binding, therefore halting the lipid peroxidation process cycle. subsequently, it transforms into a less potent radical that another antioxidant molecule can subsequently absorb (Jomova *et al*., 2023).

c. Vitamin A

Vitamin A acts on ROS by forming a vitamin A radical, which may act as an antioxidant by reacting with another radical to form a non-radical, or be regenerated as vitamin A. In excess, vitamin A may act as a pro-oxidant, promoting DNA oxidation (Didier *et al*., 2023).

I.5.2.2. Polyphenols

Phenolic compounds are a family of molecules widely distributed in the plant reign. Phenolic compounds (phenolic acids, flavonoids and tannins) form the most important group of phytochemical compounds in plants. They possess more than two aromatic -OH groups and at least one aromatic benzene ring. They are biologically active molecules (Cartea *et al*., 2010). Recent research on phenolic compounds in general is very advanced, due to their diverse activities as vasoconstrictors, anti-inflammatories, enzyme inhibitors, antimicrobials, antioxidants and anti-free radicals (Jakubczyk *et al*., 2020). Polyphenols are capable of scavenging radical species and chelating transition metals such as iron and copper, which catalyze oxidation. The beneficial effects of polyphenols are of particular interest to phytotherapy (Lakey-Beitia *et al*., 2021).

a. Phenolic acids

Phenolic acids are found in a number of agricultural and medicinal plants. These compounds are derived from two distinct subgroups, hydroxycinnamic acids, the most abundant of which are caffeic acid, ferulic acid, chlorogenic acid and hydroxybenzoic acids, but the most widespread are salicylic acid and gallic acid. They are considered phytochemicals with prebiotic, antioxidant and anti-inflammatory effects (Salau *et al*., 2020). Some phenolic acids have anticancer effects, such as ferulic acid and caffeic acid, which prevent the formation of lung cancer in mice, and gallic acid, which inhibits the formation of oesophageal cancer in rats (Gomes *et al*., 2003).

b. Flavonoïdes

Flavonoids represent a very wide range of natural compounds belonging to the polyphenol family. They are virtually universal plant pigments, and are partly responsible for the coloring of flowers, fruit and sometimes leaves (Liu *et al*., 2022). Flavonoids all have the same basic chemical structure, with a carbon skeleton of 15 carbon atoms consisting of two aromatic rings (A) and (B) linked by a C3 chain, and the 3-carbon bridge between the two phenyls generally forming a third pyran ring (Castellano *et al*., 2013). Flavonoids play a number of roles, such as protecting tissues against the harmful effects of UV radiation, and also helping to defend plants against pathogenic micro-organisms.

c. Tannins

Tannins are hydrosoluble phenolic compounds with a molecular weight of between 500 and 3000 Da. They have high antioxidant capacities due to their phenol nuclei, which enable them to trap free radicals and inactivate pro-oxidant ions (Dehghanian *et al*., 2022). Studies have shown that many tannins have anti-inflammatory, antifungal, anti-tumor, antiviral and antidiarrheal properties (Fraga-Corral *et al*., 2021).

- **Hydrolysable tannins:** Compounds known as hydrolyzable tannins are those that have a core of glucose or another polyol esterified with either hexahydroxydiphenic acid, also known as ellagitannins, or gallic acid, also known as gallotannins (Macáková *et al*., 2014).

- **Condensed tannins:** Condensed tannins consist of flavan-3-ol nuclei forming oligomers or polymers. They are also known as proanthocyanidins because, in hot ethanol solutions, they break down into anthocyanidins. (Qi *et al*., 2023).

Forming insoluble complexes with proteins, polysaccharides, nucleic acids, and alkaloids is one of tannin's distinctive characteristics. Within this broad category, tannins display several distinct bioactivities that are frequently connected to their antioxidant capacity (Haslam, 2007).

II. Inflammation

Inflammation is the response of vascularized living tissue to an internal or external stimuli, such as an infection or damage (Chen *et al*., 2018). When tissue homeostasis is compromised, the innate and adaptive immune systems' defense against pathogenic intruders causes inflammation, which is a ubiquitous phenomenon. (Ashley *et al*., 2012). Since inflammation drives the immune system to eradicate the infection and repair tissue damage, it often has a favorable effect (Megha *et al*., 2021). When a pathogen is aggressive, persistent, or occurs in a particular place, or when the inflammatory process is not properly controlled, or when the cells involved exhibit abnormalities in terms of quantity or quality, inflammation can occasionally be harmful. (Jain *et al*., 2015). Inflammation is accompanied by the synthesis of several inflammatory mediators such as cytokines, leukotrienes, and prostaglandins (Noack and Kolopp-Sarda, 2018). In addition, inflammatory cells can produce reactive oxygen species (ROS) and nitrogen, which can trigger toxic oxidation reactions, leading to tissue damage (Majdalawieh and Fayyad, 2015). The inflammatory responses may be localized or systemic, specific or non-specific to the antigen (Wang *et al*., 2015), it can be acute or chronic; the first is an immediate, short-term response that generally passes completely on its own or with treatment. However, it can develop into a chronic inflammation, which can be linked to a variety of diseases (Oronsky *et al*., 2022).

II.1. Acute inflammation

Acute inflammation is a short-term reaction to unpleasant stimuli. It is distinguished by an increase in the movement of plasma and blood leukocytes into injured tissues. A cascade of metabolic processes initiates the inflammatory response (Banerjee, 2014). The appearance of signs is often abrupt and followed by severe vasculo-exudative symptoms. It is recognized by four major symptoms: redness, heat, swelling, and pain (Miyasaka and Takatsu, 2016). Acute

inflammatory reactions have three major phases. the vascular phase, the cellular phase, and the resolution phase (Arulselvan *et al*., 2016).

II.1.1. Vascular phase

Acute inflammation is a typical occurrence in vascularized tissues. Microvascular permeability is an early indicator of a change in vascular flow and vessel caliber (Santos and Reis, 2010). The main signs of inflammation are illustrated in Figure 4. Pain occurs due to activation of nociceptors which become sensitized with a lower threshold for activation. Redness is due to local vasodilation with increased blood flow to the affected area. Warmth, due to a combination of increased local blood flow and increased cellular metabolic activity. Swelling occurs due to increased capillary permeability, with plasma extravasation and localized edema (Gebhart and Schmidt, 2013).

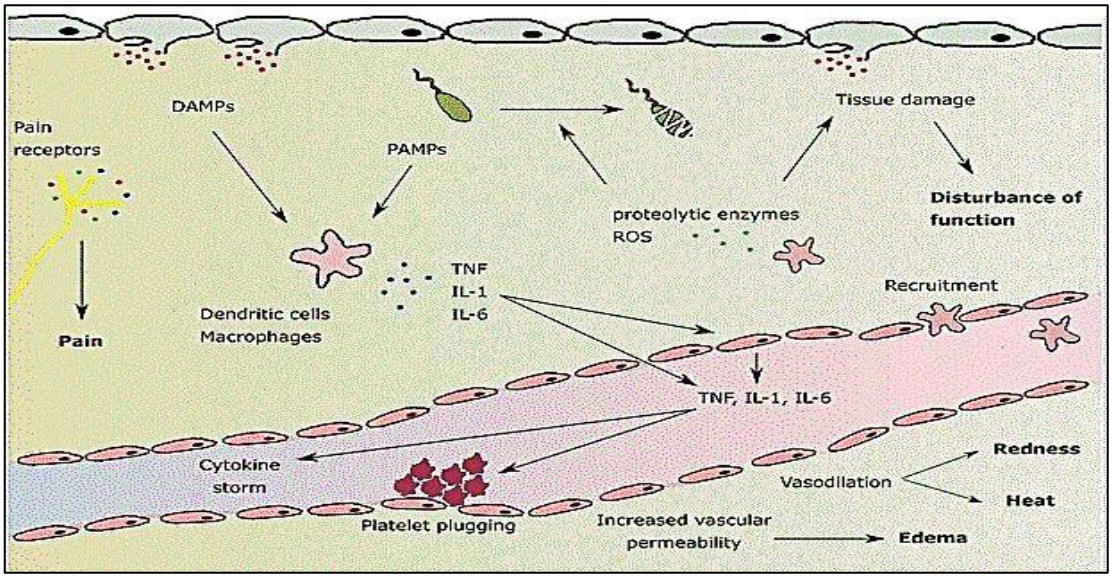

Figure 4: Tissue and vascular changes during a local inflammatory event; the cardinal signs of inflammation (Varela *et al*., 2018).

The vascular phase comprises three basic changes: active congestion, inflammatory edema and leukocyte diapedesis (Lee and Surh, 2012a).

Active congestion is caused by a vasodilation that occurs after a brief period of vasoconstriction, lasting only a few seconds and triggered by the sympathetic nervous system (Zeghal and Sahnoun, 2013). A few seconds of vasoconstriction will interrupt platelet mobility in the circulation and activate them, causing the release of mediators such as serotonin and histamine (Chaudhary *et al*., 2022). The release of vasoactive factors causes significant vasodilation,

resulting in an increase in blood flow. This early hemodynamic change in inflammation, combined with the increased permeability of the microvasculature, causes a slowing of circulation and stasis, known as active congestion (Vohr, 2016). The increase in microcirculatory flow somewhat explains the appearance of heat and redness (Jacobi *et al*., 2006).

Inflammatory edema develops when an excessive quantity of exsudat, a liquid made up of water and plasmic proteins, accumulates in the interstitial tissue or serous cavities. It is induced by a rise in hydrostatic pressure as a result of vasodilation, as well as an increase in the permeability of the walls of tiny capillaries due to the action of chemical mediators such as histamine. Plasmatic exudation promotes tissue distension and increased pressure on local nerve terminals. This clarifies the symptoms of tumescence and discomfort (Dorward *et al*., 2012).

Diapedesis, also known as transendothelial migration, as illustrated in Figure 5, is the process by which leukocytes migrate through endothelial cells. This transendothelial migration takes place in four sequential steps: capture, rolling, cell adhesion and diapedesis (Muller, 2011).

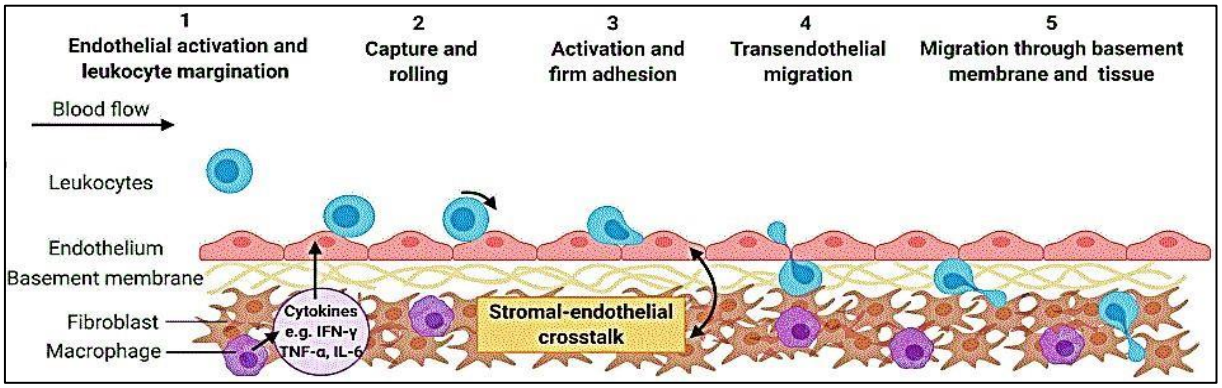

Figure 5: Leucocytes migrate from blood vessels to the inflammatory site (Manning *et al*., 2021).

Polynuclear cells are the first to migrate, followed by monocytes and lymphocytes. Leukocytes migrate out of the microcirculation and accumulate in the lesion site, triggering the cellular phase (Steinmann *et al*., 2013).

II.1.2. Cellular phase

Endothelial cells and circulating leucocytes are the main players in this phase, which comes after the vascular reaction. Extravascular interstitial leucocyte inflow is characteristic; within the first 6 to 24 hours, polynucleated cells move to the inflammatory site, and over the next 24

to 48 hours, monocytes and lymphocytes follow. Then, utilizing a gradient of chemical concentrations known as chemoattractant as guidance, they head straight towards the source of the inflammation (Wagner and Roth, 2000). The pathogen is eliminated at the inflammatory site by phagocytosis and the release of hydrolytic enzymes, such as collagenase, elastase, and protease. On the other hand, macrophages facilitate the removal of cellular and tissue waste and the cleansing of the inflammatory environment (McCarty *et al*., 2012).

II.1.3. Resolution phase

The resolution or regeneration phase is characterized by the elimination or detachment of the causative agent and cellular and tissue debris from the inflammatory environment, either internally (phagocytosis and pinocytosis), or externally through natural orifices or abscess creation. The severity of healing depends on the amount of chemical substances lost during the acute phase (Weill and Batteux, 2003). The conjunctival tissue is rebuilt by collagen synthesis, cellular multiplication (fibroblasts), and vascular neogenesis from persisting or peripherally located capillaries. The healing of a wound entails cell proliferation, active cell migration, and the creation of extracellular matrix (Lambole and Upendra, 2012). The duration of wound healing and repair is determined by the size and depth of the lesion. Several cells interact in this complex process, including endothelial cells, fibroplasts and keratinocytes (Eming *et al*., 2014). Vasoconstriction occurs during the inflammatory phase, which encourages the release of inflammatory mediators. the proliferative phase, which is marked by angiogenesis and fibroblasts primarily producing granulation tissue. Tensile strength is increased during the remodelling phase, which is marked by the reformulation and enhancement of collagen fibre components (Rodrigues *et al*., 2019).

The overproduction of connective tissue during cicatrix development leads to hypertrophic cicatrices and cysts. Additionally, a lack of deterrence might lead to the persistence of inflammatory phenomena, resulting in chronic inflammation (Murti and Kumar, 2012).

II.2. Chronic inflammation

Chronic inflammation is a failure of acute inflammation and is also caused by some autoimmune diseases. It is characterized by a protracted duration (Lawrence and Gilroy, 2007). As demonstrated in Figure 6, chronic inflammation is an inflammation that has no tendency to heal spontaneously and evolves by persisting or worsening for several months or years (Noack

and Kolopp-Sarda, 2018). The cellular and vascular phases of inflammation coexist throughout its course, in contrast to acute inflammation, when they occur independently (Howcroft *et al*., 2013). During chronic inflammation, a variety of intracellular signaling pathways are activated, including cell surface receptors, tyrosine kinases, and transcription factors, resulting in the overexpression of proinflammatory genes (de Lavor *et al*., 2018). It is associated with the invasion of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis (Kumar *et al*., 2013).

Unlike in acute inflammation, vascular and cellular phases do not follow one another, but coexist throughout the evolution of this inflammation. Chronic inflammation often leads to loss of tissue or organ function. Phenomena of tissue destruction and attempted repair are also present (Lee and Surh, 2012).

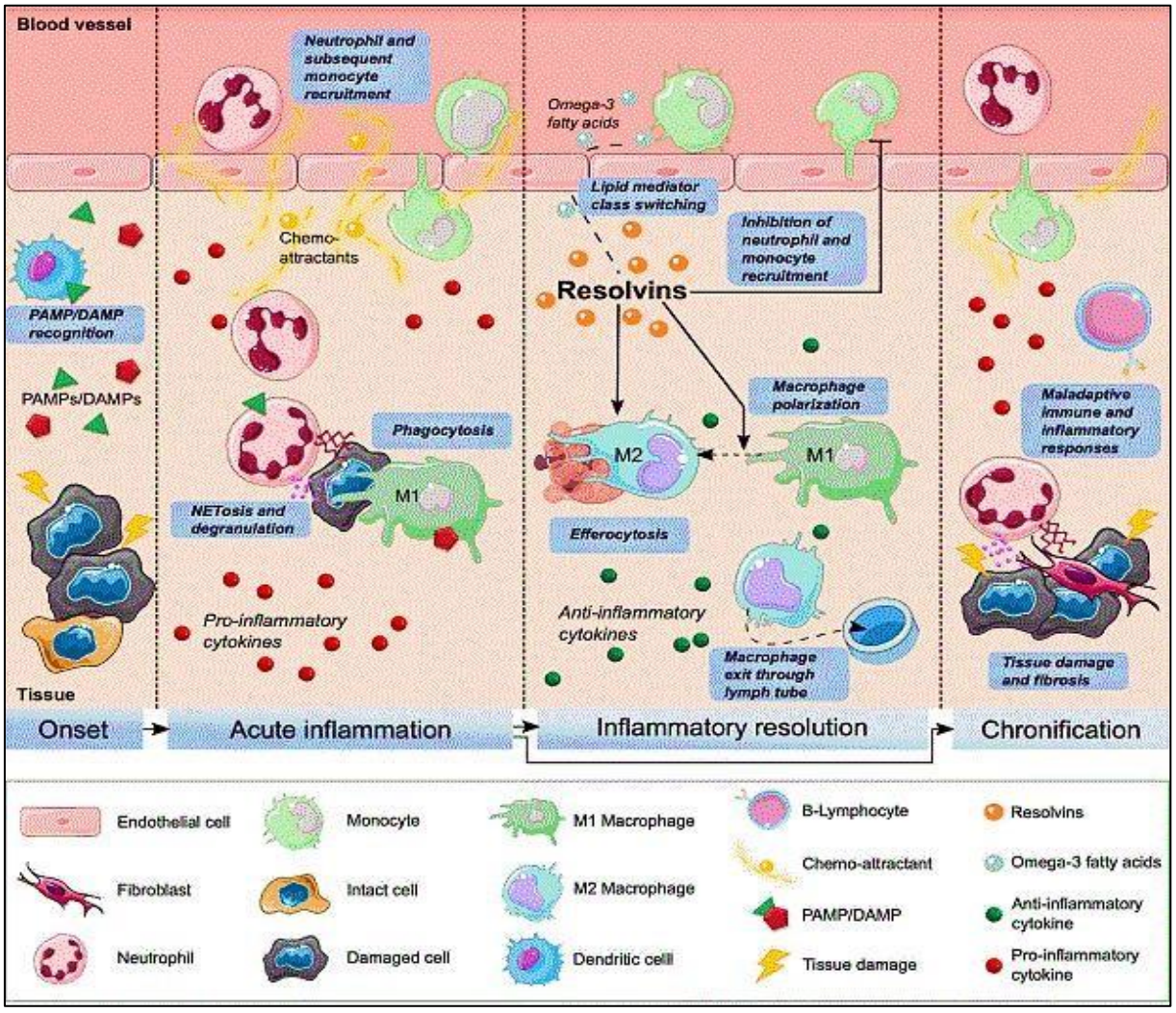

Figure 6: The different possible outcomes of acute inflammation (Centanni *et al*., 2023).

II.3. Treatment of inflammation

II.3.1. Nonsteroidal anti-inflammatory drugs

Worldwide, one of the most popular treatment classes is that of nonsteroidal anti-inflammatory medicines (NSAIDs). Along with their anti-inflammatory qualities, these drugs offer analgesic and antipyretic effects. Cyclooxygenase inhibition, which results in prostaglandin production, is primarily responsible for the effects of NSAIDs. Depending on whether salicylated AINS is used, this inhibition may be irreversible or absent from other AINS (Sohail *et al*., 2023). Unfortunately, there are often undesirable side effects, such gastrointestinal problems, associated with using synthetic anti-inflammatory medicines (Drina, 2017a).

The impossibility of dissociating the therapeutic effects of NSAIDs from their adverse effects is explained by their mechanism of action. NSAIDs competitively inhibit the biotransformation of arachidonic acid into prostaglandin H_2 (PGH_2) by cyclooxygenase. This inhibition makes it impossible for PGH_2 to be converted into prostanoids by the specific isomerases
Cyclooxygenase exists in two forms: cyclooxygenase 2 (COX_2) and cyclooxygenase 1 (COX_1) (Gunaydin and Bilge, 2018). COX_2 is inducible, and its synthesis is stimulated by TNFα and interleukin 1. It is involved in inflammation and some processes associated with cell proliferation. COX_1, on the other hand, is a constitutive enzyme present in most tissues, and is involved in protection of the gastric mucosa, platelet aggregation, vascular homeostasis and maintenance of hydrosodium balance (Gandhi *et al*., 2017). Inhibition of COX_1, due to a lack of specificity of action when NSAIDs are used, is the cause of the adverse effects of these drugs. This calls for the development of COX_2-selective NSAIDs (Tréchot and Jouzeau, 2014).

II.3.2. Steroidal anti-inflammatory drugs

Steroidal anti-inflammatories (SIAs) or glucocorticoids are synthetic molecules derived from natural hormones (cortisone and cortisol) or hemi-synthesized from animal or plant extracts. They have anti-inflammatory, analgesic and immunosuppressive properties (Coutinho and Chapman, 2011). Glucocorticoids act at multiple levels on all phases of inflammation, whether acute or chronic. In addition, they strongly reduce the migration of polymorphonuclear cells and monocytes/macrophages to the inflammatory site, as well as the production of inflammatory mediators such as histamine serotonin, bradykinins, cytokines and superoxide ions (Centanni *et al*., 2023). Glucocorticoids are also responsible for reducing capillary

permeability and increasing osteoclast activity, leading to bone fragility (De Lucena and Rangel, 2018). Glucocorticoids are the most effective treatment for chronic inflammatory diseases such as rheumatoid arthritis and autoimmune diseases (Kiranmayi *et al*., 2018). However, they have the disadvantage of reducing the body's defenses and causing disorders that can be acute (arterial hypertension, deregulation of natural glucocorticoid synthesis.) or chronic, such as osteoporosis, cataracts and weight gain (Strehl *et al*., 2011).

II.3.3. Anti-inflammatory drugs of plant origin

Many diseases have been described as being associated with inflammatory processes. Currently available anti-inflammatory therapy is often ineffective or causes intolerable side-effects. New anti-inflammatory substances are therefore still urgently needed. Plants were the first source of remedies in human history (Fürst and Zündorf, 2014). Medicinal plants are widely used by humans for their many biological benefits. These actions are attributed to their abundance of bioactive secondary metabolites. Since its chemical characteriszation in the 19th century, plantbased bioactive compounds have supported the creation of pharmaceuticals (Riaz *et al*., 2023). Numerous investigations *in vitro* and *in vivo* have shown that phenolic compounds have antiinflammatory properties. Secondary metabolites, unlike pharmaceutical substances, operate on several targets rather than just one receptor or signaling pathway. These active compounds can limit arachidonic acid metabolism by obstructing the cyclooxygenase and lipoxygenase pathways (Calixto *et al*., 2000). Bio-compounds can exert anti-inflammatory effects, notably through radical scavenging, regulation of cellular activities in inflammatory cells and modulation of the activities of enzymes involved in arachidonic acid metabolism, notably phospholipase A2, cyclooxygenase and arginine metabolism (Hussain *et al*., 2016).

III. Pain and analgesics

III.1. Pain

III.1.1. Definition

The definition of pain given by the International Association for the Study of Pain (IASP) is "an unpleasant sensory and emotional experience, associated with or described in terms of actual or potential tissue damage"(Wranker *et al*., 2014). The term pain refers to a spectrum of sensations with very different characteristics, ranging in intensity from unpleasant to

unbearable. Since suffering causes mental discomfort, man has always sought a way to eliminate or reduce pain (Jang *et al*., 2020).

The prostaglandins formed by an enzyme called cyclooxygenase 2 (COX_2), secreted by damaged cells, induce the sensation of pain by connecting to G protein receptors and increasing the amount of cyclic adenosine monophosphate (cAMP) in the cells.

III.1.2. Pain classification

Depending on the duration of the evolution, pain can be divided into two classes

III.1.2.1. Acute pain

Acute pain often has an obvious cause, for example following trauma, surgery, or the onset of a well-recognized disease process (e.g. myocardial ischaemia, pancreatitis). It may be of nociceptive or neuropathic origin. Acute pain lasts less than 3 months and plays some useful positive role such as to provide a warming of tissue damage and inducing immobilization to allow appropriate healing (Gupta *et al*., 2010).

III.1.2.2. Chronic pain

Chronic pain is defined as pain lasting longer than the expected healing period, which is often practically defined as pain lasting longer than three to six months (definitions vary across clinical and research contexts). As opposed to acute pain, chronic pain is no longer adaptive. It has been considered to stem from a 'faulty alarm system' – activating the body's physiological systems inappropriately, when there is no acute threat (Timmers *et al*., 2019).

III.2. Analgesic activity

III.2.1. Definition

Analgesic activity is defined as a loss of sensitivity to pain, and analgesic as a drug or substance that prevents or reduces the sensation of pain (El Geziry *et al*., 2018). Nowadays, non-steroidal anti-inflammatory drugs are used to control pain. Their analgesic action is produced rapidly, but their side-effects are the main drawback of their use. These drugs cause digestive system

dysfunction, pruritus, blurred vision, dizziness, skin rashes and liver damage (Thombre *et al*., 2019).

III.2.2. Sources of analgesics

Analgesics are used to relieve pain without loss of consciousness, by acting on the central and/or peripheral nervous system. They are divided into two classes: synthetic analgesics (nonmorphinics and morphinics) and natural analgesics (Deshmukh *et al*., 2014).

III.2.2.1. Non-morphine analgesics

Non-morphine analgesics include all non-opioid pain-relieving drugs. They are used in perioperative care, as well as by chronic pain caregivers. They act primarily by inhibiting cyclooxygenase, an enzyme responsible for a cascade of reactions that, among other things, cause pain. Acetylsalicylic acid prevents sensitization of nociceptive nerve endings to algogenic substances (Gupta *et al*., 2010).

III.2.2.2. Morphine analgesics (opioids)

Opioids are natural, semi-synthetic, or synthetic chemicals psychotropic opiate whose effects are similar to those of morphine, without being chemically related (Al-Onizi *et al*., 2023).

Morphine analgesics are known for their analgesic properties, targeting receptors expressed throughout the afferent pain pathway, including the peripheral nerve endings of primary afferent neurons. Opioid analgesics are of low, intermediate or high efficacy, and are used for the relief of mild, moderate or severe chronic pain. Among the low-efficacy opioids, codeine is used to relieve mild to moderate pain. Morphine is a high-efficacy opioid analgesic used to relieve severe pain (Mathews *et al*., 2015).

III.2.2.3. Natural analgesics

The use of natural products, mainly medicinal plants, is one of the oldest therapies used by mankind (Hani Mutlak, 2015). In recent years, people have been eager to use herbal medicines because of their lower complications and fewer side effects than synthetic drugs. With the growing demand for medicinal plants and related compounds, phytopharmaceutical studies and the use of these remedies for pain management have multiplied worldwide (Kala *et al*., 2006).

it is therefore necessary to search for bioactive compounds from natural products, in particular from medicinal plants, for use as alternative analgesics with little or no side effects (Ezeja *et al*., 2011). Among analgesic alkaloids, the well-known Morphine is isolated from *Papaver somniferum* opium poppy and mitragynine from *Mitragyna speciosa* (Vadhel *et al*., 2023). Quinoline alkaloids, ribalinine and methylisoplatydesmine isolated from the aerial parts of *Skimmia laureola* (Rutaceae) have been shown to be Acetylcholinesterase inibitors (Gondwal *et al*., 2015). In addition, *Esenbeckia leiocarpa* (Rutaceae) is a major source of leptomerin and kokusaginin (Cardoso-Lopes *et al*., 2010).

IV. Stomach

IV.1. Anatomy of the stomach

The stomach is an expandable, J-shaped organ, located in the left subphrenic lodge, and varies in size depending on replenishment, averaging 25 cm in length and 12 cm in width. Extending from the esophagus to the duodenum in the upper left abdomen, between the liver (right), spleen (left), pancreas (back), diaphragm (top) and transverse colon (bottom), as illustrated figure 7 the stomach is subdivided into four regions: The cardia, which surrounds the outlet of the esophagus into the stomach; The gastric fundus, which is the region above the orifice of the cardia; The body of the stomach, the largest region, is the middle portion that extends downward into the funnel-shaped pyloric portion; The pyloric portion, which is the distal portion of the stomach and is divided into the pyloric antrum and the pyloric canal (Karnul and Murthy, 2022).

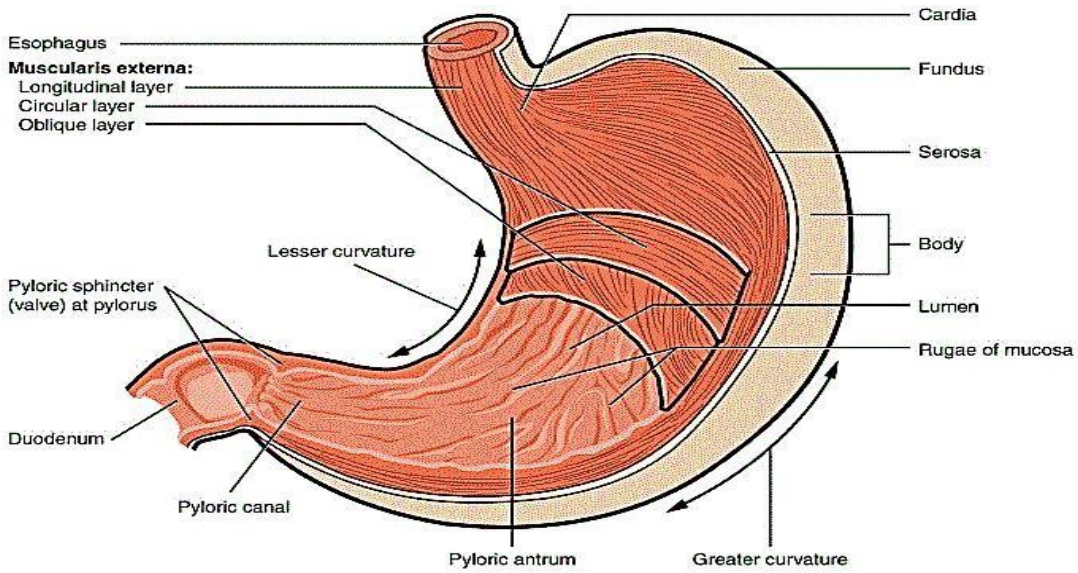

Figure 7: Anatomy of the stomach (Massey *et al*., 2019).

IV.2. Stomach histology

The stomach has three types of muscle: longitudinal, oblique and circular, which provide the contractions needed to knead food and send it to the small intestine. The gastric wall consists of a monostratified glandular epithelium with numerous invaginations that gives rise to the gastric glands. Histologically, it comprises 4 superimposed layers, from the inside out: The mucosa: made up of a simple cylindrical epithelium (formed by the mucoid, principal and parietal cells), the chorion (connective tissue, blood and lymphatic vessels) and the muscularus mucosae. Submucosa: a layer of loose connective tissue containing blood vessels, neurofibers and numerous free cells (lymphocytes, plasma cells, etc.). Muscular: composed of muscle fibers arranged in three tunics: inner oblique layer, middle circular layer and longitudinal layer. externe. The serosa is the outermost protective layer, allowing the organ to glide freely (Wilson and Stevenson, 2019).

IV.3. Physiology of the stomach

The physiological importance of the stomach is linked to a triple function. A motor function: firstly, the stomach is the first reservoir for food, in which it is stored and mixed by mechanical stirring with gastric juice, ensuring its transformation into a semi-liquid "chyme" until it is evacuated into the small intestine (Levin, 2023). In addition, a secretory function dominated by chlorhydro-peptic secretion ensures food digestion and even the destruction of most ingested bacteria. Finally, the mucus secreted by the stomach forms a protective layer against gastric acidity (Martinsen *et al*., 2019).

IV.6.1. Gastric secretions

The main role of the stomach is to transform food into a semi-liquid chyme state, making it acceptable to the intestine. The agent of this transformation is gastric juice, which is secreted by the gastric glands. In addition, the stomach secretes a mucus that forms a protective layer for the gastric mucosa against gastric acidity, and lubricates the ingesta, facilitating its downstream passage. The mucosa is different in the fundus and body of the stomach, and in the pyloric antrum (Iijima *et al*., 2009).

- **Fundic mucosa:** Comprising large, shallow crypts, these glands are composed of three cell types (Figure 8): The principal cells produce pepsinogen, the inactive form of the proteolytic enzyme pepsin. The principal cells are found mainly in the basal regions of the gastric glands. Parietal cells (border cells), scattered throughout the main cells, secrete hydrochloric acid and intrinsic factor which enables absorption of vitamin B12 in the small intestine. The few endocrine cells, the D cells, secrete somatostatin (Tortora. and Derrickson, 2010).
- **Pyloric mucosa:** It has deep, narrow crypts and 2 cell types: Collar mucus cells, found in the upper, or "collar", part of the glands, produce one type of mucus. The precise function of this mucus is not yet known. Endocrine cells, G cells (secrete gastrin), D cells (somatostatin) (Wilson and Stevenson, 2019).

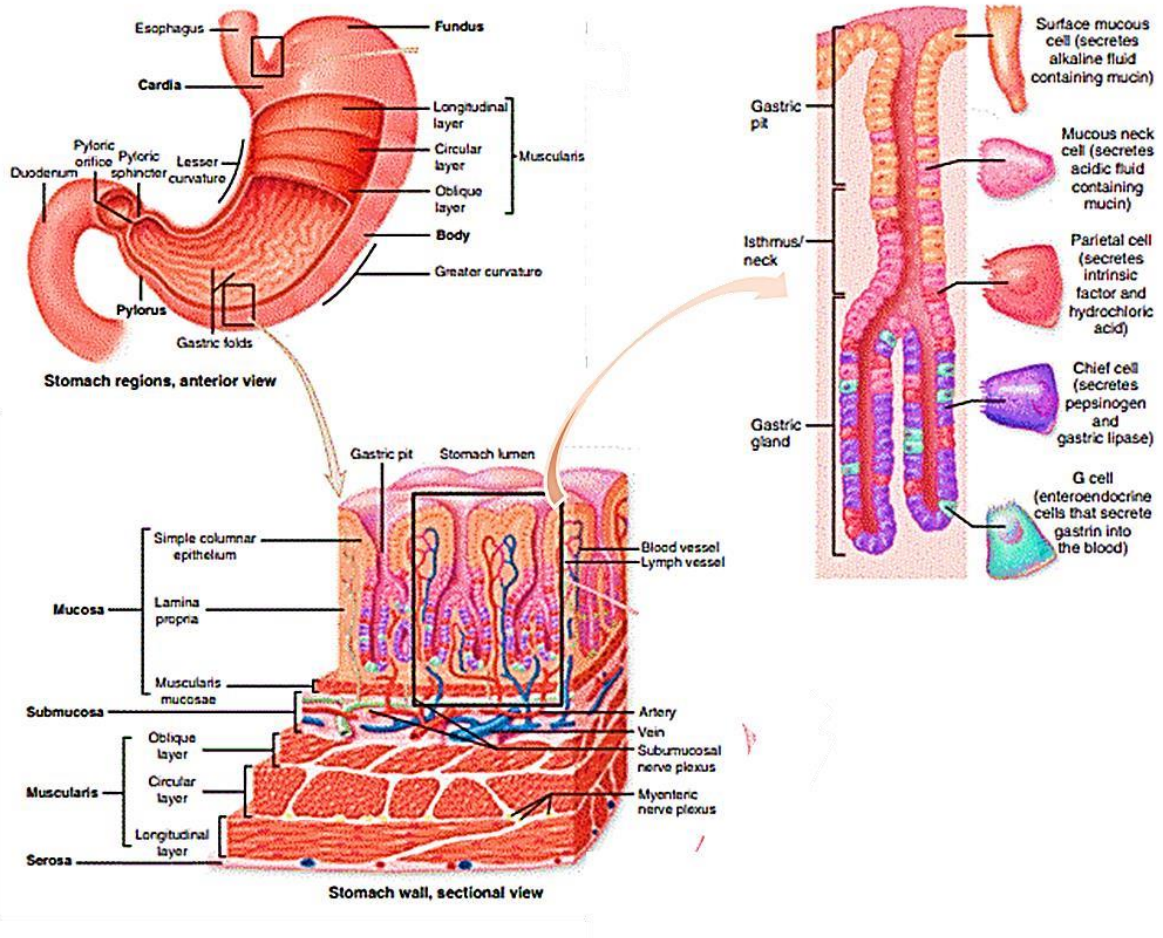

Figure 8: Cross section of the gastric mucosa, with its gastric glands and the types of cells that make up it (Wilson and Stevenson, 2019).

IV.6.2. Gastric juice

When food reaches the stomach, the stomach wall expands and the pH of the gastric contents increases. These changes trigger nerve impulses that stimulate the flow of gastric juice, a colorless, clear, acidic liquid (pH between 1.5 and 2.5) containing: mineral substances including hydrochloric acid and bicarbonates, and organic substances including mucus, pepsinogenes I and II and gastric lipase (Massey *et al*., 2019).

IV.4. Regulation of gastric acid secretion

Food intake stimulates acid secretion in an abrupt and sustained plateau fashion for two hours, followed by a gradual return to basal levels; this process is also governed by nervous and hormonal mechanisms. HCl secretion is stimulated by three chemical substances, all of which act via second messenger systems (cAMP and Ca^{2+}) (Johnson, 2019).

IV.4.1. Gastric acid secretion stimulants

Gastric acid secretion is regulated by three primary components that activate the parietal cell: histamine, gastrin, and acetylcholine (ACh). Optimum acid secretion requires the synergistic action of all three mediators (Bitziou and Patel, 2012).

Histaminocytes or enterocromaffin-like cells (ECL) located in the gastric mucosa, play a crucial role in the regulation of gastric acid secretion by releasing histamine, which acts paracrine by binding to H_2 receptors on parietal cells (adenylcyclase-coupled receptors) to increase intracellular cAMP (Waldum *et al*., 2019). G cells produce Gastrin, which acts endocrinally on the parietal cells themselves and on histaminocytes, stimulating histamine release. Gastrin receptors are coupled to a G protein which activates phospholipase C, leading to an increase in cytosolic Ca^{2+} (Craven, 2016). Acetylcholine released by stimulation of the vagus nerve, it acts directly on parietal cells (M_3 receptors) and indirectly by stimulating histaminocytes and gastrin cells (Soll and Walsh, 1979; Takeuchi *et al*., 2016).

IV.4.2. Gastric acid secretion inhibitors

The stomach and duodenum act in tandem: when chyme enters the duodenum, receptors in the duodenal wall react, triggering the enterogastric reflex which inhibits gastric secretion (Kelly, 2004). These include: Somatostatin produced by D cells, acts paracrine, inhibits gastric

secretion of all substances (Rindi *et al*., 2018). Secretin acts endocrine, inhibiting gastric secretion and motility during the gastric phase of secretion (Schubert, 2008). Prostaglandins have an action paracrine (receptors coupled to an adenylate cyclase-inhibiting G protein) (Post *et al*., 1998). Gastric inhibitory peptide (GIP): produced in the duodenal mucosa, inhibits HCl production (minor effect) (Meier, 2006). Vasoactive intestinal peptide (VIP): produced in the duodenal mucosa, inhibits HCl production in the stomach (Hällgren, 1997).

IV.5. Protective mechanism of the gastric mucosa

In the physiological state, the concentration of hydrochloric acid in the stomach is a million times higher than in plasma, so how does the stomach manage to resist it without itself falling victim to it (Werme *et al*., 2015). Various mechanisms known as the "gastric mucosal barrier" are responsible for this resistance (Tarnawski *et al*., 2012). This barrier comprises different lines of defenses.

IV.5.1. Mucus layer

The Mucus layer is a continuous film in the form of a visco-elastic gel covering the entire stomach wall and acts as the first line of defense for the gastric mucosa, providing chemical and physical protection against gastric acidity by neutralizing the H+ ions in contact, thanks to its high HCO_3^- content. Mucus can also protect the stomach against autodigestion by gastric juice enzymes, notably pepsin (Ichikawa and Ishihar, 2011).

IV.5.2. Prostaglandins

Prostaglandins secreted by the gastric mucosa play a potential role in stimulating bicarbonate secretion, promote blood flow, can inhibit gastric acid secretion and provide dramatic protection against a wide variety of agents which cause acute mucosal damage (Takeuchi *et al*., 2010).

IV.5.3. Blood flow

Blood flow protects the mucosa by supplying various nutrients, notably oxygen and bicarbonates for gastric cell function, and is also necessary for the rapid evacuation of residual H^+ ions through increased blood flow (Abdel-Salam *et al*., 2001).

IV.5.4. Epithelial barrier

The defensive properties of the epithelial barrier are ensured on the one hand by the presence of tight junctions linking epithelial cells (ECs), which strongly prevent the entry of H+ ions, and on the other hand ECs can regenerate rapidly by two mechanisms (Förster, 2008).

Continuous regeneration involves the proliferation and differentiation of gastric mucosal stem cells (Hoffmann, 2008). This restitution involves the migration of adjacent cells to the site of injury for repair and takes place within minutes of the appearance of an injury (Terano *et al*., 2001).

IV.6. Gastric ulcer

Gastric ulcers, are defined as disturbances in the integrity of the gastric mucosa. It results from an imbalance between protective and aggressive factors in the stomach (Escobedo-Hinojosa *et al*., 2018). Anatomopathologically, it is characterized by a loss of substance from the gastric mucosa, which may extend to the muscularis propria, and generally accompanied by abdominal pain resulting from contact between the acid secreted by the stomach and the wounds. A distinction is made according to the depth of the lesion: an abrasion, an erosion or a true ulcer (Lanas and Chan, 2017).

Nowadays, gastric ulcers are considered to be one of the most frequent conditions of the gastrointestinal tract caused by various factors, such as smoking, stress, inappropriate diet, and deficiencies in gastroprotective mechanisms are closely linked to the development of gastric mucosal ulcerations which can also be due to drugs, hence the interest in finding a natural therapeutic alternative derived from plants (Zhang *et al*., 2020).

IV.6.1. Pathophysiology and etiology of ulcers

The aggressive chlorhydro-peptic action is reinforced by the interplay of other pathogenic factors, which can be classified as endogenous and exogenous (Benia and Amroune, 2006).

IV.6.1.1. Endogenous factors

They are mainly represented by genetic factors, acid hypersecretion and gastric motricity disorders.

- **Genetic factors**: Ulcerative disease is a sex-linked recessive inherited disorder, affecting men more often. In addition, the prevalence of this condition is higher in subjects with blood group (O) (Bouarioua *et al*., 2007).

- **Hypersecretion of acid**: Hypersecretion of acid may be the result of an increase in parietal cell mass or an increase in parietal cell sensitivity to various stimuli (gastrin), which is a pathogenic factor in subjects suffering from this problem (Roberts-Thomson, 2021)

- **Gastric motor disorders**: In stomach ulcers, evacuation is slowed down, leading to gastric stasis. This delay can accentuate gastrin release and acid secretion, reinforcing the aggression. The same applies to duodeno-gastric reflux. Bile salts and lysolecithins alter the gastric mucosa and cause gastritis due to their high concentrations, breaking down the gastric mucosal barrier. As a result, the flow of H^+ ions and the reflux of Na+ ions increases, the potential difference falls and blood flow to the mucosa is altered (Lake *et al*., 2021).

IV.6.1.2. Exogenous factors

Several exogenous factors can be implicated in ulcer disease (Figure 9), including:

- **Helicobacter pylori infection**: Helicobacter pylori infection is most often acquired in childhood and depends on hygienic conditions. This gram-negative bacillus is able to resist gastric acidity thanks to its high urease activity. Bacterial urease hydrolyzes gastric urea and produces ammonia, which buffers HCl and creates a microenvironment favorable to bacterial survival. The bacteria penetrate the mucus layer and colonize the surface of superficial gastric cells, mainly located in the antrum. This contact induces the release of chemokines, notably interleukin 8 (IL8), which attracts and activates polynuclears and macrophages (Roberts-
Thomson, 2021). Pro-inflammatory cytokines such as TNFα and IFNγ are also released during this inflammatory process. In addition, the activation of complement via the alternative pathway and the release of chemical mediators lead to micro-vascular disruption. This inflammation may persist throughout the subject's life, unless eradicated (Werme *et al*., 2015).

- **Non-steroidal anti-inflammatory drugs**: Low-dose non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain and inflammation. However, their use is often limited by serious ulcer complications (Drina, 2017). The properties of these

antiinflammatories are based on the inhibition of cyclooxygenases (COX 1 and 2), enzymes whose main role is the transformation of arachidonic acid into prostaglandins. Inhibition of their synthesis can alter the mucosa's defense mechanisms, leading to reduced blood flow, adhesion of polynuclear cells to the lining, endothelial damage and reduced mucosal blood flow, thereby promoting an inflammatory process. The latter is amplified by anti-inflammatory-stimulated TNFα production in macrophages (Vane *et al*., 1998).

- **Tobacco**: Smoking is a factor in the formation of peptic ulcers, where numerous effects appear to be produced by tobacco consumption, including reduced salivary concentration and blood flow, as well as increased acid, gastrin and pepsinogen secretion. Smoking can also multiply the risk of ulcers and round subjects more resistant to treatment, so smokers are twice as likely to develop an ulcer as non-smokers. Smoking appears to lower the defenses of the gastric mucosa (Maity *et al*., 2003).

- **Diet**: Nutritional management aims to avoid foods that have been suggested as ulcerogenic, such as sugar, which increases gastric acidity. Spicy foods, tea, coffee and all caffeine-based beverages have the capacity to increase acid secretion by acting on the H+ / K+ ATPase pump (Chen *et al*., 2023).

- **Stress**: The sensitivity of the mucosa to stress has long been recognized. The relationship between stress and ulcers is established via a neuro-hormonal pathway, through changes in vascularization that lead to chlorhydropeptic hypersecretion, diverting circulation via arteriovenous shunts (Konturek *et al*., 1992).

- **Alcohol**: Several studies have demonstrated that ethanol is capable of inducing hemorrhagic lesions and erosions in the gastric mucosa. The genesis of these lesions is attributed to a decrease in mucin levels and a reduction in local blood circulation (Yu *et al*., 2020). As a result, vascular permeability increases, leading to hyperemic foci and digestive hemorrhaging. In addition, wine and beer further stimulate acid secretion in response to hypergastrinemia (Chi *et al*., 2021).

