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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا إلا ما علمتنا

إنك أنت العليم الحكيم

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صدق الله العظيم

Dedication

And

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DEDICATION

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ABSTRACTS

ملخص

النبتة .*Sanguisorba minor* L المعروفة باسم البرنبيرون الصغيرة، يستخدم تقليديا في الطب لعلاج العديد من الأمراض, تهدف هذه الدراسة الى التقييم العلمي للاستخدامات الطبية الشعبية و ذلك من خلال تحديد التركيب الكيميائي للنبات، وفحص التأثيرات المضادة للأكسدة ، الالتهاب والقرحة المعدية من خلال نماذج في المخبر وكذا عند الحيوان، وكذلك التأكد من سلامتها. تم الحصول على المستخلص المائي (SMAE) والإيثانولي (SMCE) عن طريق الغلي والنقع وتم الحصول على المردود بنسبة 8.67٪ و 12.6٪ على التوالي . أظهر التحليل الكمي أن المستخلص الإيثانولي يحتوي على أكبر كمية من البوليفينولات الكلي بكمية تقدر 623.33 ±0.01 مكغروغرام مكافئ حمض الغاليك/مغ من المستخلص الجاف، على خلاف المستخلص المائي الذي كان يحتوي على الكمية الأكبر من الفلافونوييدات حيث قدرت 15.46 ±0.00 مكغروغرام مكافئ الكيرسيتين/مغ من المستخلص الجاف. خضع المستخلصان لتحليل نوعي وكمي لمكوناتهما الفينولية بواسطة HPLC-DAD، تم تحديد 17 مركبًا نشطًا بيولوجيًا في المستخلصين, تم تقييم النشاط المضاد للأكسدة في المختبر باستخدام عدة تقنيات بما في ذلك جذور DPPH و hydroxyl (OH) و nitric oxide و ABTS وقدرة الارتباط بايونات الحديد الثنائية واختبار القدرة الاختزالية.من خلال النتائج اظهر المستخلص الايثانولي فعالية كبيرة في طريقة استخلاب المعادن بتركيز تثبيط قدر ب 23.77±0.00 مغ/مل، بالمقابل اظهر المستخلص المائي فعالية مع اختبار جذر الهيدروكسيل بتركيز قدر ب28±0.00مغ/مل. تم تقييم النشاط المضاد للالتهابات من خلال تثبيط تحلل البروتينات(BSA) واظهرت النتائج أن كلا المستخلصين لهما تأثير يعتمد على الجرعة . أدى تناول 150 مغ/كغ من كلا المستخلصين عن طريق الفم إلى انخفاض كبير في التشنجات التي يسببها حمض الأسيتيك، بينما أدت جرعة 150 مغ/كغ إلى انخفاض الوذمة التي يسببها الغزيلين وزيت الكروتون والكاراجينان عند الفئران. تم اختبار السمية الحادة وفقًا لتوصيات منظمة التعاون الاقتصادي والتنمية باستخدام جرعتين (2 غ/كغ و 5 غ/كغ)، ولم يلاحظ أي تغيير في السلوك أو حالات وفاة، مما يشير إلى أن مستخلص المائي يمكن اعتباره غير سام،كما تم تقييم النشاط المضاد لالتهاب المعدة على نموذج التهاب المعدة الناجم عن الإيثانول في كلا المستخلصين على الجرذان بجرعات (100 و 250 و 500 مغ/كغ). أظهرت النتائج أن كلا المستخلصين وفرا حماية للغشاء المخاطي . في الأخير استخلصنا ان مستخلصات نبتة .*Sanguisorba minor* L تتمتع بخصائص مضادة للالتهابات، للأكسدة ومضادة أيضا للقرحة مثيرة للاهتمام.

الكلمات المفتاحية: .*Sanguisorba minor* L ، النشاط المضاد للأكسدة، البوليفينولات ، HPLC-DAD، قرحة المعدة.

Abstract

The plant *Sanguisorba minor* L. (*S. minor*) commonly known as salad burnet and historically has been used in traditional medicine for the treatment of numerous ailments. This study intends to evaluate the phytochemical composition and examining its antioxidant, anti-inflammatory, and antiulcer effects through *in vitro* and *in vivo* models, as well as assessing its safety. *S. minor* aqueous (SMAE) and *S. minor* ethanolic (SMCE) extracts were obtained by decoction and maceration methods, resulting in yields of 8.67 and 12.6%, respectively. Quantitative investigation revealed that SMCE has the highest overall polyphenol content with a value of 623.33 ±0.01 µg gallic acid equivalent, whereas the SMAE displayed the highest flavonoid concentration with a value of 15.46 ±0.00 µg quercetin equivalent/mg dry extract, respectively. Both extracts were subjected to qualitative and quantitative analysis of their phenolic constituents using HPLC-DAD analysis. Seventeen bioactive components were identified in two extracts. The *in vitro* antioxidant activity was evaluated using various assays models, including DPPH, hydroxyl, nitric oxide, ABTS radicals scavenging, ferrous ion chelation, reducing power and the phosphomolybdate assays. Metal chelating showed that SMCE was the effective extract with IC_{50} of 23.77±0.00 µg/mL, conversely in the hydroxyl radical test the SMAE showed significant inhibition with IC_{50} of 28±0.00 µg/mL . The *in vitro* anti-inflammatory activity was evaluated by the protein denaturation inhibition assay, revealing that both extracts demonstrated dose-dependent effects. The oral administration of both extracts (150 mg/kg) markedly suppressed acetic acid-induced nociception. At the dose of 150 mg/kg, the two extracts was greatly inhibit edema development caused by xylene, croton oil, and carrageenan effects. Acute toxicity was assessed in accordance with OECD recommendations utilizing two limit doses (2 and 5 g/kg). No behavioral changes or fatalities were noted, suggesting that SAME can be regarded as non-toxic. The gastroprotective efficacy of both extracts was assessed using ethanol-induced stomach ulceration in rats at dosages of 100, 250, and 500 mg/kg. The findings show that the mucosa was protected against ulceration in a dose-dependent manner by both SMCE and SMAE. To sum up, our study showed that *Sanguisorba minor* L. extracts had very promising antioxidant, anti-inflammatory, and antiulcerogenic properties.

Keywords: *Sanguisorba minor* L., polyphenols, antioxidants, HPLC-DAD analysis, gastric ulcer, toxicity

Résumé

La plante *Sanguisorba minor* L., communément appelée petite pimprenelle, a été traditionnellement utilisé en médecine pour le traitement de nombreuse affections .Cette étude a pour objectif d'évaluer scientifiquement les usage ethnomédicaux en caractérisant la composition phytochimique et en examinant les effets antioxydants ,anti-inflammatoires et antiulcéreux à travers des modèles *in vitro* et *in vivo*, ainsi s'assurer son sécurité .L'extrait aqueux (SMAE) et éthanolique (SMCE) son obtenue par décoction et macération avec des rendement de 8.67% et 12.6%, respectivement .L'analyse quantitative a révélé que le SMCE contenait le plus grand quantité des polyphénols totaux (623.33 ± 0.01µg équivalent d'acide gallique/mg d'extrait sec)a l'inverse le SMAE contient la quantité élevé des flavonoïdes (15.46± 0.00 µg équivalent de quercetine /mg extrait sec). Les deux extraits ont été soumis a l'analyse qualitative et quantitative de leurs constituants phénoliques par HPLC-DAD .17 composées bioactive ont été identifier pour les deux extraits. L'activité antioxydante *in vitro* a été évaluer par plusieurs techniques inclus le radical DPPH, hydroxyl (OH), oxide nitrite (NO), et ABTS, la chélation du fer ferreux, le pouvoir réducteur, et la méthode de phosphomolybdate. La chélation des métaux a montré que SMCE était l'extrait efficace avec un IC_{50} de 23.77 ±0.00 µg/mL, a l'inverse dans le test du radical hydroxyl l'extrai SMAE a montré une inhibition importante avec un IC_{50} de 28 ±0.00 µg/mL. L'activité anti-inflammatoire a été évalué par l'inhibition de la dénaturation des protéines (BSA) et les résultats montre que les deux extraits ont un effet dose dépendante. L'administration orale de 150 mg/kg des deux extraits réduit significativement les contorsions induites par l'acide acétique, tandis que la dose 150 mg/kg diminuent l'œdème causé par le xylène, l'huile de croton et le carragenine. La toxicité aigüe a été évalué conformément aux recommandations de l'OCDE en utilisant deux doses (2 g/kg et 5 g/kg), aucun changement comportemental ni cas de mortalité n'a été remarqué, suggérant que l'extrait SMAE peut considérer comme non toxique. L'activité gastroprotectrice a été évaluée sur un modèle d'ulcération gastrite induite par l'éthanol chez les rats à des doses de (100,250 et 500 mg/kg). Les résultats montre que les deux extrait ont offert une protection dose dépendante de la muqueuse. On conclue que notre étude a montré que l'extrait de la plante *Sanguisorba minor* L. présente des propriétés anti-inflammatoires, antioxydant et antiulcéreuse intéressantes.

Mots clés: *Sanguisoba minor* L., polyphenols, activité antioxydante, HPLC-DAD, ulcère gastrique, toxicité.

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ABBREVIATIONS

ABBREVIATIONS

SMAE: *Sanguisorba minor* aqueous extract
SMCE: *Sanguisorba minor* etahnolic extract
ABTS: 2.2′-Azino-bis-(3-ethylbenzenothiazoline-6-sulfonic acid)
AMPK: AMP-activated protein kinase
BH_4: Tetrahydrobiopterin
BHT: Butyl hydroxyl toluene
CAT: Catalase
CM: Circular muscle
COX: Cyclooxygenase
DPPH: 1, 1-Diphenyl-2-picryl-hydrazyl
DW: Dried weight.
EDTA: Ethylenediaminetetraacetic acid
EGCG: Epigallocatechin gallate
eNOS: Endothelial nitric oxide synthase
EPSPs: Excitatory postsynaptic potential
GI: Gastrointestinal
GPx: Glutathione peroxidase
GR: GSH reductase
GSH: Glutathione
IL: Interleukin
iNOS: Inducible nitric oxide synthase
LD_{50}: Lethal dose, 50%
LDL: Low-density lipoprotein
LM: Longitudinal muscle
$LOO^{\bullet}$: Lipid peroxyl
LOOH: Lipid peroxide
LOX: Lipoxygenase
LPO: Lipid peroxidation
LTP: Long-term potentiation
MPO: Myeloperoxidase
NADPH: Nicotinamide adenine dinucleotide phosphate
NANC: Non-adrenergic non-cholinergic
NFkB: Nuclear factor kB
NFTs: Neurofibrillary tangles
NK: Neurokinin
nNOS: Neuronal nitric oxide
N_2O_3: Dinitrogen trioxide
NOS: Nitric oxide synthase
NSAIDs: Non-steroidal anti-inflammatory drugs
NTS: Nucleus tractus solitarius
$ONOO^-$: Peroxynitrite
PGs: Prostaglandins

PKC: Protein kinase C
RNS: Reactive nitrogen species
RO•: Alkoxyl
ROO•: Peroxyl
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TBA: Thiobarbituric acid
TCA: Trichloroacetic acid
TFC: Total flavonoid content
TNF: Tumor necrosis factor
TPC: Total phenolics content
HPLC-DAD: High performance liquid chromatography-Diode-Array
V: Vessel.
VIP: Vasoactive intestinal peptide

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Introduction

Introduction

The natural environment has served as a source of health for humanity from the beginning of time. The selection of medicinal herbs was deliberate, informed by experiential knowledge of their utilization. The usage of medicinal plant has significantly increased in the last many years, presumably owing to their cultural significance, availability, and cost-effectiveness (Thomford *et al*., 2015). The therapeutic application of plants remains prevalent in certain nation; particularly in under develop nations, despite advancements in pharmacology (Tabuti *et al*., 2003). Africa's largest nation is Algeria. It is distinguished by its variety of medicinal and aromatic flora. Algeria's flora is abundant comprising over 4300 species and subspecies of vascular plants (Kouider *et al*., 2019). Inflammation represents as a natural protective response to biological chemical or viral assaults. This immune response may be detrimental due to the pathogen's aggressiveness, its persistence, and irregularities in the regulation and synthesis of inflammation-related cells. These inflammatory processes play a role in the onset of various human illness such as diabetes, arthritis and allergies. An important factor in both initiation and persistence of inflammation is oxidative damage, hence facilitating the emergence and/or continuation of several diseases.