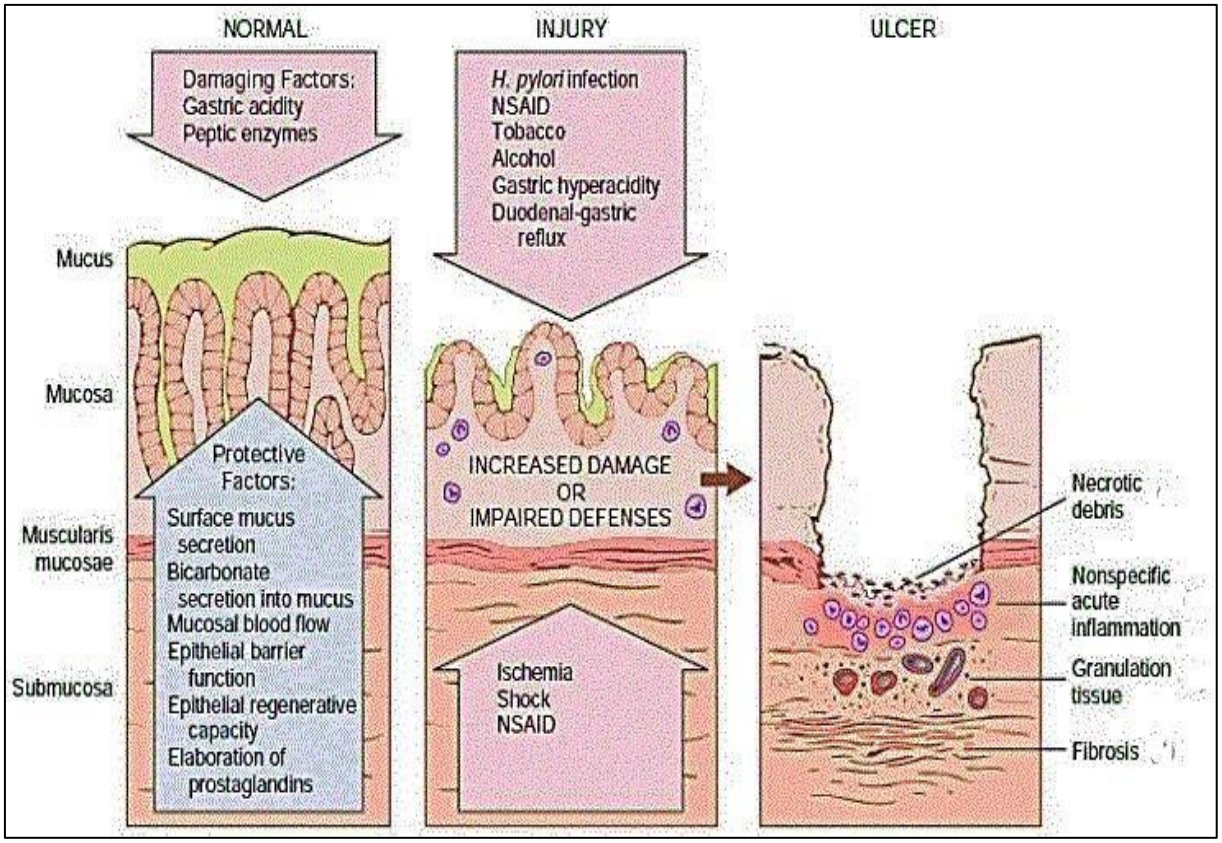

Figure 9: Physiopathophysiology of chronic peptic ulcer in stomach (Yadav *et al*., 2021).

IV.6.2. Treatment of gastric ulcer

IV.6.2.1. Medical treatment

A wide range of medications is available for the treatment of gastric ulcer, aimed at soothing pain and healing ulceration by reducing the aggressive power of acid secretion and reinforcing mucosal defense: Prostaglandin analogues, proton pump inhibitors, H2-receptor antagonists, antacids, etc (He *et al*., 2023).

Eradication of *H pylori* is achieved by combining antisecretory agents with antibiotics. Based on triple therapy, capable of eradicating *H. pylori* in 90% of cases, a proton pump inhibitor (PPI) and antibiotic biotherapy (Marcus *et al*., 2016).

IV.6.2.2. Phytotherapy of ulcers

Nowadays, medicinal plants are of therapeutic importance and constitute a veritable natural pharmacy for maintaining our health, thanks to their wealth of secondary metabolites, namely phenolic compounds, which are characterized by a wide range of biochemical and

pharmacological effects, including antioxidant and anti-inflammatory properties (Sun and Shahrajabian, 2023). Flavonoids show gastroprotective, anti-secretory, and antioxidant properties. Alkaloids and terpenoids also display gastroprotective qualities, influencing pH levels and ulcer indices. Tannins and saponins, present in numerous plants, exhibit antiulcerogenic potential by modulating acid secretion (Shahzad *et al*., 2024), indeed these can be used as an alternative or additive agent to current therapy. Consequently, these compounds may have a more effective and less toxic therapeutic potential for the treatment of peptic ulcers (Sumbul *et al*., 2011).

Polyphenols act in the gastrointestinal tract either as antiulcer, antisecretory or antioxidant agents, notably flavonoids and tannins (Dhiman *et al*., 2016). The most important mechanism of action responsible for flavonoids' anti-ulcer activity is their antioxidant properties, which involve the reduction of free radicals, chelation of ions and transition metals, inhibition of oxidative enzymes, enhancement of protein and non-protein antioxidants and reduction of lipid peroxidation (De Lira Mota *et al*., 2009).

Tannins are used in medicine primarily for their astringent properties; they react with proteins in tissue layers, precipitating at the ulcer site, forming a protective layer (tannin-protein / tannin polysaccharide complex) that prevents the absorption of toxic substances and promotes resistance to the action of proteolytic enzymes, thus tannins have the property of inhibiting stomach proteases such as pepsin (De Jesus *et al*., 2012).

V. Molecular docking

V.1. Definition

Docking is the term given to molecular simulations in which different chemical, physical and biological approaches are combined to study, at the atomic level, the interactions between two molecules (Figure 10): one, the target, a protein with one or more specific active sites and a known three-dimensional structure. The other, the ligand, is a small, flexible molecule. This enables us to determine the most favorable conformations for ligand binding to the target, leading to the formation of the most stable ligand-protein complex (Meng *et al*., 2011).

The technique is used to forecast and examine how a substance or molecule interacts to a target protein is called molecular docking. With the aid of this computational method, it is possible to

simulate the drug's molecular interactions with the target protein in order to comprehend their atomic-level interactions and assess their potential pharmacological actions (Permatasari *et al*., 2024).

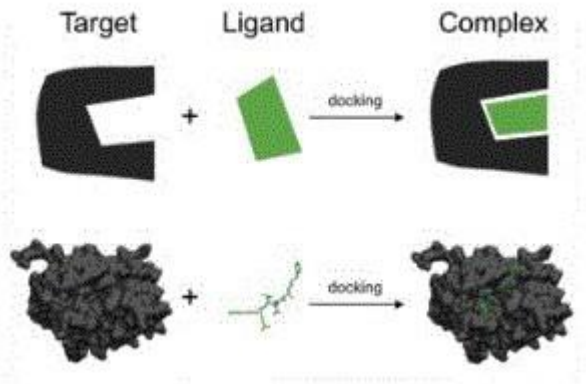

Figure 10: Representative scheme of molecular docking mechanism (Dnyandev *et al*., 2021).

V.2. Purpose of molecular docking

Docking is a molecular modeling technique that predicts the preferred direction of one molecule to another as they jump towards each other to form a stable compound. The direction of rotation can be carried to predict the strength of involvement or bond affinity linking two molecules together, for example, as a function of score (Dnyandev *et al*., 2021). Thus, the aim of molecular docking is to achieve an optimized conformation for both protein and ligand, and the fundamental direction between protein and ligand so that the free energy of the general method is minimized (Ferreira *et al*., 2015).

V.3. Principle

Docking is used to predict the structure of the intermolecular complex resulting from the association between two or more molecules. It can also be used to determine how a ligand (small molecule) interacts with a receptor (macromolecule), and to calculate the binding energy between them. It also helps determine which candidate ligand will interact best with a target receptor (Mohanty and Mohanty, 2023).

V.4. Docking tools

The tools most commonly used in docking are: a small molecule called a ligand. A biological target of therapeutic interest, called a protein, and a docking program (Pinzi and Rastelli, 2019).

V.4.1. Ligand

A ligand is an atom, ion or molecule carrying chemical functions that enable it to bind to one or more central atoms or ions. The term ligand is most often used in coordination chemistry and organometallic chemistry (branches of inorganic chemistry). In biology, a ligand (from the Latin ligand Um, binding) is a molecule that binds reversibly to a targeted macromolecule, protein or nucleic acid, generally playing a functional role: structural stabilization, catalysis, modulation of enzymatic activity, transmission of a signal. This term, widely used in the study of proteins, designates molecules that react non-covalently and specifically with the protein and play a role in its functions. The binding of a ligand to a receptor protein often modifies the latter's conformation, i.e. its three-dimensional (3D) structure. The energy associated with the intermolecular interactions formed between the protein and its ligand enhances this conformational change. This structural modification can thus potentially modulate its functional state and activity (Ahmad *et al*., 2006).

V.4.2. Receptor

A receptor is a macromolecule, most often a protein. The Protein Data Bank (PDB) is a worldwide repository of information on the three-dimensional structure of biological macromolecules: proteins, essentially, and nucleic acids. These molecules come from all biological kingdoms. Three-dimensional structures are derived mainly from X-ray diffraction analysis, others from nuclear magnetic resonance (NMR) or molecular modeling. The various three-dimensional conformations can be downloaded as pdb extensions, readable by docking software and containing various information about the protein in question. For example: the name of the receptor, the team that solved the structure, the experimental method, etc (Burley *et al*., 2017).

V.4.3. Program

Software is a set of programs that enables a computer or computer system to perform a particular task or function, processes and rules, and possibly documentation, relating to the operation of a data processing system (as opposed to hardware). The aim of a molecular docking program is to correctly predict the mode of interaction between two chemical entities, and to recognize which of these is the best. At present, several molecular docking programs

(commercial and non-commercial) are available: Auto Dock,AutoDockVina,GOLD, FlexX, DOCK and ICM. (Jacob *et al*., 2012).

VI. Selected plant *Anabasis articulata*

VI.1. Family of Chenopodiaceae

The Chenopodiaceae are a family of roughly 100 genera and 1500 species of herbs and shrubs found worldwide. It has a worldwide distribution, most members of the Chenopodiaceae family are halophytes, and they occur predominantly in arid and semi-arid environments (Akopian *et al*., 2020). Small trees or plants that are annual, subshrubs, or shrubs are uncommon perennials. There is indumentum of vesicular hairs (furfuraceous or farinose), ramified (dendroid), stellate, and infrequently glandular hairs or glabrous plants. Stems and branches are occasionally jointed (articulate). The leaf blades are flattened, terete, semiterete, or, in certain species, reduced to scales. The leaves are alternating, opposing, exstipulate, petiolate, or sessile. Monochlamydeous, bisexual, or unisexual flowers. If present, there may be one or two lanceolate, navicular, or scale-like bractlets (Gelin *et al*., 2003). One of the more common and widely distributed weeds in the world, *Chenopodium album* L. has been around since prehistoric times and is occasionally used as a medicinal herb. In the Andes, *chenopodium quinoa* willd. is a staple pseudo-cereal; occasionally, people have also eaten the fruits of other genus species. *Spinacia* is a popular herb for pot and salads. *Beta* is consumed as a vegetable (garden beet), used as a pot herb (Swiss chard), and may be a source of glucose and sucrose (sugar beet). For the purpose of making soap and glass, a number of genera, most notably Salsola and Salicorma, have been used as sources of soda (Clemants, 1992).

VI.2. Botanical description of *Anabasis articulata*

Anabasis articulata as illustrated in Figure 11 is an endemic Saharan plant with a low bush, a thick, tortuous stump and a very light bluish-green color. Twigs are deciduous and fall to the base of the plant. Opposite leaves have a very short free part, obtuse or ending in a whitish point. The pinkish-white flowers are isolated in the axils of each leaf. The fruit is surrounded by three wings due to the dilation of three of the sepals. This species is common in stony soils throughout the Sahara, right up to the southern Sahara (Ozenda, 2004).

VI.3. Classification

Systematic position according to (Quézel and Santa, 1963) of *A. articulata* Mok. Forsk was as following:

Phylum: Phanerogams or Spermaphytes
Subphylum: Angiosperms
Class: Eudicots
Subclass: Preasteriaceae
Order: Caryophyllales
Families: Chenopodiaceae
Genus: Anabasis
Species: *Anabasis articulata* (Forsk.) Moq.

Noms vernaculaires: El-Ajrem.

Figure 11: *Anabasis articulata* (Forsk.) Moq. A: plant, B and C : leaves and leaves (Self photo)

VI.4. Traditional uses

Many Saharan plant species have been used in traditional medicine by the indigenous population to treat fever, diarrhea, diabetes, asthma, rheumatism and cancer. Plants in the Chenopodiaceae family are used for their wealth of bioactive substances. The Anabasis genus thrives in stony, sandy wadis widely traveled by camels and goats (Chopra, 1956).

The continued use of traditional medicine is attributable not only to cultural reasons and poverty, but also to the ineffectiveness of many existing drugs (Senhaji *et al*., 2020), with much interest in the biologically active compounds present in plants and herbs for their safety and efficacy in the prevention and/or treatment of human diseases (Djeridane *et al*., 2015).

In Algeria, Syria, Egypt and Iraq, *A. articulata* is commonly used to treat asthma, fever, eczema and kidney infections in Western medicine (Khamees *et al*., 2019) and has also been used as a plaster to treat scabies (Nouidjem *et al*., 2021).

This species is a remedy for the treatment of diabetes (Kambouche *et al*., 2009), and also has cholinergic properties (Tilyabaev and Abduvakhabov, 1998). The aerial parts are used in decoction and as a poultice to treat dermatoses, skin diseases (eczema), headaches and fever (Hammiche and Maiza, 2006).

VI.5. Pharmaceutical activities

Few research reported medicinal properties of *A. articulata*. According to Abdallaha *et al*., (2014), the methanolic crude extract of the aerial portion has shown the strongest antiinflammatory efficacy in rats. Gamal *et al*., 2022(a) found that triterpenoids from the aerial parts of *Anabasis articulata* (Forssk) Moq: gastroprotective effect *in vivo* with *in silico* studies, cytotoxic and antimicrobial activities. Ben Menni *et al*., (2022) reported an antiproliferative, an Antioxidant, an anti-tyrosinase, an anti-acetylcholinesterase and an anti-inflammatory propreties of Sterols of *A. articulata.*

A. articulata has been also reported to have medicinal properties as antioxidant (BelyagoubiBenhammou *et al*., 2019) and antimicrobial activities (Bouaziz *et al*., 2009), antiangiogenic activity (Abdulsahib *et al*., 2016),

and anti-arthritic effect (Abdulhusain *et al*., 2022). Also, several research have evaluated the effect of *A. articulata* on diabetes (Kambouche *et al*., 2009; Metwally *et al*., 2012; Djeridane *et al*., 2015). Abdulsahib, (2022) investigated the beneficial impact of *A. articulata* stem extract on decreasing intraocular pressure in a glaucoma rat model.

VI.6. Chemical composition

Few studies have been carried out on the phytochemistry of *A. articulata* (Benhammou *et al*., 2013; Ghembaza *et al*., 2016). These authors highlighted the presence of flavonoids, tannins, saponins, alkaloids, carbohydrates and/or glycosides, coumarins and triterpenes in extracts of this species. Other work carried out on different parts of *A. articulata*, twigs and roots of species from Bechar (Algeria) by Benhammou *et al*., (2013) revealed that the latter contains flavonoids, flavonols, tannins, carotenoids, saponosides with varying intensities, but also the presence of alkaloids on the twig part. Other work carried out by Abdulsahib *et al*., (2013) on *A. articulata* stems from Bagdad shows the presence of tannins, saponosides, alkaloids, resins, flavones with the absence of coumarins, terpenes and steroids.

Previous phytochemical study on *A. articulata* led to the isolation of four known saponins: 3O-glucopyranosyl of(stigmasterol, ß-sitosterol, sitostanol), 3-O-[ß-D- the glucopyranosyl] oleanolic acid , 3-O-[ß-D-glucopyranosyl -28-O-ß-D - xylopyranosyl] oleanolic acid, in addition to proceric acid (Metwally *et al*., 2012). Chromatography analysis of successive fractions revealed the isolation of β-sitosterol from its unsaponifiable fraction, caffeine form its methylene chloride fraction, and 4-acetoxy phenol from the ethyl acetate one (Gamal *et al*., 2022b).

Materials & Methods

I. Material

I.1. Survey area

The wilaya of M'sila is located in the South-East of Algiers at 248 Km; it extends over an area of 18175 Km². Limited to the North by the wilayas: Bouira, Bordj Bou-Arreridj and Setif, to the East by Batna and Biskra, to the West by Djelfa and Medea and to the South by Djelfa and Biskra.

El Mergueb reserve (Figure 12) is located about 180 km south of Algiers, among the high steppic plains of the Hodna. It covers an area of 16,481 ha, straddling the communes of Sidi Hadjeres, Ain El Hadjel and Sidi Ammer (Boudjadja *et al*., 2010). It is located in the steppe routes of the highlands of Algeria, it extends between the coordinates of Lambert relative to the topographic map of, Aïn El-Hadjel to (1/50.000, following: X (608.5 and 626.7) km and Y (243.6−263.8) km. The three experimental stations lie between latitudes (35°36'12,6''N & 35°35'05,7''N) and longitudes (03°56'23,8''E & 03°58'08,7''E) with an altitude of 575 and 634 m located in the municipality of Ain EL-Hadjel (Adjabi *et al*., 2019).

The climate of the investigation area is continental, subject in part to Saharan influences. Summer is hot and dry while the winter is very cold, with low rainfall is irregular, it is of the order of 100 to 250 mm/year and with an average annual temperature of 15.8 °C (Le Houerou, 1995).

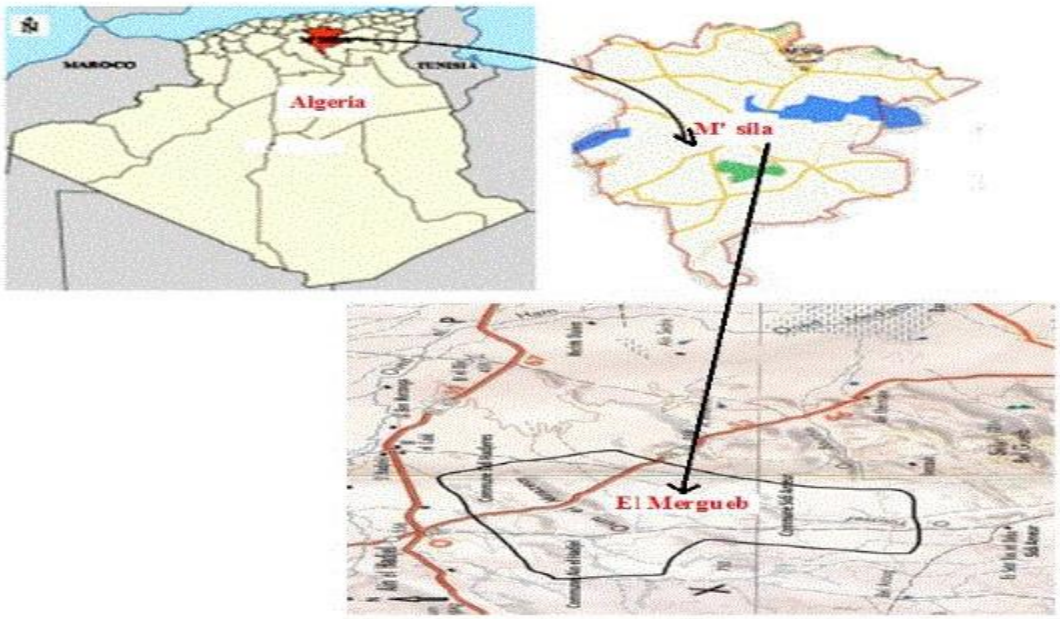

Figure 12: Geographical location of the El Mergueb nature reserve (Algeria) (Makhlouf *et al*., 2024).

I.2. Plant material

A. articulata was harvested in October–November 2020 during the flowering period in El Mergueb natural reserve in M'sila province (North Algeria). Professor Oujhih B., a renowned taxonomist at the University of Batna identified this plant. The laboratory received a voucher specimen with the identification number SNV 0045-2020. An electric mixer was used to crush and powder the *A. articulata* flowers and leaves after they had been air-dried.

I.3. Chemicals

All analytical grade chemicals were utilized in this study were procured form E. Merck, Germany.

$ABTS^+$ 2.2′-Azino-bis-(3-ethylbenzenothiazoline-6-sulfonic acid), acetate buffer, adrénaline, alcian blue, aluminum trichloride ($AlCl_3$), ammonium molybdate, ascorbic acid, aspirin, αamylase, bovine serum albumin (BSA), Butylated hydroxytoluene (BHT), Coomassie Blue G_{250}, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethylenediaminetetraacetic acid (EDTA), ferric chloride, ferrous ammonium sulphate, ferrozine, Folin-Ciocalteu reagent, gallic acid, glibenclamide, hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), indomethacin, iodine, linoleic acid, magnesium chloride ($MgCl_2$), Nw-nitro-L-arginine methyl ester hydrochloride (L-NAME), omeprazole, phenanthroline, phosphate buffer (PBS), potassium iodide, potassium ferricyanide, potassium persulfate, quercetin, sodium acetate, sodium bicarbonate solution (Na_2CO_3), sodium carbonate, sodium chloride, sodium dodecyl sulfate, sodium phosphate, sodium salicylate, sodium salicylate, starch, sucrose, sulfuric acid, tannic acid, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tris-HCL, Tween 40, xylene, β-carotene.

I.4. Animal material

Albino mice and rats (weighing 18–30 and 180–200 g, respectively). They were maintained in polycarbonate cages during 7 days in normal laboratory conditions (12/12 h light/dark cycle, 23 ± 2 °C with free access to food and water) before the beginning of the experimentation. The animal studies were conducted after obtaining clearance from Institutional Ethic Committee, and the experiments were conducted in strict compliance according to ethical principles and provided by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

II. Methods

II.1. Ethnobotanical survey in “El-Mergueb” nature reserve

In order to identify and establish a list of plants used in traditional medicine due to their pharmacological properties, an ethnobotanical survey was carried out using a questionnaire (Figure 13) distributed to villagers and people with knowledge on the use of medicinal plants

through face-to-face interviews, with the aim of collecting accurate information on therapeutic practices by the population. Our study was conducted from June to January 2021 spread over 182 questionnaire cards. The information is divided into two sections; the first concerns the informant as the sole owner of the information, while the second gathers information concerning the medicinal plants such as local names, plant parts used, medicinal use and preparation.

Section A: Questions about the informant: sex, age, level of education, origin of ethnobotanical knowledge.

Variables	**Categories**
Gender	Female Male
Age	[20years-35years] [36years-50years] [51years-60years] >60years
Educational level	Illiterate Primary College Secondary University
Origin of ethnobotanical knowledge	Family knowledge Herbalist Scientific research.

Section B: Questions concerning the medicinal plant: uses of the medicinal plant, the part of the plant used, the pathology treated.

Plant name		Part used	Mode of use	Therapeutic property	Spontaneous/ cultivated / Toxicity	Biological activities
Scientific name	Vernacular name					

Figure 13: Questionnaire card of survey (Makhlouf *et al*., 2024)

The identity of each plant species mentioned by the herbalists was verified and confirmed by botanists of the Department and by a bibliography (Quézel and Santa, 1962-1963) and (Ozenda, 1977). A medicinal use was accepted as valid only if it was mentioned by at least three independent interviewees (Al-Qura'n, 2009). The Use Value UV is the Data analysis that was

calculated according to the following formula UV=ΣU/n, where U is the number of used reports cited by each informant for a given plant species and n is the total number of informants interviewed for a given plant. It demonstrates the relative importance of plants known locally (Ouelbani *et al*., 2016).

II.2. Ethnomedicinal survey of *A. articulata*

An ethnopharmacological study was carried out to collect as much as we could about this plant's applications, 118 persons (herbalists and members of the general population) were surveyed in M'sila for one month. The surveys included the therapeutic use, the used plant, the mode of preparation and the efficacy of the indicated plant treatments.

II.3. Preparation of extracts

II.3.1. Preparation of decocted extract

The decoction extract of the flowers and leaves of *A. articulata* (DEAA)is prepared according to Ferreira *et al*., (2006). Briefly, 100 g of powder is boiled for 10 minutes in 1000 mL of distilled water. The mixture is filtered with muslin and then with the wattman filter paper. The filtrate is poured into glass plates to dry in the oven (37°C). The plates are already weighed empty and after drying to calculate the yield, the powder obtained is scraped and kept in clean bottles protected with aluminum foil for later use.

II.3.2. Preparation of the crude ethanolic extract

Extracts was obtained according to the method of Markham, (1982) the flowers and leaves of the plant powder after homogenized mixed with 1L ethanol-water (70:30 v/v) kept under agitation for 5 days resulting solution was filtered to obtain the first filtrate, This procedure was repeated on the residue using water-methanol (50:50 v/v) under agitation for 2 days to obtain the last filtrate. After 7 days the mixture was filtered. The first and last filtrates were combined. The solvent was evaporated using vacuum rotary evaporator at 40 C° to get ethanolic extract of *A. articulata* (EEAA).

II.3.3. Fractionation of the crude ethanolic extract

Fractions were obtained according to the method of Markham, (1982). The concentrated hydroethanolic solution was washed with hexane several times until a clear upper layer of hexane was obtained. The lower layer was then extracted successively with chloroform, ethyl

acetate and n-butanol. The solvent of each fraction was evaporated using vacuum rotary evaporator at 40 C° to obtain: chloroform fraction (ChFA), ethyl acetate fraction (EAFA) n-butanol fraction (nBFA) and aqueous fraction (AqFA).

II.3.4. Calculation of the plant extraction yield

The yield of the plant extract is calculated according to Radunić *et al*., (2015), it is the ratio between the weight of the extract and the weight of the treated plant. The yield which is expressed as a percentage has been calculated by the following formula: $Y = W_E / W_P \times 100$ Where: Y= Yield of the extract in percentage, W_E = Weight of the extract, W_P= Weight of the plant.

II.4. Quantitative phytochemical analysis

II.4.1. Determination of total phenolic content

The content of phenolic compounds in plant extracts was estimated spectrophotometrically using the Folin-Ciocalteu reagent method described by Slinkard and Singleton, (1977). The procedure consists of adding 500 µL of Folin-Ciocalteu reagent (diluted 10 times) to 100 µL of the extract or gallic acid. After 4 minutes, 400 µL of 7.5% sodium bicarbonate solution (Na_2CO_3) is added. After 90 minutes of incubation in the dark at room temperature, the absorbance is read at 765 nm. The concentration of total polyphenols was calculated from the regression equation of the gallic acid calibration curve at different concentrations (Figure 14). The results are expressed in micrograms of gallic acid equivalent per milligram (mg EAG/g of extract).

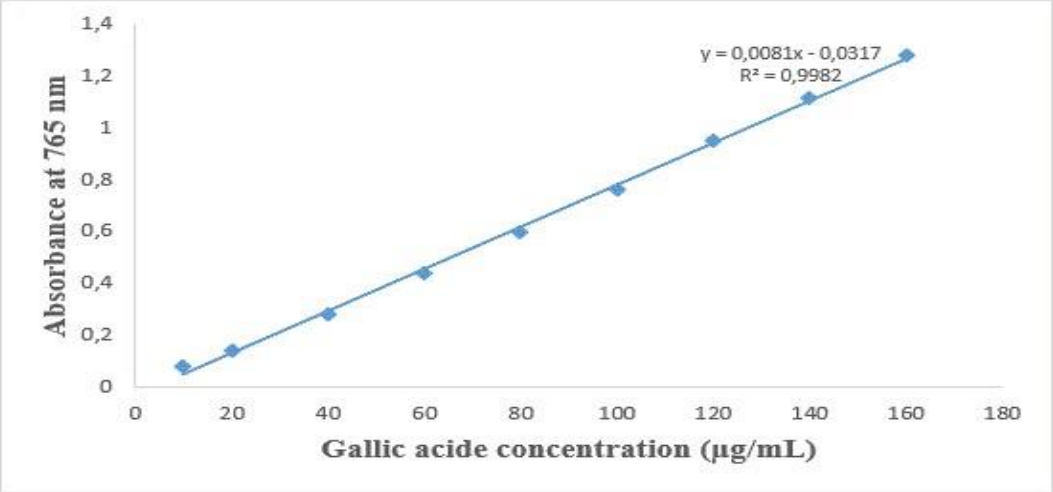

Figure 14: Standard curve of gallic acid for the determination of total polyphenols. The statistics were presented as mean with (n = 3).

II.4.2. Determination of flavonoids contents

Quantitative determination of flavonoids is performed using the aluminum trichloride method Bahorun *et al*., (1996). Briefly, 1 mL of the $AlCl_3$ solution (2%) is added to 1 mL of the extracts solution at different prepared concentrations in the appropriate solvent. After 10 min of incubation, the absorbance is read at 430 nm. The concentration of flavonoids in the extracts is deduced from a calibration line established with quercetin and is expressed in mg of equivalents of quercetin per g of extract (mg EQ/g of extract) as mentioned in Figure 15.

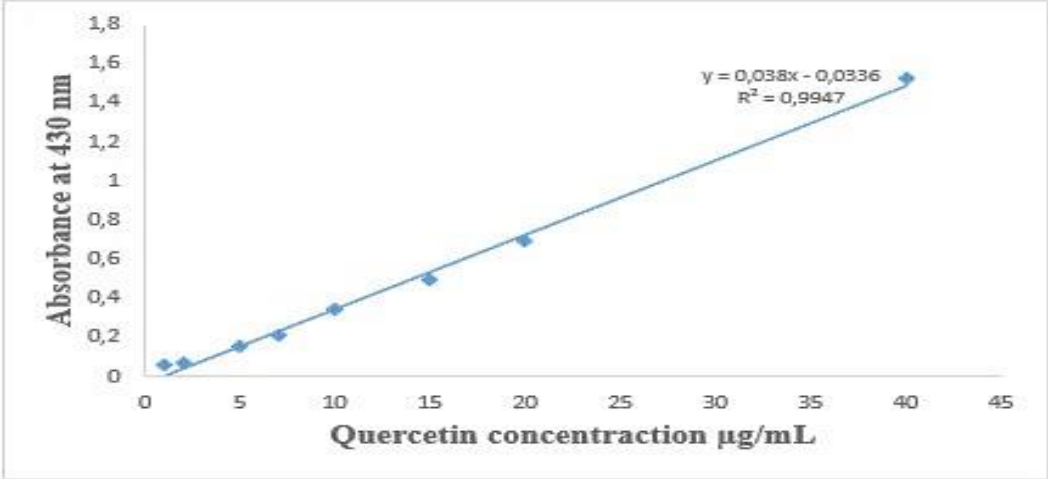

Figure 15: Standard curve of quercetin for the determination of flavonoids. The statistics were presented as mean with (n = 3).

II.4.3. Determination of total tannin content

The total content of tannins in extracts was estimated using Folin–Ciocalteu reagent and tannic acid as standard, according to the method described by (Prasanth *et al*., 2017). The reaction mixture was made by combining 0.5mL of each extract, 2.5 mL of 10% FolinCiocalteu's reagent mixed with water, in addition to add 2.5 mL of 7.5% Na_2CO_3. The samples had been incubated in a thermostat at 45 C for 45 min in darkness. Absorbance was then measured at 765 nm and the calibration curve was established using different concentrations of tannic acid (25-300 µg/mL). Total tannin content was expressed as mg of tannic acid equivalents per g of extract (mg TAE/g E of extract) using standard curve of tannic acid (Figure 16).

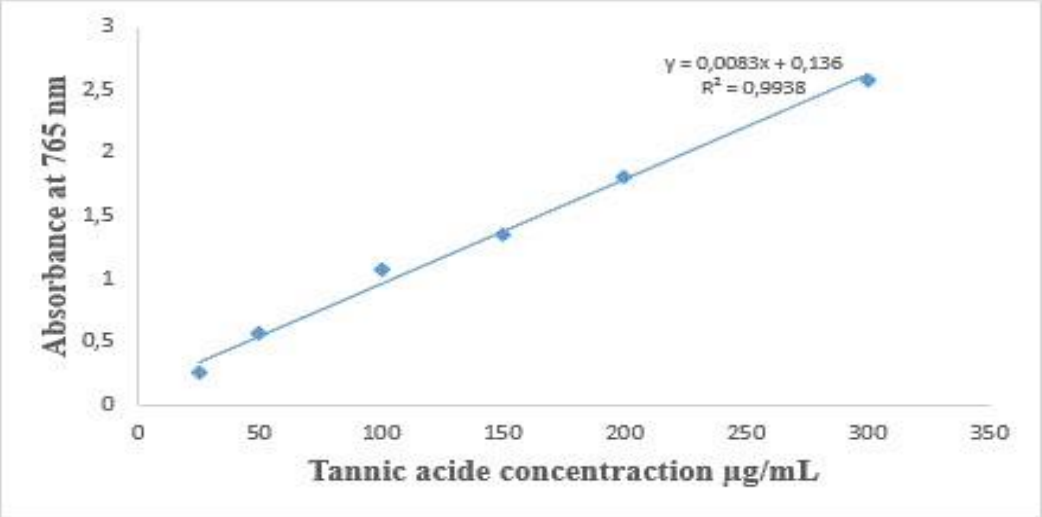

Figure 16: Standard curve of tannic acid for the determination of tannins. The statistics were presented as mean with (n = 3).

II.5. Liquid Chromatography-Mass Spectrometry Analysis LC-MS-MS

The phenolic compounds in samples were analyzed using the method reported by Ben Amor *et al*., (2022). The equipment used were Shimadzu 8040 Ultra-High Sensitivity (UPLC-ESI-MSMS) using UFMS technology, and it was outfitted with binary bump Nexera XR LC-20AD. For optimization of polyphenols standards, we used direct injection without column.

The ESI parameters were as follows: 230 KPs for the CID gas; −6.00 Kv for the conversion dynode; 350 °C for the interface temperature; 250 °C for the DL temperature; 3.00 L/min for the nebulizing gas flow; 400 °C for the heat block; and 15.00 L/min for the drying gas flow. Each standard was made in concentration of 500 g/L of methanol. Both negative and positive ions were employed with the ion trap mass spectrometer in MRM mode (multiple reaction monitoring). The mobile phase was constituted of water, 0.1% formic acid, and 70% methanol. 6 µL was the injection volume, and the flow rate was 0.3 mL/min.

The Ultra-force C18 column (I.D. 2.5 mm 100 mm, 1.8 m particle size; Restek) was used to separate the samples, and the oven temperature was adjusted at 25 °C. Isocratic elution was applied with 0.1% formic acid and methanol (30:70, v/v). The flow rate was 0.30 mL/min, and the injection volume was10 mL.

Polyphenols standards used are acetylsalicylic acid, Benzoic acid, Cennamic acid, Coumaric acid, Folic acid, Gallic acid, Maleic acid, Valinic acid, Ascorbic acid, Beta carotene, Caffeic acid, Catechol, Chrysine, Coumarin 4-hydroxy, Epicatchin, Hesperitin, Hydroxy anisol butyl, Hydroxy toluene butyl, Lawson,

II.6. Estimation of *in vitro* antioxidant activities

II.6.1. Total antioxidant capacity

Total antioxidant capacity was evaluated with the assay based on the reduction of Mo (VI) to Mo(V) by the extracts and subsequent formation of a green phosphate/Mo(V) complex at acid pH Prieto *et al*., (1999). An aliquot (0.1 mL) of plant extracts were combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in at 95 ◦C for 90 min in darkness. then the mixture was allowed to cool to room temperature, and the absorbance was measured at 765 nm against a blank.

II.6.2. DPPH radical scavenging assay

The scavenging activity of the DPPH was measured according to the protocol described by (Blois, 1958) with slight modification. The principle of this method is the reduction of the violet DPPH (2,2-diphenyl-1-picrylhydrazyl) to 2,2-diphenyl-1-picrylhydrazine of yellow color. DPPH absorbs at 517 nm, but when reduced by an antioxidant, its absorption decreases. Briefly, a 0.4 mM solution of DPPH prepared in 100 mL methanol and 800 µL of this solution was added to 200 µL of sample of extracts at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. BHT were used as standards.

Radical scavenging activity % = ($A_{control} - A_{sample} / A_{control}$)

$A_{control}$: Absorbance of the control (without sample). A_{sample}: Absorbance of the reagent with extract.

II.6.3. ABTS radical scavenging assay

According to Re *et al*., (1999) $ABTS^{+}$ radical cation was generated by a reaction of 7 mM ABTS with 2.45 Mm potassium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature. The solution was then diluted by mixing ABTS solution with methanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm. Then, 50 µL of the sample of extracts was mixed with 1mL of $ABTS^{+}$ solution and kept for 10 min at room temperature. The absorbance of reaction mixture was measured at 734 nm using spectrophotometer.

ABTS radical scavenging activity % = ($A_{control} - A_{sample} / A_{control}$) × 100

$A_{control}$: absorbance of ABTS radical and ethanol (without sample). A_{sample}: Absorbance of the ABTS with extract or standards.

II.6.4. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extractives was determined by the ability of different fraction extract to scavenge the hydroxyl radicals using the method of Smirnoff and Cumbes, (1989) with slight modification. The reaction mixture contained 100 µL of extracts or standard antioxidant, 0,5 mL of $FeSO_4$ (1.5 mM), 0,35 mL of H_2O_2 (6 mM), 0.15 mL of sodium salicylate (20 mM) and varying concentrations of plant extracts and standard. The mixtures were kept in a water bath at 37 °C for 1h, after which the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The percentage of hydroxyl radical scavenging activity was calculated according to the following formula:

Hydroxyl radical Scavenging % = $[1-(A_{sample1}-A_{sample2})/A_{control}] \times 100$.

Where, $A_{control}$ was the absorbance of the control (without sample) and $A_{sample1}$ was the absorbance in the presence of the sample; $A_{sample2}$ was the absorbance without sodium salicylate.

II.6.5. Determination of the scavenging effect on hydrogen peroxide

One of the most common methods for assessing the trapping capacity of hydrogen peroxide is based on the uptake of this molecule in the UV domain. The potential for trapping hydrogen peroxide in *A. articulata* extracts is estimated according to Mukhopadhyay *et al*., (2016). 250 µL ferrous ammonium sulphate (2 mM) is added to 1.5 mL of different extracts concentrations. The mixture is shaken vigorously, then 62.5 µL of hydrogen peroxide (5 mM) are added and the mixture is shaken and left at laboratory temperature for 5 min. Then, 1.5 mL of 1.10-phenanthroline (1 mM) were added to each tube. The mixture is incubated for 10 minutes at room temperature. Finally, the absorbance of the reaction medium is measured at 510 nm. The same procedure was repeated for ascorbic acid which is used as a standard. hydrogen peroxide Scavenging % = $[1-(A_{sample1}-A_{sample2})/A_{control}] \times 100$.

Where, $A_{control}$ was the absorbance of the control (without sample) and $A_{sample1}$ was the absorbance in the presence of the sample; $A_{sample2}$ was the absorbance without sodium salicylate.

II.6.6. Ferrous ion chelating activity

Ferrous ion chelating activity was measured following the method of Decker and Welch,

(1990). The reaction mixture (1.5 mL) contained 500 µL of extracts dilutions or EDTA, 100 µL $FeCl_2$ (0.6 mmol/L in water) and 900 µL methanol. The control contained all the reaction reagents except the extract and EDTA. The mixture was shaken well and allowed to react at room temperature for 5 min and 100 µL ferrozine (5 mmol/L in methanol) was then added. The absorbance of the Fe^{2+}- ferrozine complex was measured at 562 nm.

The chelating activity % = ($A_{control} - A_{sample} / A_{control}$)

$A_{control}$: Absorbance of the control (without sample). A_{sample}: Absorbance of the reagent with extract.

II.6.7. Reducing power

The reducing power of *A. articulata* extracts was determined according to the method of Chung *et al*., (2005). A 0.1 mL aliquot of different concentrations of extracts or BHT was mixed with an equal volume of 0.2 mol/L phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min to reduce ferricyanide into ferrocyanide. After that, 0.25 mL trichloroacetic acid was added into the mixture to stop the reaction, and the mixture was centrifuged at 3000 r/ min for 10 min. The supernatant (0.25 mL) was added into distilled water (0.25 mL) and 0.1% ferric chloride (0.5 mL), and then the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates an increase in reducing power.

II.6.8. β-Carotene bleaching assay

This assay is based on the capacity of antioxidant molecules to inhibit β-carotene oxidative degradation that is caused by oxidative compounds of linoleic acid according to the method of Kartal *et al*., (2007). β-carotene/linoleic acid emulsion was prepared by mixing 0.5 mg of ßcarotene in 1 mL of chloroform, 25 µl of linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated 40 °C using a vacuum evaporator. Then 100 mL of oxygenated distilled water was added with vigorous shaking. To an aliquot of 2.5 mL of this emulsion, 350 µL of extracts or the reference antioxidant (BHT) were added and well mixed. The absorbance was recorded after 0, 1, 2, 4, 6 and 24 hours at 490 nm. A negative control consisted of 2.5 mL distilled water or solvent instead of extract or reference antioxidant. All samples were assayed in triplicate. The percentage of inhibition was calculated using the following equation:

Inhibition % = $A_t / A_{t0} \times 100$.

Where A_t was the absorbance of the test sample at time (t) and A_{t0} was the absorbance of the test sample at a time t_0.

II.7. Estimation of *in vitro* anti-inflammatory activity

II.7.1. Inhibition egg Albumin denaturation

The protein denaturation experiment was carried out using as reported by Bouaziz *et al*., (2020). The volume of egg white was adjusted with buffer solution Tris-HCl (20 Mm, pH= 6.8) to obtain a dilution solution of 1: 100. The solution was gently stirred for 10 min followed by filtration. Identical volumes were transferred to tubes then extracts or aspirin were added, and the tubes were incubated at 74° C for 15 min. The absorption was measured at 650 nm and the following % inhibition of protein denaturation was calculated:

$$\text{Inhibition \%} = [(A_{\text{control}} - A_{\text{treated}})/ A_{\text{treated}}] \times 100.$$

II.7.2. Inhibition of denaturation of Bovine Serum Albumin

The heat denaturation of Bovine Serum Albumin was measured *in vitro* by the method of Kandikattu *et al*., (2013). A volume of 1 mL of extracts was mixed with 1 mL of bovine serum albumin solution (0.2%) prepared in Tris-HCl buffer (50 mM, pH 6.6). The mixture was made to stand for 15 minutes at 37° before being heated in bathwater for 5 minutes at 72°C. After cooling to room temperature, the absorbance was measured at 660 nm using a UV-visible spectrophotometer. As a reference, aspirin was employed. The extract's anti denaturation action on BSA was provided as inhibition percentages determined using the formula below:

$$\text{Inhibition \%} = (Ab_{\text{control}} - Ab_{\text{sample}} / Ab_{\text{control}}) \times 100$$

Where, I: The inhibition percentage, Ab_{sample}: absorbance of the test sample, and Ab_{control}: absorbance of control.

II.8. Estimation of *in silico* activity

The molecular docking study of the organic molecules that have been extracted from the plant was performed using AutoDock Vina program to complement and confirm the results in the experimental section for the *in vitro* and *in vivo* activities. The protein used to simulate this activity, named COX-2, has been obtained from the RCSB database with the code [3LN0](Wang *et al*., 2010). The active site of the protein used is delimited by a box of dimensions: a volume of 80 × 80 × 80 $Å^3$ and a center x, y, z: 42, 32 and 32, respectively.

To ensure an effective simulation, the ligands (7 molecules: gallic acid, anthron, beta carotene, butylated hydroxytoluene (BHT), butylhydroxyanisole (BHA), myricetin and rutin were

initially prepared by optimizing them using the Gaussian program (Frisch *et al*., 2008) to achieve a stable geometry with minimum energy. Next, the receptors (proteins) for the two activities were prepared using Discovery Studio. This involved removing water molecules and heteroatoms, and adding polar hydrogen atoms and Kollman charges ("Life Sciences and Material Sciences | BIOVIA – Dassault Systèmes," 2022.).Afterwards, molecular docking simulations were conducted using the AutoDock Vina program (Trott and Olson, 2010).

II.9. *In vivo* investigation

II.9.1. Acute Toxicity Study

The toxicity of extracts at a single dose was assessed on mice, according to the Organization of Economic Co-operation and Development (OECD, 2001). The mice are males of *Swiss* race weighing between 25 and 32 grams. The animals were divided into three groups with five animals.

Four treated groups were orally given the decoction extract of *A. articulata* (DEAA) and the ethanolic extract (EEAA) in a single dose of 2000 mg/kg and 5000 mg/kg body weight, while the control group administered only water vehicle. The day one of the dosing was taken as Day 0 whereas the day of sacrifice was labeled as Day 14. Individual lots of mice were monitored for 4 hours after treatment to detect behavioral and physiological changes in comparison to the control. Gross behavioral and toxic effects were observed at short intervals for 24 h, then daily for the next 14 days to detect late effects.

The following parameters were calculated:

Mortality, Body weight, Macroscopic examination and Relative organ weight.

- **Mortality:** During the fourteen days period, all the animals were detected daily for clinical signs and mortality patterns.
- **Body weight:** The body weight of each mouse was calculated using a sensitive balance during study period, once on day 0, day 7 and day14 of experiment.
- **Relative Organ Weight:** On day 14 of the study period, all the animals were euthanized by exsanguination under anesthesia of chloroform. Diverse organs namely the heart, liver, kidneys and spleen were cautiously dissected out and weighed in grams (absolute organ weight). The relative organ weight of each animal was then calculated as follows:

Relative organ weight = (absolute organ weight (G) / body weight of mouse on sacrifice day (G)) × 100.

- **Histopathology stud of liver and kidney:** After weight measurement, both of liver and kidney were fixed in 10% formalin, dehydrated in ascending degrees of ethanol (70–100%), cleared in xylene and finally embedded in paraffin. Afterward, thin sections of 5 µm were stained with hematoxylin-eosin and examined with a light microscope. Digital images recorded at 10 x magnification.

II.9.2. Estimation of *in vivo* anti-inflammatory activity

Three procedures were used to examine the anti-inflammatory activity of DEAA and EEAA: Carrageenan induced paw edema in rats, croton oil, and xylene induced ear edema in mice.

II.9.2.1. Carrageenan-induced rat paw oedema

The anti-inflammatory effect of ethanolic and decocted extracts against carrageenan induced acute paw edema in rats was conducted according to the method described previously (Tsai and Lin, 1998). Seven groups of 5 rats each were treated with DEAA (50, 100 and 200 mg/kg, p. o.), EEAA (100 and 200 mg/kg, p. o.), indomethacin (10 mg/kg, p. o.) and distilled water (10ml/kg, p. o.). One hour later after administration, acute inflammation was produced by the subplantar injection of 0.1 ml of (1% carrageenan in normal saline) in the left hind paw of the rats. The thickness of each paw was measured using a digital caliper. The measures were carried out at 0 h (V_0: before carrageenan injection) and 1, 2, 3, 4, 5 and 6 h after induction of inflammation (V_T). The difference between V_T (1, 2, 3, 4, 5 and 6 h) and V_0 was taken as the edema volume value. The following formula was used to determine the percentages of inhibition:

$$\text{Inhibition \%} = [(V_{(t)} - V_{(t0)})_{\text{control}} - (V_{(t)} - V_{(t0)})_{\text{treated group}}] / (V_{(t)} - V_{(t0)})_{\text{control}} \times 100.$$

II.9.2.2. Croton oil induced ear edema in mice

The mouse ear edema was obtained by topical application of croton oil and carried out according to the method described by Dulcetti Junior *et al*., (2004) with slight modifications. croton oil was dissolved in a 5% acetone solution (v/v) and 10 µL were applied with a micropipette to both anterior and posterior surfaces of the right ear. The left ear (control) received the vehicle (acetone 80, distilled water 20, v/v). Groups of mice (n = 5) received orally

the doses of DEAA (100 and 200 mg/Kg), EEAA (100 and 200 mg/Kg), vehicle (distilled water) or indomethacin (10 mg/Kg), 1 h before croton oil application. 6 h later, thickness of each ear was measured using a digital caliper and the anti-inflammatory activity was expressed as the % of the edema inhibition using the following formula:

Inhibition % = 100 × (D_n–D)/D_n where D is the difference of ear edema thickness in the treated group and D_n is the difference of ear edema thickness in the negative group.

II.9.2.3. Xylene-induced ear edema in mice

The xylene-induced ear edema test was carried out according to the method of Manouze *et al*., (2017) with slight modifications. Briefly, each group (five animals per group) was given by gavage one dose (100 or 200 mg/kg) of DEAA, 100 or 200 mg/kg of EEAA, indomethacin (10 mg/kg) or vehicle (10 mL/kg) 1 h before induction of ear edema by topical application of 0.02 mL xylene on the inner and outer surfaces of the right ear. The left ear was used as a control. One hour after xylene application, the thickness of the ear was measured with a digital caliper before and 2 h after the xylene application. The inhibition percentage of ear edema was computed as follows:
Inhibition % = 100 × (D_n–D)/D_n, where D is the difference of ear edema thickness in the treated group and D_n is the difference of ear edema thickness in the negative group.

II.9.3. Analgesic activity

The Koster *et al*., (1959) approach was used to assess analgesic effectiveness against acetic acid-induced pain. was used for this activity. The mice were divided into six groups of five mice each and fasted overnight. The animals were treated with Aspirin (150 mg/kg, p.o.), saline solution (10 ml/kg, p.o.) and *A. articulata* (100 and 200 mg/kg, p.o.). The mice were treated with acetic acid (0.6%, v/v in saline, 10 ml/kg, i.p.) one hour after the above treatment was carried out. The number of abdominal writhes (full extension of both hind paws) was cumulatively counted every 5 min over a period of 25 min immediately after the acetic acid injection. The analgesic effect was expressed as reduction percentage of writhing by using the following formula:

$\%Inhibition = (N_{CN} - N_t) / N_{CN} \times 100$

N_{CN}: number of writhings of the negative controlnumber of contortion of the negative control.
N_t number of contortion batch test or the positive control.

II.9.4. Antiulcer potential of plant extracts

II.9.4.1. Ethanol induced gastric ulcer

The antiulcer activity of the decocted (DEAA) and the ethanolic extract (EEAA) of the flowers and leaves of *A. articulata* was determined following the method used by Abdulla *et al*., (2010). Ulcers were induced by administering ethanol. All the animals (rats 5/group) were deprived of food for 24 hours prior to the experiment but were allowed free access of drinking water up to 1 hour prior to the experiment. Group I: ulcer control group was orally administered with distilled water (5 mL/kg); Group II was treated with omeprazole (20 mg/kg) as reference drug; Groups III, IV and V were treated with (50, 100 and 200 mg/kg) of DEAA and (50, 100 and 200) mg/kg of EEAA. One hour after this pre-treatment, all the groups of rats received a dose of absolute ethanol (2.5 mL/kg) orally. The animals were sacrificed by cervical dislocation after 30 min of necrotizing agent administration.

According to Bouaziz *et al*., (2020) the evaluation of macroscopic gastric lesion using the percentage of ulceration was calculated applying the following formula:

% ulceration = (total ulcerated area / total mucosal area) × 100.

The total area of the lesions and the total area of the stomach was measured using Image J software.

II.9.4.2. Gastric ulcer induced by ethanol in animals pretreated with L-NAME, glibenclamide and indomethacin

Separate experiments were conducted to examine the roles of a non-selective inhibitor of nitric oxide synthase: Nw-nitro-L-arginine methyl ester hydrochloride (L-NAME), KATP channel blocker: Glibenclamide or a non-steroidal anti-inflammatory drug, which inhibits the PGE2 synthesis: Indomethacin.

According to Ribeiro *et al*., (2016), rats were fasted for 18 h with water ad libitum up to 1 h before the experiment were distributed into 8 groups of 5 animals each. Three groups were pretreated before ulcer induction with L-NAME (10 mg/kg IP.), glibenclamide (10 mg/kg IP.) or indomethacin (10 mg/kg IP.). The groups were pre-treated with these antagonists or inhibitor 30 min before receiving DEAA (200 mg/kg) by gavage. Two groups, the fourth group that received only DEAA (200 mg/kg) and the fifth group received only vehicle (10 mL/kg, p.o.)

were also included. Forty-five minutes later, all animals received absolute ethanol (4 mL/kg, p.o.) to induce ulcer. One hour after ethanol treatment, all animals were sacrificed.

a. Collection of the gastric juice and measurement of gastric acidity

After the animals were sacrificed, their stomachs were removed. The stomach contents were collected, centrifuged (1000 tr/min, 10 min) at 4C, and the pH values were determined using a digital pH meter.

b. Macroscopic gastric lesion evaluation

After collection of the gastric juice, the stomach of each animal and their stomach were removed, opened along the greater curvature, washed with cold saline, and then flattened stomach samples were photographed. The total area of the lesions and the total area of the stomach was measured using Image J software (Bouaziz *et al*., 2020).

The percentage of ulceration is calculated according to the following formula:

% ulceration = (total ulcerated area / total mucosal area) × 100.

c. Determination of adhered mucus to gastric wall

The modified method of Corne, (1974) was utilized for determination of gastric mucus. Segment of the glandular region of the stomach was weighed and transferred to a test tube containing 7 mL of 0.1% Alcian blue (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8). After two consecutive rinses with 5 mL of sucrose (0.25 M), 5 mL of $MgCl_2$ (0.5 M) was added in each test tube for the extraction of mucus content with the dye. The glandular segment remained in this solution for 2 h, with intermittent agitation. After that 4 mL of the resultant blue solution was agitated vigorously with 4 mL of ethyl ether until the formation of an emulsion and was centrifuged for 10 min at 3600 tr/min. The absorbance of the supernatant was measured at 598 nm using a spectrophotometer. The concentration of Alcian blue was calculated through a calibration curve (Figure 17) and the results were expressed in mg of Alcian blue/g of glandular tissue.

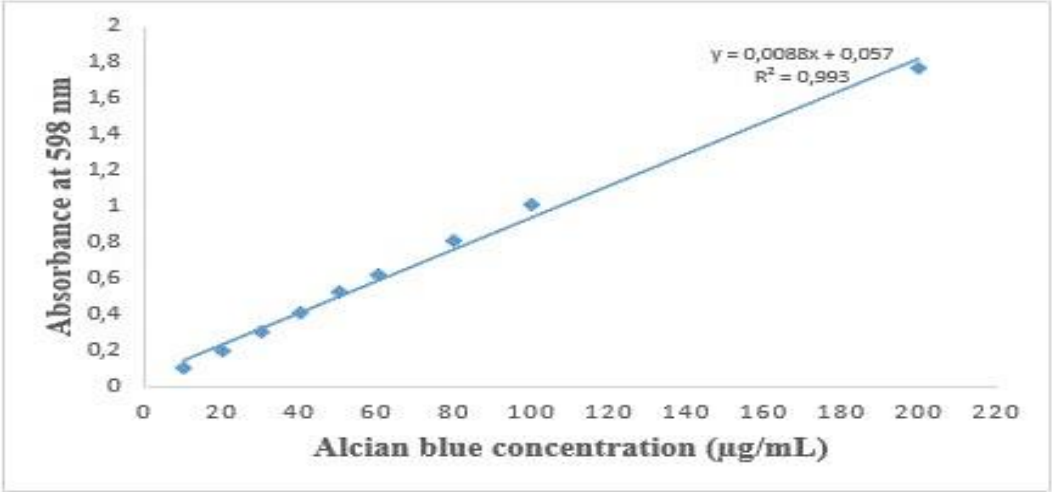

Figure 17: Standard curve of alcian blue for the determination of mucus in rat gastric tissue. The statistics were presented as mean with (n = 3).

d. Histopathological evaluation of gastric lesions

For histopathological examination, the tissues were fixed in 10% formalin solution. Then, the formalin-fixed stomach specimens were embedded in paraffin wax and serially sectioned (3–5 μm) and further stained with hematoxylin and eosin. The stained tissues were observed for pathological changes using light microscopy (Almasaudi *et al*., 2016).

e. *in vivo* antioxidant activity

i. Preparation of homogenate

Gastric tissue from treated and control rats were homogenate in lysis buffer (0.25 M sucrose, 0.05 M Tris-HCL, 1mM EDTA, pH 7.4). After 24 h incubation at -20° C, samples were centrifuged at 4,000 rpm for 15 min. The supernatant was collected and stored at -60°C until use (Pallotti and Lenaz, 2007).

ii. Total protein determination

Protein determination was carried out using the Bradford method (Bradford, 1976). The principle of this method is based on the adsorption of a dye (Coomassie Blue G_{250}) in an acidic methanolic medium. The dye adsorbs proteins, forming a complex with an adsorption peak those changes from red to blue. This adsorption takes place mainly via ionic bonds with basic amino acids (arginine, histidine and lysine) and hydrophobic interactions (hydrophobic amino acids).

iii. Standard range preparation and total protein assay

Protein assay was carried out using the Bradford method (Bradford, 1976). The experimental procedure involves mixing 20 µL of each sample with 3 mL of Bradford reagent. Tubes are gently shaken, then incubated for 5 min at room temperature. Absorbance is measured at 595nm. Beforehand, we made a standard range using Nine increasing concentrations of bovine serum albumin (BSA) ranging from 0 to 10 µg/µL prepared from a stock solution (1mg/mL). The total protein concentration in µg/µl was deduced from the equation of the straight line given by the BSA standard range illustrated in Figure 18.

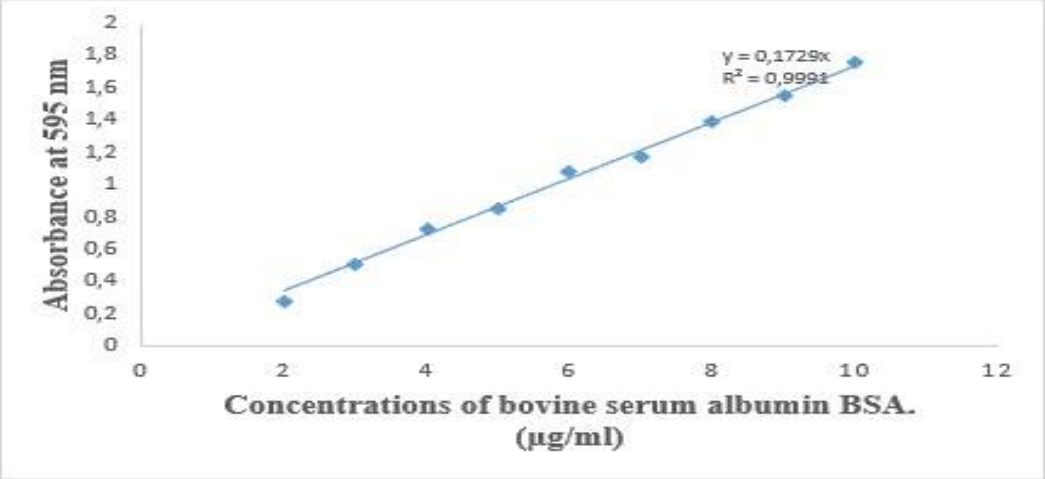

Figure 18: Standard curve of bovine serum albumin (BSA) for the determination of total protein. The statistics were presented as mean with (n = 3).

iv. Determination of superoxide dismutase (SOD) activity

According to Misra and Fridovich, (1972), adrenaline in the presence of superoxide anion O_2 •- spontaneously oxidizes to adrenochrome which is a colored compound that absorbs at 480 nm. SOD, whose role is to reduce the O_2 anion •- inhibits this reaction (Polle *et al*., 1989).

The determination of the SOD activity consists of mixing 640 µL of sodium carbonate (Na_2CO_3 (0.05M, pH=10.2) with 160 µL of adrenaline. This tube is used to set the spectrophotometer zero to 480nm. A volume of 500 µg of total protein is added. The optical density is measured every 30 seconds for 10 min.

A unit of activity of SOD is defined as the amount of enzyme capable of inhibiting the formation of adrenochrome by 50%. The results are then expressed in IU/min/µg of total protein.

v. Assessment of catalase activity (CAT)

Catalase is a hemical redox that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen (Aebi, 1974). It is mainly present in peroxisomes, but also in mitochondria and cytoplasms. The volume equivalent to 500 µg of total protein is mixed with 500 µL

phosphate buffer (PBS). This mixture is used to set the spectrometer zero to 240 nm. The reaction is then triggered by adding to the previous mixture 100 µL of H_2O_2 (200mmol/l) and the DO reading was performed every 15 seconds for 2 minutes. Catalase activity is then measured using the following formula:

CAT activity in µmol/seconds/µg protein = absorbance × (dilution factor) / (duration in seconds × molar extinction coefficient (H_2O_2) × amount of total protein in µg).

vi. Measurement of lipid peroxidation (MDA).

Tissue malondialdehyde (MDA) (mmol/L) was determined using the double heating method of Draper and Hadley, (1990). A reaction mixture containing 8.1% sodium dodecyl sulfate, 20% acetate buffer (pH 3.5) and 0.8% thiobarbituric acid (TBA) was mixed well with 500 µg of total protein for 3 min and then incubated at 95°C for 60 min. After chilling, the TBA-reactive substance (MDA) was extracted with 1 ml of H_2O and 2.5 ml of an n-butanol: pyridine mixture (15:1, v/v). The upper organic layer containing the MDA, which was produced by lipid peroxidation, was measured at 532 nm. The concentration of MDA was calculated using the molar extinction coefficient (ε MDA-TBA), The results were expressed as nmole of MDA/ g of total protein.

II.10. Statistical analysis

Statistical comparisons were performed using Graph Pad Prism (version 7.00 for Windows). using one-way analysis of variance (ANOVA) followed by Dunnet's and t test of student. For *in vitro* experiments, the statistics were presented as mean ± standard deviation (SD) with (n = 3), and as mean ± standard error of the mean (SEM) with (n = 5) for *in vivo* experiments with (n = 5) when the p-value was less than 0.05.

Results

I. Ethnobotanical survey of El-Mergueb natural reserve

I.1. Demographic features of the informants

For all interviewers, gender, age, profession, and background information were recorded. According to Table 1, the local informants (n = 182) comprised 69% women and 31% men were interviewed.

The age group that formed the majority of informants was the 51 years-60 years age group contributing 37%, followed by the 36 years-50 years and informants over 60 years. The highest age respondents provide more reliable information because they hold much of the ancestral knowledge that is part of the oral tradition. The least participative age group was the 20 years35 years which had 9%.

Users of medicinal herbs with no educational background accounted for 30% of the total (Table 1), while those with a university education accounted for 21%. Those with only a primary, middle, or secondary education account for 13%, 17%, and 19% of the population, respectively.

The majority of ethnobotanical knowledge came from family, with 65 percent being passed down through generations. The rest were herbalists (20%) and scientific research (15%).

Table 1: Demographic profile of informants interviewed (N = 182).

Variables	Categories	Total	Percentage (%)
Gender	Women Men	126 56	69% 31%
Age	[20years-35years] [36years-50years] [51years-60years] >60years	17 51 67 47	9% 28% 37% 26%
Educational level	Illiterate Primary College Secondary University	15 24 31 38 74	30% 13% 17% 19% 21%
Origin of ethnobotanical knowledge	Family knowledge Herbalist Scientific research.	118 37 27	65% 20% 15%

I.2. Diversity of medicinal plants

The research area's floristic diversity, as well as a renewed interest in phytotherapy encouraged us to identify the therapeutic plants of El Mergueb Natural Reserve. An ethnobotanical survey and analysis of the flora of the research region were conducted in this area. A total of 46 taxa belonging to 23 different families were recorded (Figure 20 and Table 2). All of the species mentioned are spontaneous, according to the flora of Algeria (Quézel and Santa, 1962), with the exception of the following species *Pinus halepensis* Mill., *Pistacia atlantica* Desf., *Pistacia lentiscus* L., *Nerium oleander* L., *Juniperus phoenicea* L., *Eucalyptus globulus* Labill., *Avena sativa* L., *Hordeum vulgare* L., *Triticum vulgare* L. and *Populus nigra* L. which are cultivated. As illustrated in Figure 19, the most represented families were Asteraceae with 9 medicinal plant species, followed by Poaceae (6 species) and Lamiaceae (4 species). This high proportion could be explained by the high representation of these families in El-Mergueb Natural Reserve flora because of the ecological factors that favour the development and adaptation of the majority of their species.

As seen in Table 2 species with high used values (UV) were *Artemisia herba-alba* Asso (0.58), *Stipa tenacissima* L. (0.42), *Artemisia campestris* L. (0.3), *Zyziphus lotus* (UV = 0.16), *Marrubium vulgare* L. (UV = 0.14). This means that these species are the most important medicinal plants used in folk medicine by the population of El Mergueb to treat ailments, which might be due to their vast distribution in the area study.

Thapsia garganica L., *Nerium oleander* L., *Colocynthis vulgaris* L., *Retama retam* Webb., *Asparagus stipularis* L., *Thymelaea hirsute* L. and *Peganum harmala* L. are the poisonous plants identified by the interwieur

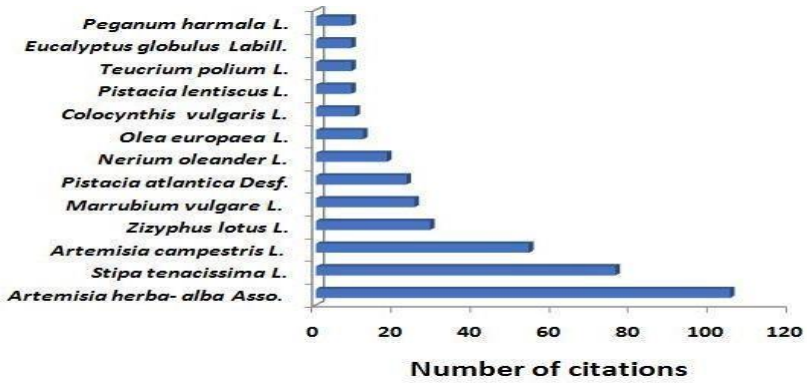

Figure 19: The most commonly cited medicinal plants of the study area.

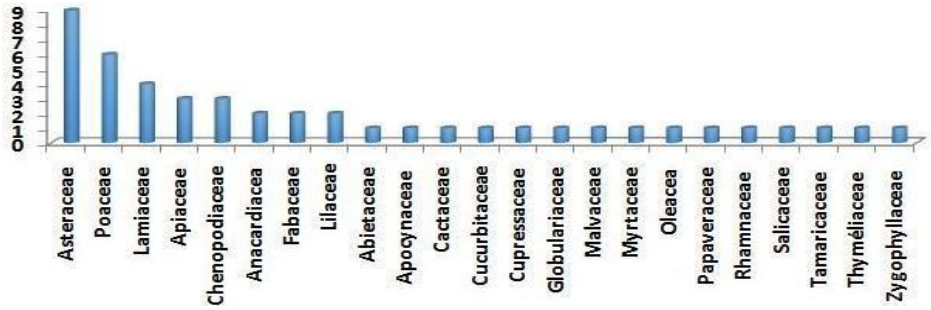

Figure 20: Number of total species per botanical family.

Table 2: Medicinal flora of El Mergueb natural reserve.

Scientific name	Vernacular name	Part used	Mode of use	Therapeutic property	S/C/T	UV
Abietaceae *Pinus halepensis* Mill.	الصنوبر الحلبي Esanaouber Alhalabi	Seeds	Cataplasm	Respiratory diseases rheumatic diseases hemorrhoids	C	0.04
Anacardiaceae *Pistacia atlantica* Desf.	البطمة El Batma	Leaves fruits roots	Infusion decoction	Digestive diseases respiratory diseases eye diseases Antitussif antipyretic	C	0.13
Pistacia lentiscus L.	الضّرو Edharou	Leaves Fruits roots	Infusion, decoction	Antiseptic expectorant diuretic	C	0.05
Apiaceae						
Bunium bulbocastanum L.	تالغودة Talghouda	Fruits	Powder, Decoction	Intestinal gas thrombosis worms	S	0.02
Thapsia garganica L.	بونافع Bounafaa	Aerial parts Roots	Cataplasm, Decoction	Rheumatic pain bronchitis sprains	S /T	0.03
Pituranthos chloranthus L.	القزيح El Kezzih	Leaves Flowers	Infusion	Indigestion abdominal pain	S	0.02
Apocynaceae *Nerium oleander* L.	الدفلة Eddafla	Aerial parts	Fumigationpowder	Respiratory disease heart disease bone pain eczema	C /T	0.10
Asteraceae *Anthemis arvensis* L.	البابونج ElBabounedj	flowers	Decoction	Stomach pain urinary tract infections. Menstruation	S	0.03

Anvillea radiata L.	النقد Ennakd	Aerial parts	Infusion	Abdominal pain female genital infections	S	0.02
Artemisia absinthium L.	شجرة مريم Chadjeret Meriem	Leaves flowers	Decoction	Digestive disorders gastric ulcer deworming	S	0.03
Artemisia campestris L.	التقفت Ettgouft	Leaves	Infusion powder cataplasm.	Digestive disorders menstruation pain antihemorrhagic snake bites	S	0.3
Artemisia herba-alba Asso.	الشيح Echih	Aerial parts roots	Infusion, decoction, powder and cataplasm	gastric ulcer anti-diarrheic anti-spasmodic, indigestions deworming	S	0.58
Inula viscosa L.	أمقرمان Amegraman	Leaves	Powder and cataplasm	Rheumatic pain anti-septic healing fractures deworming	S	0.04
Scolymus hispanicus L.	قرنينة Garnina	Leaves	Decoction	Liver and bowel diseases	S	0.04
Scorzonera laciniata L.	تالمة Talma	Leaves roots	Infusion	Abdominal pain intestinal gas.	S	0.02
Scorzonera undulate L	قيز guiz	Aerial parts roots	Infusion	Against snakebites.	S	0.02
Cactaceae						
Opuntia ficusindica L	الهندي ElHendi	Petals stem fruits	Cataplasm	Antiteigne against dermatosis	S	0.03
Chenopodiaceae						
Atriplex halimus L.	القطف El Gtaf	Leaves fruits	Decoction	Dry wounds diuretic	S	0.03
Anabasis articulata	العجرم El Adjrem	Aerial parts	plaster and infusion.	Camel's Scab Gale antidiabetic	S	0.04
Arthrophytums coparium L.	الرمث Erramth	Aerial parts	Decoction and cataplasm	Indigestion and stings of scorpion and dermatoses.	S	0.03
Cucurbitaceae						
Colocynthis vulgaris L.	الحدج El Hadj	Fruits	Decoction and pomade	Rheumatic pain against hepatitis.	S /T	0.06

Cupressaceae *Juniperus phoenicea* L.	العرعار ElAraar	Aerial parts	Infusion	Antiparasitic Antiseptic Eczema abdominal pain	C	0.03
Fabaceae *Retama raetam* Webb.	الرتم Ertem	Aerial parts	Infusion	Healing against eye irritation, antidiarrhea abdominal pain Antirheumatic	S /T	0.04
Medicago minima L.	الحسكة El haska	Fruits Leaves	Powder decoction	Mouth and bladder diseases	S	0.02
Globulariaceae *Globularia alypum* L.	تسلغة Tassalgha	Aerial parts	Infusion powder	Anti-gastralgic against wounds pains of menstruation	S	0.02
Lamiaceae *Ajuga iva* L.	شندقورة Chandgoura	Aerial parts	Infusion	Antiseptic, antirheumatic regulatation of the menstrual cycle.	S	0.03
Marrubium vulgare L.	تيمريوت Timerriout	Leaves	Infusion decoction	Expectorant, Diuretic Antiseptic	S	0.14
Mentha pulegium L.	الفليو Elfliou	Aerial parts	Decoction	Against sunburn allergy of the nose	S	0.03
Teucrium polium L.	الجعيدة Eljaada	Aerial parts	Infusion	Hypoglycemic, Abdominal, pain colic stomach ulcer	S	0.05
Lilaceae *Asparagus stipularis* L.	عنب الذئب Ain Eddib	Aerial parts	Decoction	Antispasmodic,analgesic, sedative, emollient	S /T	0.04
Asphodelus microcarpus L.	البروا ق Elborouag	Leaves	Decoction cataplasm	Diuretic, antirheumatic.	S	0.02
Malvaceae *Malva parviflora* L.	الخبيز Elkhobiz	Aerial parts roots	Infusion	Antiseptic constipation hemorrhoids headaches	S	0.04

Myrtaceae *Eucalyptus globulus* Labill.	الكاليتوس ElKalitous	Leaves	Fumigation	anti-infectious, antiseptic asthma	C	0.05
Oleacea *Olea europaea L.*	الزيتون Azzaitoun	Leaves, Fruits andHuile	Infusion	Diuretic Hyperglycemea hypertension	S	0.07
Papaveraceae *Papaver rhoeas* L.	بن نعمان Ben Naman	Flowers	Infusion	Eczem Migraines digestive disorders inflammation.	S	0.03
Poaceae						
Avena stiva L.	الخرطال Khourtal	Seeds	Powder	Diabetes liver abscesses diuretic	C	0.03
Hordeum vulgare L.	الشعير Echair	Seeds	Powder	kidney stones diabetes stomach ache rheumatism	C	0.02
Stipa tenacissima L.	الحلفاء El Halfa	Leaves	Infusion	Chronic scalp ulcers diabetes	S	0.42
Stipa capensis L.	الصمعاء Essameaa	Leaves	Infusion	Digestive problems antiseptic	S	0.03
Triticum repens L.	النجم Ennajm	Rhizome	Decoction	Abdominal pain liver and rat diseases vomiting	S	0.02
Triticum vulgare L. ou Triticum aestivum L.	القمح Elkamh	Seeds	Powder	asthenia anemia convalescence stomach pain	C	0.03
Rhamnaceae *Zizyphus lotus* L.	السدر ة Essidra	Leaves fruits roots	Decoction	Sedative Diuretic	S	0.16
Salicaceae *Populus nigra* L.	الصفصاف Essafsaf	Aerial parts roots	Decoction powder	Respiratory pathology urinary tract pathology	C	0.02

Tamaricaceae *Tamarix aphylla* L.	الطرفة Etarfa	Leaves twigs	Decoction	Fungicide digestive problem	S	0.04
Thyméliaceae *Thymelaea hirsute L.*	المثنان Elmathnan	Leaves	Infusion	kidney stones laxative	S / T	0.03
Zygophyllaceae *Peganum harmala L.*	الحرمل ElHarmel	Aerial parts	Decoction pomade Fumigation	Digestive problems Antirheumatic Fever	S / T	0.05

I.3. Preparation methods

Several medicinal plants preparation methods are used in traditional medicine to fight the different kind of ailments such as decoction, infusion, grinding, maceration, powder and cataplasm. Data analysis showed that infusion is the most common preparation method used by the population studied (42.6%), followed by decoction (27.4%), powder (13%), cataplasm 10%, fumigation (4.4%), pomade (1.6%), and plaster (1%) (Figure 21).

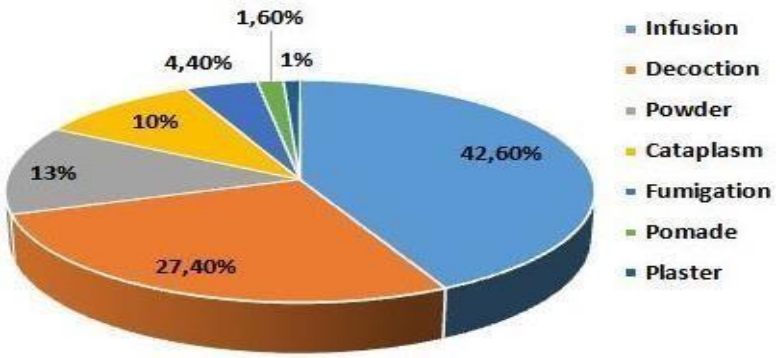

Figure 21: Modes of preparation.

I.4. Parts of the medicinal plants used

Almost all different parts of plants were reported to be used as a drug by the traditional healers and local people as illustrated in Figure 22, but leaves were the most common (39.6%), followed by the aerial part (25.6%), roots (17.4%), fruits (7.3%), seeds (4%), flowers (2.5%), oil (1.2%), twigs (1%), rhizome (0.5%), petals (0.5%) and 0.2% for stems.

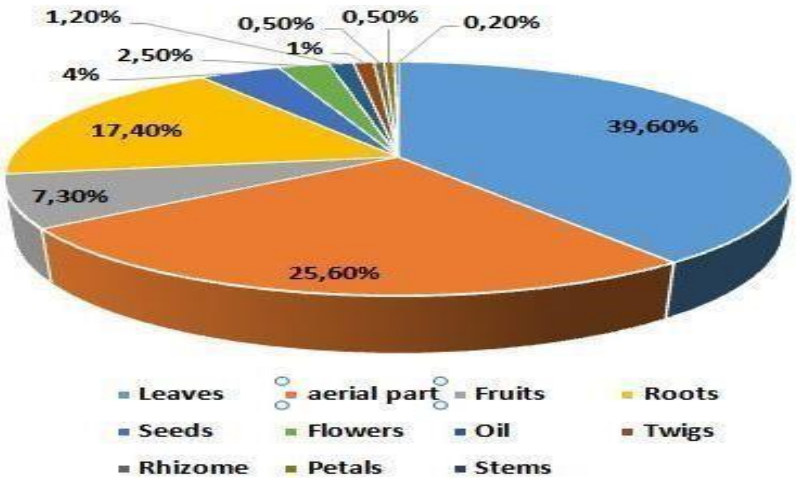

Figure 22: Distribution of the parts of the plant used.

I.5. Ethnomedicinal survey of *A. articulata*

The Ethnopharmacological survey was carried out using a questionnaire to determine the different medicinal uses of this plant. In Table 3, the survey's findings are displayed.

Table 3: Ethno-medicinal survey data of therapeutic use, part used plant and mode of preparation of *A. articulata.*

Data information	Description	Number of citations	Frequency (%)
Scientific name	*Anabasis articulata* (Forssk) Moq.	None	None
Nomenclature name	Ajrem Echannan	118 65	100 55.08
Therapeutic use	Rheumatism Diabetes Dermatosis Abdominal pain	73 51 33 12	61.86 43.22 27.96 10.16
Part of plant used	Aerial part Root flowers and leaves Stems	19 66 81 9	13.55 55.93 68.64 7.62
Mode of preparation	Decoction Cataplasm	63 106	53.38 89.83

II. Quantitative phytochemical analysis

II.1. Extract yields, total phenolic, total flavonoids and total tannins contents

Table 4 illustrates the preliminarily phytochemical analysis of several extracts of *A. Articulata*. The yields of the various fraction extracts ranged from 7.6 to 42.2% for the chloroform fraction, ethyl acetate fraction, ethanolic extract, n-butanol fraction, and aqueous fraction, respectively.

When the yields of the different extracts were compared, the chloroform fraction had the lowest extraction yield (7.6%). While the aqueous fraction had the highest yield (42.2%).

Table 4: Yield, Total phenolics content, total flavonoids content and total tannins content of the fractions of *Anabasis articulata.*

Extract	Yield (%)	TPC (mg GAE/gDE)	TFC (mg QE/gDE)	TTC (mg TAE/gDE)
DEAA	10.7	212.0±0,565	13.01 ±0.055	92.18±1.442
EEAA	15.5	396.33± 0.556	21.69± 0.154	112.49 ± 0.184
ChFA	7.6	497.98±0.377	79.89±0.789	140.19±2.135
EAFA	10.6	325.14±0.498	25.10±0.795	117.01±1.740
nBFA	29.1	404.15±0.958	44.48±0.933	162.89±2.103
AqFA	42.2	178.23±1.049	7.50±0.277	58.58±1.607

Values expressed as means±SD. of three parallel measurements. TPC: Total phenolic content. TFC: Total flavonoids content. TTC: Total tannins content. GAE: Gallic acid equivalent. QE: Quercetin equivalent. TAE: Tannic acid equivalent. DEAA: decoction extract of *Anabasis articulata.* EEAA: ethanolic extract of *Anabasis articulata.* ChFA: Chloroform fraction. EAFA: Ethyl Acetate action. nBFA: n-Butanol fraction. AqFA: aqueous fraction.

Total phenolic component content in fraction extracts ranged from 178.23 mg to 497.98 mg/g, as estimated by the calibration curve regression equation (y = 0.0081x - 0.0317) and expressed in gallic acid equivalents (GAE). The extracts' flavonoid content (mg/gDE), as estimated by the regression equation of the calibration curve (y = 0.038x - 0.0336) and expressed in Quercetin equivalents, ranged from 7.50 to 79.89 mg/g. The extracts' tannin concentration (mg/gDE) ranged from 58.58 to 162.89 mg/gDE, as estimated by the calibration curve's regression equation (y = 0.0083x + 0.136) and expressed in tannic acid equivalents.

The results given in Table 3 showed that chloroform fraction indicating highest content for total phenolics and flavonoids (497.98±0.377 and 79.89±0.789 mg/gDE, respectively). n-butanol fraction reported the highest content for tannins 162.89±2.103 mg/gDE.

II.2. Liquid Chromatography-Mass Spectrometry Analysis LC-MS-MS

Table 5 displays the phenolic chemicals that LC-MS-MS determined for the *A. articulata* fractions. The pics of molecules identified in fractions were illustrated in Figure 23. Anthrone, Beta carotene, Butyledhydroxyanisole (BHA), Butylatedhydroxytoluene (BHT), Gallic acid, Myricetin and Rutin were the biomolecules detected.

Table 5: LC-MS-MS-determined phenolic compounds of the fractions of *A. articulata.*

Coumpond name	Charge +/-	Precursor m/z	Product ion m/z	ChFA	EAFA	nBFA
Anthrone	+	195.1500	177.200 165.250	ND	D	ND
Beta carotene	+	537.200	282.500 199.25	D	D	D
Butyled hydroxyanisole BHA	+	181.100	99.15 81.05	ND	D	D
Butylated hydroxytoluene BHT	+	221	161.3 203.25	D	D	D
Gallic acid	-	168.800	125.100	D	D	D
Myricetin	+	46.15	336.2	D	D	D
Rutin	+	611.200	73.200 282.200	D	D	D

D: detected, ND: not detected,ChFA :Chloroform fraction, EAFA : Ethyl Acetate fraction,nBFA: n-Butanol fraction

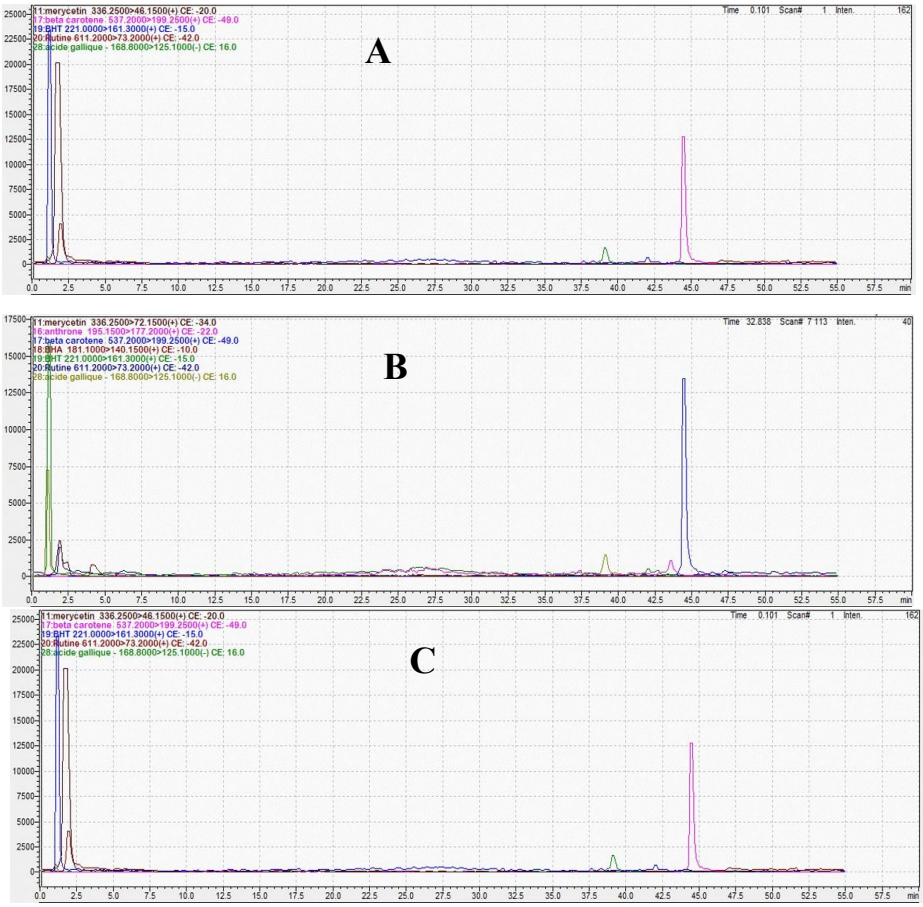

Figure 23: LC/MS-MS chromatograms obtained in the multiple reaction monitoring (MRM) mode of polyphenols detected in the fractions of *A. articulata*. **A:** Chloroform fraction. **B:** Ethyl Acetate fraction. **C:** n-Butanol fraction.

III. *In vitro* antioxidant estimation

In this study eight colorimetric methods wich are: TAC, DPPH•, $ABTS^{+}$, Hydroxyl radical, hydrogen peroxide, Ferrous ion chelating activity, Reducing power and β-carotene tests are used to evaluate the antioxidant capacity of *A. articulata* extract. The results from the antioxidant activity evaluation are presented in Table 06, Figure 24, 25 and 26.

The Total antioxidant capacity TAC of plant extracts was expressed to evaluate the total antioxidant capacity. The phosphomolybdenum approach was based on the antioxidant component reducing molybdenum (VI) to molybdenum (V) and forming a green phosphate/Mo (V) combination. The Chloroform fraction (ChFA) revealed the highest total antioxidant capacity (EC_{50}=0.150±0.0017mg/mL) while the lowest capacity was found in AqFA (EC_{50}=0.495±0.0068mg/mL). For the other extracts, the values of EC_{50} were 0.182±0.0041, 0.202±0.0015 and 0.344±0.0037mg/mL for nBFA, EAFA and EEAA, respectively. The EC_{50} of the standard Vit C was 0.002±0.003mg/mL.

DPPH radical scavenging test measures the ability of antioxidants to scavenge the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH), which reacts with hydrogen or electron donors to form the yellow-colored diphenyl picrylhydrazine. The findings demonstrated that each extract had a significant amount of DPPH radical-scavenging activity. The best free radical scavenging activity were exerted by EEAA with IC_{50}= 0.074±0.003mg/mL, in the second position ChFA with (0.082±0.0012) had a similar radical scavenging activity compared to nBFA with IC_{50}=0.083±0.0016mg/mL, while an IC_{50} of 0.026±0.0006mg/mL for the standard.

In ABTS radical scavenging activity, nBFA and EEAA had stongest ABTS radical scavenging activity with a comparable value of IC_{50} (0.012±0.0004; 0.016±0.0002mg/mL respectively) with the IC_{50} of the standard BHT 0.015±0.0003mg/mL. ChFA EAFA in the second place followed by DEAA and AqFA with an IC_{50} of 0.021±0.0007, 0.022±0.0008, 0.036±0.0008 and 0.039±0.0006mg/mL respectively.

The assessment of the hydroxyl radical scavenging activity showed an important anti-radical effect as follows in a decreasing order: ChFA (IC_{50} = 0.361±0.0087 mg/mL) >nBFA (IC_{50} = 0.422±0.0032 mg/mL) > EAFA (IC_{50} = 0.509±0.0278 mg/mL) >AqFA (IC_{50} = 0.782±0.0092 mg/mL) >EEAA (IC_{50} = 0.802±0.006mg/mL).

Hydrogen peroxide scavenging activity, in phenanthroline assay, the antioxidant activity of ChFA (EC_{50}=0.361±0.0087mg/mL) was similar to that in nBFA and in EAFA (EC_{50}=0.422±0.0032; EC_{50}=0.509±0.0278mg/mL, respectively), and highest than DEAA, AqFA and EEAA compared to the Vit C (0.010 ± 0.0004mg/mL).

Table 6: Antioxidant capacities using TAC (mg/mL), DPPH (mg/mL), ABTS (mg/mL), Hydroxyl radical scavenging (mg/mL) and hydrogen peroxide scavenging of the fractions of *Anabasis articulata.*

Extract/ Standard	EC_{50} (mg/mL)	IC_{50} (mg/mL)	IC_{50} (mg/mL)	EC_{50} (mg/mL)	IC_{50} (mg/mL)
	Total antioxidant capacity	**DPPH radical scavenging activity**	**ABTS radical scavenging activity**	**Hydroxyl radical scavenging activity**	**hydrogen peroxide scavenging activity**
DEAA	0.409±0.006****	0.170±0.0006****	0.036±0.0008****	0.769±0.0007****	3.374±0.051****
EEAA	0.344±0.004****	0.074±0.003****	0.016±0.0002^{ns}	0.802±0.006****	2.124±0.086****
ChFA	0.151±0.0017****	0.082±0.0012****	0.021±0.0007****	0.361±0.0087****	1.777±0.0402****
EAFA	0.202±0.0015****	0.119±0.0044****	0.022±0.0008****	0.509±0.0278****	2.446±0.0230****
nBfa	0.182±0.0041****	0.083±0.0016****	0.012±0.0004***	0.422±0.0032****	2.071±0.0218****
AqFA	0.496±0.0068****	0.438±0.0088****	0.039±0.0006****	0.782±0.0092****	5.163±0.0190****
Vit C	0.002±0.003	-	-	0.010 ± 0.0004	0.017 ± 0.0013
BHT	-	0.026±0.0006	0.015±0.0003	-	-

Results were expressed as means + SD, n=3. **** p< 0,0001, the comparison was realized against correspondent standard. DEAA: decoction extract of Anabasis articulata. EEAA: ethanolic extract of Anabasis articulata. ChFA: Chloroform fraction. EAFA: Ethyl Acetate fraction. nBFA: n-Butanol fraction. AqFA: aqueous fraction.

In Ferrous ion chelating activity there is a similarity between metal-chelating activities of EAFA and decoction extract 0.014±0.002 and 0.073±0.002mg/mL. The activity of these two extracts were higher than nBFA, ChFA, AqFA and EEAA (0.19±0.008, 1.11±0.03, 1.4±0.004,

2.29±0.11mg/mL, respectively) the comparison was done with the EDTA as standard (0.003±0.0006mg/mL.).

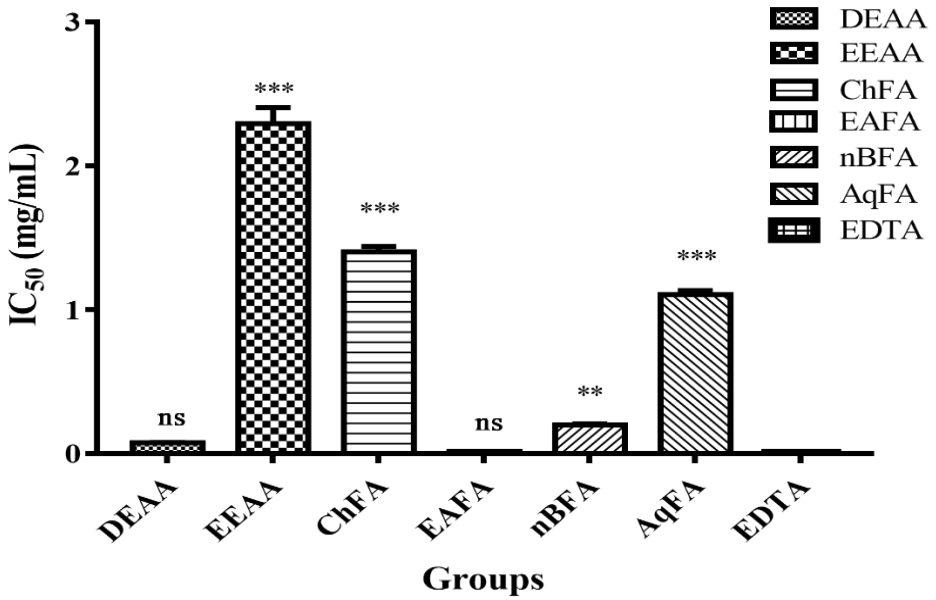

Figure 24: Ferrous ion chelating activity of plants extract of *Anabasis articulata* compared to EDTA. Results were expressed as means + SD, n=3. *** p< 0,001, ** p< 0,01 and ns: no significant.

In Reducing power, EAFA and ChFA with an IC_{50} of 0.188±0.0008 and 0,314±0.016mg/mL exhibited a best reducing power compared to the BHT as a standard 0.0556±0.006mg/mL. A similar value of IC_{50} of nBFA and EEAA (0.457±0.0256 and 0.497±0.024mg/mL) followed by AqFA with 0.6910±0.039mg/mL and DEAA with 0.9±0.014mg/mL.