The latter arises when the equilibrium of redox hemostasis is upset. This leads to the excessive generation and/or inadequate removal of free radicals, which are highly reactive molecules composed of either oxygen or nitrogen. This imbalance induces oxidative damage to multiple cellular molecules that leads to necrosis or apoptosis. Oxidative stress is involved in the aging and in the development of several illness, including as cardiovascular disorders, inflammatory conditions and cancer (Belaich *et al*., 2016). The administration of nonsteroidal anti-inflammatory medications often leads to considerable side effects, including gastrointestinal, renal, and asthmatic complications (Soubrier *et al.,* 2013). On the other hand, prolonged utilization of synthetic substances in cosmetic, pharmaceutical sector and food has been linked to teratogenic, mutagenic, and carcinogenic effects (Panicker *et al*., 2014).

Numerous individuals around endure the persistent condition of stomach ulcers (Kangwan *et al*., 2014), the condition is easily transmissible and challenging to treat, posing significant risks to human health and perhaps resulting in mortality. The treatment can be challenging, contingent upon the etiological agent and the ulcer's circumstances. The therapy with contemporary medicine is costly, posing a challenge of the patient and is not devoid of

adverse consequences. To address these issues, individuals globally are utilizing the advantages of natural products to manage these conditions. Natural products use for illness prevention and treatment has a long history in Algeria and stretches back thousands of years. A common perennial herb in Asia, Europe and North Africa, *Sanguisorba minor* L. belongs at the Rosaceae family (Cirovic *et al*., 2019). It called also salad burnet and has been employed to treat a number of illness including fever, conjunctivitis and diarrhea in traditional medicine (Viano *et al.*, 1999; Ferreira *et al.,* 2006). This species' aerial portions had an anti-ulcerogenic action, which is consistent with research on plants in the Sanguisorba genus. In addition to their therapeutic properties, *S. minor's* roots and aerial portions are edible and typically eaten in mixed vegetable salads (Zhao *et al.,* 2017; Cirovic *et al.,* 2019). Due to its rich polyphenols, *S. minor* is regarded as one of the most promising dietary treatment (Guarrera *et al*., 2013).

The aim of this study was to scientifically validate the traditional medicinal uses of this species by investigating its antioxidant, anti-inflammatory, and anti-ulcerogenic activities through both in vitro and in vivo experiments, while also assessing its toxicity profile to evaluate its safety and potential therapeutic value.

Literature review

Literature review

1. Inflammation

The inflammatory response is the reaction of living, vascularized tissue to physical, chemical or biological aggression, aimed at preserving its integrity (Medzhitov, 2008). It encompasses multiple biological systems that engage at different times and intensities: biochemical reactions, cellular activation, coagulation, and fibrinolysis, all aimed at neutralizing or removing the foreign substance (Ashley *et al*., 2012).

Inflammation is typically an advantageous process. The pathogen's aggressiveness, persistence, the site of inflammation, or aberrant regulation of the inflammatory process can all make it dangerous at times. Based on the length of time ant the dynamics of the inflammatory process, it is divided into two group: acute inflammatory reaction and chronic inflammatory reaction (Singh *et al.,* 2019). Edema, pain heat, and redness are the four common symptoms of acute inflammation, which is an instantaneous reaction that can last anywhere from a few days to a few weeks and frequently has a fast beginning. It is separated into four stages: the cellular phase (immune cell mobilization), the immediate vascular phase (local microcirculation change), and the last two stages of resolution and healing (tissue repair in a few days). Inflammatory cells infiltrate uncontrollably and persistently if the inflammation is not resolved, eventually progressing to chronic inflammation (**Figure 1**), (Noack and kolopp-Sarda, 2018). Moreover, varieties of disorders are brought on by persistent inflammation.

Moreover, varieties of disorders are brought on by persistent inflammation. Persistent cellular infiltration leads to tissue damage and hyperplasia. Numerous inflammatory mediators are released as a result of inflammatory response. Leukocytes are drawn to the site of inflammation, their adherence to the endothelium is enhanced, vascular permeability is increased, and platelet aggregation is increased (Medzhitov, 2008). Moreover, macrophages make up the majority of the cellular infiltration at the site of inflammation in the chronic inflammation (**Figure 2**). However, macrophages activate fibroblasts to produce collagen and activate other macrophage and lymphocytes. Consequently, there is a great deal of scarring, collagenous scarring, fibrosis, and necrosis (Ward, 2010).

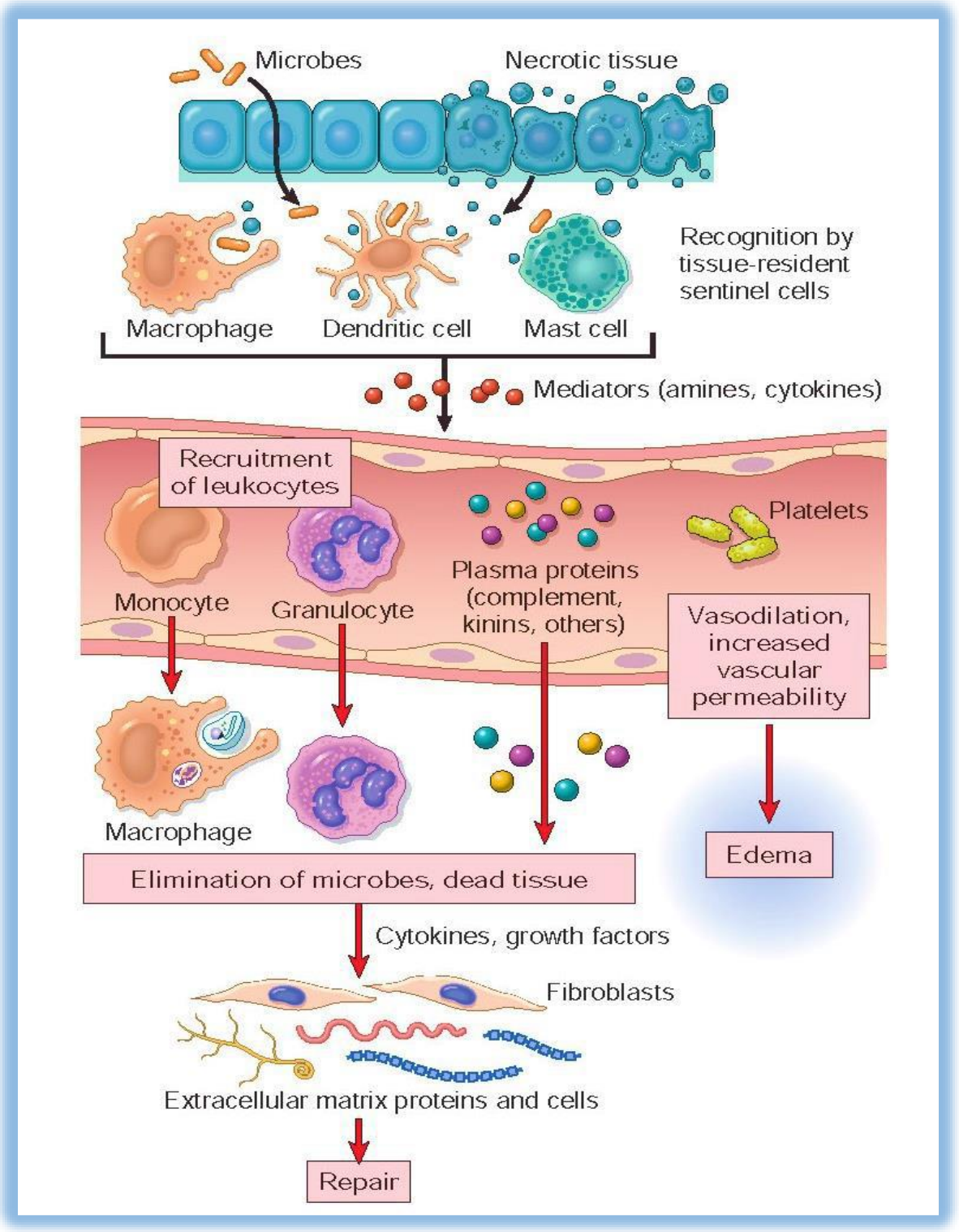

Figure 1: Acute inflammation progression (Kumar *et al.,* 2015)

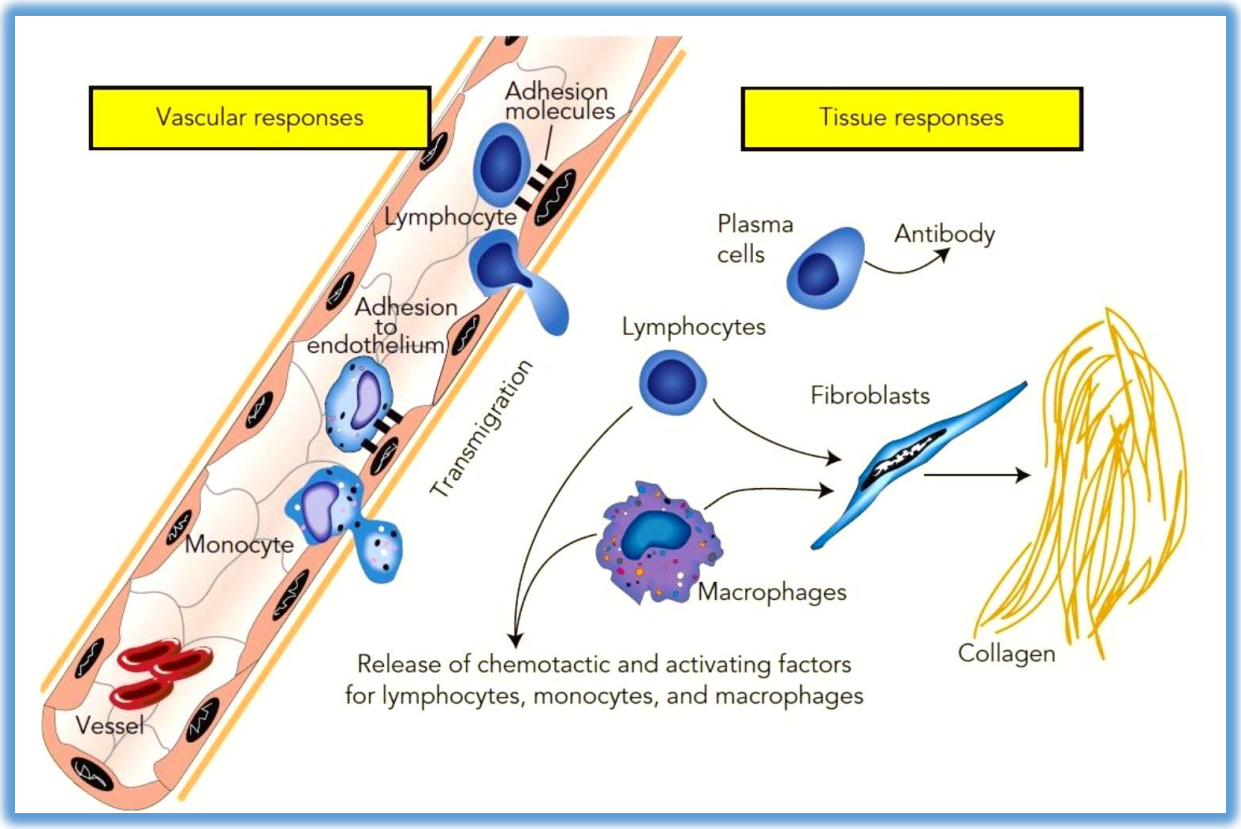

Figure 2: Cell types and mechanisms involved in chronic inflammation (Ward, 2010).

1.1. Treatment of inflammation

Anti-inflammatory drugs are well-established treatment that continue to be among the most commonly utilized in therapeutic practice. They are classified into diverse pharmacological categories and operate through distinct biochemical pathways.

Nonsteroidal anti-inflammatory medications (NSAIDs) primarily function by suppressing prostaglandin synthesis via the blockade of cyclooxygenase (COX), (**Figure 3**). COX exists in two isoforms: COX-1, referred to as constitutive COX, participate in the synthesis of protective physiological prostaglandins in the gastric mucosa and kidneys; COX-2, known as inducible COX, arises in inflammatory sites and promotes the productions of pro-inflammatory prostaglandins with harmful effects (Becker and Monassier, 2018).