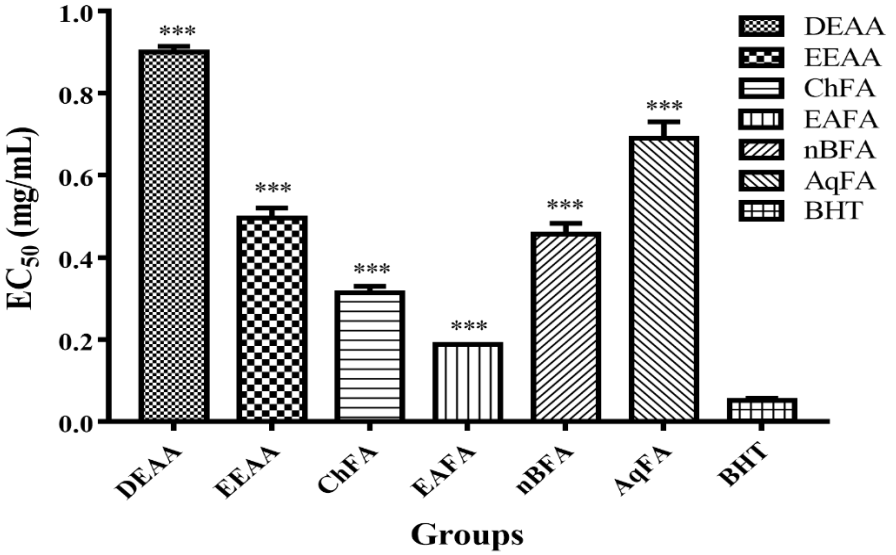

Figure 25: Reducing power activities of plants extract of *Anabasis articulata* compared to BHT. Results were expressed as means + SD, n=3. *** p< 0.001, ** p< 0.01, ns: no significant.

In β-carotene bleaching, all extracts compared to the standard BHT (93.18±1.743%) exhibited a significant antioxidant activity as represented in Figure 26. EAFA, nBFA and AqFA are the best inhibitors of β-carotene oxidation within 24 hours (85.19±0.803, 85.12±0.793 and

83.93±1.271%), followed by ChFA, EEAA and DEAA; 80.590±1.032, 79.8954±0.447 and 79.834±1.033%, respectively.

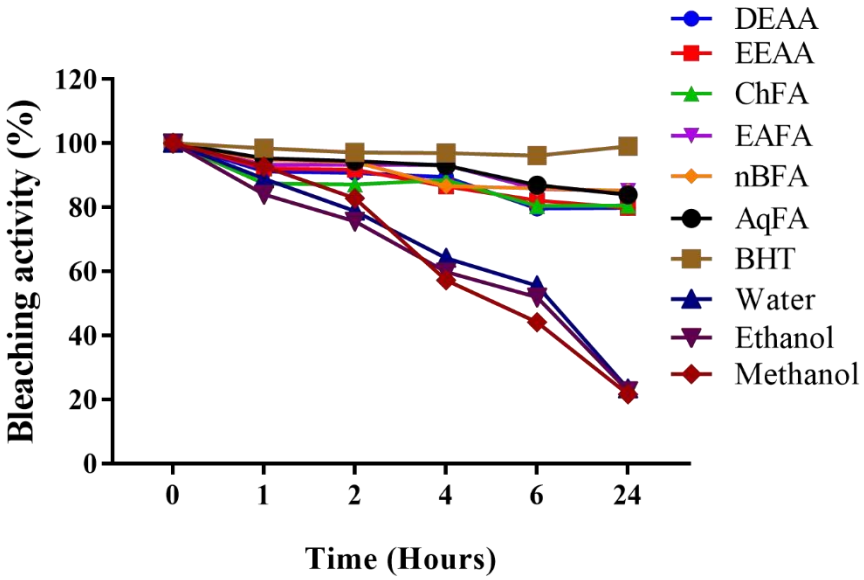

Figure 26: Kinetics of B-carotene bleaching activity (%) in the presence of DEAA: decoction extract of anabasis articulata, EEAA: ethanolic extract of anabasis articulata, ChFA: chloroform fraction, EAFA: Ethyl Acetate fraction, nBFA: n-butanol fraction, Aqueous fraction of Anabasis articulata, water, ethanol, methanol and BHT butylated hydroxytoluene during 24H. BHT was used as antioxidant reference.

IV. Estimation of *in vitro* anti-inflammatory activity

In vitro anti-inflammatory effect of extracts was evaluated utilizing egg albumin denaturation and denaturation of Bovine Serum Albumin assays with Aspirin serving as the reference drug in each case. The results are summarized in Figure 27 and 28.

The results revealed that when compared to the common anti-inflammatory medication aspirin, all extracts demonstrated a good inhibition of protein denaturation in both test in a concentration-dependent way. The percentage of inhibition was within the range from 29.71% to 92.17 % at the concentration range of 0.0625to 0.5mg/ml for egg albumin denaturation test (Figure 27) and from 59.18% to 86.31 % at the range of concentration of 0.5 to 5 mg/ml for BSA denaturation test (Figure 28). Aspirin as reference drug exhibited a higher inhibition capacity.

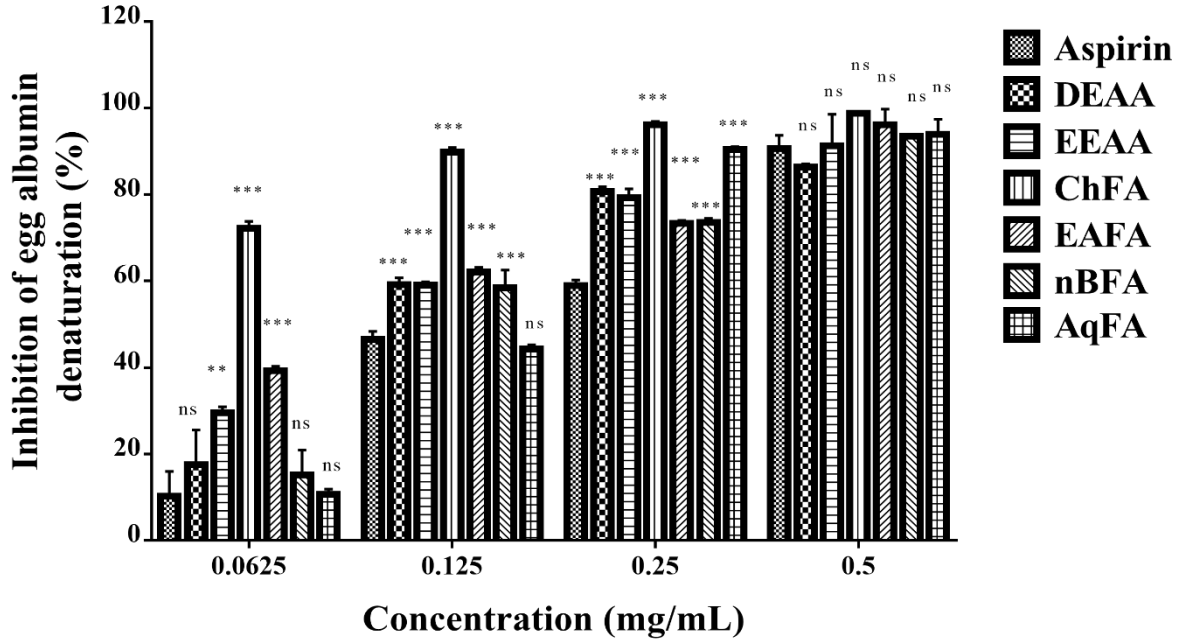

Figure 27: Egg albumin denaturation test of extracts compared to Aspirin. Values are means±SD with n=3. ns: no significant difference, ** P<0.01, *** P<0.001.

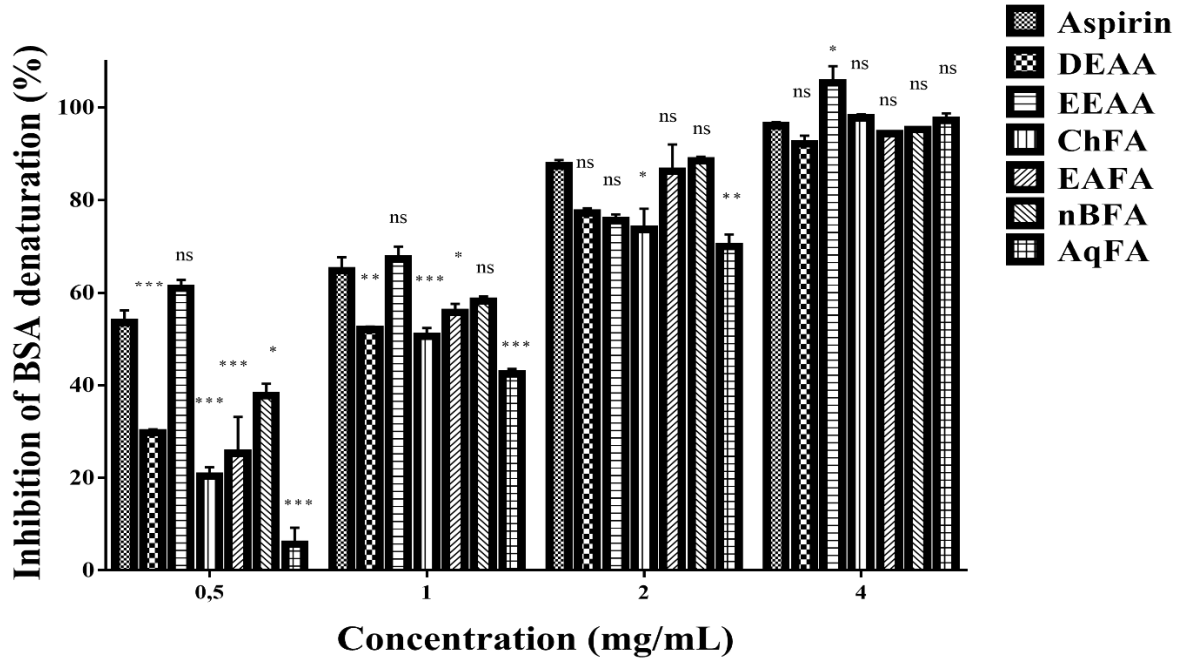

Figure 28: Anti-inflammatory test *in vitro* denaturation of bovine serum Albumin assay compared to Aspirin. Values are means±SD with n=3. ns: no significant difference, ** P<0.01, *** P<0.001.

V. Estimation of *in silico* activity

Molecular docking stud were carried out using AutoDock Vina program to determine whether the seven compounds detected provided compared to the usual (Indomethacin) an antiinflammatory impact.

Figure 29 shows the 3 Dimension structure of the inflammatory protein COX_2 while Figure30 and 31 illustrated the result of molecular docking identified by 2 Dimension and 3 Dimension detailed binding site of each ligand into the receptor. The result allowed us to determine the binding energy between the protein and various positions of the ligand. A negative value of the binding energy suggests a likelihood of binding between the ligand and the receptor.

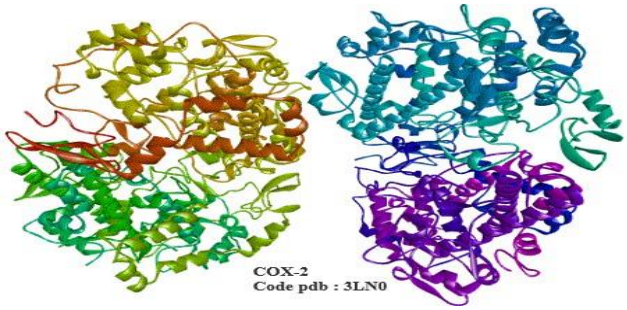

Figure 29: 3D structure of the inflammatory protein COX_2 [PDB ID: 3LN0].

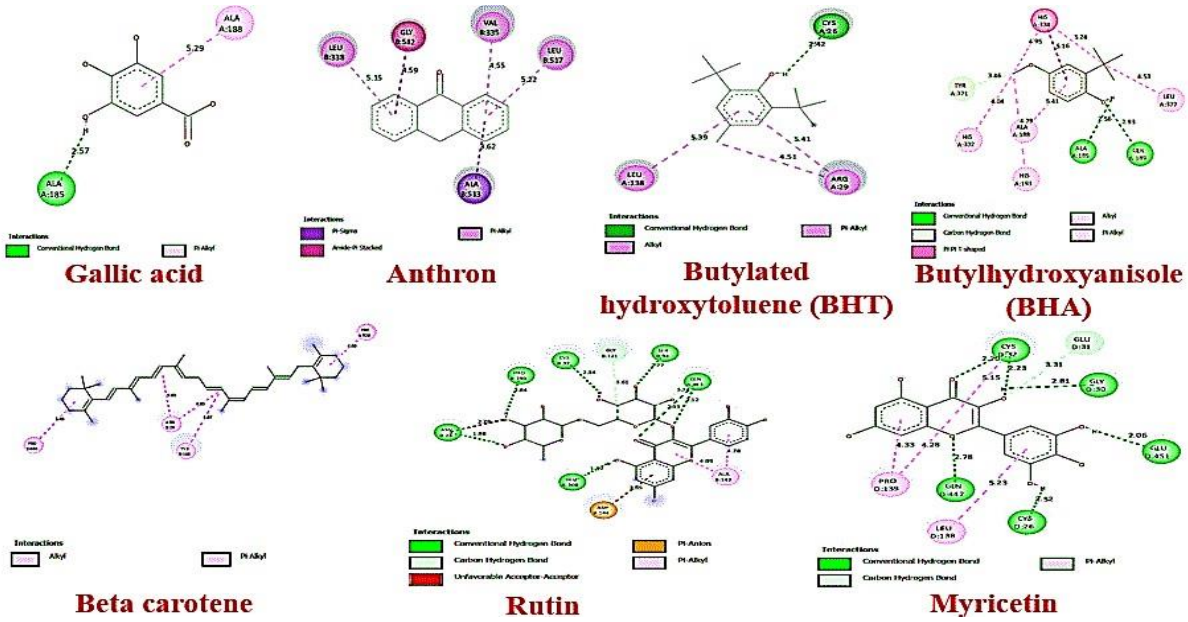

Figure 30: 2D detailed binding site of each ligand into the receptor [PDB ID: 3LN0].

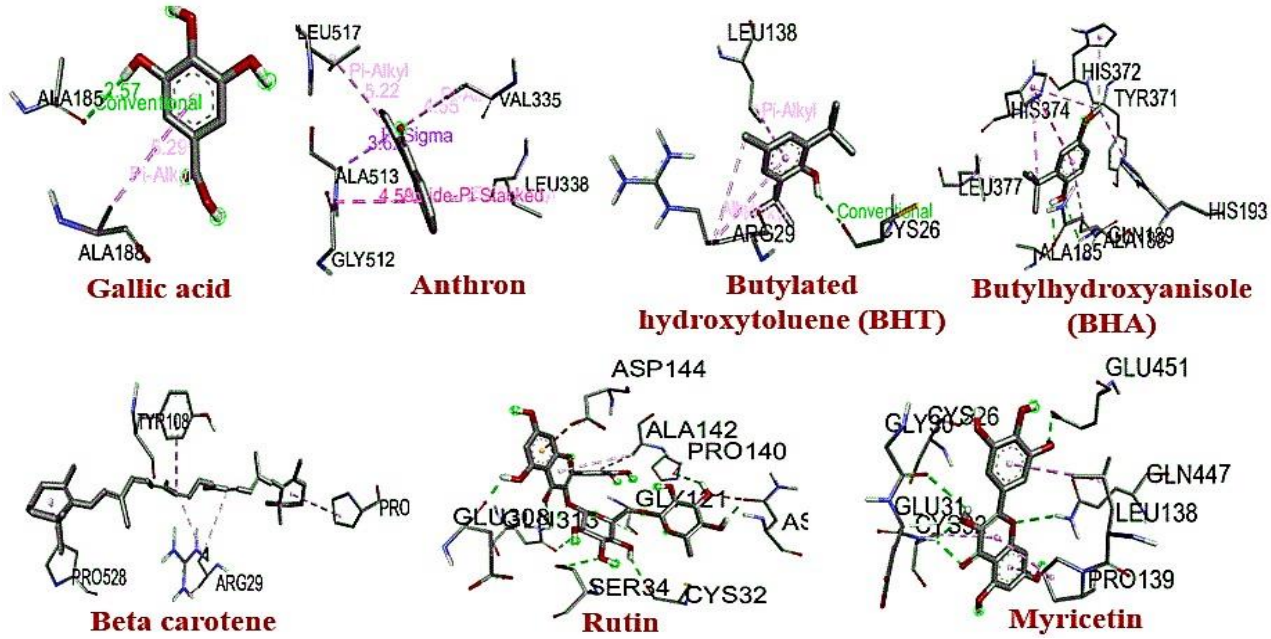

Figure 31: 3D detailed binding site of each ligand into the receptor [PDB ID: 3LN0].

Table 7 gathers the results obtained from molecular docking regarding the binding energy and inhibition constant for the 7 bio-compounds that showed interaction with the protein. We notice that the two ligands named Rutin and Myricetin have the best affinity, with values of -10.4 kcal.Mol^{-1} and -9.9 kcal.Mol^{-1}, respectively, as well as a low inhibition constant of 0.023 µM and 0.055 µM, respectively. Additionally, we performed molecular docking using a commercialized drug named: Indomethacin. According to Table 7, we observe that Beta carotene, Myricetin, and Rutin have shown favorable results compared to the drug (Indomethacin).

Table 7: Result of affinity and inhibition constant of ligands with the same protein.

	Anti-inflammatoryactivity	
	Protein: COX_2 (3LN0)	
Biomolecules	ΔG (kcal.Mol^{-1})	Ki (µM)
Gallicacid	-6.5	17.19
Anthron	-8.6	0.49
Beta carotene	-8.8	0.35
Butylatedhydroxytoluene (BHT)	-7.4	3.76
Butylhydroxyanisole (BHA)	-6.9	8.75
Myricetin	-9.9	0.055
Rutin	-10.4	0.023
Indometacine (standard)	-8.7	0.42

VI. *In vivo* investigation

V.1. Acute Toxicity Study

V.1.1. Mortality:

The results of the acute toxicity study showed that after 14 days of observation DEAA and EEAA given to mice in single doses of 2000 and 5000 mg/Kg body weight (b.w.) resulted in no mortality or morbidity. Moreover, the animals did not exhibit any toxic effects or behavioral or morphological changes.

V.1.2. Effect on body weight

The changes in the body weight of control and mice treated with the extracts DEAA and EEAA during the 14 days of treatment are presented in Figure 32. The body weight gain showed no significant difference (P>0.05) between the control and treated groups. However, the body weight of mice at 24, 48, 72 h, and 7 days (P>0.05) after DEAA 5000 mg/kg administration exhibited a decrease but statistically not significant as compared to the time of initial treatment. No significant changes were observed in the body weights in the treated groups compared to that of the control group.

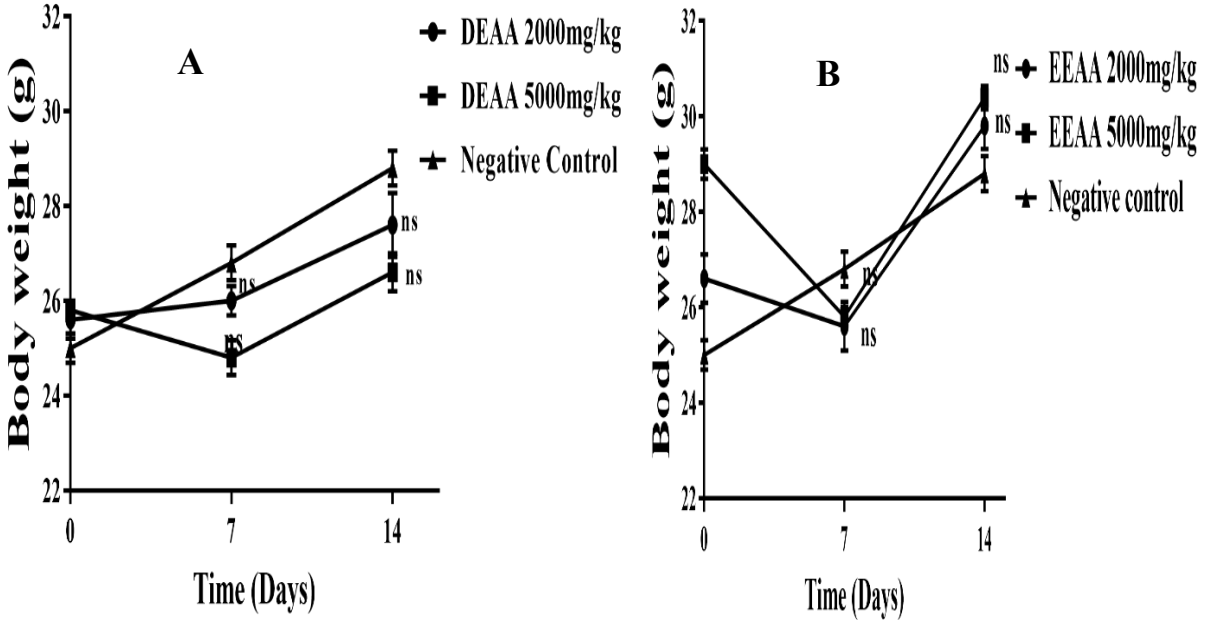

Figure 32: A: Body weight changes in the treated mice with DEAA extract. **B:** Body weight changes in the treated mice with EEAA extract and their controls at days zero, seven and fourteen in the acute toxicity experiment of *Anabasis articulata* extracts compared to negative control. Results are expressed as the mean±SEM (n=5 for each extract). ns – no significant difference.

V.1.3. Effect on organ relative weight

The vital organs (liver, kidneys, heart, and spleen) of mice were weighed after 14 days of administration at doses of 2000 and 5000 mg/kg of the two extracts (Figure 33 and 34). The liver and kidney of the group treated with 5000 mg/kg showed a statistically significant increase in weight ($P<0.001$, $P<0.01$, respectively) compared to the control group. However, the heart and spleen showed no alteration in the weight of these organs compared to the control group.

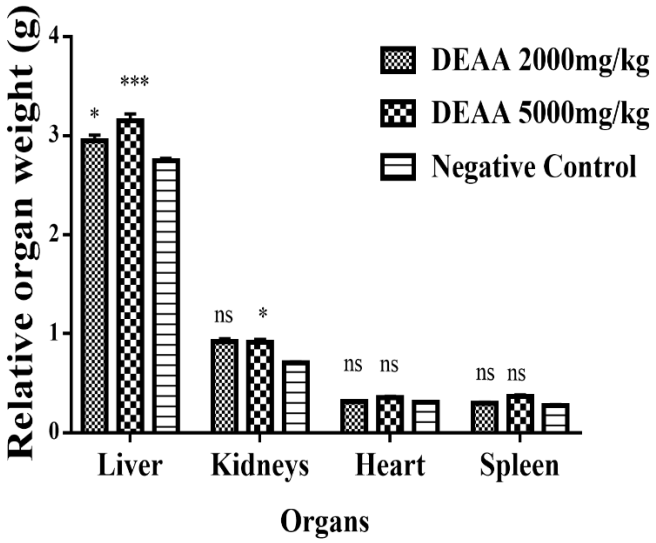

Figure 33: Relative organ weights of mice receiving DEAA after 14 days of acute toxicity experiment compared to negative control. Results are expressed as the mean±SEM (n = 5). ns – no significant difference; *P<0.05, *** P<0.001.

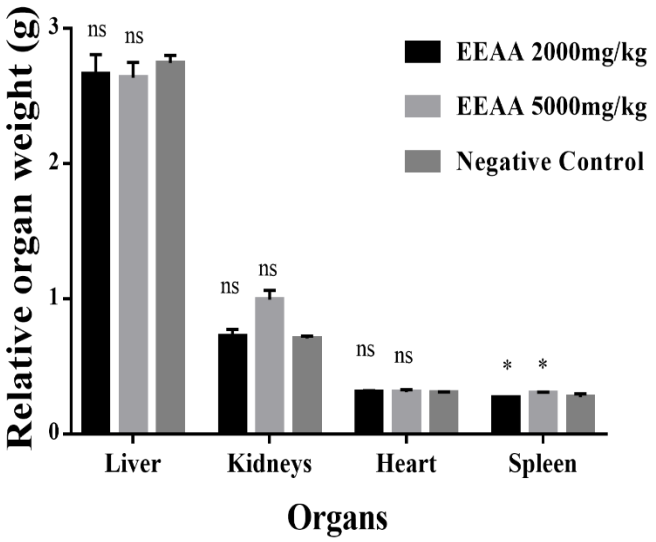

Figure 34: Relative organ weights of mice receiving EEAA after 14 days of acute toxicity experiment compared to negative control. Results are expressed as the mean±SEM (n = 5). ns – no significant difference; *P<0.05.

V.1.4. Histopathological investigation

The histopathological study of the selected organs in the acute toxicity test in mice shows no remarkable change when comparing treated group received 2000 mg/kg of DEAA, EEAA and control group). Figure 35 displayed a normal hepatic lobule composed of hepatocytes arranged in hepatic cords separated by sinusoids that drain into central vein lymphatic vessels. The same was observed for kidneys, normal glomerulus, bowman's space, proximal collecting tubules and distal collecting tubules. However treated group received 5000 mg/kg of DEAA or EEAA showed normal structures in liver and kidney but with general vascular congestions.

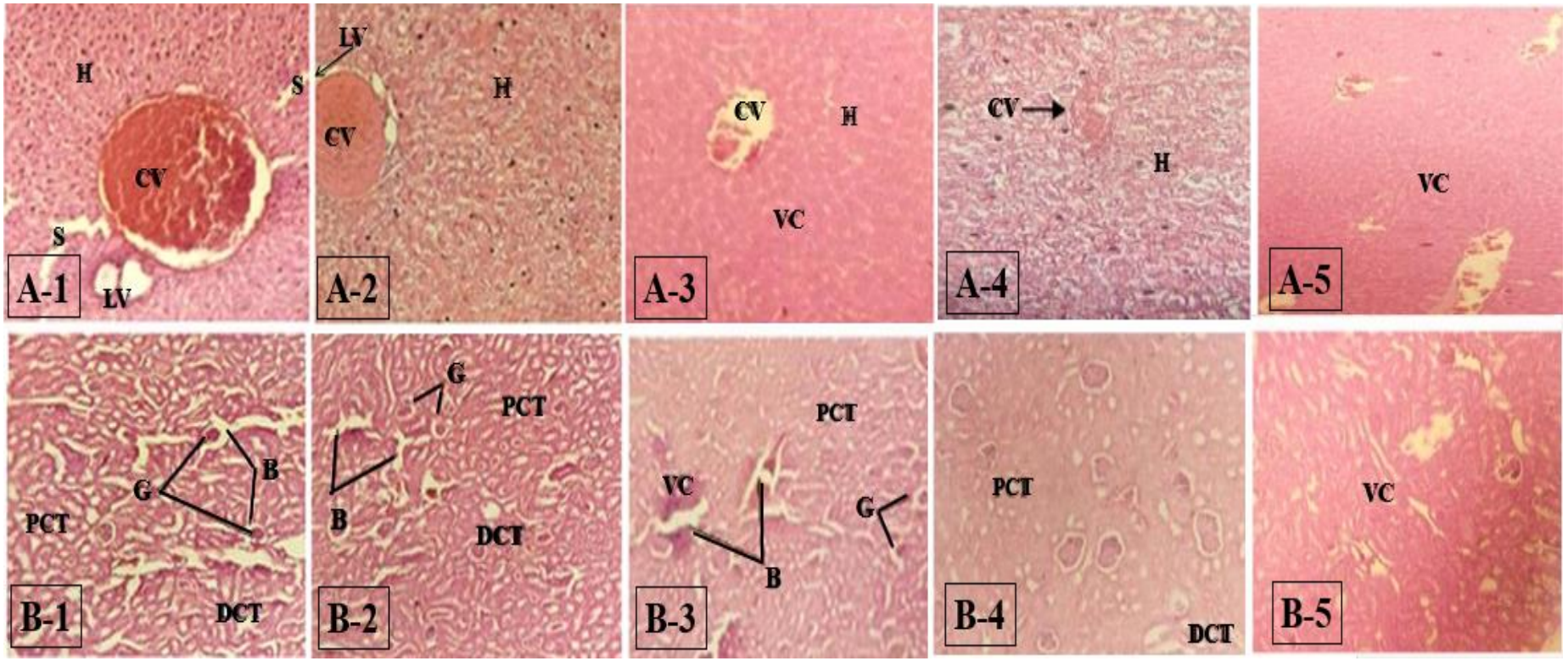

Figure 35: Histopathological study of liver (A) and kidney (B) tissues of mice tested in acute toxicity with single dose of 2000 and 5000 mg/kg of DEAA and EEAA. H & E stain (×20).H and E – hematoxylin-eosin; A-1 – Section of the liver of the control; A-2 – Section of the liver of a 2000 mg/kg DEAA-treated animal; A-3 – Section of the liver of a 5000 mg/kg DEAA treated representative A-4 – Section of the liver of a 2000 mg/kg EEAA treated representative A-5 – Section of the liver of a 5000 mg/kg EEAA treated representative; B-1 – Section of the kidney of the control; B-2 – Section of the kidney of a 2000 mg/kg DEAA treated animal; B-3 – Section of the kidney of a 5000 mg/kg DEAA treated, B-4 – Section of the kidney of a 2000 mg/kg EEAA treated, B-5 – Section of the kidney of a 5000 mg/kg EEAA treated. CV –central vein; H – hepatocyte; S – sinusoids; VC – vascular congestions; LV – lymphatic vessels; G – glomerulus; B – bowman's space; PCT – proximal collecting tubules; DCT – distal collecting tubules; VC – vascular congestions.

V.2. Estimation of *in vivo* anti-inflammatory activity

Three procedures were used to examine the anti-inflammatory activity of EEAA: Carrageenan induced paw edema in rats, croton oil, and xylene induced ear edema in mice.

V.2.1. Carrageenan-induced rat paw oedema

Three doses of 50, 100, and 200 mg/kg were tested to evaluate the anti-inflammatory activity of DEAA and EEAA. The results obtained were compared to those of the standard indomethacin (10 mg/kg). Figure 36-A and 37-A show the variations in paw thickness (mm) concerning carrageenan-induced paw edema over 6h following the administration of standard and three doses of each extract. In carrageenan-administered animals, the paw edema increased gradually and reached a peak at 6h (5.98±0.18 mm). However, in animals treated with DEAA and EEAA doses, the carrageenan-induced inflammation was reduced compared to the control group. The administration of indomethacin significantly prevented the progression of the inflammation at the plantar level of the rat's paw at 2 h and presented the lowest edema compared to the control at 6h.

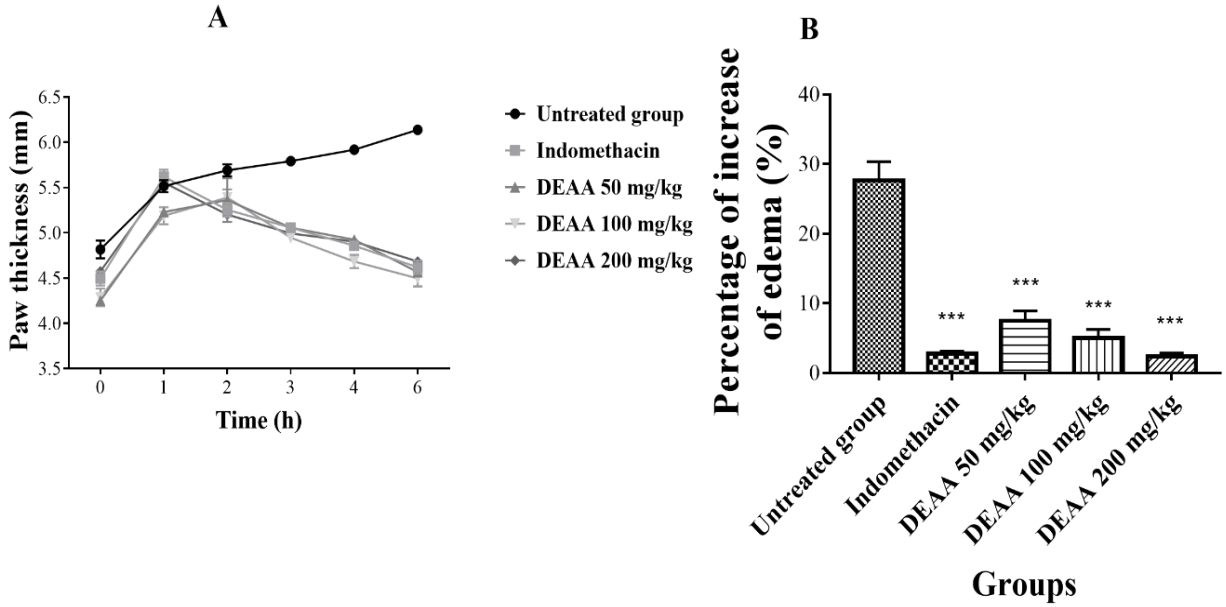

Figure 36: Anti-inflammatory effect of DEAA on carrageenan-induced paw edema in rats.
A – Changes in paw thickness (mm). **B** – Percentage of increase of edema. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Results are expressed as the mean±SEM (n = 5). ns – no significant difference, ***P<0.01 were considered significant when compared to the untreated group.

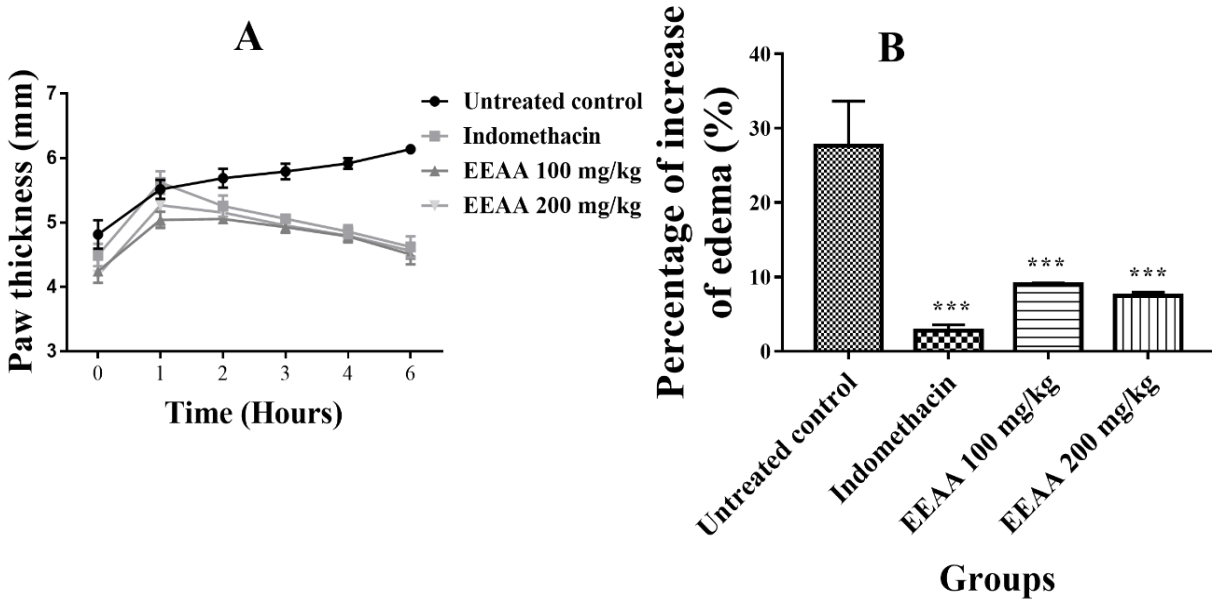

Figure 37: Anti-inflammatory effect of EEAA on carrageenan-induced paw edema in rats. **A** – Changes in paw thickness (mm). **B** – Percentage of inhibition of edema. EEAA – Ethanolic extract of the flowers and leaves of *A. articulata*. Results are expressed as the mean±SEM (n = 5). ns – no significant difference, ***P<0.01 were considered significant when compared to the untreated group.

As shown in Figure 36-B and 37-B illustrate the percentage of edema increase after 6h in rats receiving DEAA showed a similar effect at an oral dose of 200 mg/kg (2.41±0.48%) compared to indomethacin-treated group (2.77±0.37%). The effect was less noticeable (7.49±1.43%) at 50 mg/kg. The inhibition of paw edema in rats receiving EEAA showed an activity at oral doses of 100 and 200 mg/kg of 67.515±0.383% and 77.926±1.733%, respectively compared to indomethacin treated group with percentage of inhibition of 89.984±1.323%.

V.2.2. Croton oil induced ear edema in mice

Croton oil induced ear edema in mice test measures a variation in ear plug volume after the injection of an irritation agent in order to determine the inflammatory response. The findings of this investigation are shown in Figure 38 and 39.

DEAA at doses of 100 and 200 mg/kg caused inhibition of edema induced by croton oil in mice at 6 h with percentages of inhibition of 68.59±0.84% and 77.58±0.64%, respectively, while 80.68±1.25%inhibition was recorded with the nonsteroidal anti-inflammatory drug indomethacin (Figure 38).

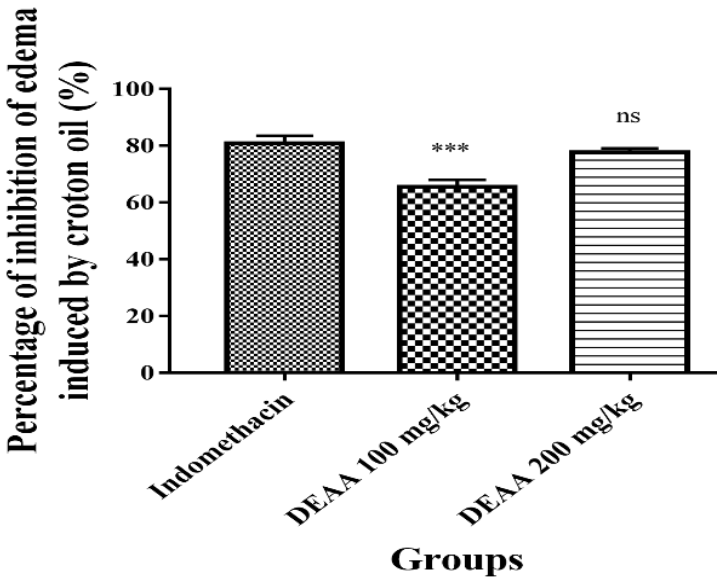

Figure 38: Percentage of reducing inflammation of DEAA in mice with croton oil-induced ear edoema. Values are presented as means±SEM. *P<0.05, *** P<0.001 compared to indomethacin.

Figure 39 illustrates that edema is effectively reduced by 65.340±1.154% and 76.193±1.135% at doses of 100 and 200 mg/kg of EEAA, respectively. Indomethacin, employed as a positive control, has an estimated 80.68±1.258% anti-inflammatory effect.

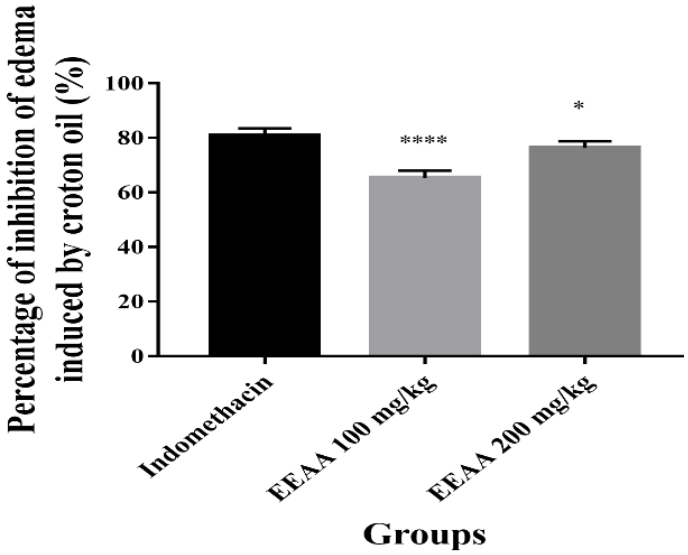

Figure 39: Percentage of reducing inflammation of EEAA in mice with croton oil-induced ear edoema. Values are presented as means±SEM. *P<0.05, *** P<0.001 compared to indomethacin.

V.2.3. Xylene-induced ear edema in mice

As illustrated in Figure 40, DEAA at doses of 100 and 200 mg/kg effectively reduced the edema in Anti-inflammatory activity using Xylene-induced ear edema in mice to 70.25±0.59% and

81.74±0.36%, respectively. Indomethacin used as a positive control promoted an antiinflammatory reduction to 82.98±0.36%.

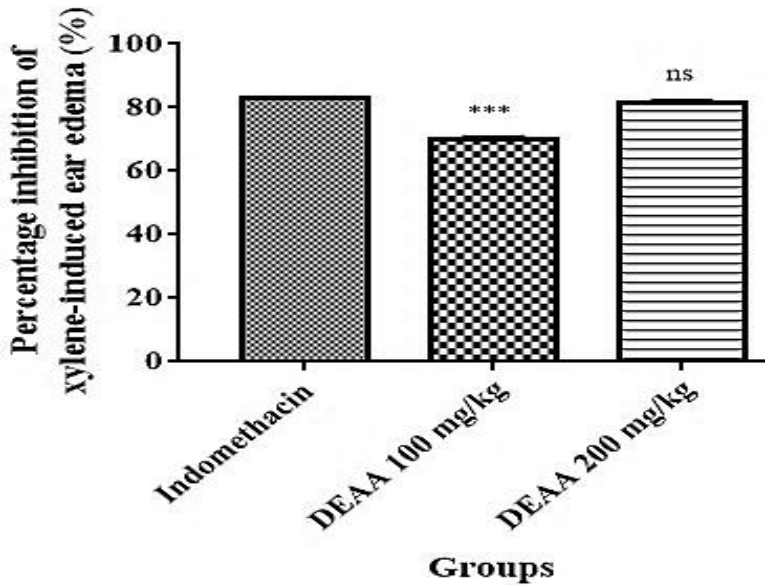

Figure 40: Anti-inflammatory effect of DEAA on xylene-induced ear edema in mice. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are expressed as means±SEM (n = 5). ns – no significant difference, *** P< 0.001 were considered significant when compared to indomethacin.

Figure 41 displays the results of the activity of anti-inflammation of EEAA in mice with xyleneinduced ear edoema. The edema is successfully reduced by EEAA at doses of 100 and 200 mg/kg, at 67.83±0.65% and 70.65±3.19%, respectively. The concurrent anti-inflammatory impact of indomethacin, the positive control, was assessed to be 82.98±0.36%.

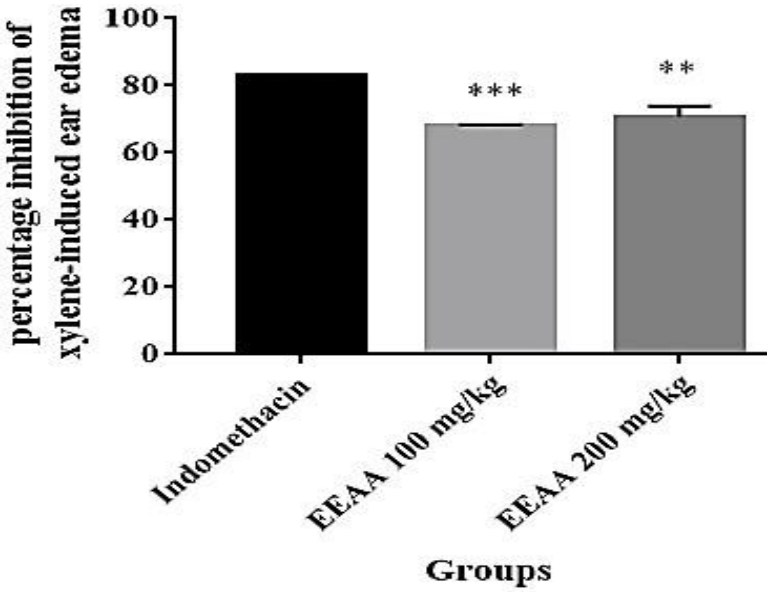

Figure 41: Percentage of reducing inflammation of EEAA in mice with xylene-induced ear edoema. Values are presented as means±SEM. **P<0.01, *** P<0.001 were considered significant when compared to indomethacin.

V.3. Estimation of analgesic activity

Intraperitoneal injection of 0.6% acetic acid induced a pain syndrome in mice by stimulation of nociceptive receptors. Oral administration of aqueous and ethanolic extracts of *A. articulata* (DEAA and EEAA) at doses of 100 mg/kg and 200 mg/kg or Aspirin were tested to evaluate the onset of pain syndrome. The results of the analgesic test representing the number of cramps and percentage of inhibition are shown in Figure 42 and 43. The results show that acetic acid induced an average of 80 cramps counted after 30 minutes in the untreated control lot. Indeed, the number of abdominal cramps decreased from 80.667±15.968 for the negative control to 32.6±2.227 for EEAA 100 mg/kg, 29.6±3.544 for DEAA 100 mg/kg, 27.8±1.068 and 24±1.924 for EEAA and DEAA 200 mg/kg respectively, compared to 11.833±1.701 for Aspirin.

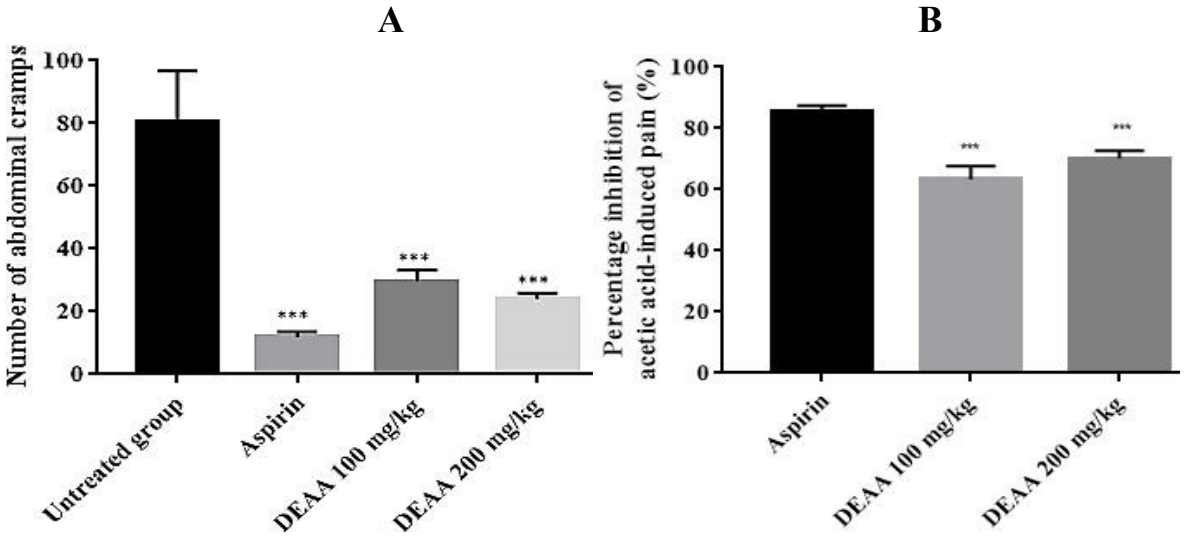

Figure 42: Analgesic effect of DEAA. **A:** Nombre of abdominal cramps. **B:** Percentage inhibition of acetic acid-induced pain (%). Values are expressed as means±SEM (n = 5). *** P< 0.001 were considered significant when compared to Aspirin.