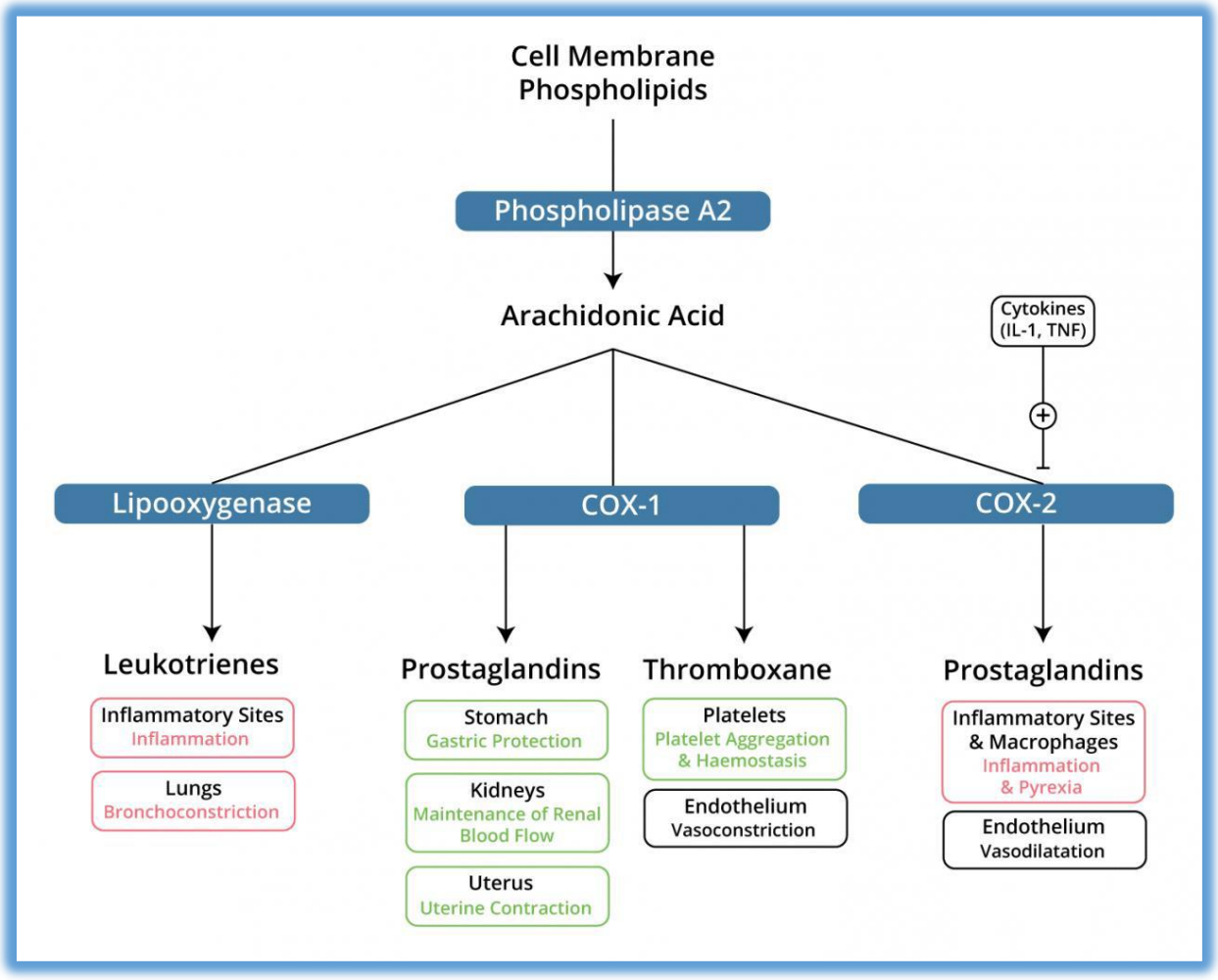

Figure 3: Mechanism of action and effects of nonsteroidan anti-inflammatory drugs (Aradhyula et al., 2024)

Steroidal anti-inflammatory drugs (SAIDs), also referred to as glucocorticoids , are synthetic analogues of cortisone. They form a very structurally uniform group with significant anti-inflammatory activity, as well as immunomodulatory and antiallergic characteristics (Mallem and Gogny, 2014). Corticosteroids interact with cytosolic receptors. They generally exert their effects through genomic mechanisms by influencing DNA transcription into RNA and regulating messenger RNAs post-transcriptionally **(Figure 4).** These glucocorticoids exert effects on several targets, encompassing both humoral and cellular mechanisms. They interact with cytokines, chemokines, adhesion molecules, enzymes, cell proliferation factors, and apoptosis regulators at a humoral level (Quatrini and Ugolini, 2021).

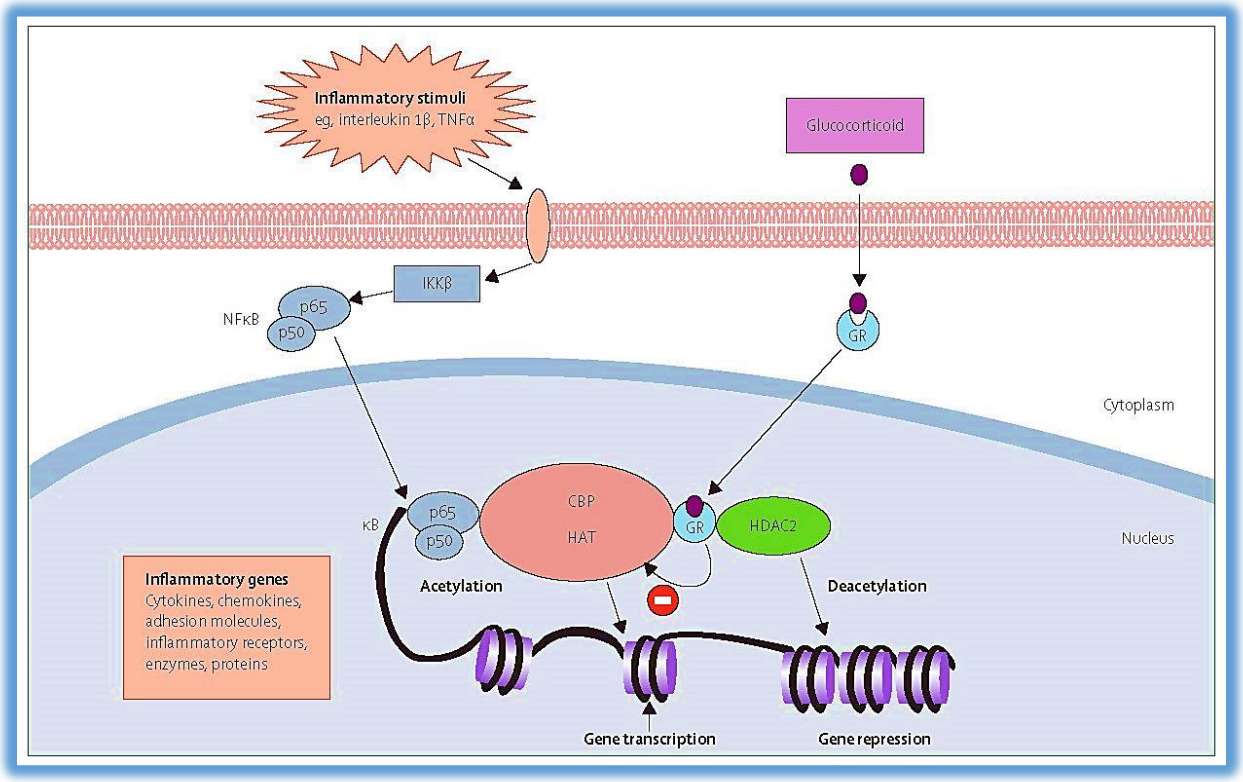

Figure 4: Mode of action of glucocorticoids (Barnes and Adock, 2009)

1.2. Plants substances as anti-inflammatory

Medicinal herbs are extensively utilized in traditional medicine globally to cure or alleviate various inflammatory illnesses. These plant's anti-inflammatory properties are mostly due the quantity of secondary metabolites, such as flavonoids, polyphenols, sterols and carotenoids (Iwalewa *et al*., 2007). Various *in vitro* and *in vivo* research have confirmed the anti-inflammatory activity of phenolics compounds. In contrast, to pharmacological agents that operate through a specific receptor or signaling pathway. Polyphenols demonstrate various mechanisms of action that target distinct pathway. These active compounds demonstrate anti-inflammatory properties by influencing many pathways, such as inhibiting arachidonic acid metabolism, suppressing cytokine production, downregulating adhesion molecule expression, blocking NF-$_k$B activation, and diminishing ROS (Zainali *et al.,* 2017; Jamtsho *et al.,* 2024).

1.3. Oxidative stress and inflammation

There is strong correlation between inflammation and oxidative stress, according to research of Zbadi *et al.* (*2018*). Activated phagocytes release reactive oxygen, chlorine species, and reactive nitrogen, such as (H_2O_2), (OH.), (NO.), and (HOCl), during inflammation in order to eradicate invasive microorganisms (Fialkaw *et al.,* 2007). Chronic inflammation causes an

increase in the creation of the reactive species, which feeds the inflammatory process and facilitating the emergence of chronic illnesses (Bardelčíková *et al*., 2023). ROS in chronic inflammatory disorders not only inflict direct cytotoxic destruction resulting in molecular, cellular, and tissue injury, but also significantly regulate the synthesis of additional inflammatory mediators and, in specific situations, affect lymphocyte differentiation (Reimund, 2002). Activated inflammatory cells release cytokines (IL-1, IL-6, TNFα, IFNγ), which subsequently stimulate the production of ROS by neutrophils, macrophages, and epithelial cells (Li *et al*., 2016). This interaction creates a beneficial relationship between inflammatory mediators and reactive oxidants. Oxidative stress-induced inflammatory mediators exacerbate the inflammatory response. Conversely, inflammation results in oxidative stress and damages cells by producing ROS and other oxidizing chemicals (Biswas, 2015), (Figure 5).

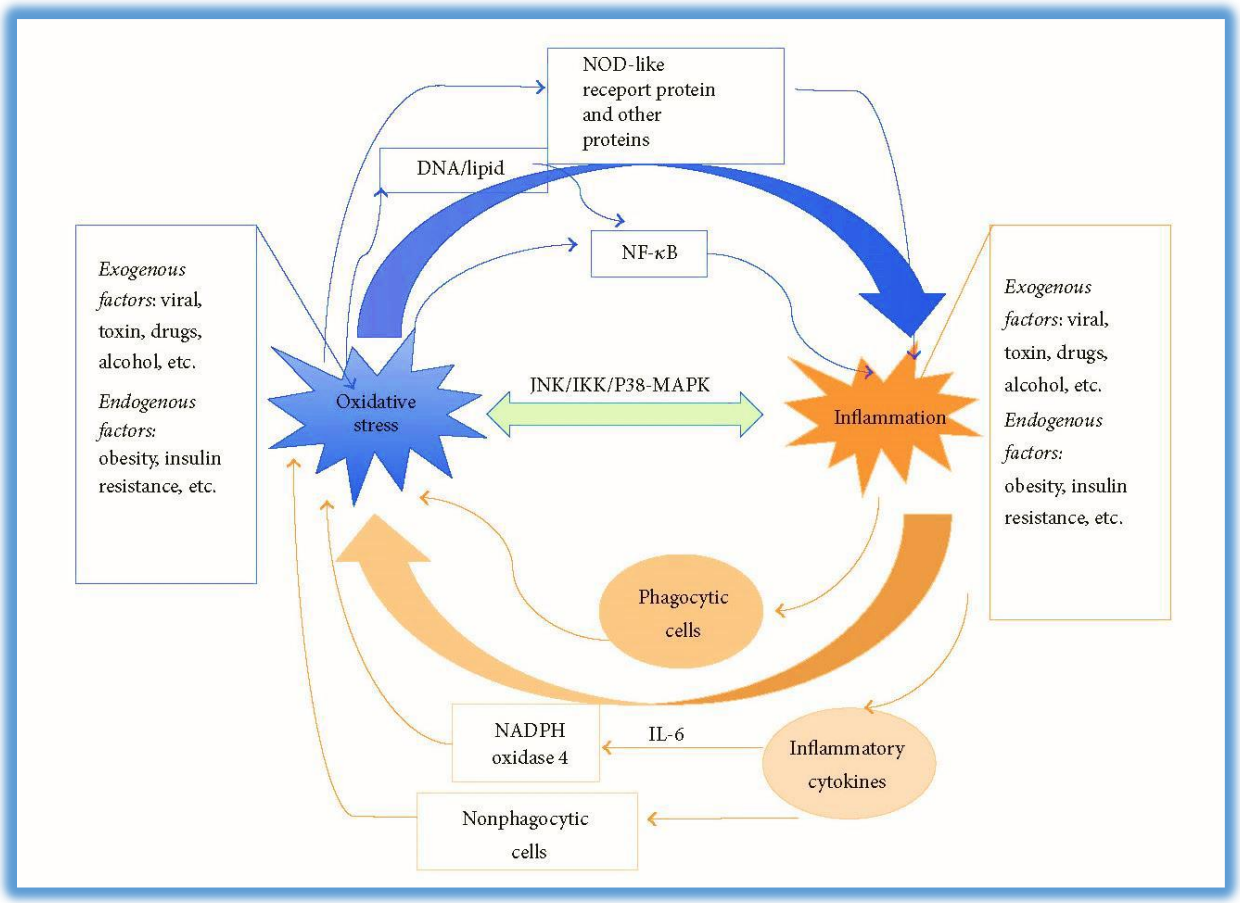

Figure 5: Interplay oxidative stress and inflammation (Li et al., 2016).