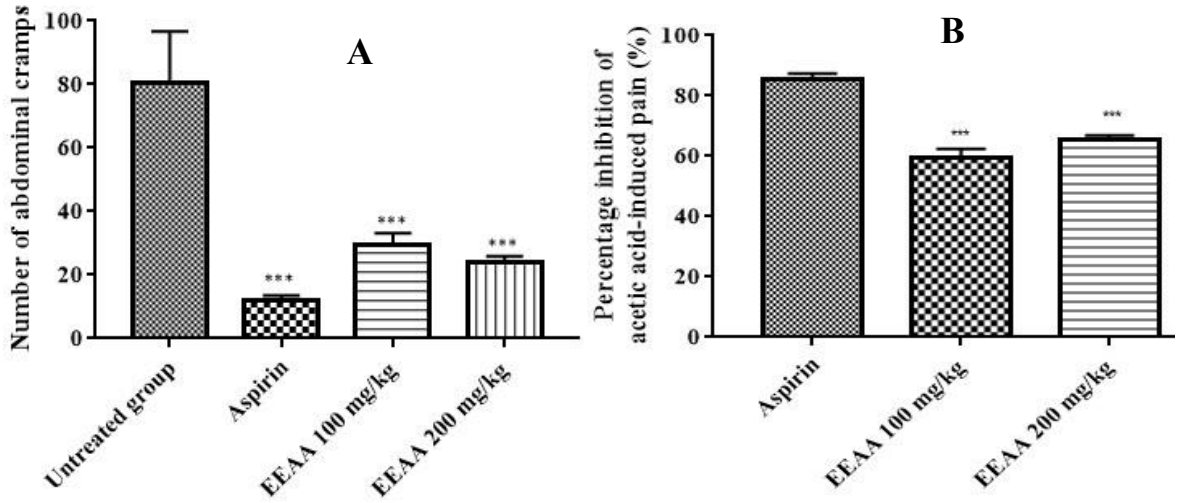

Figure 43: Analgesic effect of EEAA. **A:** Nombre of abdominal cramps. **B:** Percentage inhibition of acetic acid-induced pain (%). Values are expressed as means±SEM (n = 5). *** P< 0.001 were considered significant when compared to Aspirin.

Statistical analysis revealed significant difference between the positive control (Aspirin) and all extracts, suggesting that the aqueous and ethanolic extract of *A. articulata* used at this dose has good effect as the standard analgesic.

V.4. Antiulcer activity

V.4.1. Ethanol induced gastric ulcer

Intragastric administration of absolute ethanol to the untreated group of rats (negative control) received vehicle produced large bandlike hemorrhagic erosions in the glandular stomach. Pretreatment with DEAA at the tested doses (50,100 and 200mg/kg) and EEAA at the tested doses (200, 400 and 600 mg/kg. p.o.) and Omeprazole (20 mg/kg) offered different degrees of protection to the mucosa against all such damage caused by ethanol (Figure 44 and 45). Oral administration of 50, 100 and 200 mg/kg DEAA induced a percentage of ulceration of 37.75±6.28%, 16.034±1.308% et 5.16±0.33%, compared to negative control (45.92±1.87%) respectively. However, 33.52±1.34%, 20.46±0.98% et 12,67±0.26% were observed following the administration of 200, 400 and 600 mg/kg of EEAA respectively compared to group pretreated with omeprazole (positive control) with 4.54±2.22% of ulceration.

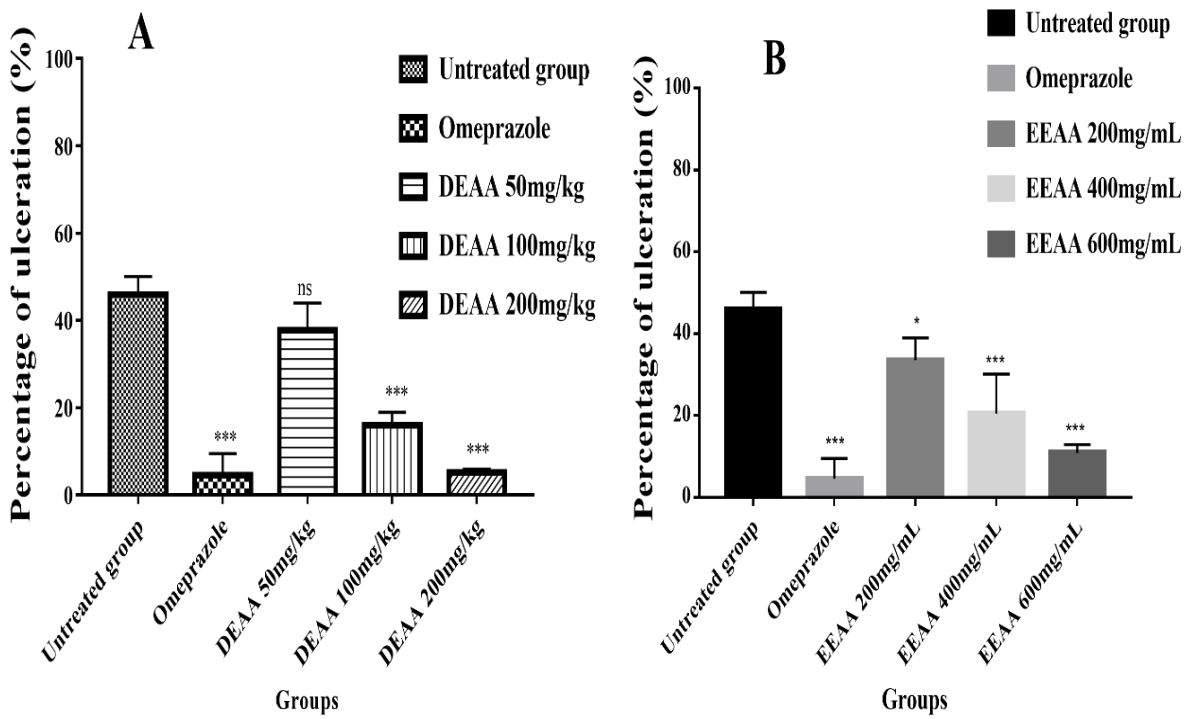

Figure 44: Ethanol induced gastric ulcer. **A:** percentage of inhibition of ulceration pretreated with DEAA. **B:** percentage of inhibition of ulceration pretreated with EEAA. ns – no significant difference, * P< 0.01. *** P< 0.001 were considered significant when compared to Omeprazole.

V.4.2. Macroscopic examination

The assay revealed an effect of ethanol on gastric tissues in the presence of DEAA or EEAA extracts at different doses, and the results are shown in Figure 45. Absolute ethanol produced extensive visible hemorrhagic necrosis of gastric mucosa in the untreated group (the vehicle) (Figure 45.B). The positive control group treated with omeprazole 20mg/kg showed milder injuries to the gastric mucosa (Figure 45.C) compared to the untreated group. However, oral administration of DEAA (Figure 45.D) and EEAA (Figure 45.E) effectively reversed the ethanol-induced gastric injury in a dose-dependent manner, with significant reduction of the gastric ulcer area. The protective properties of the DEAA extract at its highest dose (200 mg/kg) appeared similar to the positive group (Figure 45.E3), while the highest dose (600 mg/kg) from EEAA showed a best protective activity than to the positive group (Figure 45.D3).

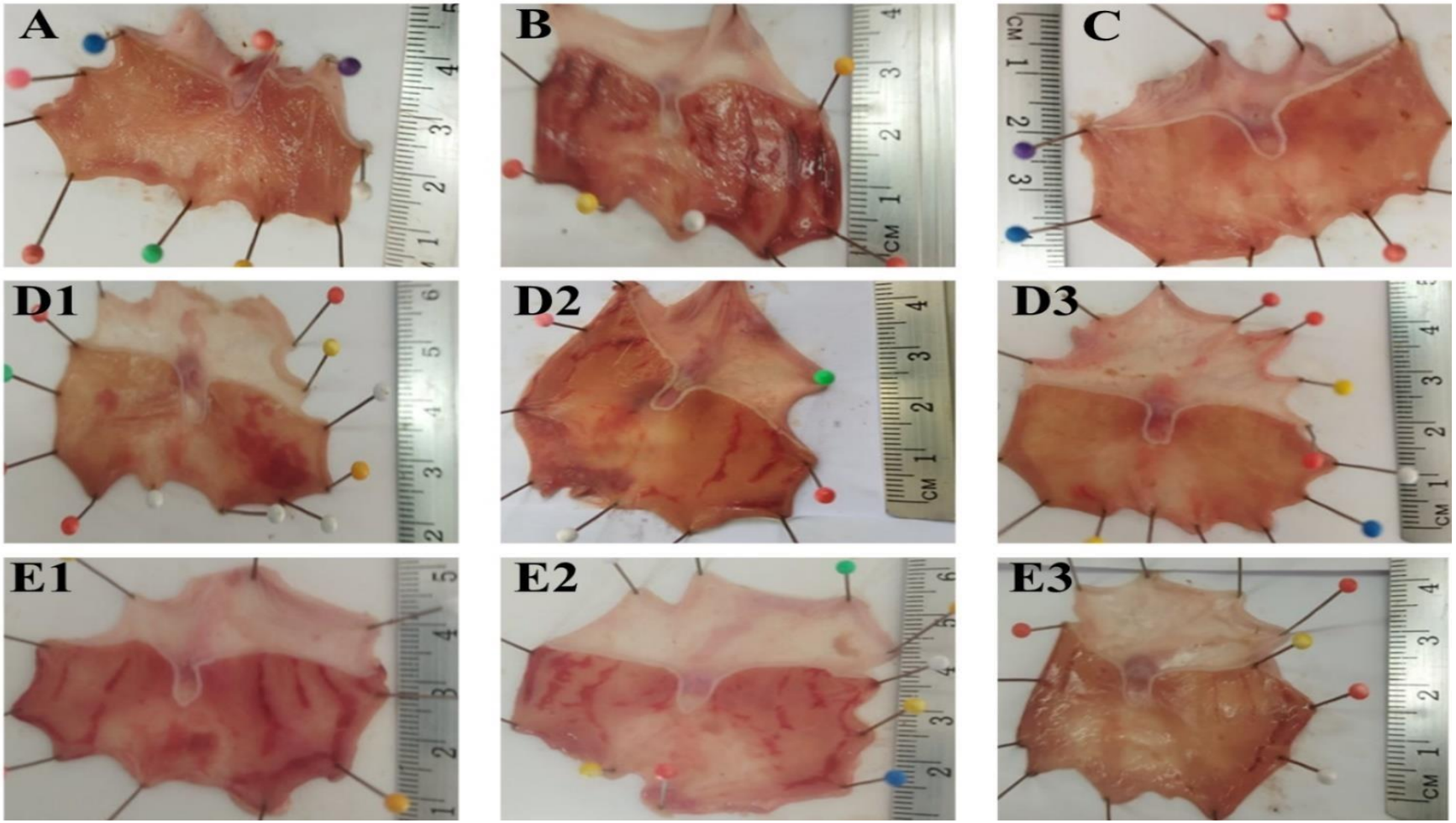

Figure 45: Macroscopic examination of stomach of gastric ulcer induction by ethanol. **A**: Normal group. **B**: The group pre-treated with vehicle as negative control. **C**: The group pretreated with Omeprazole (20 mg/kg) as positive control. **D1**, **D2** and **D3**: The group pretreated with DEAA 50, 100 and 200 mg/kg, respectively. **E1**, **E2** and **E3**: The group pretreated with EEAA 200, 400 and 600 mg/kg, respectively.

V.4.3. Mechanism of gastroprotective effect

In a series of experiments, the mechanisms of gastroprotective effect of DEAA were evaluated at a dose of 200 mg/kg to rats pretreated with L-NAME, glibenclamide and indomethacin.

V.4.3.1. Measurement of gastric acidity

Figure 46 illustrates the effect of intragastric administration of the extract obtained by decoction of *A. articulata* (DEAA 200mg/kg), omeprazole (20 mg/kg p. o.), vehicle (untreated group) and groups pretreated with L-NAME, glibenclamide and indomethacin without and with DEAA 200mg/kg on gastric juice acidity in rats.

The *per os* administration of the DEAA extract (200 mg/kg) in animals pre-treated with LNAME, glibenclamide and indomethacin reduce significatively the acidity of the gastric juice (pH = 3.85±0.35, 3.64±0.26, 4.09±0.33). The result of acidity in normal group (4.89±0.08) was similar that of the omeprazole group with a value of pH equal to 4.66±0.21 (ns – no significant difference). Whereas pH of the gastric juice was acid for the groups pre-treated with L-NAME, glibenclamide and indomethacin without the extract DEAA 200mg/kg (2.25±0.36, 2.11±0.18, 2.97±0.29, respectively).

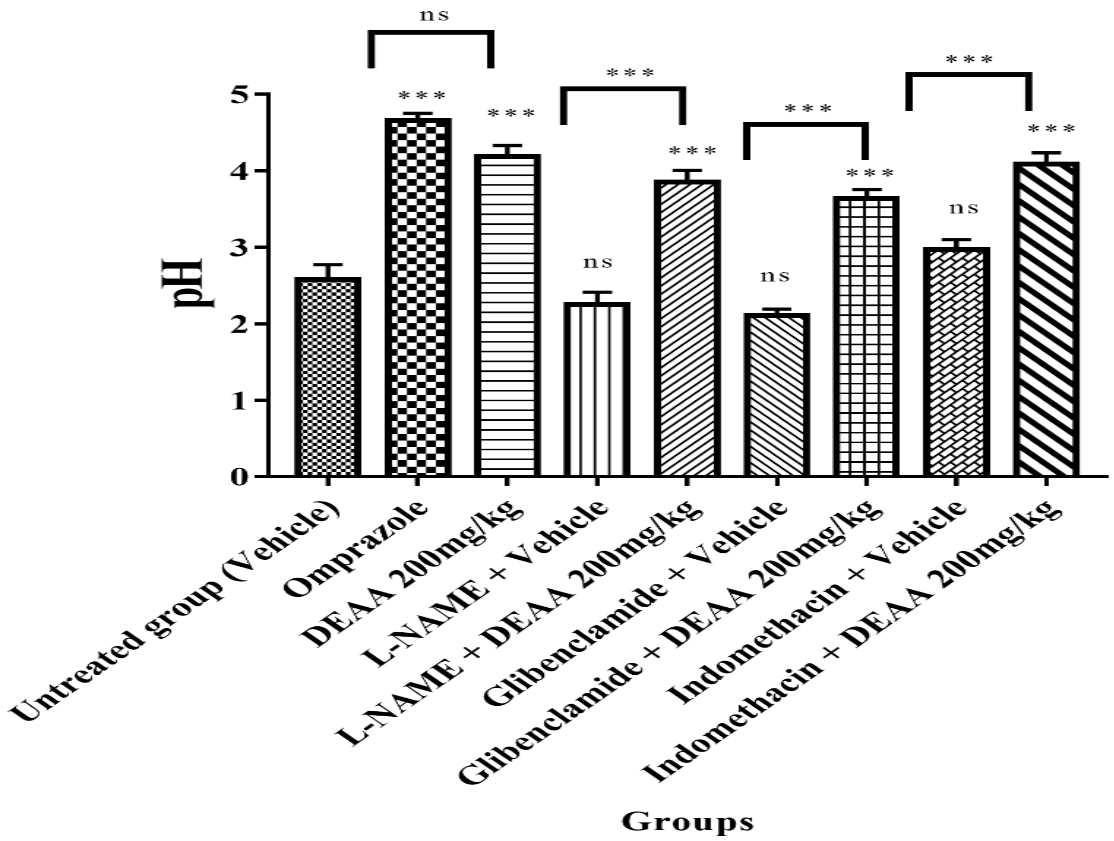

Figure 46: Measurement of gastric acidity in presence and absence of DEAA 200 mg/kg for rats pretreated with pharmacological substances: L-NAME, glibenclamide and indomethacin compared to untreated group. The results were expressed as means±SEM, ns – no significant difference, n=5. *** p< 0.001.

V.4.3.2. Macroscopic gastric lesion evaluation of mechanism

The macroscopic examination of DEAA (200mg/kg) effect in absence or presence of different pharmacological substances: L-NAME, glibenclamide and indomethacin., on ethanol-induced gastric mucosa damage is shown in Figure 47.

The protective properties of the DEAA 200mg/kg (Figure 47.D) appeared similar to the positive group (Figure 47.B) compared to the negative control (Figure 47.C).

L-NAME caused severe damage to the gastric mucosa when administered without DEAA (Figure 47.E1). However, in the presence of DEAA 200mg/kg (Figure 47.E2), the extent of damage was reduced, resulting in moderate lesions compared to the negative control.

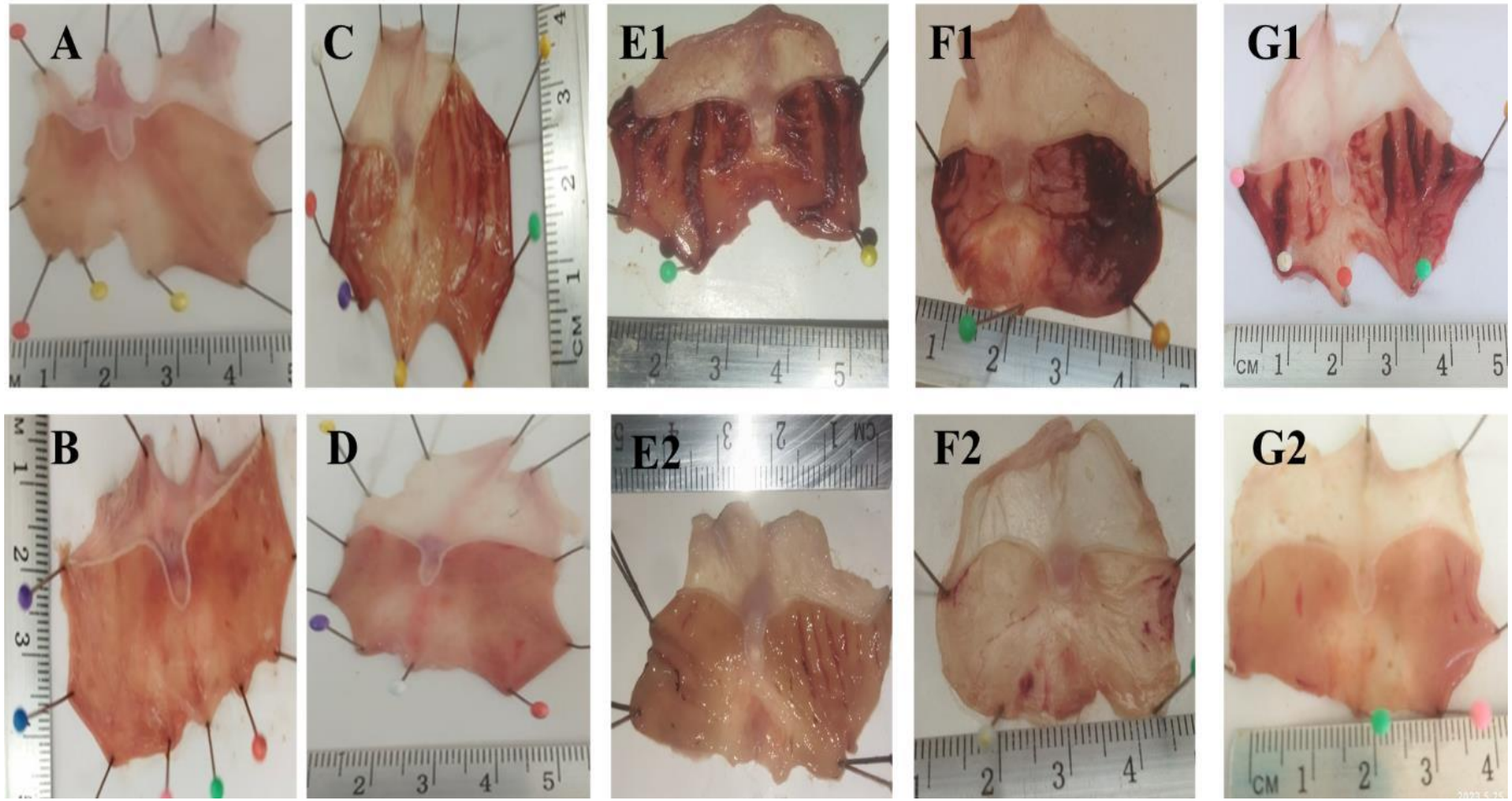

Figure 47: Gastric ulcer induced by absolute ethanol in animals pretreated with L-NAME, glibenclamide and indomethacin. **A:** Normal group. **B:** The group pretreated with Omeprazole (20 mg/kg) as positive control. **C**: The group pre-treated with vehicle as negativecontrol. **D:** The group pre-treated with DEAA 200 mg/kg. **E1**, **F1** and **G1**: The group pre-treated with L- NAME, glibenclamide or indomethacin respectively in absence of DEAA 200mg/kg. **E2**, **F2** and **G2**: The group pre-treated with L-NAME, glibenclamide or indomethacin, respectively in presence of DEAA 200mg/kg.

Glibenclamide induced extensive stomach mucosal injury (Figure 47.F1) compared to the ethanol-induced ulcer negative group. However. pretreatment with both glibenclamide and DEAA provided good protection (Figure 47.F2). This effect was similar to the control positive group (omeprazole) (Figure 47.F2).

The administration of indomethacin provided a moderate to severe gastric injury against ulcer caused by ethanol (Figure 47.G1). Whereas the pre-treatment with both indomethacin and DEAA provides a milder effect (Figure 47.G2) compared to that in presence of indomethacin alone. The protection of DEAA against the ulcer caused by the ethanol in presence of indomethacin was similar to the control positive group (omeprazole) and with DEAA alone.

V.4.3.3. Histopathological evaluation of gastric lesions

In the present study, ethanol administration to rats induced macroscopic lesions of gastric tissue, such as petechiae, hemorrhages and edemas. 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 48).

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 48.A). However, 30 min after of exposure to ethanol, rats presented damage to gastric tissue at a microscopic level. Histopathological injury caused by ethanol administration is characterized by severe detachment of the surface epithelium (dep), edema, formation of hemorrhagic and gastric lesions, and an inflammatory process characterized by neutrophil infiltration (in) (Figure 48.C). In contrast, the stomach of a rat in the positive control group. pretreated with omeprazole (Figure 48.B), maintains an intact histological mucosa structure, with mild edema, infiltration of inflammatory cells (in), and mildly dilated blood vessels in the submucosa compared to the ulcer control rats. Rats pre-treated with DEAA 200mg/kg (Figure 48.D) exhibited good protection, characterized by reduced or of edema and leukocyte infiltration.

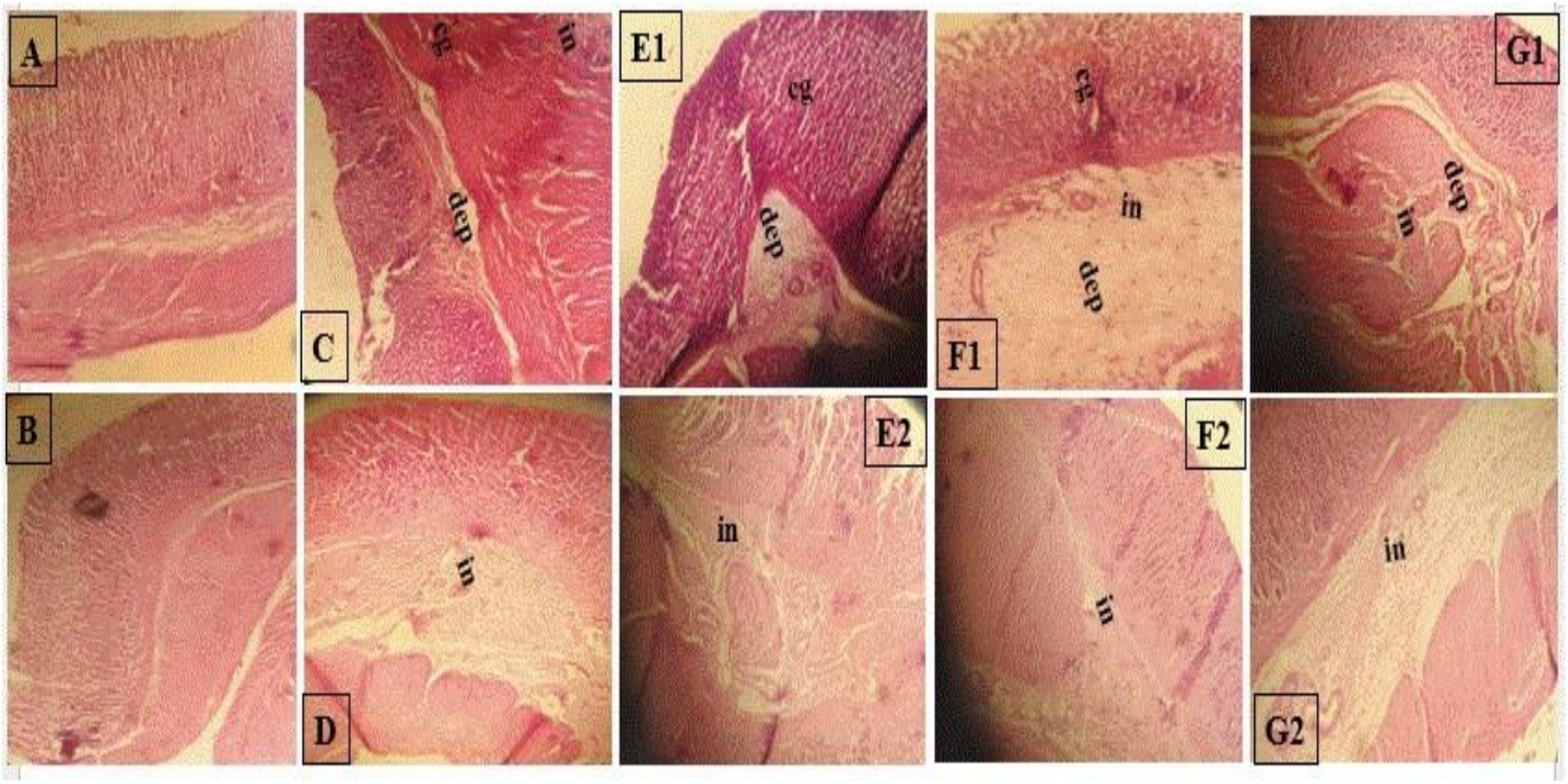

Figure 48: Histological Evaluation of Gastric Lesions in gastric ulcer induced by absolute ethanol in animals pretreated with L-NAME, glibenclamide and indomethacin. **A:** Normal group. **B:** The group pretreated with Omeprazole (20 mg/kg) as positive control. **C**: The group pre-treated with vehicle as negative control. **D:** The group pre-treated with DEAA 200 mg/kg. **E1**, **F1** and **G1**: The group pre-treated with L-NAME, glibenclamide or indomethacin respectively in absence of DEAA 200mg/kg. **E2**, **F2** and **G2**: The group pre-treated with L-NAME, glibenclamide or indomethacin, respectively in presence of DEAA 200mg/kg. (dep): detachment of the surface epithelium, (in) infiltration of inflammatory cells and (cg): congestion.

Histological observation confirms the ability of DEAA at dose 200 mg/kg to reduce ethanolinduce gastric damage in the superficial layer of the gastric mucosa and further highly significantly reduced edema and leucocytes infiltration in submucosa.

There are severe submucosal oedema and leucocytes infiltration in rats pretreated with LNAME (Figure 48.E1) alone and in rats pretreated with both L-NAME and DEAA (Figure 48.E2), compared to the negative control (Figure 48.C). In rats pretreated with glibenclamide (Figure 48.F1); there is a moderate disruption to the surface epithelium and significant leucocytes infiltration and congestion (cg) of blood vessels in the submucosal layer, compared to that seen in the vehicle as negative control rats. However, the glibenclamide in the presence of DEAA extract (Figure 48.F2) significantly reduced the edema, leucocytes infiltration and the congestion of blood vessels in the submucosal compared to glibenclamide effect in absence of DEAA. Indomethacin administration in the presence of DEAA extract (Figure 48.G2) significantly reduced leucocytes infiltration and the congestion of blood vessels in the submucosal compared to indomethacin effect in absence of DEAA administration (Figure 48.G1).

V.4.3.4. Evaluation of percentage of ulceration

The mechanisms of DEAA 200mg/kg gastroprotective effect are illustrated in Figure 49. Pretreatment with DEAA in the presence of L-NAME, glibenclamide and indomethacin offered highly significant reducing of the injuries of ulcers caused by ethanol (9.16±1.12, 11.16±1.29, 5.46±1.93%, respectively) compared to the groups in the presence of L-NAME, glibenclamide and indomethacin without the pre-treatment of DEAA (57.92±1.17, 69.92±2.17, 48.16±1.82%, respectively). However, there was significant difference between the gastroprotective effects of groups pre-treated with L-NAME, glibenclamide and indomethacin in absence of DEAA compared to the omeprazole group and with the group received DEAA (200mg/kg).

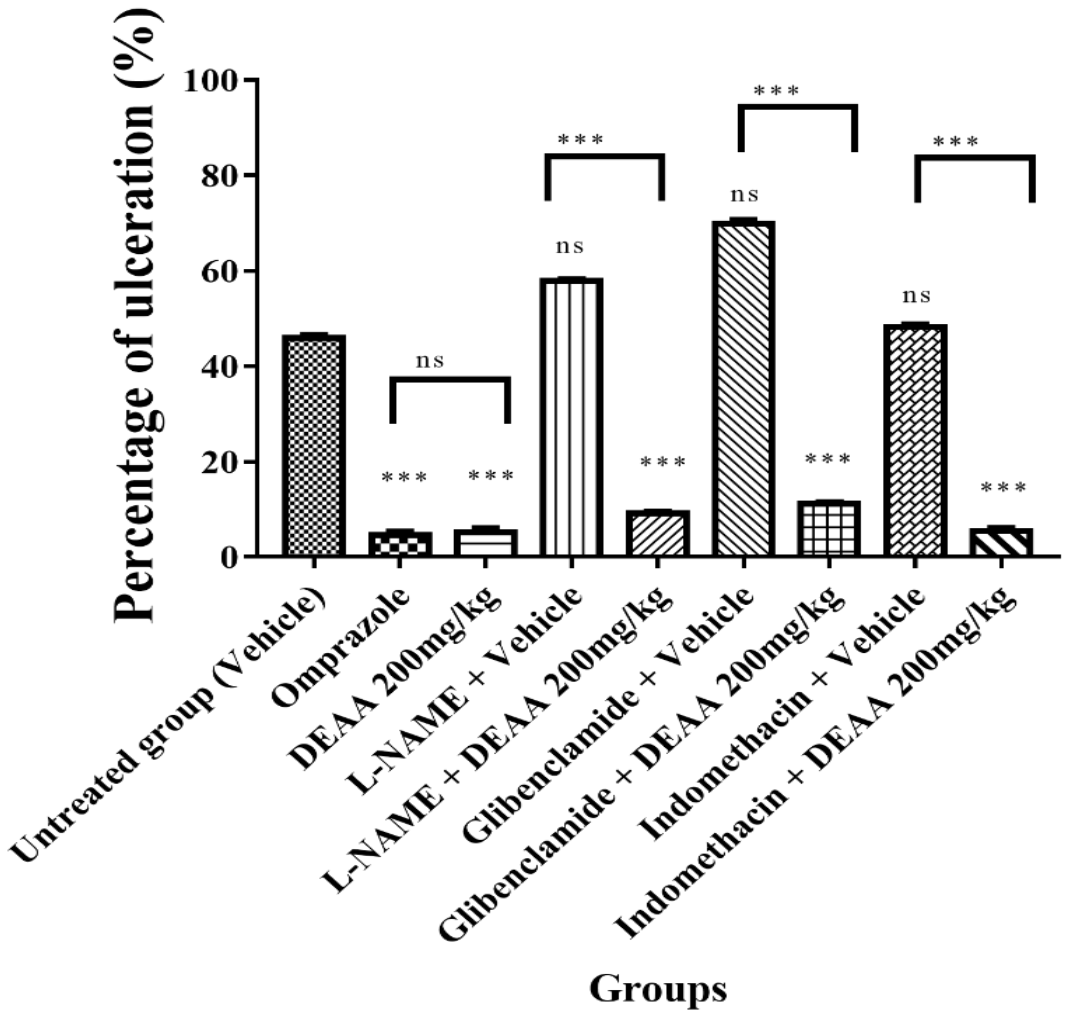

Figure 49: Gastroprotective effect of DEAA at a dose of 200 mg/kg in the presence of different pharmacological substances: L-NAME, glibenclamide and indomethacin compared to untreated group. The results were expressed as means±SEM, ns – no significant difference, n=5. *** $p < 0.001$.

V.4.3.5. Determination of adhered mucus to gastric wall

The effects of DEAA (200mg/kg) on gastric mucus content are shown in Figure 50. When DEAA (200mg/kg) was combined with different pharmacological substances (L-NAME, glibenclamide and indomethacin) increased the production of gastric mucus content (190±2.01, 176±2.11, 212±2.61 µg/g, respectively) compared to the vehicle of each group (89.43±4.12, 63.12±2.54, 95.65±3.12 µg/g tissue). This increase of the gastric mucus content had a very highly significantly ulcer protective effect.

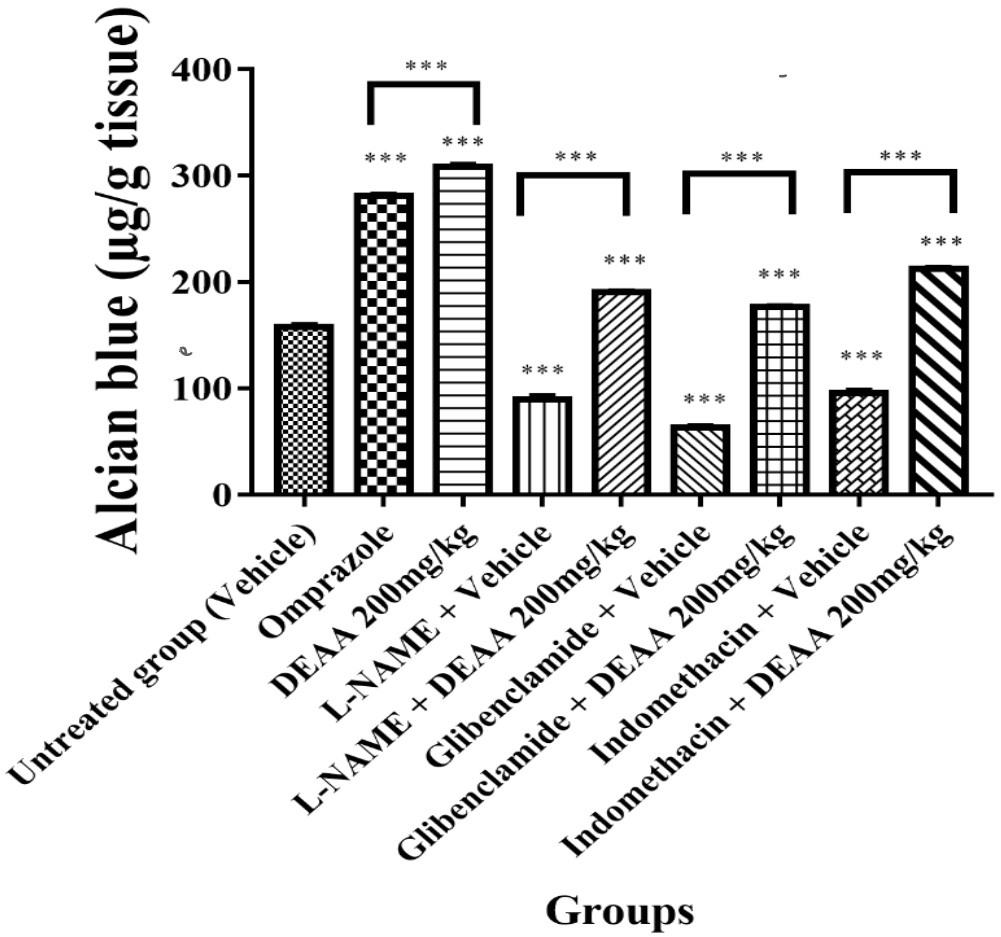

Figure 50: Determination of adhered mucus to gastric wall in presence and absence of DEAA 200 mg/kg for rats pretreated with pharmacological substances: L-NAME, glibenclamide and indomethacin compared to untreated group. The results were expressed as means±SEM, n=5. *** $p< 0.001$.

V.4.3.6. Evaluation of *in vivo* antioxidant activity

The evaluation of the effect of absence and presence of DEAA 200mg/kg in pre-treated rats with different pharmacological substances on CAT activity, SOD activity and MDA level of stomach tissue in ethanol-induced gastric mucosal lesion was illustrated in Table 8.

V.4.3.6.1. Determination of superoxide dismutase (SOD) activity

In the assessment of superoxide dismutase (SOD) activity; the statistical analysis showed in all groups treated with DEAA (200mg/kg) significant increase of the values of SOD (1.309±0.11 IU/min/µg of total protein for DEAA (200 mg/kg), 1.204±0.32 IU/min/µg of total protein for Indomethacin+DEAA (200mg/kg), 1.138±1.38 IU/min/µg of total protein for

Glibenclamide+DEAA (200mg/kg) and 1.008±1.01 IU/min/µg of total protein for L-NAME +DEAA (200mg/kg) compared to the vehicle treated animal groups 0.821±0.78 ; 0.712±0.27; 0.613±0.54; 0.454±1.43 IU/min/µg of total protein for Indomethacin+DEAA (200mg/kg), Untreated group, Glibenclamide+ Vehicle, L-NAME+Vehicle, respectively.

V.4.3.6.2. Assessment of catalase activity (CAT)

The statistical analysis of the assessment of catalase activity (CAT) revealed a significant decrease in catalase in gastric tissue in all groups untreated with DEAA200mg/kg and the values were 1.12±0.89; 1.25±1.33; 1.43±0.67; 1.98±0.65 µmol/seconds/ µg of total protein (L-NAME
+Vehicle, Glibenclamide+ Vehicle, Indomethacin+ Vehicle and Untreated group). While LNAME+DEAA200mg/kg, Glibenclamide+DEAA200mg/kg, Indomethacin+DEAA200mg/kg, DEAA200 mg/kg and the omeprazole at 20 mg/kg groups were able to significantly reversed the decrease on CAT activity induced by ethanol where the values were 2.08±2.13; 2.31±1.35;
2.65±0.07; 2.74±0.29 and 2.92±0.21 µmol/seconds/ µg of total protein respectively.

V.4.3.6.1. Measurement of lipid peroxidation

MDA estimation, the ethanol groups in absence of DEAA200mg/kg showed a change on oxidative markers with an increase on lipid peroxidation (MDA) with 49.32±0.53 nmol/µg of total protein for Glibenclamide+Vehicle, 32.16±0.41 nmol/µg of total protein for untreated group and 31.28±0.33nmol/µg of total protein for Indomethacin+Vehicle. However, the groups which received DEAA 200mg/kg and the control positive attenuated the damage induced by ethanol. Furthermore, the extract of DEAA at 200mg/kg (17.45±0.96 nmol/µg of total protein) was able to significantly prevent the increase on lipid peroxidation better than the positive control: Omeprazole at 20mg/kg (21.03±0.09nmol/µg of total protein).

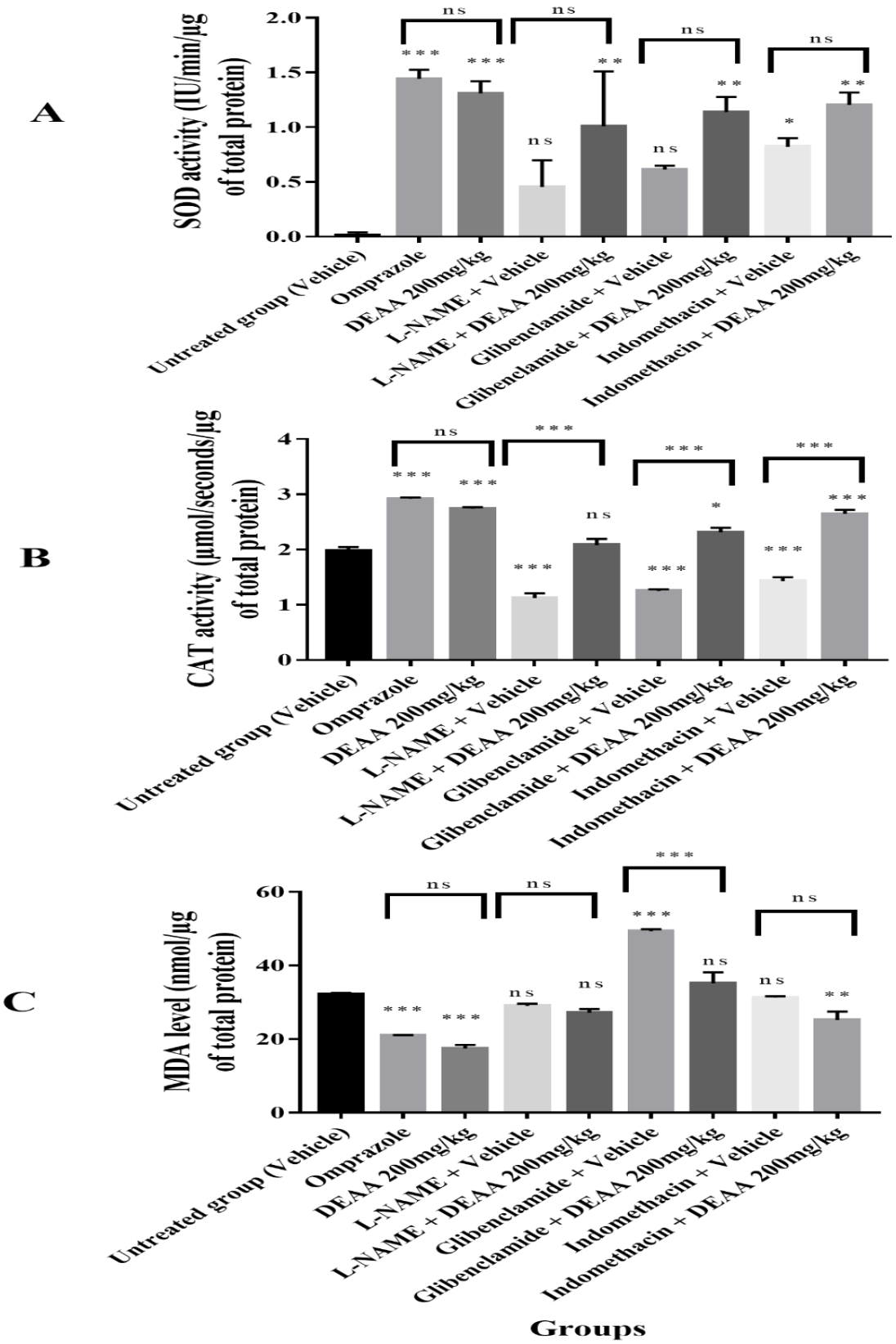

Figure 51: Evaluation of the effect of absence and presence of DEAA 200mg/kg in pretreated rats with different pharmacological substances on **A:** CAT activity, **B:** SOD activity and **C:** MDA level of stomach tissue in ethanol-induced gastric mucosal lesion compared to untreated group.

Discussion

Our study was conducted from June 2020 to January 2021 spread over 182 questionnaire cards. The information is divided into two sections: the first concerns the informant as the sole owner of the information, while the second gathers information concerning the medicinal plants such as local names, plant parts used, medicinal use and preparation. The objective of this ethnobotanical survey was to identify and establish a list of plants used in traditional medicine due to their pharmacological properties. A questionnaire (Figure 13) was distributed to villagers and people with knowledge on the use of medicinal plants through face-to-face interviews.

Of the total informants of 182 69% comprised women and 31% men were interviewed. This result is in agreement with the studies of Ramdane *et al.,* (2015); Miara *et al*., (2018) and Bouafia *et al*., (2021) who found that the majority of informants were women with the percentage of 63%, 56% and 57.14%, respectively when the authors compared medicinal plant knowledge held by males and females. This could be due to women's greater knowledge of medicinal plants and the ease with which they can share this knowledge.

The age group that formed the majority of informants was the 51 years - 60 years age group contributing 37%. The percentage of medicinal plant users older than 60 years old is similar to that found by Alalwan *et al*., (2019) where the most respondents were adults between the ages of 40 years - 60 years (62.5%) followed by (27.5%) in the age group of above 60 years. The highest age respondents provide more reliable information because they hold much of the ancestral knowledge that is part of the oral tradition. The least participative age group was the 20 years-35 years which had 9% (Table 1). The reason for lack of interest on medicinal plants within this age group could be explained by the mistrust of certain young people, who tend not to believe this herbal medicine due to the influence of modernization and exotic culture influence.

Traditional knowledge of the use of plants for medical purposes, or the interchange of information and development of herbal medicine production, might explain these findings that is why users of medicinal herbs with no educational background accounted for 30% of the total (Table 1), followed by those with a university education accounted, only a primary, middle, or secondary education account respectively. Boughrara and Belgacem, (2016) found that the

illiteracy rate was 33% among users of medicinal plants and the percentage of people who have a university level of study is 21.33%. These results are quite similar to ours.

The majority of ethnobotanical knowledge came from family, with 65 percent being passed down through generations. Our findings are consistent with those previously reported by Eddouks *et al*., (2017) and Chaachouay *et al*., (2019) who found that heritage is the primary source of knowledge acquisition for residents, accounting for approximately 45 percent of all knowledge acquisition in Morocco.

An ethnobotanical survey and analysis of the flora of the research region were conducted in El Mergueb Natural reserve. A total of 46 taxa belonging to 23 different families were recorded (Table 2). As illustrated in Figure 4, the most represented families were Asteraceae with 9 medicinal plant species, followed by Poaceae (6 species) and Lamiaceae (4 species). Our findings are similar with those of Hammiche and Maiza, (2006) who found that among 33 families listed, Asteraceae, with 12 species (15%), are the most common. This high proportion could be explained by the high representation of these families in El-Mergueb Natural Reserve flora because of the ecological factors that favour the development and adaptation of the majority of their species. According to Habib *et al*., (2020), Asteraceae, Poaceae and Brassicaceae characterize the arid and semi-arid areas in the Mediterranean regions. In Algeria, some studies highlighted the dominance of Asteraceae, Poaceae and Fabaceae in the steppe regions (Kazi Tani *et al*., 2010). This was also reported in Morocco by Fennane, (2008).

As seen in Table 2*,* species with high used values (UV) were *Artemisia herba-alba* Asso (0.58), *Stipa tenacissima* L. (0.42), *Artemisia campestris* L. (0.3), *Zyziphus lotus* (UV = 0.16), *Marrubium vulgare* L. *(*UV = 0.14). This means that these species are the most important medicinal plants used in folk medicine by the population of El Mergueb to treat ailments, which might be due to their vast distribution in the area study. *Artemisia herba-alba* was named the first specie in others studies (Nawash *et al*., 2013; Katiri *et al*., 2017). In Algerian traditional medicine, *Artimisia herba-alba* is one of the most commonly utilized herbs. This plant is used to cure gastrointestinal problems including diarrhea and abdominal discomfort, as well as to heal exterior wounds (Khennouf *et al*., 2010; Boudjelal *et al*., 2013; Miara *et al*., 2018), diabetes (Bouasla and Bouasla, 2017), cancer, and respiratory system diseases (Ouelbani *et al*., 2016).

The second most plant cited is *Stipa tenacissima* which is recommended as a hypoglycemic and in the treatment of persistent scalp ulcers, cleaning the ashes. *Artemisia campestris* is used for digestive disorders, stomach discomfort, nausea, and menstrual cramps. vulnerary, antihemorrhagic poultice whereas, *Zyziphus lotus* is used to treat anti-inflammatory diseases (Benderradji *et al*., 2014). *Marrubium vulgare* is utilized for diabetes therapy, digestive problems, stomach pain, fever, migraine, wound, cardiovascular diseases, respiratory and liver diseases, allergies, leishmaniose, febrifuge, vermifuge, anti-emetic, tonic, analgesic, antispasmodic (Miara *et al*., 2019).

Locals are aware of the dangers of poisonous plants and use extreme caution while utilizing plants such as *Thapsia garganica* L. (Mohamed Ibrahim *et al*., 2018), *Nerium oleander* L. (Aslani *et al*., 2004), *Colocynthis vulgaris* L. (Adam Sakine *et al*., 2011), *Retama retam* Webb. (Algandaby, 2015), *Asparagus stipularis* L., *Thymelaea hirsute* L., *Peganum harmala* L. (Nenaah, 2011). Toxicity of therapeutic plants can be attributed to their active chemical combinations, interactions with other herbs and medicines, or intrinsic toxicity. Plants contain complex combinations of terpenes, alkaloids, saponins, and other compounds, which increases the potential of unpleasant responses to any of them or the cumulative or synergistic effects of chemical interactions (Saad *et al*., 2006).

Several medicinal plants preparation methods are used in traditional medicine to fight the different kind of ailments such as decoction, infusion, grinding, maceration, powder and cataplasm. Data analysis showed that infusion is the most common preparation method used by the population studied (42.6%) (Figure 20). This result agrees with findings of previous studies. DemiRci and Özhatay, (2012) reported that the infusion is generally the used preparation method, accounting for 41.8% of the recorded species, followed by decoction (23.9%). However, Chaachouay *et al*.,(2019) found that the percentage of infusion was 53.9% followed by decoction (22.1%). It seems to us that decoction and infusion in water are the most widely used methods given the ease of their preparation and administration by users, as it was similarly mentioned in other studies (Hammiche and Maiza, 2006; Benítez *et al*., 2010; Boudjelal *et al*., 2013; Fakchich and Elachouri, 2014; Sargin *et al*., 2015)

Almost all different parts of plants were reported to be used as a drug by the traditional healers and local people, but leaves were the most common (39.6%) (Figure 21). This result is consistent with other studies that have shown that leaves are the most preferred part of plant for

the preparation of remedies. Miara *et al*., (2018) indicated that the leaves are the plant part most commonly used as herbal remedies (29%). Unspecified aerial parts are also often used (17%), as well as fruits and seeds (12 and 11% respectively). This result might be explained by the fact that leaves are the most commonly utilized since they are readily available. It was also shown that the respondents had a strong preference for utilizing leaves to identify and differentiate therapeutic plants. As a result, if the leaf is a crucial component in plant identification and has frequent and simple access, it should be employed more than other plant parts. This conclusion is in line with the results of Akerreta *et al*., (2007). Despite, it has been well established that all parts of the plants are rich in active compounds, the local population most often uses leaves in their preparations, which could be explained by the fact that the totality of the knowledge acquired by the population of the region is traditional and transmitted empirically from one generation to the next. In addition, the leaves are very abundant and their harvest is very easy (Sargin *et al*., 2015; Yemele *et al*., 2015). Furthermore, since the leaves are main photosynthetic organ of plants, bioactive phytochemicals are concentrated in the leaves (Benarba *et al*., 2015).

The main fieldwork of ethno-medicinal survey of *A. articulata* was to collect information by native people of the study area (M'sila). Two vernacular names are used to indicate *A. articulata*: Ajrem and Echannan. Data from the ethnopharmacological survey showed that 100% of people know the plant by the name of Ajrem. The main treated disease reported is anti-inflammatory disease, rheumatism, with a frequency of citations of 61.86% of the 118 total citations. It is followed by diabetes with a frequency of citations of 43.22% and another common disease reported by the people is the use of the plant to treat dermatosis and abdominal pain with a frequency of citation of 27.96% and 10.17%, respectively. Flowers and leaves were the most used plant component (68.64%), followed by the roots (55.93%). However, the main form of preparation was cataplasm (89.83%).

In the present study the yields of the various fraction extracts ranged from 7.6 to 42.2%. According to Benhammou *et al*., (2013), the root of *A. articulata* methanolic extract, ethyl acetate fraction, and butanolic fraction yielded lower values than our results. Solvent extractability is primarily determined by the component's solubility in the solvent (Dhanani *et al*., 2017). Compared to other species of Chenopodiaceae family, the values reported for *Atriplex halimus* (Benhammou *et al*., 2009) and *Arthrophytum scoparium* were (19.87%) according to Dehimi *et al*., (2020).

A high-yield extract with minimal alterations to its functional qualities is the result of a successful extraction procedure. Numerous studies have shown that different extraction methods result in diverse biological activity from the extracts. Mainly, a compound's solubility in a solvent determines the solvent's capacity for extraction (Hmidani *et al*., 2019). Many studies point out that the percentage of extraction yield depends mainly on the extraction procedure, in particular, the temperature used during the extraction, the polarity of the compounds of the extract, the solvent ratio and the methods of extraction (maceration, decoction, evaporation) (Markom *et al*., 2007; Wijngaard *et al*., 2012). Because hightemperature water disrupts cells and encourages the penetration and solubilization of solvent molecules, aqueous extraction is more effective at higher temperatures (De La Guardia and Armenta, 2011).

Secondary plant metabolites represent major groups of plant constituents that work predominantly as antioxidants or scavenger of free radicals. In this study, the amounts of total phenolics, flavonoids and tannins in *A. articulata* extracts were carried out. The total phenolics and the total content of tannins in extracts were determined according to Folin-Ciocalteu method using gallic acid and tannic acid as a standard respectively. Whereas, the flavonoids content was estimated by the $AlCl_3$ method and expressed as milligram of qurcetin equivalents per gram of extracts.

The results given in Table 4 showed that chloroform fraction indicating highest content for total phenolics and flavonoids (497.98±0.377 and 79.89±0.789 mg/gE, respectively). n-butanol fraction reported the highest content for tannins 162.89±2.103 mg/gE. The amount of total phenolics and flavonoids compounds in different species of Chenopodiaceae family; *Atriplex halimus* and *A. articulata* reported by Belyagoubi-Benhammou *et al*. (2014) and in *Agatophora alopecuroide*, *A. articulata*, *Hammada elegans*, *Salsola baryosma*, *Salsola vermiculata* reported by Djeridane *et al*. (2015) were lower than those found for *A. articulata* extracts in this study. In terms of total tannins content, all extracts demonstrates a higher TTC than that published by Berrani *et al*. (2019) for methanolic extracts of aerial parts and roots of *A. aretioides* Coss. & Moq.

There are various explanations for variations in the phenolic content. Many intrinsic factors such as plant varieties (genetic), plant parts. environmental circumstances (soil, climate irrigation, temperature range, exposure to diseases and pests), cultural techniques, harvest

season, drying methods, handling and storage factors can also impact plant phenolic content during the plant development cycle (Moghaddam *et al*., 2018).

Furthermore, genetic variety, biological characteristics, and environmental factors may influence the quantity of flavonoids in plants. Phenolic compounds are more soluble in polar organic solvents because they include a hydroxyl group (Aryal *et al*., 2019).

LC-MS-MS was used to qualitatively identify the secondary metabolites found in the nButanol, ethyl acetate, and chloroform fractions of *A. articulata*. The ion trap mass spectrometer was used in multiple reaction monitoring (MRM) mode, utilising both positive and negative ions. Information on 26 Polyphenols standards and whether they were detected or not.

In the present study Seven metabolites were identified, Anthrone, Beta carotene, Butylated hydroxy anisole, Butylatedhydroxytoluene, Gallic acid, Myricetin and Rutin. Chromatography analysis of successive fractions of the ethanolic extract of areal parts of *A. articulata*. Metwally *et al*., (2012) isolated β-sitosterol from the unsaponifiable fraction of *A. articulata*, caffeine from the methylene chloride fraction, and 4-acetoxy phenol from the ethyl acetate fraction using chromatography analysis. These isolates were structurally elucidated using IR, EI-MS, 1H NMR, and ^{13}C NMR methods (Gamal *et al*., 2022b). However, the chromatographic analysis of the MeOH extracts of *Anabasis aretioides* Coss. & Moq. revealed the identification of 25 phenolic compounds (Berrani *et al*., 2019) two of them: Gallic acid and Rutin were detected in the present study in the three studied fractions.

Studies on antioxidant capacity and determination of secondary metabolites involving Saharan Algerian plants are scarce. In fact, no studies about the extract obtained by decoction of flowers and leaves of *A. articulata* was investigated. Antioxidant capacities of plant extracts not only depend on extract composition but also on the conditions of the test used (Dudonné *et al*., 2009). It is crucial to use multiple tests to evaluate the extract composition, which acts through various mechanisms such as preventing chain initiation, binding transition metal ion catalysts, breaking down peroxides, reductive capacity, scavenging radicals, and stopping hydrogen abstraction (Amorati *et al*., 2013) that is why the antioxidant abilities of the studied extract were evaluated using different methods.

In this study eight colorimetric methods which are TAC, DPPH•, ABTS, Hydroxyl radical, hydrogen peroxide scavarg test, Ferrous ion chelating activity, Reducing power and β-carotene bleaching assay are used to evaluate the antioxidant capacity of our natural extract. The results from the antioxidant activity evaluation are presented in Table 6, Figure 24, 25 and 26.

Total Antioxidant Capacity TAC was determined by the phosphomolybdate method. This method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm according to Prieto *et al*., (1999). The present work showed that the extracts reveal a high total antioxidant capacity. The results showed that the overall antioxidant capacity was highest in the chloroform fraction (ChFA) (EC_{50}=0.150±0.0017 mg/mL) and lowest in the aqueous fraction (EC50=0.495±0.0068 mg/mL). The corresponding EC_{50} values for the other extracts were 0.182±0.0041, 0.202±0.0015, and 0.344±0.0037 mg/mL for nBFA, EAFA, and EEAA, in that order. An EC_{50} was 0.002±0.003 mg/mL for the standard (vitamin C). This could be attributed to the presence of phenolic compounds. Tannins and flavonoids may also be responsible for this good activity. According to Hayat *et al*., (2020) secondary metabolites such as polyphenols and flavonoids make a significant contribution to the total antioxidant activity of many medicinal plants. Moreover, antioxidant capacity depends not only on the phenolic compound content, but also on the cholic composition, the total number of hydroxyl groups and their position on the aromatic ring (Shi *et al*., 2022).

DPPH radical scavenging method is common spectrophotometric procedure for determining antioxidant capacity of extracts and it is based on the ability of DPPH radical to decolorize in the presence of antioxidants by accepting an electron or hydrogen donated by an antioxidant compound (Bouaziz *et al*., 2015). Results showed that all extracts exhibited an important activity in scavenging DPPH radicals (Table 6). The extract with the highest free radical scavenging activity were EEAA (IC_{50} = 0.074±0.003 mg/mL), ChFA (IC_{50} = 0.082±0.0012) in the second position, and nBFA (IC_{50} = 0.083±0.0016 mg/mL) in the third position, with an IC_{50} of 0.026±0.0006 mg/mL for the standard. The antioxidant activity of plant extract is most likely related to the amount of polyphenols present and may also vary depending on the quality of polyphenols and flavonoids (Li *et al*., 2012). Antioxidant structural conformation determines the mechanism of the response between antioxidants and DPPH. The structural properties of the antioxidant molecule can affect the DPPH radical, resulting in a decrease in the DPPH

number that is equivalent to the amount of hydroxyl groups in the antioxidant component (Yamauchi *et al*., 2024).

The ABTS radical scavenging activity and the DPPH technique have the same mechanistic response. Both rely on the movement of electrons (Platzer *et al*., 2021). The ABTS test is a decolourising assay that involves the straight radical into monocation, which has a long wavelength absorption spectrum without the participation of any intermediate radical (Suseela *et al*., 2010). The ABTS scavenging power was greater than the DPPH radical scavenging capability in all of our extracts. The highest ABTS radical scavenging activity was demonstrated by nBFA and EEAA, whose IC50 values were similar to the standard BHT's (0.015±0.0003 mg/mL; 0.012±0.0004 and 0.016±0.0002 mg/mL, respectively). After DEAA and AqFA, ChFA EAFA came in second with IC50 values of 0.021±0.0007, 0.022±0.0008, 0.036±0.0008, and 0.039±0.0006 mg/mL, respectively.

The solubility of the stereo selectivity of the extract's radicals in diverse systems impacts the extract's capacity to react against distinct radicals (Ashafa *et al*., 2010). There was a correlation found between phenolic contents and the ABTS scavenging effect. Polyphenols' strong ability to scavenge radicals would demonstrate their antioxidative and other biological properties (Basu and Maier, 2016).

Hydroxyl radical scavenging activity of the extractives was determined by the ability of different extracts to scavenge the hydroxyl radicals wich is a strong reactive oxygen species. In the biological system, it interacts with the polyunsaturated fatty acids in the cell membrane, causing harm. In biological systems, it is crucial to the toxicity caused by ROS since it interacts and inactivates or damages proteins, lipids, DNA, and RNA (Jomova *et al*., 2023).

The evaluation of the hydroxyl radical scavenging activity revealed a significant anti-radical impact, which is listed in decreasing order as follows: IC_{50} values for ChFA, nBFA, EAFA, and AqFA, respectively, are as follows: IC_{50} values for ChFA, nBFA, and EAFA, IC_{50} values for 0.422, 0.0032, 0.509, and 0.0278 mg/mL, 0.782, 0.0092 mg/mL, and 0.802, 0.006 mg/mL, EEAA. Antioxidants provide a significant barrier against radical-mediated toxicity by preventing free radical damage. They primarily serve as free radical scavengers, chain-breaking antioxidants, metal chelators, reducing agents, oxidative enzyme inhibitors, and singlet oxygen quenchers (Choe and Min, 2009). Secondary metabolic may operate as antioxidants since chain

breaking antioxidants act by scavenging radicals, usually hydroxyl radicals, and so preventing the cascade of oxidative events that leads to destruction of lipid membranes, proteins, and DNA (Lü *et al*., 2010).

Hydrogen peroxide scavenging activity one of the most common tests for assessing the trapping capacity of hydrogen peroxide which is based on the uptake of this molecule in the UV domain. Although hydrogen peroxide is not a highly reactive substance, it can be harmful at times. Hydrogen peroxide is a weak oxidising agent that directly inhibits a few enzymes, often by oxidising crucial thiol-groups (- SH). It has the ability to rapidly enter cell membranes. Once inside, it may combine with Fe^{2+} and Cu^{2+} ions to create hydroxyl radicals, which may be responsible for many of its harmful effects (Sunitha, 2016).

In comparison to Vit C (0.010 ± 0.0004 mg/mL), the antioxidant activity of ChFA (EC50=0.361±0.0087 mg/mL) was higher than that of DEAA, AqFA, and EEAA, and comparable to that of nBFA and EAFA (EC50=0.422±0.0032 and 0.509±0.0278 mg/mL, respectively). The present study revealed good scavenging activity suggested that phenolics compounds were responsible for this antioxidant activity. Antioxidants prevent the initiation of oxidation that occurs by inhibiting superoxide anion production and degrading hydrogen peroxide (Sun *et al*., 2009). Numerous studies have shown that there is a positive correlation between total phenols and hydrogen peroxide scavenging ability, and this tendency seems to be present in many plant species (Sroka and Cisowski, 2003; Kumar *et al*., 2017).

Metal chelating ability is crucial because it lowers the concentration of the catalyzing transition metal in lipid peroxidation, which generates the initial few radicals and initiates the radicalmediated oxidative chain reactions in biological systems. Chelating compounds that create bonds with metals have been shown to be efficient secondary antioxidants because they diminish the redox potential (Gulcin and Alwasel, 2022).

The ferrous ion chelating activity of EAFA and decoction extract (0.014±0.002 and 0.073±0.002 mg/mL) exhibit comparable metal-chelating capabilities. When compared to nBFA, ChFA, AqFA, and EEAA (0.19±0.008, 1.11±0.03, 1.4±0.004, and 2.29±0.11 mg/mL, respectively), these two extracts had greater activity. The standard used in the comparison was EDTA (0.003±0.0006 mg/mL). According to the literature, various investigations indicated that polyphenols and flavonoids can be efficient chelators for iron and copper with a certain

structure and functional groups (Halliwell, 2007). Furthermore, it was reported that the compounds with structures containing two or more of the following functional groups: – OH, –SH, –COOH, $–PO_3H_2$, –C=O, $–NR_2$, –S– and –O– have a pronounced metal chelating activity (BiÇek *et al*., 2023).

The reduction of ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) is the method by which the reducing power of extracts is evaluated (Chung *et al*., 2005). The transformation of ferric iron to ferrous iron was determined as reducing capacity. The reducing capacity of polyphenols may represent an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties (Pulido *et al*., 2000).

The extracts had a lesser lowering capacity than the standard, according to the results. When compared to the BHT at a standard 0.051±0.006, EAFA and ChFA showed the best reducing power, with IC50 values of 0.188±0.0008 and 0.314±0.016 mg/mL, respectively. AqFA had an IC50 of 0.6910±0.039 mg/mL and DEAA had an IC50 of 0.9±0.014 mg/mL, with nBFA and EEAA having comparable values of 0.457±0.0256 and 0.497±0.024 mg/ml, respectively. The presence of phenolic compounds in these extracts may be explained by their reducing power. Relations between Fe^{3+} reducing activity and total phenol content have been reported in the literature (Djenidi *et al*., 2020).

β-carotene bleaching tests include giving a hydrogen atom to suppress lipid peroxidation. Biomolecules with antioxidant properties can neutralise linoleate free radicals and prevent βcarotene bleaching (Sarikurkcu *et al*., 2008). The bleaching of β-carotene is due to the radical scavenging of hydroperoxides generated during the peroxidation of linoleic acid and the prevention of linoleic acid peroxidation (Prieto *et al*., 2012). The inhibition of lipid peroxidation is due to a strong occurrence of phenolic compounds such as phenols, saponins, terpenoids and specially flavonoids and tannins. In fact, flavonoids are accounted for its free radical as well as antioxidant activity (Rao, 2016).

Figure 26 shows that all extracts have considerable antioxidant activity when compared to the standard BHT (93.18±1.743%). In terms of β-carotene oxidation, EAFA, nBFA, and AqFA have the most inhibitory effects within a day (85.19±0.803, 85.12±0.793, and 83.93±1.271%). ChFA, EEAA, and DEAA are next best at 80.590±1.032, 79.8954±0.447, and 79.834±1.033%,

respectively. Due to their active hydrogen donor capacity of hydroxyl substitution, the investigated extracts demonstrated a significant anti-radical action that is directly correlated with their total phenolic and flavonoid contents (Pourmorad *et al*., 2006; Loganayaki *et al*., 2013; Joshi *et al*., 2024).

In vitro anti-inflammatory effect of extracts was evaluated utilizing egg albumin denaturation and denaturation of bovine serum Albumin assays with Aspirin serving as the reference drug in each case.

Protein denaturation is the process by which proteins lose their tertiary and secondary structures due to the application of an external stressor substance such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. When biological proteins are denatured, they lose their biological function (Kiranmayi *et al*., 2018) and cause inflammatory diseases. Inflammation is known to be generated by the denaturation of proteins. Denatured proteins generally no longer serve their intended biological purpose. (Nouioura *et al*., 2023). All extracts were able to inhibit protein denaturation and had an effect close to standard drug. Several investigations have shown that the anti-denaturation effect may be attributable to the presence of secondary metabolites (De *et al*., 2017; Henneh *et al*., 2018). They have interesting biological activities as the active principles such as polyphenols, triterpenoids, alkaloids, steroids, sinapic acid, coumaric acid, vanillic acid, kaempferol, ferulic acid and benzoic acid, which had exhibited significant anti-inflammatory activity (Kiranmayi *et al*., 2018; Mahdjar *et al*., 2020).

Among the molecular modelling techniques, docking has emerged as a crucial tool in the field of drug discovery, helping to anticipate the specifics of the molecular interaction between a therapeutically relevant target protein and a molecule (Sharma *et al*., 2021).

Molecular docking results can be applied to assess and enhance biological experimental findings. In biological investigations, the effect of a molecule on a system's ability to operate is measured experimentally. These tests include, for example, binding assays and activity testing conducted on cells or creatures (Ali *et al*., 2023). With this combined approach, scientists may steer the creation of new molecules, assess their potential as medicines, and improve their biological characteristics. The drug development process is accelerated and improved by this, as it provides predictive information and eliminates the need for costly and time-consuming experiments (Benyahlou *et al*., 2023).

In molecular docking study using AutoDock Vina program, Figure 26 and 27 showed many hydrogen bonding connections between each ligand and the protein. Additionally, non-covalent interactions like hydrophobic and electrostatic connections were discovered. These noncovalent interactions help the ligand-protein complexes stay stable and particular, which eventually increases their effectiveness. Based on the results obtained, each of these ligands has the potential to be an effective principle in the fight against this illness, as we can expect. Additionally, the combined efforts of these 7 ligands may produce even better results. Finally, *in silico* findings support the results of the investigation study presenting action that reduces inflammation which demonstrated an excellent correlation with the experimental findings.

In acute toxicity study by gavage, no death up to 14 days of the study period. Even at the highest dose 5000 mg/kg b.w, there were no physical signs of toxicity as evidenced by normal breathing and the absence of tremors, convulsions, diarrhea, salivation and paralysis in the treated animals. This suggests that DEAA and EEAA was safe and non-toxic to mice during the monitoring period according to the OECD categorization and consequently, it was calculated that the median lethal dosage (LD_{50}) was greater than 5000 mg/kg b.w. It may be suggested that compounds from decoction and ethanolic extract of flowers and leaves of *A. articulata* extract present no acute toxicity, since no toxic effects were found after high dose administration.

Based on the OECD, (2001) classification, this indicates that DEAA and EEAA were safe and non-toxic to mice during the monitoring period. As a result, the median lethal dose of DEAA and EEAA was determined to be in excess of 5 g/kg b.w. It is possible that compounds derived from DEAA and EEAA show no acute toxicity, since harmful effects were found after high dose administration. This finding is in line with that found by Kaddour *et al*., (2019), who found that *Arthrophytum scoparium* (Chenopodiaceae) at doses of 2 and 5 g/kg did not cause any behavioural abnormalities or mortality during the essay.

The body weight gain showed no significant difference ($P>0.05$) between the control and treated groups after DEAA and EEAA administration. This result showed that DEAA and EEAA did not affect the increase in body weight of mice (Figure 32). This may be partly explained by the fact that DEAA and EEAA had no detrimental effects on appetite or food consumption. This may be that the mice grew and gained weight over time during the test since the changes in body weight from the control would reveal the no toxicity of the extract (Sireeratawong *et al*., 2008).

Relative organ weights are important indicators of target organs injury and physiological disturbances (Righi *et al*., 2021; Nouioura *et al*., 2023). The present study as illustrated in Figures 33 and 34 did not reveal any significant changes in organ weight in the heart and spleen after administration of DEAA and EEAA, however, the kidney and liver relative weights exhibited progressive increases at 5000 mg/kg, yet this increase was significant compared to control group. This increase in the liver relative weight may be due to an increased metabolic response to the extract, which may has increased hepatic activity (Aouachria *et al*., 2017).

Histopathology study of the liver and kidney (Figure 35) revealed normal hepatocytes and normal glomeruli, no alteration in the structure in treated animals compared to control except the vascular congestions that were noticed in liver and kidney tissues of animals treated with DEAA or EEAA (5000 mg/kg).The presence of congestion in liver and kidneys may be a result of a vasoconstriction action induced by the extract on the wall of blood vessels (Grande *et al*., 2017).

Three procedures were used to examine the anti-inflammatory activity of DEAA and EEAA: Carrageenan induced paw edema in rats, croton oil, and xylene induced ear edema in mice.

Tests were conducted with two doses: 100 and 200 mg/kg. The acquired findings were compared with the drug; Indomethacin (10 mg/kg). Figure 36-A and 37-A illustrated the paw thickness of inflammation during 6 h of administration of standard and two doses of DEAA and EEAA respectively. In carrageenan treated animals, after a progressive rise, the paw edema peaked at 6.138±0.023 mm after 6 hours. However, the inhibition of paw edema in rats receiving DEAA and EEAA showed inhibition at oral doses of 200 mg/kg of 91.284 ± 1.743% and 77.926 ± 1.733%, respectively compared to indomethacin treated group with percentage of inhibition of 89.984 ± 1.323% Figure 36-B and 37-B. Abdallaha *et al*., (2014) and Ben Menni *et al*., (2022) reported similar findings for *Anabasis articulata* using methanolic and the fraction for the first and using the sterols for the second. On the other hand, *Chenopodium ambrosioides* (Chenopodiaceae) extract described by Ouadja *et al*. (2021) could not reduce Carrageenaninduced paw edema in rats.

Carrageenan can cause a range of symptoms in humans that are comparable to acute inflammation, including fast edoema, increased vascular permeability, localised telangiectasia, and plasma extravasation (Dai *et al*., 2019). A well-known and often used model of

inflammation in rats is carrageenan-mediated paw edoema, which results in a biphasic phase in the inflammatory pathway (Ben Khedir *et al*., 2016).This inflammation is characterised by the successive release of many inflammatory chemicals. The initial phase (1-3 hours) contains serotonin, histamine, and bradykinin, whereas the latter phase (3-6 hours) contains prostaglandins. Inflammatory mediators and reactive oxygen species are both elevated at the final phase of inflammation. NSAIDs often do not suppress the first phase of inflammation (Necas and Bartosikova, 2013).

Croton-oil is extracted from *Croton tiglium* L. seeds (Salatino *et al*., 2007) and it causes topic irritation and edema when applied topically (Al Amin *et al*., 2012). This test measures a variation in ear plug volume after the injection of an irritation agent in order to determine the inflammatory response. The findings of this investigation are shown in (Figure 38 and 39).

Edema is effectively reduced by 77.58±0.64% and 76.193±1.135% at dose of 200 mg/kg of DEAA and EEAA, respectively. Indomethacin, employed as a positive control has an estimated 80.68±1.258% anti-inflammatory effect. Croton oil-induced dermatitis exemplifies an acute inflammatory reaction at its peak. This test is commonly used for discovering prospective antiinflammatory medications in the management of skin conditions (Mo *et al*., 2013). The 12-Otetra-decanoyl-13-phorbol acetate (TPA) molecule is what gives croton oil its phlogogenic properties. TPA causes inflammation that manifests as a significant increase in blood vessel permeability, neutrophils infiltration, edoema, and the generation of mediators of inflammation (Karbab *et al*., 2020). According to Pérez *et al*., (2011), TPA-induced mouse ear edoema was effectively prevented by the essential oil of *Chenopodium album* L. (Chenopodiaceae).

The oedematization caused by xylene in mice's ears resembles oedematization that occurs during the early phases of acute inflammation (Torres-Rêgo *et al*., 2016). Following topical application of xylene, signs of acute inflammation include significant vaso-dilation, skin edoema, and inflammatory cell infiltration (Kim *et al*., 2007).An increase in ear oedema caused by xylene was considerably and dose-dependently reduced by the plant extract.

Figures 40 and 41 display the results of this experiment. The edoema is successfully reduced by DEAA and EEAA at dosage of 200 mg/kg, at 81.74±0.36% and 70.65±3.19%, respectively. The concurrent anti-inflammatory impact of indomethacin, the positive control, was assessed to be 82.98%.

Both extracts have anti-inflammatory properties which may be attributed to the presence of steroids, alkaloids, and flavonoids presents in extracts. The phytochemical studies of *A. articulata* revealed a variety of secondary metabolites, including phenolics (Gamal *et al*., 2022b), alkaloids (Belyagoubi-Benhammou *et al*., 2019) and saponin ssuch as 3-O-glucopyranosyl of stigmasterol, ßeta-sitosterol, sitostanol, 3-O-(ßeta-D-the gluco-pyranosyl] oleanolicacid, 3-O-[ßeta-D-gluco-pyranosyl -28-O-ßeta-D- xylopyranosyl] oleanolicacid, in addition to procericacid (Metwally *et al*., 2012).

The impact of ethanolic and decocted extracts of *A. articulata* on peripheral nociceptive sensitivity was tested in mice using an analgesic activity test. In mice, an intraperitoneal injection of 6% acetic acid triggered a cramp-like response characterised by waves of contraction and elongation of the abdominal muscles, followed by extension of the hind legs (Yemitan and Adeyemi, 2017). In our investigation, decocted and ethanolic extracts significantly ($P < 0.001$) decreased the number of acetic acid-induced abdominal contractions or twists in a dose-dependent manner. Extracts have a dose-dependent analgesic effect, which is consistent with previous research (Qnais, 2011; Bhat *et al*., 2014; Zaheer *et al*., 2018). The mechanism of pain beginning arises from a tissue damage that causes the release of various chemical mediators like as histamine and serotonin in the intraperitoneal fluid, which excite nociceptive receptors situated at the peritoneal (Uddin Chy *et al*., 2021). Acetic acid-induced contractions are also caused by the generation and release of algogenic mediators via cyclooxygenases (COX) and prostaglandin biosynthesis, particularly PGE_2 generated by COX_1. (Acha *et al*., 2019). These then stimulate pain nerve terminals, because abdominal contraction is associated to sensitisation of nociceptive receptors to prostaglandins. (Jang *et al*., 2020).

Acetylsalicylic acid treatment can decrease the algogenic impact of acetic acid by reducing the production of pain mediators in peripheral tissues and inhibiting COX_1 and COX_2 activity (Freye, E., 2008). The mechanism of action of analgesics may involve inhibition of lipo- and cyclooxygenases as well as the synthesis of inflammatory mediators and prostaglandins (PGE2 and PGE2α) (Dou *et al*., 2004). *A. articulata* extract exhibits analgesic efficacy, based on the data obtained. Because the extracts contained alkaloids and phenolic components, they may have some peripheral analgesic effect. In other therapeutic plants, these substances are known to have analgesic effects (Annegowda *et al*., 2012; Sen *et al*., 2013; Harchaoui *et al*., 2018).

According to Franke *et al*., (2005), the most prevalent cause of stomach ulcers is alcohol consumption. Excessive alcohol consumption raises the risk of stomach mucosal injury. The rat model of ethanol-induced gastric mucosal injury is commonly used in research to test drugs with putative anti-ulcer activities, as ethanol is the principal cause of stomach ulcers reported in people. (Beiranvand, 2022).

A gastric ulceration is a benign lesion of the mucosal epithelium that develops in the stomach as a result of exposure to excessive acid (Sabiu *et al*., 2015). The animal model of ethanolinduced gastric ulcer is a common and practical one for studying gastroprotective medications (Sistani Karampour *et al*., 2019).

In the present study as illustrated in Figure 43 and 44, the percentage of ulceration was 5.16±0.33% at dose of 200 mg/kg for the decocted extract of *Anabasis articulata* (DEAA). 12,67±0.26% was the percentage of ulceration for the ethanolic extract (EEAA) at dose of 600mg/kg. So, the oral administration of DEAA displayed protective effects against ethanol induced gastric lesions more than EEAA.

Ethanol causes disorganization in mucosal microcirculation and ischemia, creates free radicals, and releases endothelium, resulting in its ulcerogenic action (Prazeres *et al*., 2019). The considerable rise in ulcer index following oral treatment of ethanol in the ulcerated rats might be due to either free radical production or suppression of prostaglandin synthesis. (Kouitcheu Mabeku *et al*., 2017).

Flavonoids have been observed to enhance mucosal prostaglandin content. Reduced prostaglandin levels have been associated to decreased gastroprotection and increased stomach output, which are critical events in the aetiology of mucosal ulcers (Adefisayo *et al*., 2018). The preliminary phytochemical analysis of DEAA showed the presence of flavonoids. Flavonoids have been reported to possess antiulcer activity in various experimental models (Sakat *et al.*, 2012; Shahzad *et al*., 2024). Several mechanisms have been proposed to explain gastroprotective effects of flavonoids; these include increase of mucosal prostaglandin content, decrease of histamine secretion from mast cells by inhibition of histidine decarboxylase and inhibition of Helicobacor pylori growth. In addition, flavonoids have been found to be free radical scavenger; free radicals play an important role in ulcerative and erosive lesions of

gastrointestinal tract (Dhiman *et al*., 2016). So, the antiulcer activity of DEAA may be attributed to its flavonoids content.

The use of pharmacological compounds that operate on these several axes is necessary to understand the mechanism of action and to look for novel anti-ulcer drugs since the aetiology of gastric ulcers is complex and can affect multiple axes in the stomach. For this reason, rats with stomach ulcers caused by absolute ethanol were examined in the presence or absence of the selected extract DEAA at a concentration of 200 mg/kg. The animals were pretreated with L-NAME, glibenclamide, and indomethacin.

In order to understand how *A. articulata* extracts protect against ethanol-induced acute gastric mucosal lesions, we looked into the mechanisms underlying these benefits. Direct contact between ethanol and the stomach mucosa can result in damage due to the powerful mucosal damaging agent. We found that ethanol, a topical irritant that interacts directly with the stomach mucosa when any pharmacological agent is present, caused the gastric mucosa of rats to become severely hemorrhagic. Similar hemorrhagic lesions in the stomach of rats caused by this combination have been reported (Ribeiro *et al*., 2016).

The proliferative zone of stomach glands and mucosal defence mechanisms preserve the delicate balance between the epithelium and subepithelial components, which is necessary for the preservation of gastric mucosal integrity when exposed to a variety of hazardous substances (Czekaj *et al*., 2018). These processes include preserving continuous blood flow to the mucosa, secreting mucus and alkaline substances, and repairing and growing mucosal cells (Forssell, 1988). Endogenous prostaglandins, which stimulate the creation of NO, hydrogen sulphide, and carbon monoxide by endothelial cells and the mucosa, increase mucosal integrity and gastroprotection (Magierowski *et al*., 2016).

This molecule (NO) is involved in many processes in the gastrointestinal tract, such as blood flow regulation, vascular tone maintenance, mucus secretion from the stomach, mast cell activity modulation, and smooth muscle tone adjustment, which includes antral motor activity, intestinal peristaltism, and gastric emptying (Falcão *et al*., 2013).

Our findings demonstrated that the gastroprotection provided by the decocted extract of *A. articulata* reduces the reduction of NO production caused by the action of NO synthase

inhibitor (L-NAME), suggesting that the maintenance of synthesis is essential for gastric protection provided by DEAA (200mg/kg). By interacting with other mediators involved in mucosa protection, such as prostaglandins and the release of mucus and bicarbonate, NO can also modify the integrity of the stomach mucosa (Tarnawski *et al*., 2013).

Our findings indicate that the decocted extract of *A. articulata* offers gastroprotection against the effects of glibenaclide, a KATP channel blocker that prevents prostaglandin generation. Prostaglandins are also significant endogenous gastroprotective agents that regulate mucosal blood flow by promoting mucus and bicarbonate production, preventing neutrophil adhesion and activation, and enhancing epithelial cell resistance to possible cytotoxic injury (Makmun, 2005).

As a non-steroidal anti-inflammatory medicine (NSAID), indomethacin primarily causes stomach injury by preventing the formation of prostaglandins by blocking the activity of COX1 isozymes (Yokota *et al*., 2005). The stomach mucosa was successfully shielded against lesions caused by the combination of ethanol and indomethacin by oral treatment with *A. articulata* 200 mg/kg.

Glibenclamide, Indomethacin, and L-NAME pretreatments did not prevent the gastroprotection provided by DEAA 200 mg/kg. This implies that the decocted extract of *A. articulata* could function via a different mechanism under these conditions.

Ethanol is usually used to induce gastric lesions in animal models (Aziz *et al*., 2019), gastric lesions are characterised by a condition of inflammation, oxidative stress, free radical production, and apoptosis (Jasna and Draze, 2011). Ulcer indices, such as pH level and lesion count, are employed as indicators of stomach ulcers caused by toxic exposure. The acidity of the stomach discharge is mostly reliant on the pH value, hence an increase in hydrogen ion concentration in gastric juice leads to gastric injury (Aboul Naser *et al*., 2020). These observations were consistent with our findings, as evidenced by the observed decrease in stomach acidity and lesion numbers. The considerable fall in pH in the ulcerated stomach may be related to the imbalance between free radical production and scavenging ability (Repetto and Llesuy, 2002). In recent years, researchers have focused on exploring new safe and effective antiulcer medications or supplements from natural sources (Chatterjee and Bandyopadhyay, 2014; Chakraverty *et al*., 2016; Sharifi-Rad *et al*., 2018). Evidently, it was shown that the

preventive effect of Anabasis articulata extract was successful in raising the pH level and lowering the number of lesions and gastric acid discharges in rats with stomach ulcers.

An increasing amount of data points to a close relationship between elevated ROS levels and ethanol-induced stomach mucosal damage (Chen *et al*., 2021). Increased ROS provoke oxidative injury, cell death, and epithelial damage (Nakamura and Takada, 2021). According to Palle *et al*., (2018), ROS also decrease prostaglandin synthesis, which lowers mucus formation, and histamine release, which increases stomach acid output.

The first line of defence against acid is gastric mucus, which clings to the epithelium's produced bicarbonate to act as a barrier against self-digestion (Moraes *et al*., 2009). Mucus gel is made up of 5% mucin glycoproteins and 95% water, which is released by surface epithelial cells by apical ejection (Perez-Vilar and Hill, 2004).

The current investigation showed that the rat stomach's mucus content was considerably reduced by 100% ethanol treatment. But in every group that received 200 mg/kg of DEAA, this impact was substantially amplified. This implies that the potential of DEAA to raise the amount of gastric mucus in the stomach may contribute to their gastroprotective action.

According to Zakaria *et al*.,(2016) and Kim *et al*.,(2020) claim that the increased mucus secreted by the gastric mucosa cells can prevent the development of gastric ulcers by functioning as an efficient barrier to the diffusion of back hydrogen ions, enhancing the gastric juice's buffering capacity, and lowering friction in the stomach wall during peristalsis.

The analysis of stomach histology sections supported the macroscopic findings. Indeed, the histological sections show that the delivery of 100% ethanol caused significant hemorrhagic lesions. The histological alterations, which included significant disintegration of the surface epithelium, necrotic lesions profoundly piercing into the mucosa, widespread submucosal layer oedema, infiltration of inflammatory cells, and blood vessel congestion, corroborated these findings. In all groups, the ethanol-induced stomach damage was successfully reversed by oral administration of DEAA 200 mg/kg, resulting in a considerable decrease in the gastric ulcer area when compared to the negative control. Furthermore, the previously stated stomach histological alterations brought on by ethanol were lessened by DEAA 200 mg/kg.

According to our histological findings and in agreement with Aboul Naser *et al*.,(2020), ethanol damages the mucosa of the stomach, disrupts the cellular membranes of the mucosa, and damages the gastric microvessels, all of which lead to bleeding and necrosis. Furthermore, it results in epithelial cell damage and severe submucosal edoema (Park *et al*., 2008). Previous research by Orole, (2013) and Bawish *et al*., (2023) supporting the importance of plant extracts rich in flavonoids, alkaloids, and steroids as antiulcer drugs in rats model supported our data.

These results indicate that our extract is regarded as a potent gastroprotective agent; the observed gastroprotective effect may be attributed to the high phenolic and flavonoid content of *A. articulata*. The gastroprotective action of flavonoids has been explained by a number of mechanisms, including raising the prostaglandin content in mucosa and inhibiting histidine decarboxylase to reduce mast cell histamine release (Juvekar *et al*., 2012; Dhiman *et al*., 2016).

Through the generation of more ROS and the depletion of cellular antioxidants, ethanol causes oxidative stress. Because of damaging agents such superoxide anions, hydroxyl radicals, and lipid peroxides, this ROS-driven process causes lipid peroxidation and protein oxidation, which contributes to both acute and chronic ethanol-induced stomach ulcers (Wu, 2003; Sadek, 2022).

The production of oxygen free radicals is associated with stomach lesions caused by ethanol. Furthermore, the processes of lesions also depend on the generation of inflammatory mediators (Repetto and Llesuy, 2002). Furthermore, gastric mucosal lesions, bleeding, and necrotic tissue damage are brought on by stomach blood flow stasis and microvascular disruption, which aggregate and release substances that disturb tissues in a variety of tissues. Neutrophil infiltration is essential to the processes of inflammation and injury (Tarnawski *et al*., 2012) .

Rostami-Motamed *et al*., (2015) claim that there is a connection between ethanol-induced stomach tissue damage and oxygen free radical regeneration. Furthermore, the production of inflammatory mediators plays a key part in the fundamental mechanisms behind these lesions. Furthermore, abnormalities in the microvasculature and stomach blood flow result in bleeding and necrotic tissue destruction (Rahman *et al*., 2020). Damage and inflammation are mostly caused by the process of neutrophil infiltration, which is the aggregation and release of substances that disrupt tissue inside a number of tissues, including the stomach mucosa (El Badawy *et al*., 2021).

Through the generation of more ROS and the depletion of cellular antioxidants, ethanol causes oxidative stress. Mostly brought on by damaging substances including superoxide anions, hydroxyl radicals, and lipid peroxides, this ROS-driven process causes lipid peroxidation and protein oxidation, which contributes to both acute and chronic ethanol-induced stomach ulcers (Wu, 2003). Scavenging free radicals is a key mechanism in gastric ulcer healing (Musa *et al*., 2017). While stomach cells have a number of enzymatic and non-enzymatic antioxidants, such as sulfhydryl groups (NPSH), glutathione peroxidase (GPx), endogenous glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), to scavenge ROS, excessive ROS generation increases lipid peroxidation and depletes these antioxidant enzymes (Boligon *et al*., 2014). As the initial line of defence against ROS, the SOD transforms highly reactive O_2 into less reactive H_2O_2, protecting the stomach tissue from harm. We are aware that the stomach mucosal tissue has a reduction in SOD expression. The most often utilised indication of oxidative stress in cell and tissue damage is the MDA, a lipid peroxidation product (Ermis *et al*., 2023). A prior study established that ethanol leads to an increase in neutrophil generation of superoxide anions and hydroxyl radicals, which then combine with cellular lipids to generate lipid peroxides, which are then further metabolised to form malondialdehyde (MDA) (Nur Azlina *et al*., 2015). 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 (Grotto *et al*., 2009).

As demonstrated in Table 8, concurrent treatment with L-arginine in this study mitigated the effects of the combination of absolute ethanol with several pharmacological substances: LNAME, glibenclamide, and indomethacin. These effects included an increase in MDA levels and a decrease in SOD and CAT with surface epithelium lesions and submucosal inflammation. On the other hand, all groups show a reduction in acute gastric mucosal lesions, a decrease in SOD and CAT, and a rise in MDA levels with administration of DEAA 200mg/kg. These results are consistent with those reported by (Gamal *et al*., 2022). The use of non-selective NOS inhibitors, including L-NAME, worsened gastric mucosal damage and reduced stomach blood flow (Nishio *et al*., 2006).

Recently, the involvement of KATP channels in several models of gastric protection was described (Peskar *et al*., 2002; Medeiros *et al*., 2008). For this reason, it was determined that this route is involved in the gastroprotective mechanism by applying the KATP channel blocker Glibenclamide (Rios *et al*., 2010; Ribeiro *et al*., 2016).

Studies have indicated that prostaglandin-mediated gastroprotection includes the opening of ATP-sensitive potassium channels (KATP), which appears to be involved in various physiological activities in the stomach, such as blood flow control, gastric acid production, and stomach contractility (Klein-Júnior *et al*., 2012; Júnior *et al*., 2013). The gastroprotective effect of DEAA 200mg/kg against ethanol-induced stomach injury was diminished when KATP channels were inhibited with glibenclamide, indicating that KATP channels may play a role in DEAA's gastroprotective mechanism.

NSAIDs, the principal medications of choice in the treatment of inflammatory illnesses such as rheumatoid arthritis, are regarded a recognised cause of peptic ulcers in humans and animals (Watanabe *et al*., 2020). Indomethacin-induced gastric lesions are mostly produced by the enzyme cyclo-oxygenase (COX) inactivation, which lowers prostaglandin production (Chatterjee *et al*., 2012). Prostaglandins are endogenous gastroprotective chemicals that also play a role in the repair and protection of the gastric mucosa. They prevent the activation and adhesion of neutrophils, regulate mucosal blood flow through bicarbonate and mucus production, and protect epithelial cells against substances that might cause ulcers (Shahzad *et al*., 2024). Rats were pretreated with indomethacin in order to confirm that PGs were involved in the gastroprotective activity of *Anabasis articulata*; the outcome indicates that PGs are involved in the process.

The ulceration brought on by the pretreatment with L-NAME, Glibenclamide, and Indomethacin was significantly inhibited by the oral administration of DEAA 200 mg/kg, indicating that the NO, KATP channel, and prostaglandin pathways are involved in the protective mechanism provided by the *Anabasis articulata* decocted extract.

Conclusion and Perspective

This study marks the first investigation into the safety of an ethnopharmacological, analgesic and anti-inflammatory *in vivo* and *in vitro* and *in silico* properties of extracts derived from the flowers and leaves.

The present study demonstrated that *A. articulata* extracts are a rich source of phenolic compounds, Finding showed that ChFA represents the highest amount of total polyphenols and flavonoids, while n-Butanol fraction showed the highest amount of total tannins. In the present study Seven metabolites found in the n-Butanol, ethyl acetate, and chloroform fractions were identified qualitatively using LC-MS-MS: Anthrone, Beta carotene, Butylated hydroxy anisole, Butylatedhydroxytoluene, Gallic acid, Myricetin and Rutin.