2. Oxidative stress

An imbalance in redox homeostasis, resulting from the excess of ROS and RNS during aerobic metabolism compared to the efficacy of antioxidant defense mechanisms is known as oxidative stress. The defenses consist of enzymatic components, including SOD, CAT, and GSH-Px, together with non-enzymatic antioxidants like GSH reduced glutathione and vitamins E and C (Dush *et al*., 2025).

2.1. Reactive molecular species and unstable molecules

Atoms or molecules possessing one or more unpaired electrons in their outer orbitals are referred to as free radicals or unstable molecules, and this makes them extremely reactive (Liu, 2020). They serve as essential mediators in several biological processes, influencing a broad spectrum of physiological states, and include ROS and RNS, which have both advantageous and harmful effects (Hassan *et al*., 2024). Free radicals may be produced in reaction to exogenous stressors such as tobacco smoke, environmental pollutants, pesticides, and ionizing radiation. The primary intracellular sources of redox-active species, such as RNS and ROS , originate from various structures, including the mitochondrial electron chain (ETC), endoplasmic reticulum (ER), peroxisomes, and membranes-confined NADPH oxidases (Dush *et al.,* 2025), (Figure 6).

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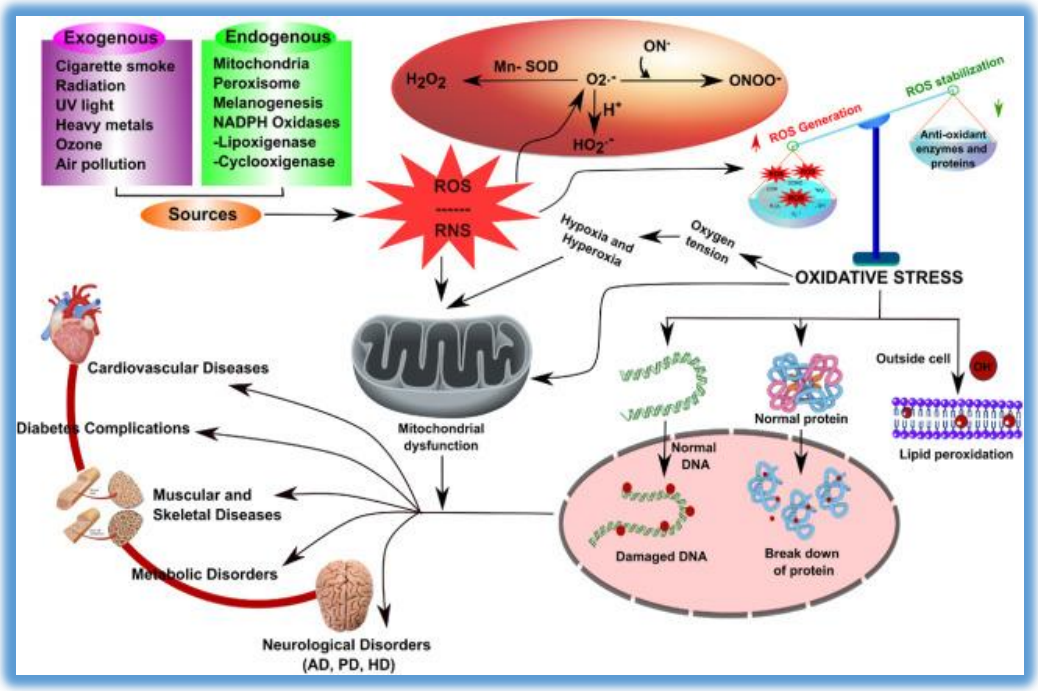

Figure 6: Cellular mechanisms of ROS/RNS species generation and response (Dush et al., 2025)

Free radicals may be in reaction to reactive oxygen species (ROS), which have a dual function: at low concentration, they provide advantageous effects like anti-atherosclerotic activity, while at high levels, they cause cellular damage, resulting in impaired endothelial function, atherosclerosis, and cardiovascular disorders (Sharifi-Rad *et al*., 2020).

2.2. Antioxidants substances

The substance that can block or postpone the oxidation of an oxidizable material at comparatively low concentration as known an antioxidant. The body depends on various endogenous antioxidants-both enzymatic (SOD, CAT, and GSH-Px) and non-enzymatic (for example uric acid, glutamate, coenzyme Q10)-alongside exogenous antioxidants to combat free radicals and their detrimental effects (Ighodaro and Akinloye, 2018), (Figure 7)

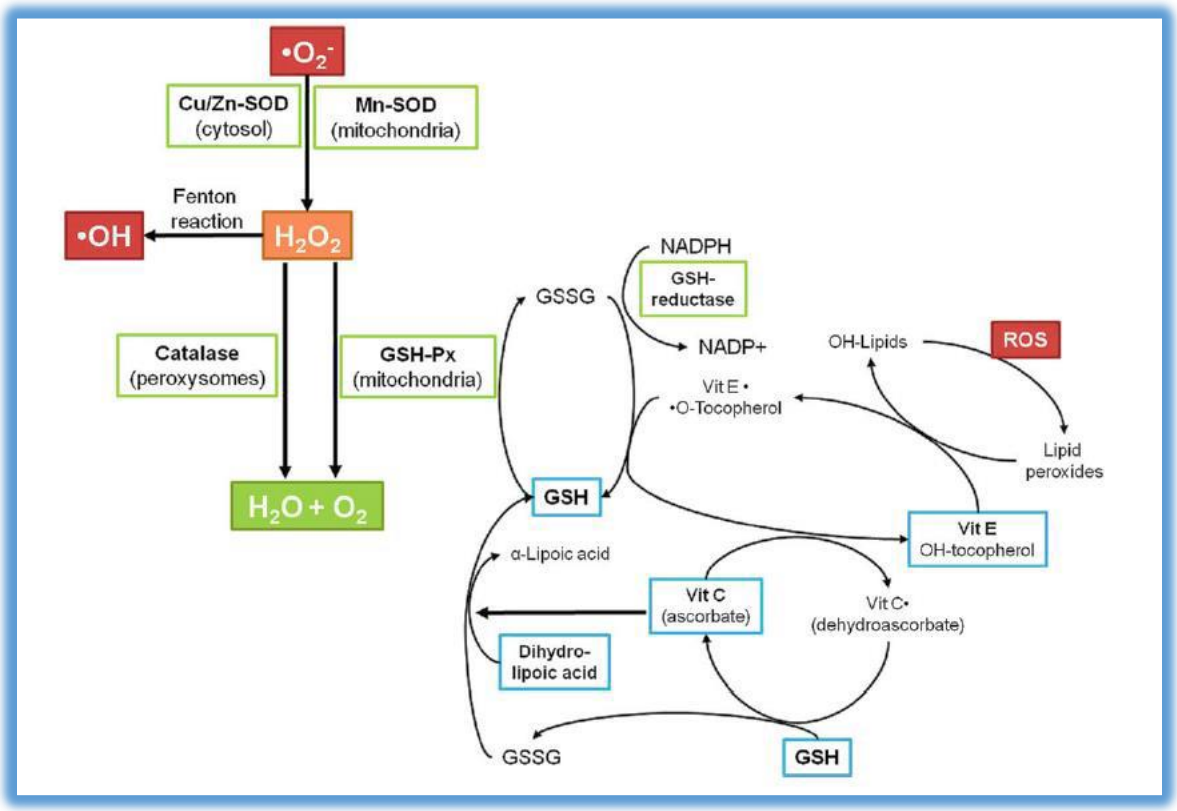

Figure 7: Cellular mechanisms of ROS/RNS species generation and response (Dush *et al*., 2025)

2.3. Polyphenols

Polyphenols are bioactive chemicals obtained from plants that exhibit a variety of biological activities. Phytochemical investigations have thus far identified more than 8000 phenolic chemicals in plants. They are categorized into several classes according on the structural attributes of their carbon framework. Phenolic chemicals, such as flavonoids, can

engage with ROS and RNS, thereby disrupting radical chain reactions and safeguarding cellular viability (Kumar and Pandey, 2013). They also exhibit chelating capabilities, allowing them to trap metal ions. Various research on animal and cellular models indicate that dietar

y polyphenols consumption may serve as a valuable adjunct for the protection and management of many diseases (Hussain *et al*., 2016).

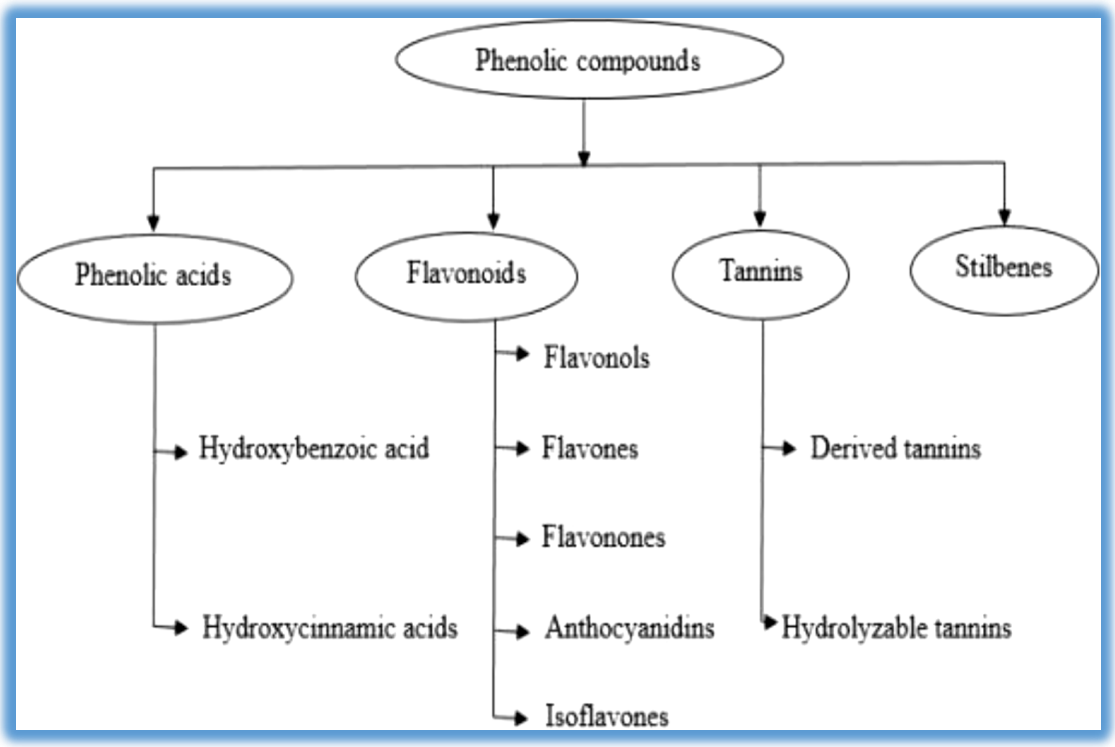

Figure 8: Primary categories of phenolic compounds (Alara et al., 2021)

3. Gastric ulcer

Gastric ulcer illness is multifaceted and intricate disorder resulting from the imbalance between protective and aggravating elements in the gastrointestinal tract (**Figure 9).** The primary aggressive elements include physiological mediators-such as stomach acid, endothelins, pepsin, reactive oxygen species, oxidants, and leukotrienes and exogenous agents like ethanol and NSAIDs. In contrast, defensive mechanisms comprise gastric mucus, bicarbonate secretion, sufficient mucosal, nitric oxide (NO), blood flow, prostaglandins (PGs) and antioxidants systems including enzyme (CAT) and peptide (glutathione, GSH) (Khan *et al*., 2023; Shahzad *et al*., 2024). The integrity of the gastro-duodenal mucosa is preserved by homeostatic balance between protective and harmful factors (Kansara and Sakhreliya, 2013).

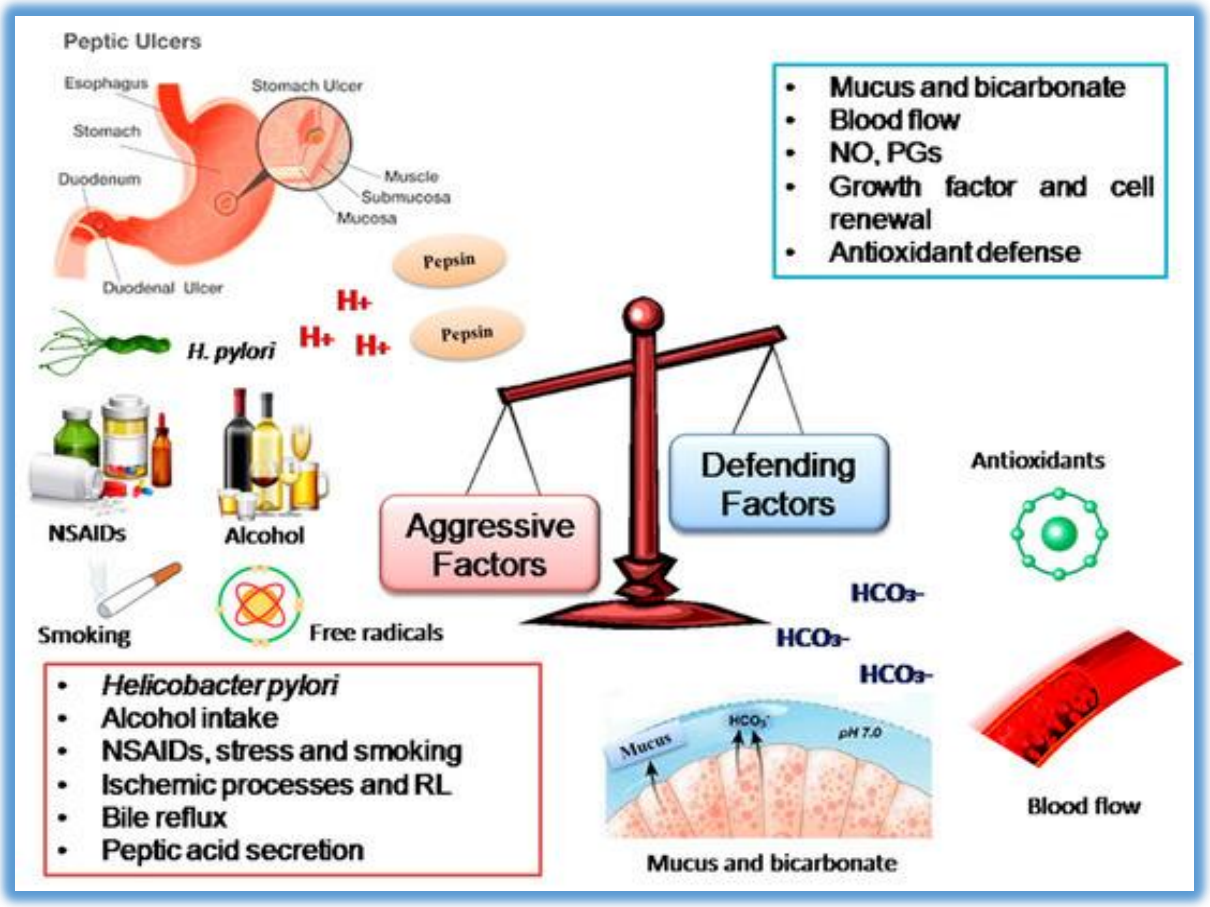

Figure 9: Providing examples of the elements that contribute to gastric ulcers (Serafim et al., 2020)

3.1. Ethanol-induced stomach ulcer model related to oxidative stress

Alcohol has already been investigated as an example for stomach ulcers. It represents one of the most widely employed models for assessing gastroprotective medications. Ethanol injures the vascular endothelial cells of the stomach mucus membrane, leading to microcirculatory disruptions and hypoxia, which subsequently induce excessive generation of oxygen radicals (Arab *et al*., 2015). ROS induce tissue injury by facilitating oxidation and lipid peroxidation. Furthermore, reactive oxygene species such as $O_2^{\cdot}$ and H_2O_2 provoke inflammation and tissue damage by causing degradation and fragmentation of cellular DNA (Suzuki *et al.,* 2011). The ethanol-generated ulcer model is a pertinent framework for investigating cytoprotective or antioxidant medecines, with its mechanisms delineated in **(Figure10**).

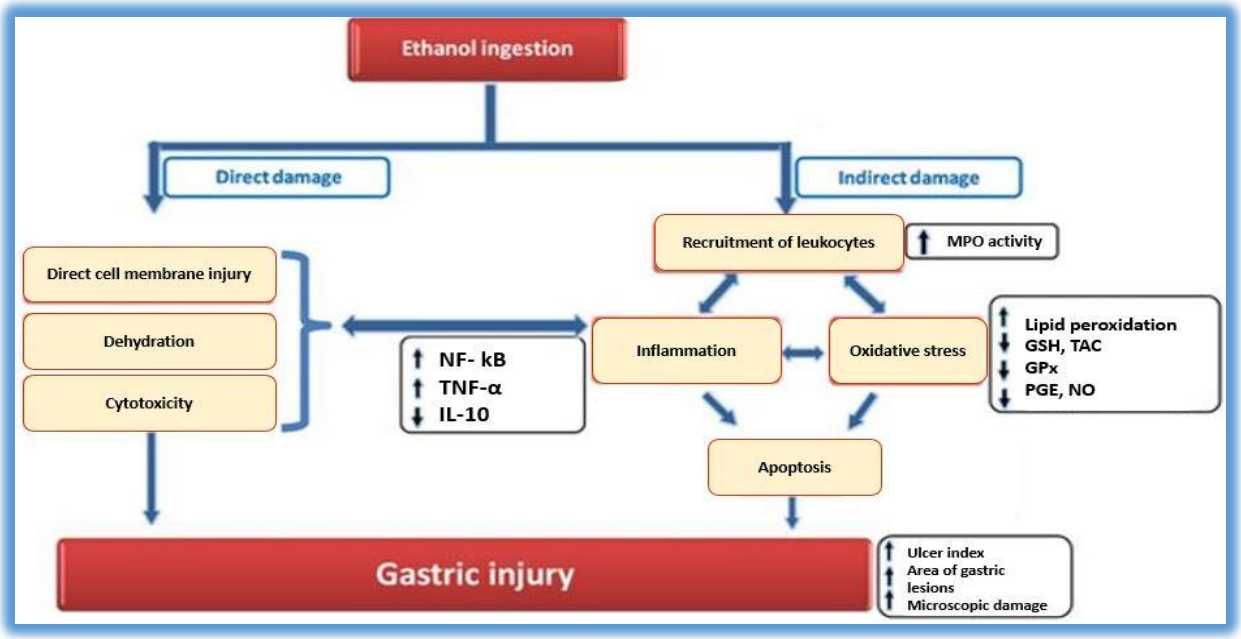

Figure 10: Primary mechanisms of ethanol-induced stomach ulceration (Arab et al., 2015).

3.2. Herbal therapy

Medicinal plants are increasingly acknowledged for their therapeutic significance, serving as a natural repository of secondary metabolites that enhance human health. Numerous studies have enhanced understanding of the efficacy and safety of medicinal products for ulcerated stomach and elucidated their processes for action in both human and experimental settings (Saurabh *et al*., 2012). Herbal drugs exert preventive effects against stomach ulcers by stimulating mucous cell proliferation, diminishing oxidative stress, and suppressing gastric acid output and K^+-ATPase activity (Gupta *et al.,* 2021). Botanical compounds, which are naturally occurring plant-derived molecules, are emerging as potential therapeutics for the treatment of stress-induced stomach ulcers. This extensive review analyses many phytochemicals-including alkaloids, glycosides, flavonoids, tannins, terpenoids, polysaccharides, and saponins-emphrazing their possible medicinal uses (Shahzad *et al.,* 2024). Some of these secondary metabolites included in gastric ulcer are in **Figure 11**.

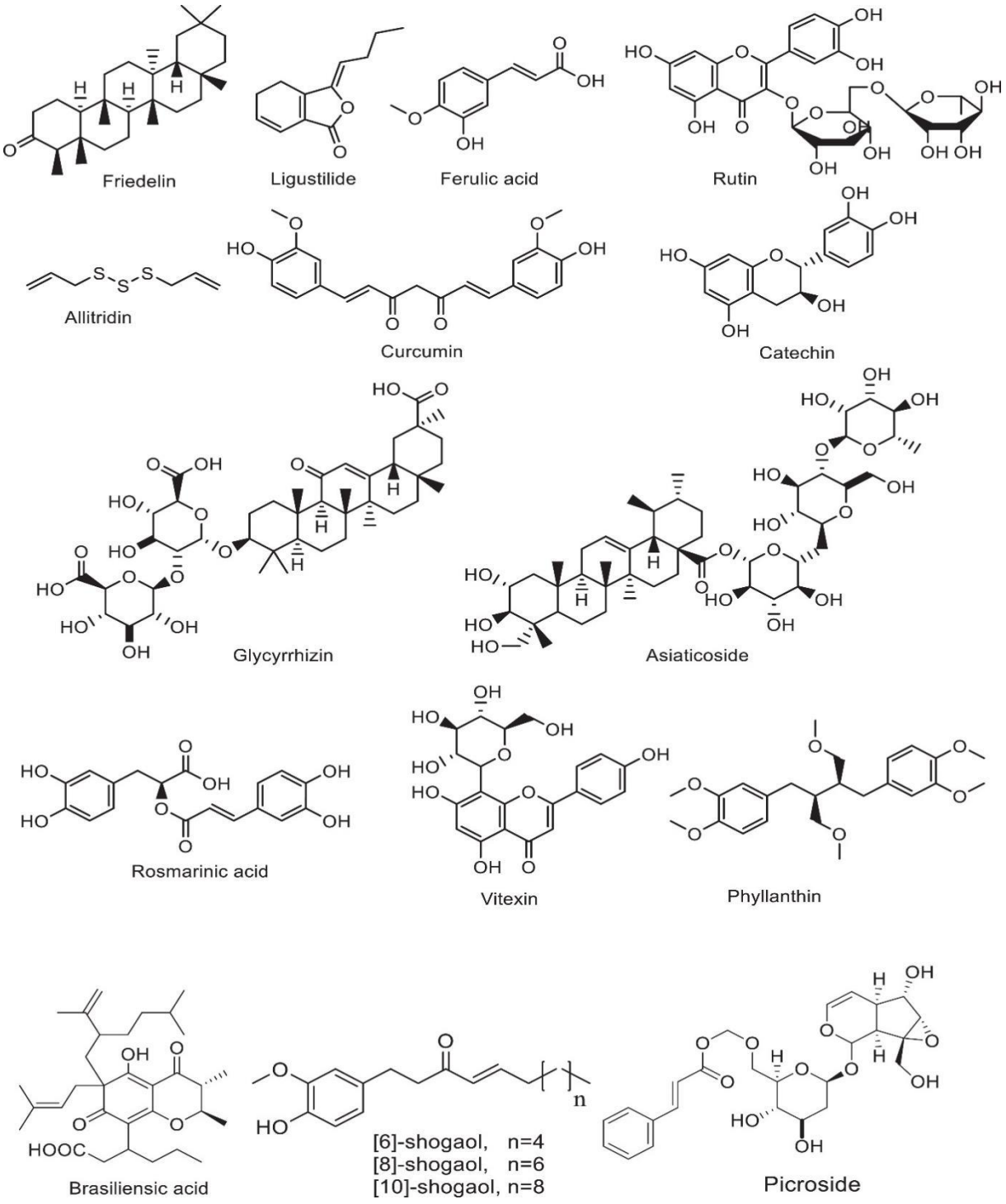

Figure 11: Bioactive phytoconstituent and their chemical structures associated with anti-ulcer properties (Gupta *et al*., 2021)

4. *Sanguisorba minor* L.

A vast pool of chemicals exists in nature that may be beneficial for drug discovery. The genus *Sanguisorba* comprises perennial plants within the Rosaceae family, encompassing around 142 species and subspecies found across East Asia and Southen Europe (Zhou *et al*., 2021; Wang *et al*., 2020). This genus is identifiable by its elongated, impariinnate leaves and short, tetramerous or trimerous blooms that are devoid of petals (Tocai *et al*., 2023).

Sanguisorba minor L. is a perennial plant highly resilient to drought conditiond (Fry *et al.*, 2018). The angular, erect stems, which may spread or lie flat, can attain lengths of 80 cm to 1 m and have leaves, segmented into 9 to 25 leaflets, characterized by a rounded and crenate morphology. The number of leaflets diminishes progressively as one ascends the stem, with the leaflest becoming increasingly pointed and serrated. The flowers, characterized by a pale green hue interspersed with purple, are clustered in dense, globular spikes and bloom from April to July, occasionally extending into August. They exhibit male, female, or hermaphroditic characteristics, possessing dispersed oval dentition. There are 15 to 30 stamens and 2 or 3 carpels, each crowned with a style equivalent in length to the calyx lobes, culminating in a light purple, brush-like stigma. The indehiscent fruit comprises two or three seeds (Andari *et al*., 2012)



Figure 12: The aboveground portion of the plant *Sanguisorba minor* L. (Toccai *et al.,* 2022)

Literature review

4.1. Taxonomy

Sanguisorba minor L. is categorized as follows:

* **Kingdom**: Plantae.