The findings from the inflammatory and analgesic investigations validate the traditional use of *A. articulata* and the *in silico* study confirm these results. The acute toxicity study suggests that the aqueous extract of the plant is safe up to a dose of 5 g/kg of mice body weight when consumed orally as a single administration. DEAA can be considered a potential source of natural compounds that can be incorporated into the human diet, alongside its potential applications as antioxidant and anti-inflammatory agents.

This study offers significant insights into the acute oral toxicity profiles of DEAA and EEAA which can greatly benefit any forthcoming *in vivo* and clinical investigations involving this plant medicine.

The findings also highlight the potential of *A. articulata* as a natural antioxidant, suggesting its possible utility as functional food ingredients and in pharmaceutical applications.

In the case of DEAA-induced gastroprotective effect, both NO and the cyclooxygenase pathways were implicated. Also, the adherent mucus normally covers the gastric mucosa and acts as a protective physical barrier against noxious luminal agents.

We evaluated several oxidant-antioxidant parameters in the rat stomach tissues in order to investigate the function of oxidative stress in our ulceration model. In line with the hypothesis that oxidative stress plays a role in the pathophysiology of ethanol-induced gastric ulcers, the experimental results demonstrated that ethanol significantly raised the MDA level. This impact was followed by a reduction in CAT and SOD activity. On the other hand, the pretreatment with DEAA significantly increased the levels of CAT and SOD and decreased MDA production, showing their antioxidant activity. Their effect on gastroprotection may be partially explained by this.

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 Gastric ulcer.

After conducting a thorough assessment of the many biological activities linked to the chosen medicinal plant, there are still a number of uncharted research directions that need to be investigated:

- ✞ It is strongly advised to do a thorough analysis of the chemical components found in the plant.
- ✞ It is highly advised to carry out comprehensive cytotoxicity tests on a variety of cell lines, with a special emphasis on cancer cell lines.
- ✞ It is crucial to investigate further the processes behind the plant's effects on stomach emptying and its gastroprotective properties.
- ✞ An important next step is to identify and characterise the active molecules causing the biological activities that have been seen.
- ✞ Utilising these plant elements' positive qualities for the food and pharmaceutical sectors presents a promising opportunity.

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ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS IN "EL-MERGUEB" NATURE RESERVE, M'SILA PROVINCE, NORTHERN ALGERIA

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Abstract. Medicinal plants are considered as a rich sources of bioactive compounds. The present study aimed to document the local knowledge of medicinal plants' use in El Mergueb natural reserve of the wilaya of M'sila, northern Algeria. The valorization of this natural heritage requires an ethnobotanical study which allows to describe the different uses of medicinal plants by the local population and to establish the catalogue of medicinal plants and their therapeutic uses. Ethnobotanical data were collected from 182 local informants through semi-structured questionnaires and open interviews. In the present study a total of 46 plant species belonging to 23 botanical families were recorded for the treatment of different ailments. Asteraceae was the most represented family with 9 species followed by Poaceae, Lamiaceae and Fabaceae. *Artemisia herba-alba* Asso (UV = 0.58), *Stipa tenacissima* L. (UV = 0.42), *Artemisia campestris* L. (UV = 0.3), *Zyziphus lotus* L. (UV = 0.16) and *Marrubium vulgare* L. (UV = 0.14) were the most frequently used by local informants, with the highest UV. Leaves (39.6%) were the most widely used plant parts. Most herbal remedies were consumed as decoction and infusion. The results obtained are very valuable source of information on the region studied and on the Algerian medicinal flora. They could serve as a database for further phytochemical and pharmacological investigations and for research into new natural sources of biomolecules beneficial to people.
Keywords: *survey, phytotherapy, diseases, semi-arid region, Algeria*

Introduction

Algeria is the largest country in the Mediterranean basin and Africa with a total area of 237 639 100 ha, distinct bioclimatic, ecological zones, and significant species diversity (Souilah *et al*., 2018). Medicinal plants have long been a part of healthcare in many parts of the world, particularly in developing nations. Traditional medicine has used medicinal plants as a cure for a variety of diseases for many years (Ekor, 2014).

Therapeutic plants with pharmacological qualities have been identified as rich sources of components with significant potential for disease prevention (Karbab *et al*., 2020). Like other countries in the world, plants have long been used in Algeria to cure a variety of diseases. The knowledge of the plants used can shed light on their qualities, which will allow future research (Hamza *et al*., 2019).

Ethnobotany is the study of human-plant relationships; several active chemicals utilized in modern medicine are derived from ethnobotanical data, which is mostly based on popular and traditional medical knowledge. This knowledge, which primarily belongs to traditional practitioners is still passed down orally putting this herbal traditional practice at risk of extinction if it is not preserved (Ouelbani *et al*., 2016).

In Algeria, many ethnobotanical and ethnopharmacological studies have recently been undertaken in different regions such as the central Sahara (Hammiche and Maiza, 2006),

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the North (Bouasla and Bouasla, 2017) and Northwest Algeria (Benarba *et al*., 2015) in order to preserve indigenous knowledge and to develop a strategy for the protection of biodiversity and particularly rare plant species, threatened or extinct in the wild from excessive human use (Bakiri *et al*., 2016). The inventory of plants is now estimated at 4449 taxa, including 3951 native taxa of which 464 are endemic (Habib *et al*., 2020). In this context, we conducted an ethnobotanical study in El Mergueb natural reserve. To our knowledge, this region has never been explored ethnopharmacologically. Therefore, this study was conducted to obtain the ethnomedicinal knowledge of the El Mergueb natural reserve and to determine the cultural importance of plant taxa, families, local names, usage parts and methods of preparation.

Material and methods

Ethnobotanical survey

Survey area

M' sila (known also as Hodna) region is situated in the central part of Northern Algeria at 351 42′ N latitude and 41 32′ E longitude. It covers a total area of 18718 km^2 (47 municipalities) (*Fig. 1*). The territory is mostly hilly with an average altitude of 500 m, semi- arid and characterized by the predominance of steppe (63% total area) (Boudjelal *et al*., 2013). El Mergueb reserve is a natural steppe space located at the western edge of the Hodna pan (*Fig. 1*). It covers an area of 16.481 ha, straddling the communes of Sidi Hadjeres, Ain El Hadjel and Sidi Ammer (Boudjadja *et al*., 2010). It extends between the coordinates of Lambert relative to the topographic map of Aïn El-Hadjel to (1/50.000, following: X (608.5 and 626.7) km and Y (243.6−263.8) km. The three experimental stations lie between latitudes (35°36'12.6"N and 35°35'05.7"N) and longitudes (03°56'23.8"E and 03°58'08.7"E) with an altitude of 575 and 634 m located in the municipality of Ain EL-Hadjel (Adjabi *et al*., 2019).

The climate of M'Sila is characterized by semi-arid and dry conditions typical of a desert region. It is continental, subject in part to the Saharan influences. According to the data from the meteorological station of M'sila (2010-2015), the variation in average monthly precipitation is irregular in a general manner. The rainyest month is September with an average of 25.6 mm, while July has the least rainy month with a value of 3.75 mm (*Fig. 2*). January is the coldest month, with a minimum of 8.41°C, while the warmest month is July, with an average maximum of 31.11°C for average monthly temperatures (*Fig. 2*). The average maximum temperature curve (M) shows that the highest maximum temperature is recorded in July with 44°C and the lowest average maximum temperatures observed in January 18.3°C. Thus, M'sila has a dry period that extends over twelve months, from January to December and it is located in the semi-arid bioclimate with a cold winter according to the Emberger rainfall ratio (Q2 = 15.62) (Merniz *et al*., 2018).

Data collection

In order to identify and establish a list of plants used in traditional medicine due to their pharmacological properties, an ethnobotanical survey was carried out using a questionnaire distributed to villagers and people with knowledge on the use of medicinal plants through face-to-face interviews, with the aim of collecting accurate information on

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therapeutic practices by the population. Our study was conducted from June 2020 to January 2021 spread over 182 questionnaire cards using probability sampling method (Ocvirk *et al*., 2019) and all the respondents were villagers from El Mergueb. The information is divided into two sections (*Fig. 3*); the first concerns the informant as the sole owner of the information, while the second gathers information concerning the medicinal plant such as local name, plant parts used, spontaneous or cultivated, toxicity, medicinal use and preparation. The identity of each plant species mentioned by the informants was verified and confirmed by a bibliography (Quézel and Santa, 1962a) and Ozenda (1977). A medicinal use was accepted as valid only if it was mentioned by at least three independent interviewees (Al-Qura'n, 2009).

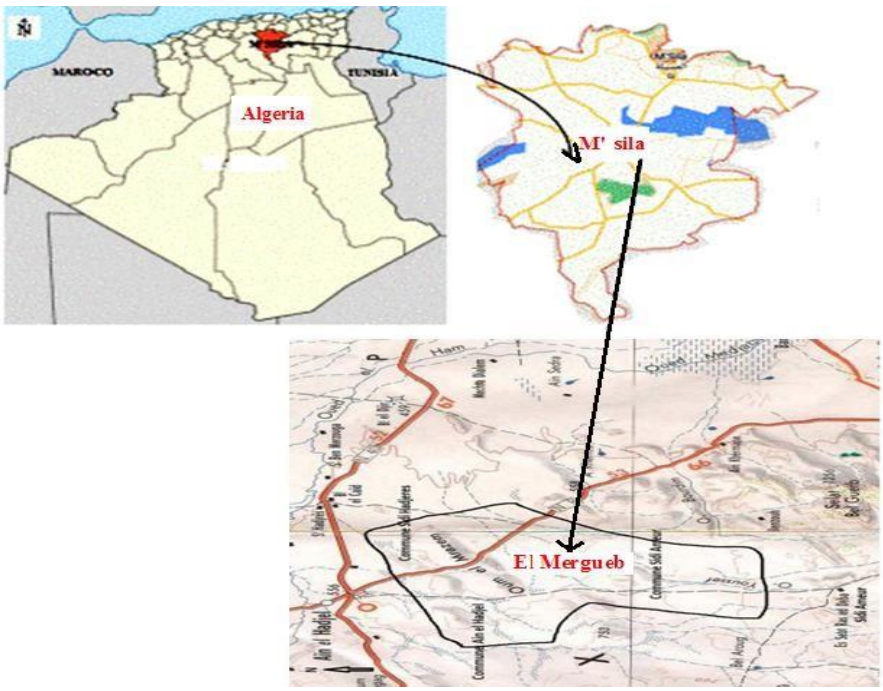

Figure 1. *Geographical location of El Mergueb nature reserve (Algeria)*

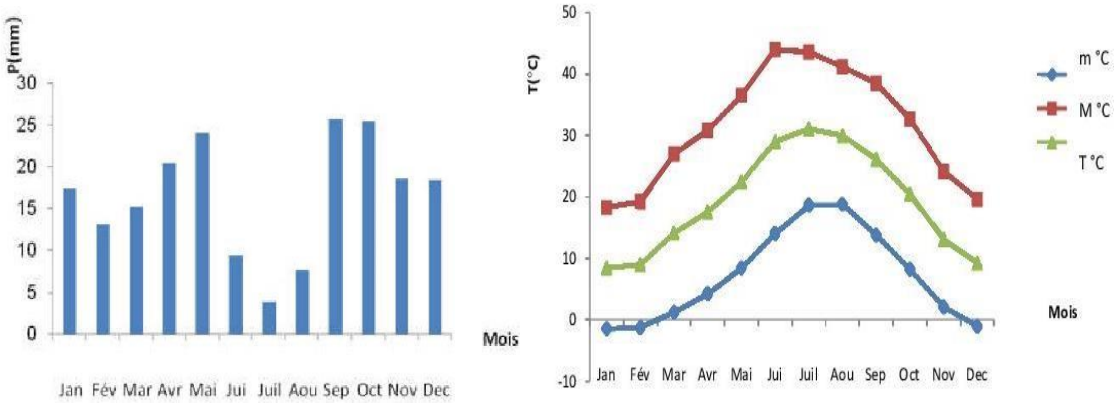

Figure 2. *Average rainfall and temperature in M'Sila, Algeria (Merniz et al., 2018)*

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Data analysis

Data obtained were spread on an Excel spreadsheet and statistically analyzed (age, gender, education level, Origin of ethnobotanical knowledge, various proportions like plant families, plant parts used, methods of use). The particular use of use value (UV) is to determine the relative significance of the local species. UV was calculated using *Equation 1.*

$$UV = \Sigma Ui/N \quad \text{(Eq.1)}$$

where Ui: number of uses reports per species, and N: total number of informants (Trotter and Logan, 1986). It demonstrates the relative importance of plants known locally (Ouelbani *et al*., 2016).

Section A: *Questions about the informant: sex, age, level of education, origin of ethnobotanical knowledge.*

Variables	**Categories**
Gender	Female Male
Age	[20years-35years] [36years-50years] [51years-60years] >60years
Educational level	Illiterate Primary College Secondary University
Origin of ethnobotanical knowledge	Family knowledge Herbalist Scientific research

Section B: *Questions concerning the medicinal plant: uses of the medicinal plant, the part of the plant used, the pathology treated.*

Plant name		Part used	Mode of use	Therapeutic property	Spontaneous/ cultivated/ Toxicity
Scientific name	Vernacular name				

Figure 3. *Questionnaire card of survey*

Results and discussion

Demographic features of the informants

For all interviewers, gender, age, profession, and background information were recorded. According to *Table 1*, the local informants (n = 182) comprised 69% women

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and 31% men were interviewed. This result is in agreement with the studies of Ramdane *et al*. (2015), Miara *et al*. (2018) and Bouafia *et al*. (2021) who found that the majority of informants were women with the percentage of 63%, 56% and 57.14%, respectively when the authors compared medicinal plant knowledge held by males and females. This could be due to women's greater knowledge of medicinal plants and the ease with which they can share this knowledge.

The age group that formed the majority of informants was the 51 years-60 years age group contributing 37%, followed by the 36 years-50 years and informants over 60 years (*Table 1*). The percentage of medicinal plant users older than 60 years old is similar to that found by Alalwan *et al*. (2019) where the most respondents were adults between the ages of 40 years –60 years (62.5%) followed by (27.5%) in the age group of above 60 years. The highest age respondents provide more reliable information because they hold much of the ancestral knowledge that is part of the oral tradition. The least participative age group was the 20 years-35 years which had 9% (*Table 1*). The reason for lack of interest on medicinal plants within this age group could be explained by the mistrust of certain young people, who tend not to believe this herbal medicine due to the influence of modernization and exotic culture influence.

Table 1. *Demographic profile of informants interviewed (N = 182)*

Variables	Categories	Total	Percentage (%)
Gender	Women Men	126 56	69% 31%
Age	[20 years-35 years] [36 years-50 years] [51 years-60 years] >60years	17 51 67 47	9% 28% 37% 26%
Educational level	Illiterate Primary College Secondary University	15 24 31 38 74	30% 13% 17% 19% 21%
Origin of ethnobotanical knowledge	Family knowledge Herbalist Scientific research.	118 37 27	65% 20% 15%

Users of medicinal herbs with no educational background accounted for 30% of the total (*Table 1*), while those with a university education accounted for 21%. Those with only a primary, middle, or secondary education account for 13%, 17%, and 19% of the population, respectively. Traditional knowledge of the use of plants for medical purposes, or the interchange of information and development of herbal medicine production, might explain these findings. Boughrara and Belgacem (2016) found that the illiteracy rate was 33% among users of medicinal plants and the percentage of people who have a university level of study is 21.33%. These results are quite similar to ours. The majority of ethnobotanical knowledge came from family, with 65 percent being passed down through

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generations. The rest were herbalists (20%) and scientific research (15%). Our findings are consistent with those previously reported by Eddouks *et al*. (2017) and Chaachouay *et al*. (2019) who found that heritage is the primary source of knowledge acquisition for residents, accounting for approximately 45 percent of all knowledge acquisition in Morocco.

Diversity of medicinal plants

The research area's floristic diversity, as well as a renewed interest in phytotherapy encouraged us to identify the therapeutic plants of El Mergueb Natural Reserve. An ethnobotanical survey and analysis of the flora of the research region were conducted in this area. A total of 46 taxa belonging to 23 different families were recorded (*Table 2*). All of the species mentioned are spontaneous, according to the flora of Algeria (Quézel and Santa, 1962b), with the exception of the following species *Pinus halepensis* Mill., *Pistacia atlantica* Desf., *Pistacia lentiscus* L., *Nerium oleander* L.*, Juniperus phoenicea* L.*, Eucalyptus globulus* Labill.*, Avena sativa* L., *Hordeum vulgare* L., *Triticum vulgare* L. and *Populus nigra* L. which are cultivated. As illustrated in *Figure 4*, the most represented families were Asteraceae with 9 medicinal plant species, followed by Poaceae (6 species) and Lamiaceae (4 species). Our findings are similar with those of Hammiche and Maiza (2006) who found that among 33 families listed, Asteraceae, with 12 species (15%), are the most common. This high proportion could be explained by the high representation of these families in El Mergueb Natural Reserve flora because of the ecological factors that favour the development and adaptation of the majority of their species. According to Habib *et al*. (2020), Asteraceae, Poaceae and Brassicaceae characterize the arid and semi-arid areas in the Mediterranean regions. In Algeria, some studies highlighted the dominance of Asteraceae, Poaceae and Fabaceae in the steppe regions (Kazi Tani *et al*., 2010). This was also reported in Morocco by Fennane (2008).

As seen in *Figure 5* species with high used values (UV) were *Artemisia herba-alba* Asso (0.58), *Stipa tenacissima* L. (0.42), *Artemisia campestris* L. (0.3), *Zyziphus lotus* (UV = 0.16), *Marrubium vulgare* L. *(*UV = 0.14). This means that these species are the most important medicinal plants used in folk medicine by the population of El Mergueb to treat ailments, which might be due to their vast distribution in the area study. *Artemisia herba-alba* was named the first specie in others studies (Katiri *et al*., 2017; Nawash *et al*., 2013). In Algerian traditional medicine, *Artimisia herba-alba* is one of the most commonly utilized herbs. This plant is used to cure gastrointestinal problems including diarrhea and abdominal discomfort, as well as to heal exterior wounds (Khennouf *et al*., 2010; Boudjelal *et al*., 2013; Miara *et al*., 2018), diabetes (Bouasla and Bouasla, 2017), cancer, and respiratory system diseases (Ouelbani *et al*., 2016). The second most plant cited is *Stipa tenacissima* which is recommended as a hypoglycemic and in the treatment of persistent scalp ulcers, cleaning the ashes. *Artemisia campestris* is used for digestive disorders, stomach discomfort, nausea, and menstrual cramps. vulnerary, anti-hemorrhagic poultice whereas, *Zyziphus lotus* is used to treat anti-inflammatory diseases (Benderradji *et al*., 2014). *Marrubium vulgare* is utilized for diabetes therapy, digestive problems, stomach pain, fever, migraine, wound, cardiovascular diseases, respiratory and liver diseases, allergies, leishmaniose, febrifuge, vermifuge, anti-emetic, tonic, analgesic, antispasmodic (Miara *et al*., 2019).

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Locals are aware of the dangers of poisonous plants and use extreme caution while utilizing plants such as *Thapsia garganica* L. (Mohamed Ibrahim *et al*., 2018), *Nerium oleander* L. (Aslani *et al*., 2004), *Colocynthis vulgaris* L. (Adam Sakine *et al*., 2011), *Retama retam* Webb. (Algandaby, 2015), *Asparagus stipularis* L., *Thymelaea hirsute* L., *Peganum harmala* L. (Nenaah, 2011). Toxicity of therapeutic plants can be attributed to their active chemical combinations, interactions with other herbs and medicines, or intrinsic toxicity. Plants contain complex combinations of terpenes, alkaloids, saponins, and other compounds, which increases the potential of unpleasant responses to any of them or the cumulative or synergistic effects of chemical interactions (Saad *et al*., 2006).

Table 2. *Medicinal flora of El Mergueb natural reserve*

Family/scientific name	Vernacular name	Part used	Mode of use	Therapeutic property	UV
Abietaceae *Pinus halepensis* Mill.	Essanaoubar alhalabi	Seeds	Cataplasm	Respiratory diseases, rheumatic diseases, hemorrhoids	0.04
Anacardiaceae *Pistacia atlantica* Desf.	Elbatma	Leaves, fruits,roots	Infusion, decoction	Digestive diseases, respiratory diseases, eye diseases, antitussive, antipyretic	0.13
Pistacia lentiscus L.	Edharou	Leaves, fruits, roots	Infusion, decoction	Antiseptic, expectorant, diuretic	0.05
Piaceae *Bunium mauritanicum* L.	Talghouda	Fruits	Powder, decoction	Intestinal gas, thrombosis, worms	0.02
Thapsia garganica L.	Bounafaa	Aerial parts, roots	Cataplasm, decoction	Rheumatic pain, bronchitis, sprains	0.03
Pituranthos chloranthus L.	Elkezzih	Leaves, flowers	Infusion	Indigestion, abdominal pain	0.02
Apocynaceae *Nerium oleander* L.	Eddafla	Aerial parts	Fumigation, powder	Respiratory disease, heart disease, bone pain, eczema	0.10
Asteraceae *Anthemis arvensis* L.	Elbabounedj	Flowers	Decoction	Abdominal pain, Menstruation pain, urinary tract infections	0.03
Anvillea radiata L.	Ennakd	Aerial parts	Infusion	Abdominal pain, female genital infections	0.02
Artemisia absinthium L.	Chadjeret Meriem	Leaves, flowers	Decoction	Digestive disorders gastric ulcer, deworming	0.03
Artemisia campestris L.	Ettgouft	Leaves	Infusion, powder, cataplasm	Digestive disorders, menstruation pain, antihemorrhagic, snake bites	0.3
Artemisia herba- alba Asso.	Echih	Aerial parts, roots	Infusion, decoction, powder, cataplasm	Gastric ulcer, anti-diarrheic, anti-spasmodic, indigestions, deworming	0.58
Inula viscose L.	Amegraman	Leaves	Powder and Cataplasm	Rheumatic pain, anti-septic healing fractures, deworming	0.04
Scolymus hispanicus L.	Garnina	Leaves	Decoction	Liver and bowel diseases	0.04
Scorzonera laciniata L.	Talma	Leaves, roots	Infusion	Abdominal pain, intestinal gas	0.02
Scorzoneraun dulate L	Uizg	Aerial parts, roots	Infusion	Against snakebites	0.02
Cactaceae *Opuntia ficus-indica* L	Elhendi	Petals, stem, fruits	Cataplasm	Antiteigne, against dermatosis	0.03
Chenopodiaceae *Atriplex halimus* L.	Elgtaf	Leaves, fruits	Decoction	Dry wounds, diuretic	0.03

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Anabasis articulata (Forssk.) Moq	Eladjrem	Aerial parts	Plaster Infusion	Antidiabetic	0.04
Arthrophytum scoparium L.	Erramth	Aerial parts	Decoction, cataplasm	Indigestion, stings of scorpion, dermatoses	0.03
Cucurbitaceae *Colocynthis vulgaris* L.	ElHadj	Fruits	Decoction, pomade	Rheumatic pain, against hepatitis	0.06
Cupressaceae *Juniperus phoenicea* L.	Elaraar	Aerial parts	Infusion	Antiparasitic, antiseptic, eczema, abdominal pain	0.03
Fabaceae *Retama raetam* Webb.	Ertem	Aerial parts	Infusion	Healing, against eye irritation, anti-diarrhea, abdominal pain, antirheumatic	0.04
Medicago minima L.	Elhaska	Fruits, leaves	Powder, decoction	Mouth and bladder diseases	0.02
Globulariaceae *Globularia alypum* L.	Tassalgha	Aerial parts	Infusion, powder	Anti-gastralgic, against wounds, menstruation pain	0.02
Lamiaceae *Ajuga iva* L.	Chandgoura	Aerial parts	Infusion	Antiseptic, antirheumatic, regulation of the menstrual cycle	0.03
Marrubium vulgare L.	Timriout	Leaves	Infusion, decoction	Expectorant, Diuretic, antiseptic	0.14
Mentha pulegium L.	Elfliou	Aerial parts	Decoction	Against sunburn, allergy of the nose	0.03
Teucrium polium L.	Eljaada	Aerial parts	Infusion	Hypoglycemic, abdominal pain, colic, stomach ulcer	0.05
Lilaceae *Asparagus stipularis* L.	Ain Eddib	Aerial parts	Decoction	Antispasmodic, analgesic, sedative, emollient	0.04
Asphodelus microcarpus L.	Elborouag	Leaves	Decoction Cataplasm	Diuretic, antirheumatic.	0.02
Malvaceae *Malva parviflora* L.	Elkhobiz	Aerial parts, roots	Infusion	Antiseptic, constipation, hemorrhoids, headaches	0.04
Myrtaceae *Eucalyptus globulus* Labill.	Elkalitous	Leaves	Fumigation	Anti-infectious, antiseptic, asthma	0.05
Oleacea *Olea europaea* L.	Azzaitoun	Leaves, fruits, oil	Infusion	Diuretic, hyperglycemia, hypertension	0.07
Papaveraceae *Papaver rhoeas* L.	Ben Naman	Flowers	Infusion	Eczema, migraines, digestive disorders, inflammation of the bladder	0.03
Poaceae *Avena stiva* L.	Khourtal	Seeds	Powder	Diabetes, liver abscesses, diuretic	0.03
Hordeum vulgare L.	Echair	Seeds	Powder	Kidney stones, diabetes, stomach ache, rheumatism	0.02
Stipa tenacissima L.	Elhalfa	Leaves	Infusion	Chronic scalp ulcers, diabetes	0.42
Stipa capensis L.	Essameaa	Leaves	Infusion	Digestive problems antiseptic	0.03
Triticum repens L.	Ennajm	Rhizome	Decoction	Abdominal pain, liver and rat diseases, vomiting	0.02
Triticum vulgare L.	Elkamh	Seeds	Powder	Asthenia	0.03
Triticum aestivum L.	Elkamh	Seeds	Powder	Anemia, convalescence, stomach pain	0.03
Rhamnaceae *Zizyphus lotus* L.	Essidra	Leaves, fruits,roots	Decoction	Sedative, diuretic	0.16
Salicaceae *Populus nigra* L.	Essafsaf	Aerial parts, roots	Decoction, powder	Respiratory pathology, urinary tract pathology	0.02

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Tamaricaceae *Tamarix aphylla* L.	Etarfa	Leaves twigs	Decoction	Fungicide, digestive problem	0.04
Thymeliaceae *Thymelaea hirsute* L.	Elmathnan	Leaves	Infusion	Kidney stones, laxative	0.03
Zygophyllaceae *Peganum harmala* L.	Elharmel	Aerial parts	Decoction pomade, fumigation	Digestive problems antirheumatic, fever	0.05

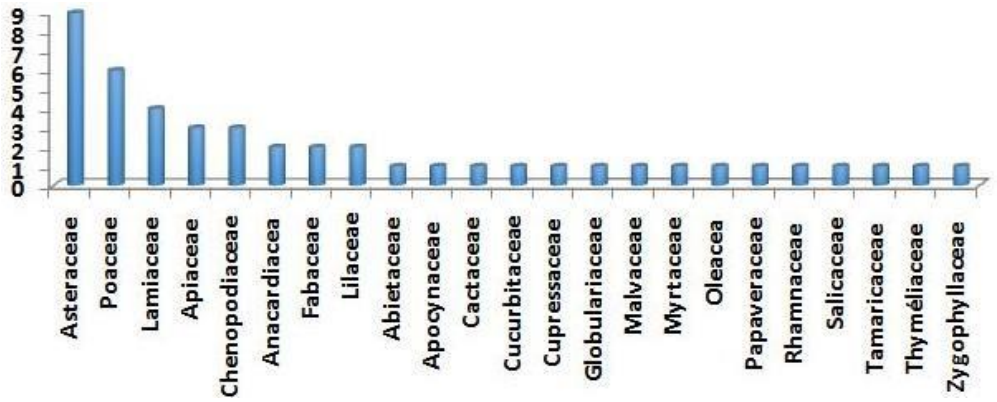

Figure 4. *Number of total species per botanical family*

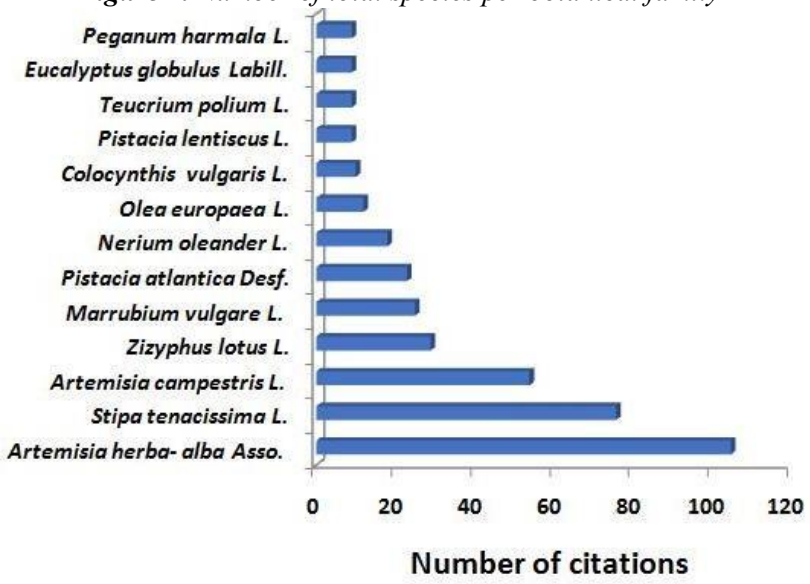

Figure 5. *The most commonly cited medicinal plants of the study area*

Preparation methods

Several medicinal plants preparation methods are used in traditional medicine to fight the different kind of ailments such as decoction, infusion, grinding, maceration, powder and cataplasm. Data analysis showed that infusion is the most common preparation method used by the population studied (42.6%), followed by decoction (27.4%), powder (13%), cataplasm 10%, fumigation (4.4%), pomade (1.6%), and plaster (1%) (*Fig. 6*). This result agrees with findings of previous studies. Demi Rci and Özhatay (2012) reported that the infusion is generally the preparation method of choice, accounting for

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41.8% of the recorded species, followed by decoction (23.9%). However, Chaachouay *et al*. (2019) found that the percentage of infusion was 53.9% followed by decoction (22.1%). It seems to us that decoction and infusion in water are the most widely used methods given the ease of their preparation and administration by users, as it was similarly mentioned in other studies (Hammiche and Maiza, 2006; Benítez *et al*., 2010; Boudjelal *et al*., 2013; Fakchich and Elachouri, 2014; Sargin *et al*., 2015).

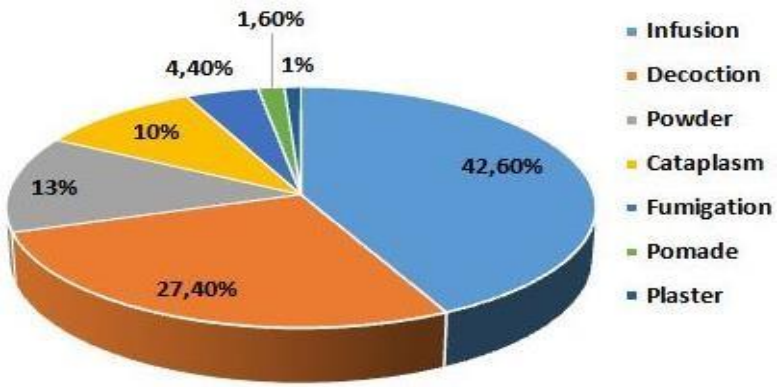

Figure 6. *Modes of preparation*

Parts of the medicinal plants used

Almost all different parts of plants were reported to be used as a drug by the traditional healers and local people, but leaves were the most common (39.6%) (*Fig. 7*), followed by the aerial part (25.6%), roots (17.4%%), fruits (7.3%), seeds (4%), flowers (2.5%), oil (1.2%), twigs (1%), rhizome (0.5%), petals (0.5%) and 0.2% for stems. This result is consistent with other studies that have shown that leaves are the most preferred part of plant for the preparation of remedies. Miara *et al*. (2018) indicated that the leaves are the plant part most commonly used as herbal remedies (29%). Unspecified aerial parts are also often used (17%), as well as fruits and seeds (12 and 11% respectively). This result might be explained by the fact that leaves are the most commonly utilized since they are readily available. It was also shown that the respondents had a strong preference for utilizing leaves to identify and differentiate therapeutic plants. As a result, if the leaf is a crucial component in plant identification and has frequent and simple access, it should be employed more than other plant parts. This conclusion is in line with the results of Akerreta *et al*. (2007). Despite, it has been well established that all parts of the plants are rich in active compounds, the local population most often uses leaves in their preparations, which could be explained by the fact that the totality of the knowledge acquired by the population of the region is traditional and transmitted empirically from one generation to the next. In addition, the leaves are very abundant and their harvest is very easy (Sargin *et al*., 2015; Yemele *et al*., 2015). Furthermore, since the leaves are main photosynthetic organ of plants, bioactive phytochemicals are concentrated in the leaves (Benarba *et al*., 2015).

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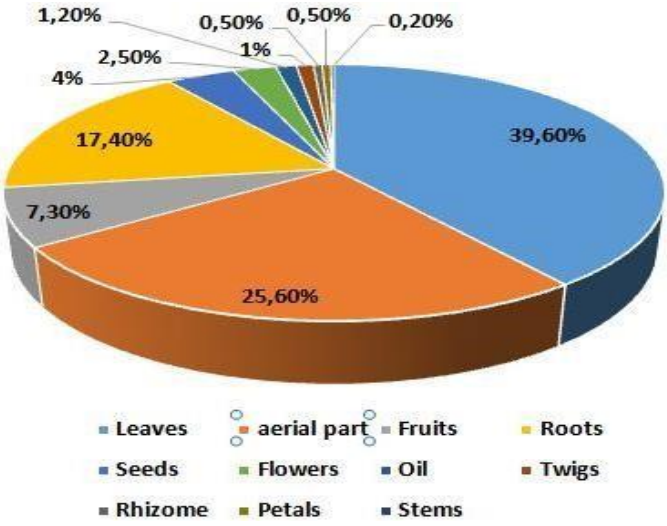

Figure 7. *Distribution of the parts of the plant used*

Conclusion

The ethnobotanical survey revealed that the study area has a great biodiversity with a variety of medicinal plants and still needs more exploration. This floral richness indicates the high potential of traditional knowledge for the development of natural derived products as affordable medicines. In our ethnobotanical study conducted in the El Mergueb reserve in M'sila, we obtained an interesting dataset, including the traditional medicinal use of 46 species belonging to 23 botanical families. Asteraceae and Poaceae were the most widely used and important families. Plants with high UV should be studied in order to isolate the bioactive compounds and validate their popular uses. Some of the plants reported in this study were well known phytochemically, pharmacologically, and clinically. Thus, they can be used for the medical or pharmaceutical purposes. Additionally, the traditional and folkloric knowledge for using medicinal plants in this area can help to discover the potential plants for specific health problems.

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Assessment of antioxidant and antiinflammatory activities and acute toxicity of the aqueous extract from a mixture of leaves and flowers of *Anabasis articulata* (Forssk.) Moq.

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Abstract: Colometric assays were used to quantify the secondary metabolites obtained by a decoction of the extract of *Anabasis articulata* (DEAA) flowers and leaves. Antioxidant activity was examined using several methods: total antioxidant capacity, the 2,2-diphenyl-1-picrylhydrazy (DPPH) radical scavenging assay, and the β-carotene bleaching assay. Single oral doses of 2000 and 5000 mg/kg body weight were administered to albino mice to assess acute toxicity. *In vitro* antiinflammatory activity was determined using the egg albumin denaturation test, and the *in vivo* inflammatory effect was assessed utilizing carrageenan, croton oil, and xylene-induced edema tests. Results showed that DEAA contained high amounts of polyphenols, flavonoids, and tannins and exhibited antioxidant activity in all tested assays. DEAA inhibited protein denaturation and did not cause any mortality or adverse effects. Oral administration of 200 mg/kg DEAA significantly reduced the edema induced by carrageenan, croton oil, and xylene. This study highlights the use of *Anabasis articulata* (Forssk.) Moq. in traditional herbal medicine. It possesses antioxidant activity and can be considered safe for oral consumption; it also has potential beneficial effects in treating diseases associated with inflammation and pain.

Keywords: *Anabasis articulata* (Forssk.) Moq.; polyphenols; antioxidants; acute toxicity; anti-inflammatory activity

INTRODUCTION

Medications designed to treat pain and inflammation often come with side effects and offer limited capacity, especially in the treatment of chronic conditions[1]. Inflammation is the reactive condition characterized by hyperemia and exudation from blood vessels, resulting in redness, heat, swelling, and pain within a tissue in reaction to physical or chemical damage or bacterial invasion [2]. It is the body's tissue response to injury and involves a complex cascade of enzyme activation, mediator release, fluid extravasations, cell migration, tissue disintegration, and repair [3]. Both steroidal antiinflammatory and nonsteroidal antiinflammatory drugs (NSAIDs) are used to mitigate inflammation. Steroids have a distinct function in the treatment of inflammatory illnesses. However, due to their toxicity, steroids are typically prescribed for short durations, except in highly critical instances where the risks are

considered acceptable. Long-term use of NSAIDs is also linked to serious adverse effects, most notably bleeding in the gastrointestinal tract [4]. Natural products exhibit high effectiveness with few adverse effects as alternatives to pharmaceutical therapies. One promising approach to finding antiinflammatory medications is to research plants

utilized in conventional medicine as antiinflammatory compounds [5].

Anabasis articulata (Forssk) Moq., commonly known as "ajrem" in Algeria, belongs to the Chenopodiaceae family and is a plant widely used in traditional medicine to treat fever, headaches, diabetes, and skin disorders, including eczema and lice [6]. The plant is a dwarf shrub, 35-110 cm in height. Stems are woody for about half the length or more, erect, or twisted. Branches have equal internodes and oppose each other; they are brittle, with mature ones displaying bark that splits and peels; the leaves are reduced to small 2-lobed cupules, villous, with

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solitary flowers measuring up to 5 mm [7]. The plant is consumed orally after decoction in water, either by itself or in combination with other medicinal herbs [8].

A. articulata has been reported to have medicinal properties due to its antioxidant and antimicrobial activities [9], antiangiogenic activity [10], antiarthritic effect [11],and antidiabetic activity [8,12,13]. Researchers have evaluated the effect of another genus of the same family. Evaluations of the antioxidant and antibacterial activities were carried out on Moroccan *Anabasis aretioïdes* Coss. &Moq. [14]. *Atriplex Halimus* Sub. Sp. *Schwein furthii* from Algeria was assessed for its *in vitro* antioxidant activity [15]; *Agatophora alopecuroide*, *Hammada elegans, Salsola baryosma*, and *Salsola vermiculata* were evaluated for their antioxidant, and α-amylase- and α-glycosidase-inhibiting activities [13]. Phytochemical studies of *A. articulata* revealed a variety of secondary metabolites, including phenolics [16], alkaloids [17], and saponins such as 3-O-glucopyranosyl of stigmasterol, ß-sitosterol, sitostanol, 3-O-[ß-D-the glucopyranosyl] oleanolic acid, 3-O-[ß-D-glucopyranosyl -28-O-ß-D-xylopyranosyl] oleanolic acid, in addition to proceric acid [12].

No scientific investigations have been performed into the antiinflammatory effect and toxicity of the aqueous extract of the *A. articulata* leaf and flower mixture obtained by decoction. In this study, we assessed the total phenolic, total flavonoid, and total tannin contents in this extract andtheir safety by examining acute toxicity. In addition, we examined the *in vitro* and *in vivo* antiinflammatory effects.

MATERIALS AND METHODS

Ethics statement

All experimental studies were approved by the Committee of the Algerian Association of Sciences in Animal Experimentation(http://aasea.asso.dz/articles/) under Law No.8808/1988, associated with veterinary medical activities and protection of animal health (NºJORA: 004/1988).Permission for experimental use was obtained from the Faculty of Nature and Life Sciences, Ferhat Abbas University of Setif 1. All procedures were performed in compliance with laws and institutional guidelines.

Nomenclature

The medicinal plant used in the present study was *A. articulata* (Forssk.) Moq., commonly called "El Ajrem", belongs to the Chenopodiaceae family. The plant was identified by Professor B.Oujhih, an expert taxonomist at the Institute of Nutrition and Agronomy, University of Batna (Algeria). A voucher specimen was deposited at the laboratory under the number SNV 0045–2020. **Chemicals and instruments**

All analytical grade chemicals utilized in this study were procured from E. Merck, Germany, including the Folin-Ciocalteu reagent, sodium bicarbonate (Na_2CO_3), gallic acid, aluminum trichloride ($AlCl_3$), quercetin, tannic acid, 2.2-diphenyl-1-picrylhydrazyl(DPPH), butylated hydroxytoluene (BHT), sulfuric acid, sodium phosphate, molybdate, ascorbic acid, β-carotene, linoleic acid, Tween 40, Tris-HCl buffer, aspirin, phosphatebuffered saline, carrageenan, indomethacin, croton oil, xylene.

Plant material

A. articulata was harvested in October-November 2020 from the El Mergueb natural reserve of M'sila province (North Algeria) at 35° 42' N latitude and 4° 32' E longitude. The flowers and leaves of *A. articulata* were air-dried in shade, crushed, and then powdered using an electric grinder.

Extraction procedure

The flower and leaves mixture of *A. articulata* powder (100 g) was extracted by decoction using 1000mL of boiled distilled water according to the method of Ferreira *et al*. [18]. The resulting mixture was subjected to magnetic agitation for 20 min. After filtration, the filtrate was evaporated to dryness to obtain DEAA. The residue was weighed to obtain the extractive yield and stored in an airtight bottle at 4°C.

Animal material

Albino mice and rats (weighing between 18-30 and 120-200 g, respectively) were keptin polycarbonate cages for7 days in standard laboratory conditions (12 h light/dark cycle, 23±2°C with free access to food and water) before the beginning of the experiment. **Quantitative phytochemical analysis**

Determination of total phenolic content

The content of phenolic compounds in the extracts was estimated spectrophotometrically using the FolinCiocalteu reagent method described by Li *et al*. [19].

The procedure entails the addition of 500 µL of FolinCiocalteu reagent (diluted 10 times) to 100 µL of the extract or gallic acid. After 4 min, 400 µL of 7.5% sodium bicarbonate solution (Na_2CO_3) was added. After 90 min of incubation in the dark at room temperature, the absorbance was read at 765 nm. The concentration of total polyphenols was calculated from the regression equation of the gallic acid calibration curve at different concentrations. The results are expressed in mg equivalent of gallic acid per g of dry extract (mg EAG/g DE). **Determination of flavonoid content**

Quantitative determination of flavonoids was performed using the aluminum trichloride method [20]. Briefly, 1 mL of the $AlCl_3$solution (2%) was added to 1 mL of the extract solution at different prepared concentrations in the appropriate solvent. At 430 nm, the absorbance was measured after 10 min of incubation. The concentration of flavonoids in the extracts was deduced from a calibration line established with quercetin and expressed in milligram equivalents of quercetin per gram of dry extract (mg EQ/g DE). **Determination of total tannin content**

The total content of tannins in the extract was estimated using the Folin-Ciocalteu reagent and tannic acid as standard, according to the method described by Prasanth *et al*. [21]. The reaction mixture was made by combining 0.5mL of extract, 2.5 mL of 10% FolinCiocalteu reagent, and 2.5 mL of 7.5% Na_2CO_3. The samples were incubated at 45C° for 45 min in darkness. Absorbance was then measured at 765 nm, and the calibration curve was established using different concentrations of tannic acid (25-300 µg/mL). Total tannin content was expressed as mg equivalent of tannic acid per g of dry extract (mg TAE/g DE).

Estimation of *in vitro* antioxidant activity

Total antioxidant capacity

Total antioxidant capacity (TAC) was evaluated with an assay based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH [22]. An aliquot (0.1 mL) of plant extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95C° for 90 min in darkness. The mixture was allowed to cool to room temperature, and the absorbance was measured at 765 nm against a blank prepared with one mL of the reagent solution and the appropriate

volume of the sample's solvent. It was then incubated in the same manner as the other samples.

DPPH radical scavenging assay

The extract's free radical scavenging activity was measured using DPPH with the modified method of Shimamura *et al*. [23]. Briefly, a 0.4-mM solution of DPPH was prepared in 100mL methanol, and 800 µL of this solution was added to 200 µL of the sample at different concentrations. The absorbance was determined at 517 nm after 30 min. BHT was used as standard.

$(Abs_{control} - Abs_{sample} / Abs_{control})$ is the percentage of radical scavenging activity;

$Abs_{control}$: Absorbance of the control (without sample).

Abs_{sample}: Absorption of the extract with the reagent.

β-Carotene bleaching assay

This assay is based on the capacity of antioxidant molecules to inhibit β-carotene oxidative degradation caused by linoleic acid oxidative compounds according to the method of Kartal *et al*. [24]. A mixture of 0.5 mg of betacarotene, 1 mL of chloroform, 25 µL of linoleic acid, and 200 mg of Tween 40 was used to create a ß-carotene/ linoleic acid emulsion. Chloroform was evaporated at 40°C using a vacuum evaporator. Afterward, 100 mL of distilled oxygenated water was added while the mixture was vigorously shaken. To an aliquot of 2.5 mL of this emulsion, 350 µL of DEAA or the reference antioxidant (BHT) was added and well mixed. The absorbance was recorded after 0, 1, 2, 4, 6, and 24 h at 490 nm. A negative control consisted of 2.5 mL distilled water or methanol instead of extract or reference antioxidant. All samples were assayed in triplicate. The percentage of inhibition was determined using the equation below:

$$\text{Inhibition } \% = Abs_{(t)} / Abs_{(t0)} \times 100.$$

where $Abs_{(t)}$ was the absorbance of the test sample at the given time t, and $Abs_{(t0)}$ was the absorbance of the test sample at a time t_0.

Estimation of *in vitro* antiinflammatory activity

The protein denaturation experiment was carried out, as reported by Bouaziz *et al*. [23]. The volume of egg white was adjusted with a buffer solution of 20 mM Tris-HCl (pH6.8) to obtain a dilution solution of 1:100. The solution was gently stirred for 10 min, followed by filtration. Identical volumes were transferred to tubes, followed by the addition of DEAA or aspirin, and the tubes were incubated at 74°C for 15 min. The absorption was measured at 650 nm, and the following % inhibition of protein denaturation was calculated: inhibition %=

[(Abs control – Abs treated)/ Abs treated] × 100.