* **Phylum**: Tracheobionta.

***Class:** Magnoliopsida.

***Order:** Rosidae**.**

***Family**: Rosaceae.

***Genus:** *Sanguisorba.*

***Species**: *Sanguisorba minor* L

4.2. Traditional medicine and biological activities

Sanguisorba minor L. was utilized in traditional medicine for treatment of various diseases including conjunctivitis, fever, and diarrhea, administered as a tincture or infusion (Viano *et al*., 1999; Paniagua-Zambrana *et al*., 2020; Ćirović *et al*., 2022). In old traditional Chinese the root of the plant have been utilized to mitigate internal hemorrhaging and gingival hemorrhage (Zhao *et al.,* 2017; Zhou *et al.,* 2021). Leaves were utilized for flavoring wine due to the belief that they could offer protection against contagious diseases (Sabbatini *et al*., 2019).

S. minor L. has also been utilized in European folk medicine for the treatment of external and internal hemorrhaging, as well as for addressing open wounds. It is also utilized in women's health for the regulation of heavy or irregular menstruation (Paniagua-Zambrana *et al.,* 2020).

In Romania, *S minor* L. is said to enhance hunger, which is why it may be incorporated into salads, cooked foods, spinach (Fierascu *et al.,* 2017). Biological studies on *S minor* L. extracts have demonstrated various pharmacological effects, including : antioxidant, anti-Alzheimer , antiviral, antimicrobial (Romojaro *et al*., 2013; Ennerfelt *et al*., 2020; Viano *et al.,* 1999; Ginovyan *et al*., 2017), neuroprotective, anti-inflammatory, and antibacterial properties (Hosseini *et al*., 2022; Arihan *et al*., 2015; Ceccanti *et al*., 2021). Anti-ulcerogenic and antifungal properties were also exhibited (Zhao *et al.,* 2017; Gatto *et al*., 2013).

4.3. Phytochemical composition

Sanguisorba minor L. contains a diverse array of chemicals, including flavonoids, tannins, triterpenes, phenols, terpenes, and fatty acids. **Table 1** summarizes the outcomes from the several research.

Table 1: The cumulative phenolic concentration in the aerial components of *S. minor* L. as reported in literature

Nº	**Compounds**	**References**
	Tannin	
1	Sanguiin H-10 derivative	(Karkanis *et al*., 2019; Tocai *et al.,* 2021)
2	Galloyl-bis-hexahydroxydiphenyl-glucoside	(Ceccanti *et al*., 2021)
3	Lambertianin C	(Karkanis *et al*., 2019; Finimundy *et al.,* 2021
4	Ellagic acid hexoside	(Ceccanti *et al*., 2021)
	Flavonoids	
5	Catechin	(Karkanis *et al*., 2019
6	Epicatechin	(Karkanis *et al*., 2019
7	Quercetin-glucuronide	(Ceccanti *et al.,* 2021; Karkanis *et al*., 2019
8	Quercetin-galloylhexoside	(Karkanis *et al*., 2019)
9	Quercetin-O-hexoside gallate(isomer1)	(Ceccanti *et al*., 2021)
10	Quercetin-O-hexoside gallate(isomer2)	(Ceccanti *et al*., 2021)
11	Quercetin –O-pentoside	(Karkanis *et al*., 2019)
12	Kaempferol 3-O-glucoside	(Ceccanti *et al*., 2021)
13	Kaempferol 3-O-hexoside	(Ceccanti *et al*., 2021)
14	Apigenin-O-deoxyhexoside	(Karkanis *et al*., 2019)
	Phenolics acids	

15	Neochlorogenic acid	(Ceccanti *et al*., 2021)
16	Gallic acid glucoside	(Ceccanti *et al*., 2021)
17	Caffeoyl ester	(Ceccanti *et al*., 2021)
18	Digalloyl glucoside	(Ceccanti *et al.,* 2021)
19	Ellagic acid	(Zhou *et al.,* 2021)
20	Caffeic acid	(Zhou *et al.,* 2021)

Material and methods

Material and methods

1. Materials

1.1. Collection and identification of the plant

Professor Laouer Hocine (Laboratory of valorization of nature biological resources, Department of Biology and Vegetal Ecology, University of Setif-1 Algeria identified the plant *Sanguisorba minor* L. which was gathered in March from the eastern region of Setif-1. A voucher sample was submitted to the laboratory with the code of SNV-0035-2024. The collected samples were air-dried for a period of one week.



Figure 13: *Sanguisorba minor* L. aerial part collected from Guenzet (Setif 2023)

1.2. Animals

In the course of this study, wistar rats, specifically adult females weighing 150 to 200 g and albino mice weighing 25 to 32 g, were employed. The animals were acquired from the Pasteur Institute in Algiers. The rats and mice were kept in cages under conventional circumstances of a 12:12 hour light/dark cycle and temperature of 28 ± 1 °C for eight days before the initiation of the tests. The animals were granted free access to water and a regular feed (al libitum), while being maintained in compliance with the stipulations set forth in Animals By-Laws N° 425-2008. The experiment assays obtained approval.

2. Methods

2.1. Extraction procedure

A decoction was prepared by extracting 200g of plant with 2 L of hot distilled water, adhering to the methodology defined by Mrabti *et al*., (2018). For 25 minutes at 40 °C the mixture that emerged was subjected to magnetic agitation. Following filtration with Wathman paper, the resulting filtrate was concentrated in an oven at 45 °C for 72 hours. The ethanolic extract of *S. minor* was produced by maceration with minor modification (Karbab *et al*., 2021), the aerial part of the plant (50 g) is macerated in ethanol (7 days, 4 times), then filtered on filter paper

Wathman number 1, the first filtrate is stored in a refrigerator, at the end evaporated with a rotavap at a temperature of 42°C, subsequently it was dried at 42°C to provide the ethanolic extract (SMCE) which was stored at 4°C.

2.2. Quantitative phytochemical estimation

2.2.1. Total phenolics content (TPC)

The total concentration of phenolic compounds of the extract was assessed utilizing the Follin Ciocalteu technique (Li *et al*., 2007). A solution of 500 µL of diluted Folin Ciocalteu (1:10) was combined with 100 µL. About 400 µL of a 7.5 g in 100 mL of sodium carbonate solution was introduced after 4 minutes. The resulting combination was then incubated in the absence of the light for 120 minutes. The wavelength of absorbance was recorded at 765 nm. The quantity in each extract was quantified and expressed as µg of gallic acid equivalent (**Figure 14**)

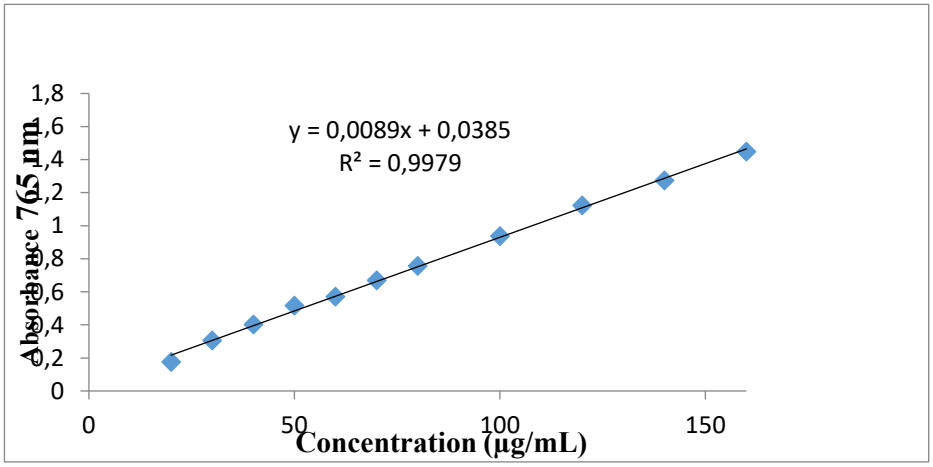

Figure 14: The standard gallic acid curve. The value are the triple mean± SD

2.2.2. Total flavonoids content (TFC)

The determination of the total quantity of flavonoid) was quantified following the methodology established by Ghedjati *et al.* (2025). An equivalent volume of aluminum chloride (2%) was combined with the extract; the blend was incubated at room temperature for 10 minutes. After incubation, the absorbance was quantified at 430nm. Utilizing the quercetin curve calibration, the concentration of the flavonoids was determined as equivalent to micrograms of quercetin per mg of dry extract (**Figure 15**).

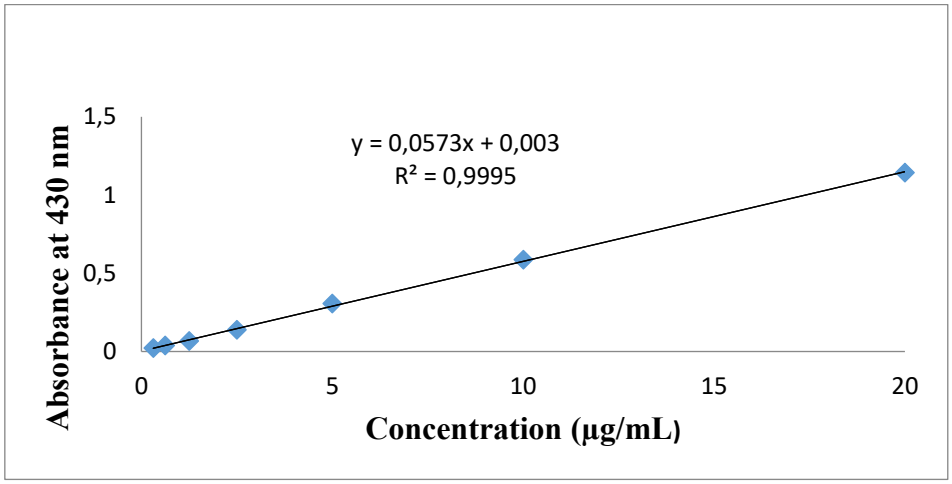

Figure 15: The standard quercetin curve. The value are the triple mean± SD

2.3. Phytochemical analysis

2.3.1. HPLC-DAD analysis

The HPLC-DAD analysis employed an Agilent 1260 series. The separation employed a Zorbax Eclipse Plus C6 column (4.6 mmx250 mmi.d., 5µm). The solution in the mobile phase consisted of 0.05% trifluoriacetic acid at a flow 0.6 mL/min and distilled water. The phase of movement was systematically programmed in a linear as outlined below: 0 min (82% A); 0-1 min (82%A); 1-11 min (75% A); 11-18 min (60%A);18-22 min(82%A); 22-24 min (82%A). The multi-wavelength sensor was measured at 280 nm. The injection volume for each sample solution was 5 microliters. The column temperature was sustained at 40°C.

2.4. Pharmacological effects

2.4.1. *In vitro* antioxidants properties

2.4.1.1. DPPH radical-scavenging assay

The DPPH test is a straightforward and effective technique that uses antioxidant molecules to decrease a stable free radical by accepting an electron or hydrogen atom. When DPPH is reduced, it turns yellow but in its radical state, it is a deep purple color. This change is correlated with a decrease in the free radical's concentration. The greater the hue change, the more DPPH is diminished, which is consistent with the extract's increased antioxidant ability. With a notable absorption band at 517 nm, DPPH is a straightforward spectrophotometric technique for determining the absorbance of species under investigation of Guendet *et al*., (1997) technique was used to assess the test. Experimental protocol 50 µL of extract and 1250 µL of DPPH solution were combined, and then the combination was allowed to stand at ambient

temperature for 30 minutes. The absorbance of the samples was subsequently measured at 517nm. The subsequent formula is employed to measure the radical scavenging capacity: %I = $[(A_{control}-A_{sample})/A_{control}]x100$

2.4.1.2. ABTS scavenging assay

The ABTS (2,2'-azino-bis (3-ethylebenzothiazoline-6-sulfonic acid) scavenging capacity was measured following the procedure outlined by (Re *et al*., 1999). About 7Mm ABTS in H_2O was reacted with 2.45 mM potassium persulfate ($K_2S_2O_{8)}$ to produce the ABTS stock solution, which was then left to stand in the dark for the 16 hours. 50µL of the SMAE or SMCE at various concentrations was combined with 1mL of this solution. At 734 nm, the absorbance was measured, with BHT as the standard.