***In vivo* investigations**

Acute toxicity study

The acute toxicity was assessed on mice according to the Organization of Economic Co-operation and Development guidelines 423 (OECD 423) [26]. The animals were randomly divided into three groups containing five male mice, each weighing between 18-30 g. Two treated groups were orally given a single dose of DEAA at 2000mg/kg and 5000mg/kg body weight, while the control group was given only distilled water. The first day of the treatment was taken as Day 0,and the day of sacrifice was Day 14. Individual lots of mice were monitored for 4 h after treatment to detect behavioral and physiological changes in comparison to the control. Gross behavioral and toxic effects were observed at short intervals for 24 h, then daily for the next 14 days to detect late effects.

The body weight of each mouse was measured during the study period, on days 0, 7, and 14. All mice were killed on day 14. The kidney, liver, heart, and spleen were weighed to determine relative organ weights and to observe macroscopic lesions. The relative organ weight of each animal was then determined using the formula:

Relative organ weight = (absolute organ weight (g) / body weight of mouse on sacrifice day (g)) × 100.

For histological examination, the liver and kidney were fixed in 10% formalin, dehydrated in ascending degrees of ethanol (70-100%), cleared in xylene, and finally embedded in paraffin. Afterward, thin sections of 5 µm were stained with hematoxylin-eosin and examined with a light microscope. Digital images recorded at 20×magnification. **Antiinflammatory activity**

Carrageenan-induced paw edema in rats

The antiinflammatory effect of DEAA against carrageenan-induced acute paw edema in rats was assessed according to the method described by Tsai and Lin [27]. Five groups of 5 rats each were treated with DEAA (50, 100, and 200 mg/kg *per os*), indomethacin (10 mg/kg *per os*), and distilled water (10mL/kg *per os*). One h after administration, acute inflammation was produced by the subplantar injection of 0.1 mL of (1%carrageenan in saline) in the left hind paw. The thickness of each paw was measured using a digital caliper. Measurement was carried out at 0 h (V_0: before carrageenan injection) and 1, 2, 3, 4, 5, and 6 h after the induction of inflammation (V_T). The difference between V_T (1, 2, 3, 4, 5, and 6 h) and V_0 was taken as the edema volume value. The percentages of increase were calculated according to the following formula:

$$\text{increase } \% = (D_n\text{-}D_0) / D_0 \times 100.$$

Croton oil-induced ear edema in mice

The mouse ear edema was induced by topical application of croton oil according to the method described in [28] with slight modifications. Croton oil was dissolved in a 5% acetone solution (v/v), and 10 µL of the solution were applied to both the anterior and posterior surfaces of the right ear with a micropipette. The left ear (control) received the vehicle (acetone 80, distilled water 20 v/v). Groups of mice (n=5) received oral doses of DEAA (100 and 200 mg/kg), vehicle (distilled water), or indomethacin (10 mg/kg) 1 h before croton oil application. Six h later, the thickness of each ear was measured using a digital caliper, and the antiinflammatory activity was expressed as the percentage of edema inhibition using the following formula:

Inhibition % = $100 \times (D_n–D)/D_n$, where D is the difference in ear edema thickness in the treated group, and D_n is the difference in ear edema thickness in the negative group.

Xylene-induced ear edema in mice

The xylene-induced ear edema test was carried out according to the method of Manouze *et al*. [29] with slight modifications. Briefly, each group (5animals per group) was given by gavage one dose (100 or 200 mg/kg) of DEAA, indomethacin (10 mg/kg), or vehicle (10 mL/kg) 1 h. The right ear's inner and outer surfaces were treated topically with 0.02 mL of xylene to cause ear edema. The left ear was used as a control. One h after xylene application, the thickness of the ear was measured with a digital caliper. The following formula was used to calculate the inhibition percentage of ear edema:

Inhibition % = $100 \times (D_n–D)/D_n$, where D is the difference inear edema thickness in the treated group, and D_n is the difference inear edema thickness in the negative group.

Statistical analysis

Statistical comparisons were performed using Graph Pad Prism (version 7.00 for Windows). One-way analysis of variance (ANOVA) followed by Dunnet's test was used for all assays except for the *in vitro* antiinflammatory effect, where the Student's t-test was used. Statistical data are presented as the mean±standard deviation (SD) with n=3, while for *in vivo* experiments data are presented as the mean±standard error of the mean (SEM) with n=5. A difference was considered significant when the P value was less than 0.05.

RESULTS

Quantitative phytochemical analysis

The mixture of the flowers and leaves of *A. articulata* extracted by decoction gave a high yield percentage (10.7%). The quantitative assessment of polyphenols, flavonoids, and tannins were estimated at 210.20±0.565mg GAE/g DE, 13±0.055mg QE/g DE and 138.27±1.442mg TAE/g DE respectively.

Estimation of *in vitro* antioxidant activity

For this test, DEAA had an EC_{50} of 0.4093±0.0058 mg/ mL compared to the reference antioxidant; ascorbic acid was noted, which proved to be more effective ($P<0.001$) with an EC_{50} of 0.002±0.003mg/mL. In the DPPH test, DEAA possesses a good scavenging activity (IC_{50} =0.170±0.0006) compared to BHT (IC_{50} = 0.026±0.0006 mg/mL). Fig. 1 shows the results of the antioxidant activity of DEAA measured by the β-carotene bleaching test. DEAA effectively prevented the bleaching of β-carotene (79.833±1.03%) compared to the standard BHT (98.11±1.05%). Results obtained from the evaluation of the antioxidant activity of DEAA indicate that the extract contains reductants capable of conferring stability to the oxidant molecules.

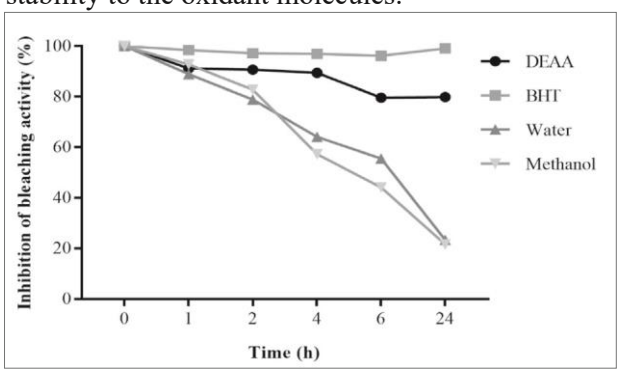


Fig. 1. Antioxidant activity of the decocted extract of *A. articulata* measured by β-carotene bleaching test. Bleaching kinetics of β-Carotene in the presence of DEAA, methanol, water, and standard BHT during 24 h. Values are presented as the mean ±SD. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction; BHT –butylated hydroxytoluene.

Estimation of *in vitro* antiinflammatory activity

DEAA was able to prevent denaturation of albumin in a concentration-dependent way. The percentage of inhibition was within arange from 29.71% to 92.17% at the concentration range of 0.0625to 0.5mg/mL (Fig. 2). Aspirin as a reference drug exhibited a higher inhibition capacity (96.03%) at the higher concentration for the denaturation of egg test.

In vivo investigation

Acute toxicity study

The results of the acute toxicity study showed that after 14 days of observation DEAA given to mice in single doses of 2000 and 5000 mg/Kg body weight (b.w.) resulted in no mortality or morbidity. Moreover, the animals did not exhibit any toxic effects or behavioral or morphological changes.

Effect on body weight and organ relative weight

The changes in the body weight of control and mice treated with the extract of *A. articulata* obtained by decoction during the 14 days of treatment are presented in Fig. 3. The body weight gain showed no significant difference ($P>0.05$) between the control and treated groups. However, the body weight of mice at 24, 48, 72 h, and 7 days ($P>0.05$) after administration of DEAA at 5000mg/kg exhibited a decrease but was statistically insignificant compared to the time of initial treatment. No significant changes were observed in the body weights in the treated groups compared to that of the control group, suggesting that DEAA had no effect on the growth of mice at the oral doses administered.

The vital organs (liver, kidneys, heart, and spleen) of mice were weighed after 14 days of administration at doses of 2000 and 5000 mg/kg (Fig. 4). The liver and kidney of the group treated with 5000 mg/kg showed a statistically significant increase in weight ($P<0.001$ and $P<0.01$, respectively) compared to the control group. However, the heart and spleen showed no alteration in weight compared to the control group (Fig. 4).

Histological analysis

The histopathological study of the selected organs in the acute toxicity test in mice showed no remarkable change when comparing the treated group that received 2000 mg/kg of DEAA and the control group. Fig. 5 shows a normal hepatic lobule composed of

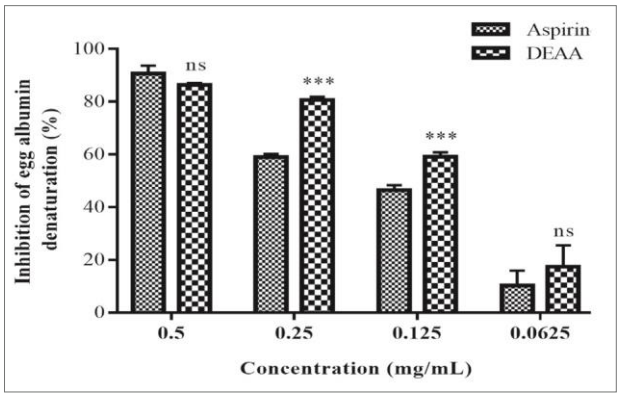


Fig. 2. *In vitro* antiinflammatory effect of DEAA according to the egg albumin denaturation test. Aspirin was used as a reference drug. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are presented as the mean±SD compared to aspirin. ns – no significant difference, *** P<0.001.

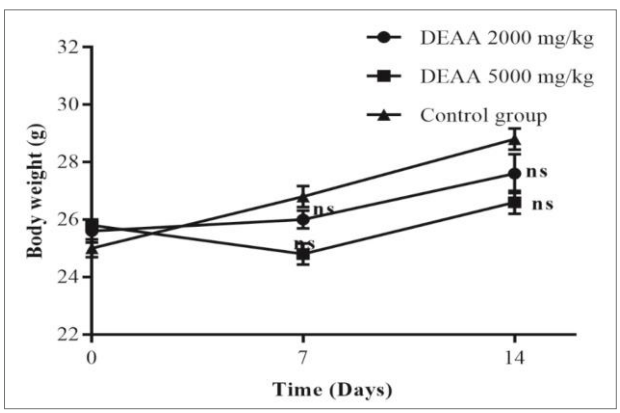


Fig. 3. Body weight changes in the treated mice and their controls at days 0, 7,and 14in the acute toxicity experiment of DEAA. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Results are expressed as the mean±SEM(n=5). ns–no significant difference.

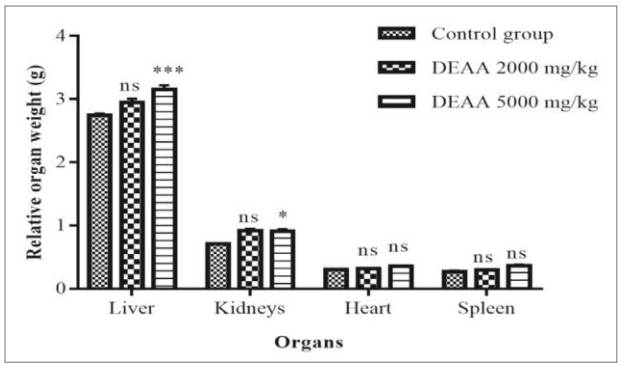


Fig. 4. Relative organ weights of mice receiving DEAA after 14 days of acute toxicity experiment. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Results are expressed as the mean ±SEM (n = 5). ns – no significant difference;
*P<0.05, *** P<0.001

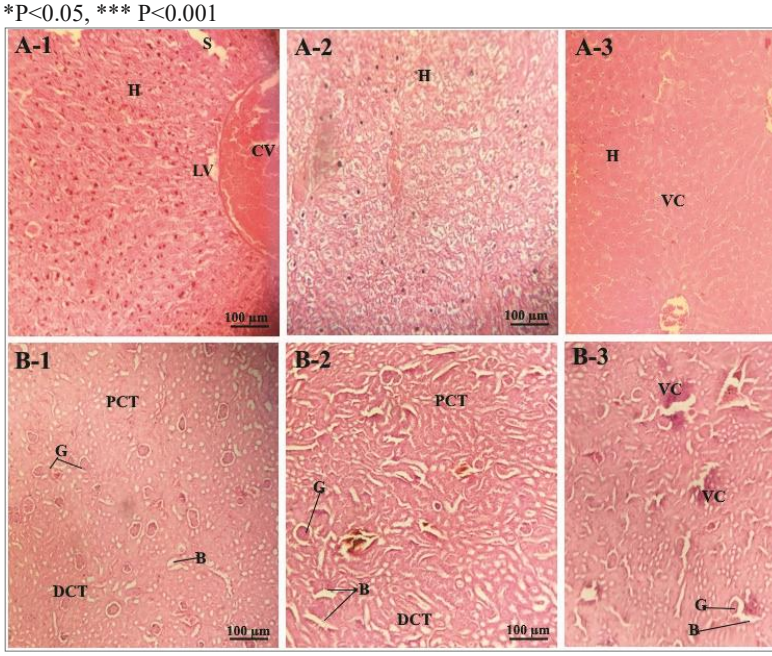
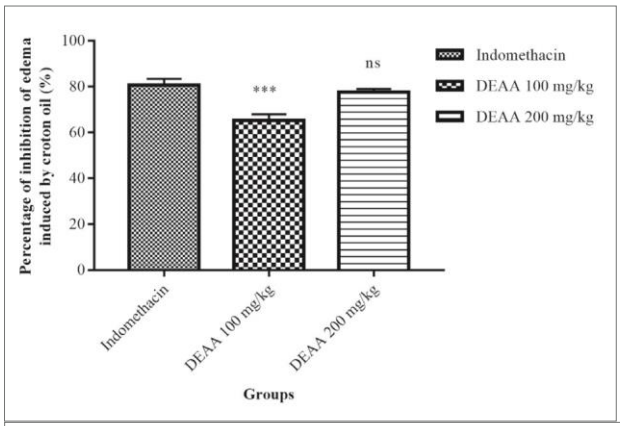
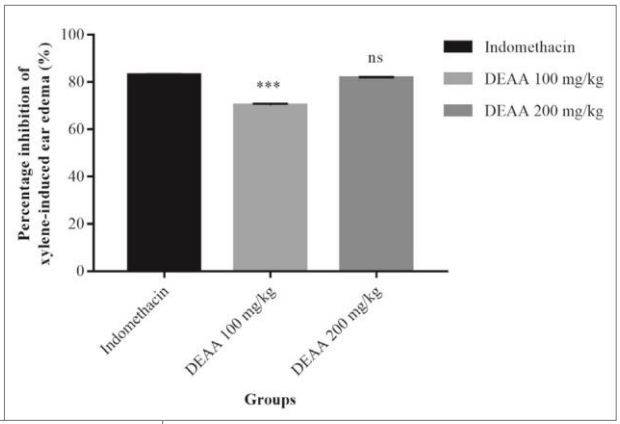


Fig. 5. Histopathological study of liver (**A**) and kidney (**B**) tissues of mice treated with a single dose of 2000 and 5000 mg/kg of DEAA. H & E stain (×20), (scale bars=100 µm). H and E – hematoxylin-eosin; **A-1**– Section of the liver of the control;**A-2**– Section of the liver of a 2000 mg/kg DEAA-treated animal;**A-3**– Section of the liver of a 5000 mg/ kg DEAA treated representative wascharacterized byvascular congestions in the entirety of the structure of the liver; **B-1**– Section of the kidney of the control; **B-2**– Section of the kidney of a 2000 mg/kg DEAA-treated animal; **B-3**– Section of the kidney of a 5000 mg/kg DEAA treated animal where vascular congestion was observed. CV –central vein; H – hepatocyte; S – sinusoids; VC – vascular congestions; LV – lymphatic vessels; G – glomerulus; B – Bowman's space; PCT – proximal collecting tubules; DCT – distal collecting tubules; VC – vascular congestions.





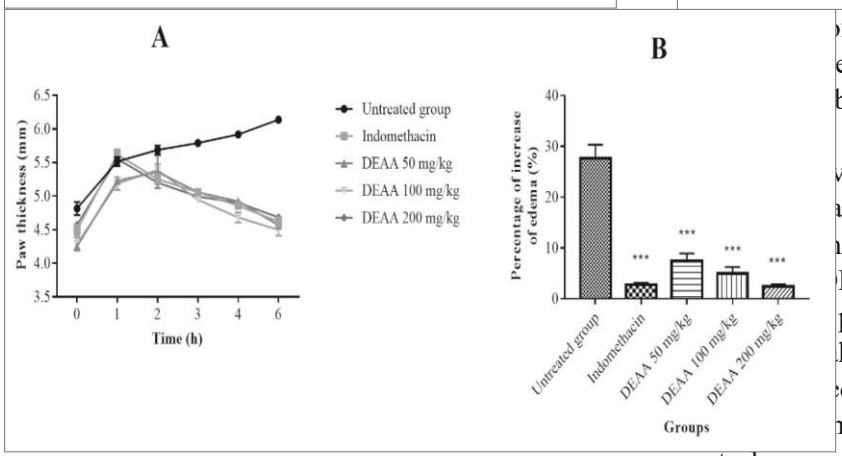


Fig. 6. Antiinflammatory effect of DEAA on carrageenan-induced paw edema in rats. **A** – Changes in paw thickness (mm). **B** – Percentage of increase of edema. DEAA – extract of the flowers and leaves of *A.articulata* obtained by decoction. Results are expressed as the mean±SEM (n = 5). ns – no significant difference, ***P<0.01 were considered significant when compared with the untreated group.

hepatocytes arranged in hepatic cords separated by sinusoids that drain into central vein lymphatic vessels. The same was observed for kidneys, normal glomerulus, Bowman's space, proximal collecting tubules, and distal collecting tubules (Fig. 5). However, in the group treated with5000 mg/kg of DEAA, the liver and kidney showed normal structures, but with vascular congestions.

Invivo antiinflammatory activity

Carrageenan-induced paw edema

mg/kg (2.41±0.48%) compared to indomethacin-treated group (2.77±0.37%). The effect was less noticeable (7.49±1.43%) at 50 mg/kg.

f 50, 100, and 200 mg/kg were tested
e antiinflammatory activity of DEAA.
btained were compared with those of
indomethacin (10 mg/kg). Fig. 6A
variations in paw thickness (mm)
rrageenan-induced paw edema over6
e administration of standard and three
EAA. In carrageenan-administered
paw edema increased gradually and
k at6 h (5.98±0.18 mm). However, in
d with DEAA doses, the carrageenan-
mmation was reduced compared to the control group. The administration of indomethacin significantly prevented the progression of the inflammation at the plantar level of the rat's paw at 2 h and presented the lowest edema compared to the control at 6h (4.72 ±0.12mm). Fig. 6B illustrates that DEAA caused a dose-dependent decrease in the percentage of paw edema. The percentage of edema increase after 6h in rats receiving DEAA showed a similar effect at an oral dose of 200

Fig. 7. Antiinflammatory effect of DEAA on croton oil-induced ear edema in mice. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are expressed as means±SEM. ns – no significant difference, *** P<0.001 were considered significant when compared with indomethacin.

Fig. 8. Antiinflammatory effect of DEAA on xylene-induced ear edema in mice. DEAA – extract of the flowers and leaves of *A. articulata* obtained by decoction. Values are expressed as means±SEM (n=5). ns – no significant difference, *** P<0.001 were considered significant when compared with indomethacin.

Croton oil-induced ear edema in mice

DEAA at doses of 100 and 200 mg/kg caused inhibition of edema induced by croton oil in mice at

6 h with percentages of inhibition of 68.59±0.84%and 77.58±0.64%, respectively, while 80.68±1.25%inhibition was recorded with the nonsteroidal antiinflammatory drug indomethacin (Fig. 7).

Xylene-induced ear edema in mice

Fig. 8. shows that DEAA at doses of 100 and 200 mg/ kg effectively reduced the edema to 70.25±0.59% and 81.74±0.36%, respectively. Indomethacin used as a positive control promoted an antiinflammatory reduction to 82.98±0.36%.

DISCUSSION

Medicinal plants are widely acknowledged as a significant source of bioactive compounds. Due to limited research in the literature, this study investigates the extract of *A. articulata* obtained by decoction. Here, we provide experimental findings related to acute toxicity and antiinflammatory and antioxidant properties. Phytochemical analysis revealed the existence of several classes of secondary metabolites in *A. articulata* leaf and flower extracts obtained by decoction. The yield of DEAA extraction was 10.7%. Compared to other species of the Chenopodiaceae family, this value was lower than the yield of 19.87% reported for *Atriplex halimus*[30]and *Arthrophytum scoparium* by Dehimi *et al*. [15]. Many studies point out that the percentage of extraction yield depends mainly on the extraction procedure, in particular, the temperature used during the extraction, the polarity of the compounds of the extract, the solvent ratio, and the methods of extraction (maceration, decoction, evaporation) [31,32].

The present study revealed that the DEAA exhibited a high concentration of phenolic compounds (TPC=210.20±0.565 GAE/g DE).This finding was better than that of Benhammou *et al*. [33], who utilized methanolic maceration extract from *A. articulata* stems (25.48±3.83 mg EAG/g DE) and roots (19.85±7.52 mg GAE/g DE). These results were consistent with those reported by El-Haci *et al*. [34] for the endemic Algerian *A. aretioides* ethanolic maceration extract of leaves (231.85±20.59 mg EAG/g DE). Concerning the total flavonoid content, the *A. articulata* decoction revealed13.01±0.055mg QE/g DE. Benhammou *et al*. [33] reported a lower total flavonoid content for stem and root extracts (3.08±0.20 and 3.80±0.06 mg QE/g DE, respectively).In contrast, the ethanolic and methanolic maceration extracts of the aerial part (leaves) of *A. aretioides* showed a high amount of flavonoid content, 132.8±24.58 and 46.68±0.74 mg CE /g DE, respectively [34]. The total tannins in our study accounted for 138.27mg TAE/g, an amount low compared to the Moroccan *A. aretioides* Coss. &Moq. that had 2.56±1.54 mg TAE/g DE and 5.93±2.42 mg TAE/g DE for the methanolic extracts of the aerial part and roots, respectively[35].

Research on the antioxidant capacity and identification of secondary metabolites from Saharan Algerian plants is scarce. Studies of the extract obtained by decoction of flowers and leaves of *A. articulata* have not been undertaken. The antioxidant capacities of plant extracts are influenced not only by their composition but also by the testing conditions used [36]. For this reason, the antioxidant capabilities of the studied extract were evaluated using different methods. The phosphomolybdate method was used to determine the total antioxidant capacity. This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The study revealed that the antioxidant activity of the leaf and flower extract was 0.4093±0.006 mg/ mL. Benhammou *et al*. [33] reported similar results in their investigation of *A. articulata* (stem and root) collected from the Bechar region (southern Algeria).

The DPPH radical scavenging method is a common spectrophotometric technique used to assess the antioxidant capacity of extracts. It relies on the ability of the DPPH radical to lose its color in the presence of antioxidants, which occurs when an antioxidant compound donates an electron or hydrogen to the DPPH radical[37]. The antioxidant activity of *A. articulata* was found to be strong,

exhibiting significant scavenging activity against DPPH, with an IC_{50}=0.1695±0.00059 mg/mL. This value was higher than that reported by Hamdoon *et al*. [38] for an ethyl acetate extract and chloroform extract of *A. articulata* from Libya (246.38±4.01 µg/mL and 416.61±6.19 µg/ mL, respectively). But, for the ethanolic extract, a similar scavenging activity with an $IC_{50}$of 149.26±3.23µg/mL was obtained. Bouaziz *et al*. [9] reported that extracts of the aerial part of Tunisian *A. oropediorum* exhibited a better scavenging efficiency against DPPH radicals with an IC_{50} of 0.70±0.80 µg/mL, 2.23±0.38 µg/mL 3.72±0.63 µg/mL, and 1.12±0.19 µg/mL for hexane, ethyl acetate, methanol, and water extracts, respectively.

The β-carotene bleaching test is based on proton transfer processes where linoleic acid, an unsaturated fatty acid, is oxidized by reactive oxygen species (ROS) created by oxygenation, which will cause discoloration. Antioxidants in the examined extracts reduce the degree of discoloration [39]. DEAA exhibited a notable ability to inhibit the bleaching of β-carotene over24 h when compared to the positive control.

Results from the assessment of DEAA antioxidant activities showed that the extract contains reductants that can provide ROS stability. For different types of plants, the extraction techniques and extraction solvents have different effects on the antioxidant activity of bioactive chemicals. The antioxidant activity of DEAA could be attributed to its flavonoid and phenolic contents that act as a strong natural free radical scavenger. Moreover, there is a positive correlation between antioxidant activity and the content of polyphenols [40,41].

Protein denaturation is the disruption of proteins' tertiary and secondary structures caused by external stressors such as a strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. When denatured, biological proteins lose their biological function [42] and cause inflammatory diseases. The ability of the decoction extract of *A.articulata* to inhibit protein denaturation was evaluated through the egg albumin denaturation technique. DEAA was able to inhibit protein denaturation and had an effect close to the standard drug at a dose of 5 mL/mg. Several investigations have shown that the anti-denaturation effect of DEAA may be attributable to secondary metabolites [43,44]. Their active components, such as polyphenols, triterpenoids, alkaloids, steroids, sinapic acid, coumaric acid, vanillic acid, kaempferol, ferulic acid, and benzoic acid, exhibit antiinflammatory activity [42,45].

Bioactive compounds derived from medicinal plants exhibit a range of beneficial biological properties. However, they can induce toxic effects, resulting in systemic toxicity, nephrotoxicity, and hepatotoxicity. Consequently, clinical complications may arise, potentially leading to carcinogenesis and severe infections [46]. The majority of serious side effects result from the misuse of herbal medications[47]. There is a lack of toxicological research in the literature regarding the aqueous extract of *A. articulata*. Therefore, we are interested in conducting DEAA acute toxicity studies to improve its safety in treating specific human disorders.

In the acute toxicity study by gavage, no death up to 14 days of the study period was observed. There were no physical signs of toxicity even at the highest dose of 5000 mg/kg b.w., as evidenced by normal breathing and the absence of tremors, convulsions, diarrhea, salivation, and paralysis in the treated animals. This suggests that DEAA was safe and non-toxic to mice during the monitoring period according to the OECD categorization, and consequently, it was calculated that the median lethal dosage (LD_{50}) of DEAA was greater than 5000 mg/ kg b.w. It could be inferred that compounds from the decoction of flowers and leaves of *A. articulata* extract present no acute toxicity since no toxic effects were observed even after high-dose administration.

No significant difference (P>0.05) was observed in body weight gain between the control and treated groups following DEAA administration. The extract did not influence the mice's weight gain throughout the study, indicating that changes in body weight

compared to the control group would have revealed any potential toxicity of the extract[48]. This may be partly explained by the fact that DEAA had no detrimental effects on appetite or food consumption.

Organ weights are important indicators of target organ injury and physiological disturbances [49,50]. The current study did not detect any notable alterations in organ weight concerning the heart and spleen. However, relative weights of the kidney and liver exhibited a gradual increase at 5000 mg/kg, with this rise proving significant compared to the control group. This increase in liver relative weight may be due to an increased metabolic response to the extract, which may have increased hepatic activity [51].

Histopathology examination of the liver and kidney revealed normal hepatocytes and glomeruli, no alteration in the structure in treated animals compared to control except for the vascular congestions noticed in liver and kidney tissues of animals treated with DEAA (5000mg/kg).The presence of congestion in the liver and kidneys may result from a vasoconstriction action induced by the extract on the wall of blood vessels [52].

The *in vivo* antiinflammatory activity of DEAA was investigated using carrageenan-induced paw edema, xylene-, and croton oil-induced ear edema models. These three approaches are widely acknowledged as pharmacological models for evaluating the acute antiinflammatory effects of natural medicines [53]. The edema produced in rat feet by injection of carrageenan is mediated by histamine and 5-hydroxytryptamine (5-HT) during the first hour, after which the enhanced vascular permeability is sustained by kinin release for the next hour. From 2 to 6 h, the mediator appears to be a prostaglandin, and its release is linked to leucocyte migration into the inflamed location [54]. Prostaglandins promote the production of inflammatory exudates during tenderness. The use of nonsteroidal antiinflammatory medicines (NSAID) such as indomethacin reduces the impact of edema formation by inhibiting cyclooxygenase activity and prostaglandin synthesis [55]. We showed that the DEAA at 100 and 200 mg/kg exhibited similar effects in the 2ndphase (3-6 h), comparable to the standard drug indomethacin. The antiinflammatory activity of the extract might act through a mechanism that involves the inhibition of cyclooxygenase (COX) associated with the inflammatory cascade induced by carrageenan [56]. The inhibition of these proinflammatory mediators by flavonoids may be one of the most important mechanisms of their antiinflammatory activity [57]. This is also in agreement with several studies claiming that many plants that contain chemical compounds such as polyphenols have powerful antiinflammatory abilities that work by inhibiting prostaglandin pathways [58].

Croton oil is obtained from the seeds of *Croton tiglium*. Externally, the oil produces topical irritation and edema. In this test, the inflammatory response is typically evaluated by measuring the change in ear plug weight at a specific time point following the application of the irritating agent [59]. Xylene is a phlogistic agent that induces ear edema as it is sensitive to COX inhibitors and is used to assess the effect of NSAID substances that inhibit prostaglandin synthesis [60]. The two tests cause immediate irritation that leads to the infiltration of inflammatory cells, vasodilation, accumulation of fluid, and edema [61]. The decoction extract of *A. articulata* at an oral dose of 200 mg/kg produced significant inhibition of edema compared to indomethacin, which is related to the release of inflammatory mediators like substance P, histamine, kinin-like substances, and prostaglandins produced by increased levels of phospholipase A2 and COX-2 [49]. The antiinflammatory activity of the extract in the two antiinflammatory ear edema models could be due to its phytochemical compounds, such as polyphenols. Several plant species rich in polyphenols, particularly flavonoids, are reported to possess important pharmacological actions, such as anti-inflammatory [62]. According to other studies, flavonoids like rutin, quercetin, luteolin, hesperidin, and bi-flavonoids yielded significant antiinflammatory activity [63, 64], and the inhibition of histamine may be important. Several studies have highlighted the efficacy of polyphenols in mitigating inflammation by reducing the release of potent

inflammatory mediators and inhibiting crucial inflammatory enzymes like COX and 5-lipoxygenase. The inhibition of these mediators is crucial in resolving inflammation and treating chronic inflammatory conditions.[65].

CONCLUSIONS

Research has been conducted on *Anabasis articulata* using different parts, such as stems and roots, to extract with polar solvents. Nonetheless, this study marks the first investigation into the antioxidant effects, toxicological profile, as well as the *in vitro* and *in vivo* antiinflammatory activity of the decoction extract derived from the flowers and leaves. The findings from the antioxidant and antiinflammatory investigations validate the traditional use of *A. articulata*. The acute toxicity study suggests that the aqueous extract of the plant is safe up to a dose of 5 g/kg of mice body weight when consumed orally as a single administration. DEAA can be considered a potential source of natural compounds that can be incorporated into the human diet, alongside its potential applications as antioxidant and antiinflammatory agents.

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Data availability: Data underlying the reported findings have been provided as a raw dataset, which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Makhlouf%20 et%20al_Dataset.pdf

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ملخص

باستخدام مجموعة متنوعة من النماذج *in vitro*، *in silico* و *in vivo*، تهدف هذه الدراسة إلى تقييم النشاطية المضادة للأكسدة والمضادة للالتهاب والمسكنات للألم والمضادة للتقرح لعدة مستخلصات من نبات العجرم *Anabasis articulata*، وهو من النباتات الجزائرية المتواجدة في المناطق القاحلة وشبه القاحلة و تنتمي إلى عائلة Chenopodiaceae تم اختيارها بعد دراسة اثنوبنباتية أجريت في محمية المرقب الطبيعية (عدد المشاركين = 182 مستجوب). في هذه الدراسة تم تحديد الجزيئات الحيوية، وتحديد كمية المستقلبات الثانوية وإجراء ثمانية اختبارات لدراسة النشاط المضاد للأكسدة للمستخلصات: إجمالي القدرة المضادة للأكسدة؛ اختبار أزاحة جذور ABTS ، DPPH، جذر الهيدروكسيل، بيروكسيد الهيدروجين، استخلاب المعادن، اختبار قوة الإرجاع، اختبار تبييض β-carotene. تم تقييم النشاطية المضاد للالتهابات في المختبر باستخدام تخريب ألبومين البيض وBSA للمستخلص المائي DEAA والمستخلص الايثانولي EEAA وأجزائه. كما تم إجراء Molecular docking باستخدامAutodock vina . تم إعطاء جرعات فموية مفردة لكل من DEAA و EEAA بتركيز 2000 و5000 ملغم/كغم من وزن الجسم للفئران البيضاء لتقييم السمية الحادة. تم تقييم التأثير المضاد للالتهابات باستخدام نموذج الوذمة induced-carrageen في الجرذان، وذمة الأذن باستخدام croton oil، وذمة الأذن المستحدثة بالxylene في الفئران. تم تحقيق نشاط مسكن عن طريق الحقن acetic acide داخل الصفاق. تم تقييم النشاط الوقائي للمعدة باستخدام تقرح المعدة المستحث بالإيثانول في الجرذان بجرعات 50 و100 و200 ملغم/كغم من DEAA و200 و400 و600 ملغم/كغم من وEEAA. كما تضمنت الدراسة الميكانيكية المعالجة المسبقة بمضادات/مثبطات مناسبة مثل: glibenclamide, indomethacin and L-NAME. تم تحديد 46 نوعًا من النباتات التي تنتمي إلى 23 عائلة نباتية لعلاج أمراض مختلفة. تم تحديد سبعة مستقلبات موجودة في أجزاء n-butanol وethyl acetate وchloroform بشكل نوعي بواسطة LC-MS-MS وهي: الأنثرون، وبيتاكاروتين، وبوتيل هيدروكسي أنيسول، وبوتيل هيدروكسي تولوين البوتيلات، وحمض الغاليك، والميريسيتين، والروتين. أظهرت النتائج أن ChFA يمثل أكبر كمية من إجمالي البوليفينول والفلافونويدات. أظهر جزء n-butanol أعلى كمية من العفص الكلي. أظهر التقييم الكمي لقدرة الكسح DPPH أن المستخلص الإيثانولي كان الأكثر نشاطاً بقيم IC_{50}= 0.074±0.003 مجم/مل، وكان لجزء الكلوروفورم (ChFA) التأثير الأقوى في نشاط ازاحت جذر الهيدروكسيل وازاحت جذر الهيدروكسيل وازاحت جذر البيروكسيد الهيدروجين بقيم EC_{50}= 0. 151 ± 0.0017 ، و IC_{50}= 0.361 ± 0.0087 و IC_{50}= 1.777 ± 0.0402 مجم/مل) ،كما كان لجزء butanol-n أعلى نشاط في اختبار ازاحت جذر ABTS بقيمة IC_{50}= 0.0007 ± 0.021مجم/مل. أظهرت جميع المستخلصات تثبيطاً جيداً لأكسدة linoleic acid. وقد ثبت وجود نشاط جيد مضاد للالتهابات في المختبر باستخدام تخريب ألبومين البيض وBSA في كل من DEAA و EEAA وأجزائها. كشف Molecular docking للجزيئات الحيوية المحددة والدواء المرجعي (indomethacin) عن تفاعل جيد مع بروتين COX_2 الالتهابي (3LN0). وأظهر اختبار السمية الحادة أن DEAA و EEAA لم يسبب الوفيات أو الآثار الضارة. وقد أدى إعطاء 100 أو 200 ملغم/كغم من DEAA و EEBA عن طريق الفم للجرذان والفئران إلى تثبيط الوذمة في الاختبارات الثلاث، مع نسب جيدة لكلا الجرعتين من كلا المستخلصين. أظهرت نتيجة النشاط المسكن تأثيراً جيداً مقارنة بالمسكن القياسي (aspirin) لنفس الجرعات والمستخلصات. كما أن الإعطاء الفموي لـ DEAA بجرعات 100 مجم/كجم أو 200 مجم/كجم يحمي الغشاء المخاطي المعدي من الإيثانول المطلق. أدت المعالجة المسبقة بجرعةL-NAME ، وهو مثبط ل nitric oxide synthesis ، أو indomethacin ، وهو مثبط لإنتاج prostaglandin، أو glibenclamide ، وهو مثبط لقنواتKATP ، إلى عكس النشاط الوقائي للمعدة DEAA 200 ملغم/كغم، عن طريق الفم. قلل DEAA بشكل فعال من إنتاج العصارة المعدية القاعدية دون أي تأثير على الحموضة الكلية. وقد اشتمل تأثير DEAA الواقي للمعدة على زيادة في الإنزيمات المضادة للأكسدة وإفراز المخاط. توفر هذه الدراسة لأول مرة الأساس العلمي للاستخدام التقليدي لهذا النبات للتأثير الوقائي للمعدة وتدعم الاستخدام التقليدي لهذا النبات لعلاج بعض الاضطرابات المرتبطة بالالتهاب والإجهاد التأكسدي. يمكن استغلال هذه النتائج في الصناعات الدوائية والغذائية ومستحضرات التجميل

الكلمات الرئيسية :العجرم *Anabasis articulata*، الإجهاد التأكسدي، Molecular docking، السمية الحادة، الالتهاب، مسكن الالم، قرحة المعدة.

Abstract

Using a variety of *in-vitro*, *in-silico* and *in vivo* models, the aim of the present study was to investigate the antioxidant, anti-inflammatory, analgesic and anti-ulcerogenic activities of several extracts of Anabasis articulata, a plant from the Algerian flora of arid and semi-arid regions belonging to the Chenopodiaceae family selected on the basis of an ethnobotanical survey (n=182 respondents) conducted at the El-Mergueb nature reserve. Identification of biomolecules, quantification of secondary metabolites and performance of eight tests to study the antioxidant activity of the extracts: Total antioxidant capacity (TAC), DPPH - 2,2-diphenyl-1picrylhydrazyl; ABTS - 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid, hydroxyl radical scavenging, metal-chelating hydrogen peroxide scavenging, reducing power test and β-carotene bleaching assay. In vitro anti-inflammatory activity was attested using the ovalbumin denaturation and BSA assay for DEAA, EEAA and their fractions. Molecular docking was performed using Autodock vina. Single oral doses of 2000 and 5000 mg/kg body weight were administered to albino mice to assess acute toxicity. The anti-inflammatory effect was assessed using the carrageenan-induced paw edema model in rats, the croton oil-induced ear edema model and the xylene-induced ear edema model in mice. Analgesic activity was achieved by intraperitoneal injection of acetic acid. Gastroprotective activity was assessed using ethanol-induced gastric ulceration in rats at doses of 50, 100 and 200 mg/kg p.o. for DEAA and 200, 400 and 600 mg/kg p.o. for EEAA. The mechanistic study involved pretreatment with appropriate antagonists/inhibitors such as glibenclamide, indomethacin and L-NAME. 46 plant species belonging to 23 botanical families were identified for the treatment of various ailments. Seven metabolites present in the n-butanol, ethyl acetate and chloroform fractions were qualitatively identified by LC-MS-MS: anthrone, beta-carotene, butylhydroxyanisole, butylhydroxytoluene, gallic acid, myricetin and rutin. The results showed that ChFA represents the largest quantity of total polyphenols and flavonoids. The n-butanol fraction showed the highest amount of total tannins. Quantitative evaluation of DPPH scavenging capacity showed that the ethanolic extract was the most active with IC50 values of 0.074±0.003 mg/mL, the chloroform fraction (ChFA) had the strongest effect in TAC, hydroxyl radical scavenging and hydrogen peroxide scavenging activity with EC50= 0.151±0.0017, IC50= 0.361±0.0087 and IC50= 1.777±0.0402 mg/mL), however, the nbutanol fraction had the highest activity in the ABTS radical scavenging test with an IC50 of 0.021±0.0007 mg/mL. All extracts showed good inhibition of linoleic acid oxidation. Good in vitro anti-inflammatory activity was attested using the ovalbumin denaturation and BSA assays for DEAA, EEAA and their fractions. Molecular docking of the identified biomolecules and the reference drug (indomethacin) revealed good interaction with the inflammatory protein COX2 (3LN0). Acute toxicity testing showed that DEAA and EEAA did not cause mortality or adverse effects. Oral administration of 100 or 200 mg/kg of DEAA and EEAA to rats and mice inhibited edema in three tests, with good percentages for both doses of both extracts. The analgesic activity result showed a good effect compared with the standard analgesic (aspirin) for the same doses and extracts. Oral administration of DEAA at doses of 100 mg/kg or 200 mg/kg protected the gastric mucosa against absolute ethanol. Pretreatment with L-NAME, an inhibitor of nitric oxide synthesis, indomethacin, an inhibitor of prostaglandin production, or glibenclamide, a KATP channel blocker, reversed the gastroprotective activity of (DEAA200 mg/kg, p.o.). DEAA effectively reduced basal gastric juice production without any effect on total acidity. The gastroprotective action of DEAA involved an increase in antioxidant enzymes and mucus secretion. This study provides for the first time the scientific basis for the traditional use of this plant for gastro-preventive effect and supports the traditional use of this plant to treat certain disorders linked to inflammation and oxidative stress. These results can be exploited in the pharmaceutical, food and cosmetics industries.

Key words: Anabasis articulata, Oxidative stress, Molecular docking, Acute toxicity, Inflammation, Analgesic, Gastric ulcer.

Résumé

En utilisant une variété de modèles *in-vitro, in-silico et in vivo,* le but de la présente étude était d'étudier les activités antioxydante, anti-inflammatoire, analgésique et anti-ulcérogène de plusieurs extraits d'*Anabasis articulata*, une plante de la flore algérienne des régions arides et semi-arides appartenant à la famille des Chenopodiaceae choisis sur la base une enquête ethnobotanique (n=182 interrogés) menée à la réserve naturelle El-Mergueb. L'identification des biomolécules, la quantification et de métabolites secondaires et la réalisation de huit tests pour l'étude de l'activité antioxydante des extraits : Capacité antioxydante totale (TAC), DPPH - 2,2-diphényl-1picrylhydrazyl; ABTS - 2,2′-azino-bis 3-éthylbenzothiazoline-6-acide sulfonique, piégeage du radical hydroxyle, piégeage du peroxyde d'hydrogène chélatrice des métaux, test du pouvoir réducteur et dosage du blanchiment du β-carotène. L'activité anti-inflammatoire *in vitro* a été attestée en utilisant la dénaturation de l'ovalbumine et le test BSA pour le DEAA, l'EEAA et leurs fractions. Le docking moléculaire a été effectuer en utilisant l'Autodock vina. Des doses orales uniques de 2000 et 5000 mg/kg de poids corporel ont été administrées à des souris albinos pour évaluer la toxicité aiguë. L'effet anti-inflammatoire a été évalué en utilisant le modèle d'œdème de la patte induit par la carragénine chez le rat, l'œdème de l'oreille induit par l'huile de croton et l'œdème de l'oreille induit par le xylène chez la souris. L'activité analgésique a été réalisée par injection intrapéritonéale d'acide acétique. L'activité gastroprotectrice a été évaluée en utilisant l'ulcération gastrique induite par l'éthanol chez les rats à des doses de 50, 100 et 200 mg/kg p.o. de DEAA et 200, 400 et 600 mg/kg p.o pour l'EEAA. L'étude mécaniste a impliqué des prétraitements avec des antagonistes/inhibiteurs appropriés tels que le glibenclamide, l'indométacine et le L-NAME. 46 espèces de plantes appartenant à 23 familles botaniques ont été recensées pour le traitement de différentes affections. Sept métabolites présents dans les fractions de n-butanol, d'acétate d'éthyle et de chloroforme ont été identifiés qualitativement par LC-MS-MS: anthrone, bêta-carotène, butylhydroxyanisole, butylhydroxytoluène, acide gallique, myricétine et rutine. Les résultats ont montré que le ChFA représente la plus grande quantité de polyphénols totaux, de flavonoïdes. La fraction n-butanol a montré la plus grande quantité de tannins totaux. L'évaluation quantitative du pouvoir de piégeage de DPPH a montré que l'extrait éthanolique était le plus actif avec des valeurs IC_{50} de 0.074±0.003 mg/mL, la fraction chloroforme (ChFA) a eu l'effet le plus fort dans le TAC, le piégeage du radical hydroxyle et l'activité de piégeage du peroxyde d'hydrogène avec EC_{50}= 0.151±0.0017, IC_{50}= 0.361±0.0087 et IC50= 1.777±0.0402 mg/mL), cependant, la fraction n-butanol a eu la plus grande activité dans le test de piégeage du radical ABTS avec un IC_{50} de 0.021±0.0007 mg/mL. Tous les extraits ont montré une bonne inhibition de l'oxydation de l'acide linolénique. Une bonne activité anti-inflammatoire *in vitro* a été attestée en utilisant la dénaturation de l'ovalbumine et le test BSA pour le DEAA, l'EEAA et leurs fractions. L'amarrage moléculaire des biomolécules identifié et le médicament de référence (l'indométacine) a révélé une bonne interaction vis à vis la protéine inflammatoire COX_2 (3LN0). Le test de toxicité aiguë a montré que DEAA et EEAA n'ont pas provoqué de mortalité ou d'effets indésirables. L'administration orale de 100 ou 200 mg/kg de DEAA et d'EEAA à des rats et à des souris a inhibé l'œdème dans trois tests avec un bon pourcentage pour les deux doses des deux extraits. Le résultat de l'activité analgésique montre un bon effet comparé à l'analgésique standard (l'aspirine) pour les mêmes doses et extraits. L'administration orale de DEAA à des doses de 100 mg/kg ou 200 mg/kg a permis de protéger la muqueuse gastrique contre l'éthanol absolu. Le prétraitement par le L-NAME, un inhibiteur de la synthèse de l'oxyde nitrique, l'indométacine, un inhibiteur de la production de prostaglandines, ou le glibenclamide, un bloqueur du canal KATP, a inversé l'activité gastroprotectrice de (DEAA200 mg/kg, p.o.). La DEAA a réduit efficacement la production basale de suc gastrique sans aucun effet sur l'acidité totale. L'action gastroprotectrice de la DEAA a impliqué une augmentation des enzymes antioxydantes et de la sécrétion de mucus. Cette étude fournit pour la première fois les bases scientifiques de l'utilisation traditionnelle de cette plante pour l'effet gastro-préventif et soutient l'utilisation traditionnelle de cette plante pour traiter certains troubles liés à l'inflammation et au stress oxydatif. Ces résultats peuvent être exploités dans le domaine des industries pharmaceutiques, alimentaires et cosmétiques.

Mots clés : *Anabasis articulata*, Stress oxydatif, Docking moléculaire, Toxicité aiguë, Inflammation, Analgésique, Ulcère gastrique.