2.4.1.3. Ferric reducing power assay

The lowering capacity of the extracts is evaluated according to the method described by Bouaziz *et al*. (2015). The development of this technique aimed to assess the ability of extract' reduction the potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}) which turns blue or green. Then, 100 µL of the SMAE or SMCE at different concentrations were combined with 100 µL of phosphate buffer (0.2 M, pH 6.6) and 100 µL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was then kept for 20 minutes at 50°C in water bath, and the reaction was terminated by adding 250 µL of 10% trichloroacetic acid. The blend underwent centrifugation a duration of 10 minutes at a speed of 3000 rpm. Lastly a volume of 500 µL of freshly made 0.1% $FeCl_3$ in water and 250 µL of resultant supernatant were combined with same volume of distilled water. At 700 nm the absorbance was quantified. EC_{50} which stands for the effective concentration at which the absorbance reaches 0.5, was used to communicate the results.

2.4.1.4. β-carotene bleaching assay

The β-carotene bleaching assay is commonly employed to evaluate the antioxidant efficacy of extracts in the field of food chemistry. The assessment of β-carotene response in the analyzed systems was conducted using the methodology of (Aslan *et al*., 2006). βcarotene/linoleic acid emulsion in water saturated in oxygen with generated peroxyl radicals (LOO-) is the main component of the reaction medium. The spectrophotometric measurement at 490 nm is utilized to assess the antioxidant activity of the extract under examination. About 0.5 mg of β-carotene was dissolved in 1mL of the solvent as chloroform and followed by adding

25 µL of linoleic acid and 200 mg of tween 40. After the chloroform was evaporated and 100 mL of distilled water saturated with oxygen was induced to the solution to form an emulsion. A volume of 175 µL standard or extract with concentration (2 mg/mL) were mixed with a volume 1250 µL of emulsion. The emulsion system was left to incubate in darkness at room temperature, with regular time intervals (t) recorded at 0, 1, 2, 4, 6, and 24 hours. Methanol and distilled water served as negative standards. The measurement of absorbance is conducted at 490 nm. The assessment of β-carotene bleaching inhibition was conducted as follows: Antioxidant activity (%)= $(Absorbance_{sample}/Absorbance_{BHT})x100$

2.4.1.5. Nitric oxide scavenging assay

The technique of Marcocci *et al*. (1994) was used to evaluate the $NO\cdot$ produced by sodium nitroprusside (SNP), with modifications. A solution containing 400µL of sodium nitrite (5mM, pH:7.3) and same quantity of extract or ascorbic acid at different concentrations was incubated for 80 min under a visible polychromic light source to generate $NO\cdot$. Furthermore, a volume of 400 µL sulfanilamide (1% prepared in acid phosphoric 5%) was introduced, followed by incubation of the mixture in darkness for 10 minutes. A volume of 400 µL of naphthyl ethylenediamine dihydrochloride (0.1%), was introduced to start the last reaction of NO^2-coopling, after a 20-minutes incubation period, absorbance was obtained at 534 nm. The subsequent formula was utilized to ascertain the proportion of nitric oxide scavenging activity.

$$NO\ scavenging\ capacity(\%)= [(A_{control}-A_{sample})/A_{control}]x100$$

2.4.1.6. Estimation of iron-chelating assay

The extract of the plant was assessed for their capacity to chelate iron and prevent the formation of the Fe^{2+}-ferrozine complex using the method established by Decker and Welch. (1990). A volume 0.25 mL of ethylenediamine tetraacetic acid (EDTA) or the extract at several concentrations was combined with 0.05 mL of $FeCl_2$ (0.6 mM)) and 0.45 mL methanol. After five minutes, the mixture reaction was started by adding 0.08 mL of ferrozine (5 mM) and left to develop at room temperature for ten minutes. The absorbance was mesuread at 562 nm. The calculation of chelating capacity was quantified as the inhibition percentage utilizing the following equation:

$$Ferrous\ chelating\ capacity\ (\%)=100x[(A_{control}-A_{sample})/A_{control}].$$

2.4.1.7. Phosphomolybdate assay

The overall antioxidant capacity of the extracts was assessed using the phosphomolybdenum technique outlined by Prieto *et al*. (1999) with modification. A volume 0.1 mL of various concentration of the extract or ascorbic acid (0.05 at 0.9 mg/mL) has been added to 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). The mixtures were 90 min in a water bath at 95°C. Upon cooled to laboratory temperature, the absorbance of the extract was measured at 765 nm.

2.4.1.8. Hydroxyl radical scavenging assay

The scavenging activity of the radical hydroxyl (OH·) was assessed utilizing the methodology outlined by Smirnoff et *al*. (1989) with a few changes, a volume 0.1 mL of different strenghts of standard or extracts was incorporated into the reaction mixture (0.5 mL $FeSO_4$, 0.35 mL H_2O_2, 0.15 mL salicylic acid). Following incubation of the mixture in oven at 37°C for 60 min, the absorbance was readied at 562 nm and the percentage inhibition was quantified using the subsequent formula:

$$OH^{\cdot} \text{ scavenging capacity (\%)} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{control}}]x100$$

2.5. Assessment of *in vitro* anti-inflammatory efficacy

The *in vitro* anti-inflammatory activity was validated using the approach outlined by Hamoudi *et al*. (2021) with slight changes. The same ratio of different concentrations of extracts and Aspirin as standard was added to 0.2% of bovine serum albumin prepared in a mixture of tris-HCl (PH 6.8), and then incubated in an oven at 37° for 15 minutes. After this step, each tube were then placed in a water bath at 72°C for 5 minutes. Following exposure to ambient air, the different concentrations are determined by spectropotometric reading at 660 nm. The protective effect of bovine serum albumin was determined using the subsequent equation:

$$\text{Protective effects (\%)} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{control}}]x100$$

2.5.1. *In vivo* anti-inflammatory activity

2.5.1.1. Carrageenan-induced paw edema

The anti-inflammatory efficacy of the SMAE and SMCE was evaluated using the carrageenan-induced paw edema model as outlined by Laroui *et al.* (2024). This study involved

36 females' rats which were categorized into four equal groups as follows: The first group serves as a positive control and is administered the reference drug (indometacin 20 mg/Kg), whereas, the subsequent group function as a negative control and is given tap water. The third and fourth groups represent the test groups receiving SMAE and SMCE at dosages of 150 and 300 mg/kg respectively. The research was conducted on rats that had fasted for 16 hours. One hour following the oral administration of various drugs (indometacin 20 mg, water and both dosages of extracts), edema was generated in the subplantar tissue of the right paw by injecting 0.01 ml of carrageenan suspension (1% made in NaCl saline solution). The thickness of the leg was determined with a calliper every hour for four hours. The anti-inflammatory impact was quantified by the percentage of edema inhibition utilizing the subsequent formula:

Inhibition of edema (%)=100x[(Paw edema of control-Paw edema of test group)/Paw edema of control]

2.5.1.2. Croton oil-induced ear edema

In this test, ear edema was evaluated by croton oil as irritant, as delineated by Cheniti *et al.* (2022) with minor alterations. For the oral treatment 30 mice are divided into four groups, 6 mice in each group: group 1, the negative control, administrated distilled water; group 2, the positive control, receives 50 mg of indometacin; the 3rd and 4th groups receive the two doses, 150 mg and 300 mg of SAME and SMCE respectively. After 1 hour of gavage of the extracts , the negative and positive controls, 15 µL of prepared croton oil (80 µL in 50% acetone-water v/v) was put on to the inner surface of the right ear. 6 hours later, the thickness of the right ear was assessed with a caliper.

For the topical assay, the 24 mice were allocated into four groups, with each group including 6 mice. The initial group was treated topically with the positive control (indomethacin), the second group with a croton oil solution, the third and fourth group with SMAE and SMCE. The proportion of edema inhibition was determined using the following formula:

I% =100 × (Dc– De)/Dc, where Dc represents the variation in ear edema thickness in the negative control group, while De represents the variation in ear edema in the SMCE and SMAE or positive control.

2.5.1.3. Xylene-induced ear edema

In this study, we used technique outlined by Mayouf *et al.* (2019), the 24 mice were allocated into four groups, with 6 mice in each group. All four groups underwent topical administration. The initial group administrated the negative control (xylene), the second group was administrated the positive control (0.5 mg indometacin), whereas the third and fourth groups received the SAME and SMCE respectively. About 30 µL/ear of xylene were administrated to each ear to cause edema in all animals, followed by the application of an equivalent volume (30 µL) of an aqueous solution comprising 0.5mg of indomethacin and SMCE, SMAE to the same ear surface, while the control group received solely xylene.

For the oral therapy, the 36 mice were divided into 6 groups, with 6 mice in each group. The first group was administrated the negative control (distilled water), while the subsequent group was given positive control (indomethacin 50 mg) and the third and fourth groups received the two doses of SMAE and SMCE (150 and 300 mg) respectively. After 1 h of treatment with positive and negative controls and both doses of SMCE we applied 30 µL of xylene to induce edema in all mice. Ear thickness was quantified using a digital caliper prior to and following a two hours application period. The percentage of inhibition is calculated using the formula:

I%=100x(Dc-De)/Dc , where Dc represents the disparity in ear edema thickness between the negative control, whereas De denotes the disparity in ear edema within the SMCE, SMAE or positive control.

2.5.1.4. Analgesic activity

The analgesic activity of both extracts was assessed using the method outlined by Zerargui *et al.* (2022), known against acetic acid-induced pain. In this test, all groups received extracts and the standard orally: the positive control group received aspirin at a concentration of 100 mg/kg, the negative control group was administrated simply distilled water and the both therapy groups were given a single dose of 150 and 300 mg/kg of SMCE and SMAE respectively. After one hour's gavage of extract and standard, all mice were administreted acetic acid (0.6%, 10 mL/kg) via intraperitoneal injection. In a cage take each mouse separately and count the number of twitches from time 0 to 30 minutes at 5 minute intervals.

The proportion of pain inhibition was calculated using the susquent formula:

Percentage of inhibition % = $100 \times [(C_{nc} - C_{tr}) / C_{nc}]$

C_{nc} represents the mean number of twitches in the negative control group, while C_{tr} represents the mean number of twitches in groups administrated varying doses of SMCE, SMCE and aspirin.

2.6. Toxicological studies

We utilized the acute toxic class technique specified in OECD standards 423 (OECD, 2001) to assess the biosafety of the SAME. The three experimental groups were formed, with each group consisting of six animals. Before administering SMAE, all animals underwent an overnight fasting period while having free access to clean tap water. The treated and control groups were weighed before to receiving a single dose of either 2000 mg/kg or 5000 mg/kg via gavage, with the control group receiving distilled water. Following administration of the extract in both doses, all animals were observed for 15, 30 min then 1, 2, 3, 4, 24 and 48 hours and subsequently on a daily basis for the next 14 days for toxicological signs, death, and other indications, including morphological, physiological, and behavioral alterations

2.6.1. Weight of the relative organs

The internal organs of the treated mice were excise and meticulously rinsed with cold physiological saline after the mice were scarified on day 14. ROW =[absolute weight of the organ(g)/the total weight of rat on sacrifice day(g)] is the formula was used to weigh the different organs (liver, lungs, spleen, stomach, heart and kidneys) and determine their proportional weights.

2.6.2 Biochemical analysis

After sacrificing the mice, heparinized tubes were used to collect the blood, which was then centrifuged for 15 minutes at 3500 rpm. Some biochemical parameters are measured from this plasma such as blood glucose, renal balance, liver balance, cholesterol and alkaline phosphatase.

2.6.3. Histological examination

Both organs were preserved in 10% formalin after gaining weight, cleared in xylene imbedded in paraffin after being dehydrated in increasing concentrations of ethanol (70-100%). Hematoxylin-eosin was then utilized to stain 5 µm thin slices. Digital pictures of the treated (liver and kidney) and control groups were taken using a DFC 945 camera that was mounted on a microscope with a 10x magnification.

2.7. Gastric ulceration in rats induced by ethanol

2.7.1. Gastroprotective effects

The gastric protection of SMAE and SMCE was approved by the ulcer induction method with ethanol outlined by Maamache *et al.* (2024). The rats were segregated into 9 groups and placed in individual cages. Each group containing 8 rats, the negative control group received distilled water, while the positive control group received omeprazole at a dosage of 100 mg/Kg, the healthy group receives nothing and the last six groups received dosages of 100, 250 and 500 mg/Kg for SMAE and SMCE respectively. The animals were starved for 18 hours and permitted access solely to sugar water until one hour prior to extract gavage. After one hour's gavage of extract and standards 1 ml/200 g, 0.5 ml/200 g a pure ethanol is delivered orally. All animals are sacrificed by cervical dislocation thirty minutes post-ethanol delivery, the stomach was excised and rinsed with NaCl, the stomachs were photographed for better vision then spread on a cork plate to determine the surface area of the lesions using the software (Image j). The percentage of the ulceration was computed for all groups using this formula:

Percentage of ulceration (%)=100x(Total ulcerated area/total mucosal area)

2.7.1.2. Histological evaluation of gastric lesions

A 10% formalin preparation was utilized to preserve a tissues for histological examination. The identical procedures for histological parts of the kidneys and liver were performed for the stomach as previously described.

2.8. Statistical analysis

Statistical analysis of the data was conducted using Graph Pad Prism (version 6.00). The data were presented as mean ± SD (standard deviation), and the IC_{50} values were ascertained by linear regression analysis. The mean± standard error of the means (SEM) was utilized to present the in vivo data. Examine To evaluate mean differences, a one-way ANOVA was employed; values $P<0.05$.

Results and Discussion

Results and discussion

1. Extraction and phytochemicals analysis

1.1. Extraction yields

The extraction process was conducted in two primary phases: decoction with distilled water and maceration employing absolute ethanol. The yield (g/g DW) as the weight proportion (%) of the final extracted material (g) relative to the initial quantity of the dry powder (g), (Table 1).

Table 1: Extraction yield percentages and color derived from the aqueous and ethanolic extracts of *Sanguisorba minor* L.

Extraction yields (g/g DW)	***Yield* (%)**	***Color***
SMAE	8.67	Brown powder
SMCE	12 .6	Green powder

The extraction yield demonstrated that SMCE exhibited the largest proportion at 12.6%, in contrast to SMAE, which recorded 8.67%. The yield of extract is a crucial issue to consider in the industrialization of beneficial extracts (Kim *et al*., 2019). When we compare our results with those of Haouam *et al*. (2023), our yield is higher for the aqueous extract with a value of 8.67%, compared to 4.28%. However, when comparing the yield of our ethanolic extract, which is 12.6% lower than that of the same family *Sanguisorba officinalis* L. with a value of 26.50% (Byun *et al*., 2021). Polar solvent that includes ethanol, methanol and water are commonly utilised to extract polar compounds. Moreover, each extraction technique seeks to enhance the overall yield and selectivity of bioactive constituents, augment the sensitivity of the bioassay by elevating the concentrations of the targeted compounds, and convert the latter into a form more conductive to yield separation and detection (Pandey *et al*., 2014). Numerous research indicate that the extraction yield proportion is mostly controlled by the extraction operation, namely the temperature employed, the polarity of the extract components, the solvent ratio and the technique of extraction methods utilized (Markom *et al.*, 2007; Wijingaard *et al*., 2012).

Phenolic compounds including phenolic acids and flavonoids constitute the predominant category of phytochemicals in plants, acknowledged for their anti-inflammatory and antioxidant characteristics (Chen *et al*., 2019). The overall polyphenol and flavonoid contents were quantified utilizing colorimetric assays with Folin-Ciocalteu reagent and aluminium trichloride, respectively. These two methods are regarded as the most effective for quantifying polyphenols and flavonoids in plants extracts due to their standardization,

simplicity and reproducibility (Djeridane *et al*., 2010). The overall flavonoid and phenolic content of SMAE and SMCE is displayed in Table 2

Table2 : Contents of total phenolics and flavonoids contents in aqueous (SMAE) and ethanolic (SMCE) extracts from *S. minor* L.

Extracts	TPC (µg GAEq / mg extract)	TFC (µg QEq / mg extract)
SMAE	549.551 ± 0.01	15.466 ± 0.00
SMCE	623.33 ± 0.01	8.71 ± 0.04

The high TPC was found in *S. minor* L. ethanolic extract with value of (623.33 ± 0.01 µg GAEq / mg extract) in contrast to the aqueous extract,which recorded (549.551 ± 0.01 µg GAEq / mg extract), however the flavonoids content is almost double in the aqueous extract with a value of (15.466 ± 0.00 µg QEq/mg extract). The amount of these chemicals in extracts from plant is contingent upon the relative polarity of the solvents employed during the extraction process (Aires *et al*., 2017).

Total phenolic tenor of leaves of ethanolic extract from *Sanguisorba minor* scop(Tocai *et al.*, 2022) is much lower (160.96 µg GAEq / mg extract) than that of aerial part of our ethanolic extract from *S. minor* L. , followed by the flowers (131.56 µg GAEq / mg extract). In another study conducted by Karkanis *et al*. (2019) ,the TPC was (137 ± 0.1 µg GAEq / mg extract) and the TFC was (83.7± 0.2 µg QEq / mg extract). Following to the research as reported by Cirovic *et al* . (2022), the polyphenol content is (132± 3.87 µg GAEq / mg extract) in the methanolic extract, whereas in the chloroform extract is lower, with a value of 67.87± 0.77 µg GAEq / mg extract). Conversely, the flavonoid content is high in the chloroform extract, with a value of (889.00± 43.30 µg QEq / mg extract) and a content of (506.50± 5.00 µg QEq / mg extract) for the methanolic extract.

The variations in phytochemical quantities may be attributed to the differing geo-climatic circumstances of the collecting sites and the experimental parameters (the utilized solvent, plant component, and the collection stade), (Haouam *et al*., 2023). Furthermore, genetic variability within plant species or among individuals of the same species strongly influences the production and composition of secondary metabolites and their bioactive properties (Bibi *et al.,* 2022).

1.2. Phytochemicals analysis

A quantitative analysis of 18 phenolic components from the both extracts of *S. minor* L. was conducted using the HPLC-DAD. The analysis results and concentrations of bioactive chemicals are presented in (**Table 3).** The concentration of polyphenols and flavonoids is very high in the SMCE, with a value of 71495.34 µg/g compared to the SMAE 22844.82 µg/g. Following HPLC-DAD, the phenolic acids identified include methyl gallate as the predominant ingredient in SMCE followed by Gallic acid, Chlorogenic acid, Rutin, Ferullic acid, Syringic acid, caffeic acid, ellagic acid, naringinin, vanillin, rosmarinic acid, kaempferol, quercetin, daidzein.

Table 3 : HPLC analysis of the overall polyphenols of the decoction and ethanolic extracts of *S.minor*

Polyphenols	**Concentrations (µg/g)**	
	Aqueous	**Ethanolic**
Gallic acid	9325.25	16778.74
Chlorogenic acid	4731.67	14018.97
Coffeic acid	696.44	2130.44
Syringic acid	456.94	2790.34
Catechin	/	/
Methyl gallate	725.25	24574.54
Rutin	/	6055.03
Ellagic acid	1670.56	939.44
Coumaric acid	/	25.94
Ferullic acid	525.79	3709.97
Vanillin	107.26	122.98
Naringinin	4549.32	172.86
Rosmarinic acid	9.42	62.37
Daidzein	4.04	13.96
Quercetin	27.56	39.11
Cinnamic acid	9.40	2.77
Kaempferol	5.97	46.84
Hesperetin	/	11.04

Conversely, in the SMAE extract the main compound is gallic acid with a concentration lower than that of the SMCE (9325.25µg/g), followed by chlorogenic acid, high content of naringenin, ellagic acid, methyl gallate, caffeic acid, ferullic acid, syringic acid, vanillin, quercetin, Daidzein, cinnamic acid, kaempferol with absence of Hesperetin. Numerous studies have previously demonstrated that several of these phenolic compounds confer favorable effects on human health owing to their antioxidant, anticancer, and anti-inflammatory properties, and are regarded as potent enzyme inhibitors. These results indicate that ethanol

utilized in maceration effectively recovered a range of phenolic compounds with significant activity. A study of Tocai *et al*. (2022) revealed that *S. minor* contains twenty-three components, including phenolic acids, tannins, and flavonoids, detected using HPLC-DAD-MS. Another study conducted by Haouam *et al*. (2023) shows seven phenolic compounds in the aqueous extract, which is significantly lower than our results, where 17 compounds were detected. The variations in chemical compositions can be attributed to the differing geo-climatic circumstances of the collecting sites and the testing parameters. The phenolic compounds found in *S. minor*, along with other ingredients, likely provide different biological functions, including antioxidant and antibacterial effects, in a synergistic way (Cristina *et al*., 2021).

1.3. *In vitro* pharmacological activity

1.3.1. Antioxidant properties

The antioxidant efficacy of *S. minor* L. extracts was assessed by six radical scavenging assays: $DPPH^{\cdot}$, $ABTS^{\cdot}$, $OH^{\cdot}$, iron-chelating, Phospomolybdate and $NO^{\cdot}$. IC_{50} was computed and presented in Table 4.

Table 4 : DPPH., ABTS.,OH.,NO., iron-chelating and phosphomolybdate assays.

	***In vitro* antioxidant estimation**					
Extract/	DPPH	ABTS	$OH^{\cdot}$	$NO^{\cdot}$	Iron-chelating	Phosphomolybdate
standard	**IC_{50}(µg/mL)**					
SMCE	11.03± 0.001*	7± 0,003*	63.93± 0.001 ^{ns}	40.31± 0.002**	230.77±0.002***	46.83 ± 0.003***
SMAE	24± 0,002***	10± 0.002***	28± 0.008***	50± 0.001***	57.32± 0.008***	72.65± 0.002***
BHT	8.81± 0.002	3,54 ± 0,006	NE	NE	NE	NE
EDTA	NE	NE	NE	NE	8.45± 0.00	NE
Ascorbic acid	NE	NE	66± 0.005	6,37± 0.006	NE	21.20± 0.002

Value are mean of triplicates ± SD ns: $p>0.05$;*$p<0.05$;**$p<0.01$;***$p<0.001$. Comparison with standards (BHT, EDTA, Ascorbic acid). NE: not evaluated. IC_{50}= sample concentration at which 50% of the free radicals activity was inhibited. SCME : Ethanolic extract of *S. minor*.SMAE: aqueous extract of *S. minor*.

Utilizing multiple assays to assess the extract's composition is essential, as it operates through diverse mechanisms including the inhibition of chain initiation, chelation of transition metal ion catalysts, decomposition of peroxides, reductive capacity, radical scavenging, and cessation of hydrogen abstraction (Amorati *et al.*, 2013). Consequently, the antioxidant properties of the examined extract were assessed using various methodologies.

Results and discussion

The DPPH radical scavenging assay evaluates the capacity of antioxidant to neutralize the stable radical 2,2-diphenil-1-picrylhydrazyl (DPPH),which interacts with hydrogen or electron donors to produce the yellow-hued diphenyl picrylhydrazine (Lang *et al*., 2024). The *S. minor* L. extracts shown significant DPPH scavenging activity. This is probably due to the presence of polyphenols. Phenolic chemicals serve as important antioxidant in food, capable of neutralizing free radicals and other oxidative species by electron or hydrogen transfer, proton loss, and chelation mechanisms (Zeb., 2020). Among these bioactive are caffeic acid, vanillin, ferulic acid, p-coumaric acid, chlorogenic acid neochlorogenic acid (Andres *et al*., 2023). *S. minor* L. possesses substantial quantities of polyphenols, thereby exhibiting significant antioxidant, anti-tumor, and antibacterial activities (Ceccanti *et al*., 2019;Lenzi *et al* ., 2019; Finimundy *et al*., 2020).

A comparison of our results with those obtained with other extract from the same species shows that they are inferior to those obtained by Haouam *et al.* (2023) with 128.30 µg/ml for the aqueous extract and 39.51 µg/ml for the butanolic extract. Similarly, Cristina *et al. (*2021) show inhibition of the DPPH radical similar to our ethanolic extract with a percentage of 94.61%.

The antiradical ability of the examined extracts was validated using the ABTS assay, a method often employed to assess the antiradical activity of plant extract. This assay evaluates the ability of an antioxidant to reduce the cationic radical $ABTS^{\cdot+}$ through electron transfer. The extract exhibiting significant antiradical action is characterized by a high polyphenol content. The results correspond with those of Haouam *et al*. (2023), who identified a strong association between polyphenol concentration and the scavenging action of *S. minor* L. extracts. Moreover, Cirovic *et al*. (2022) demonstrated that the scavenging efficacy of *S. minor* L. extracts on the $ABTS^{\cdot+}$ radical significantly fluctuates based on the solvent and extraction method employed. Chemical study of *S. minor* L. extracts revealed elevated concentration of chlorogenic acid and caffeic acid. These are phenolic acids capable of sequestering the $ABTS^{\cdot+}$ radical (Zengin *et al*., 2020).

The hydroxyl radical, the most hazardous of free radicals, is ciefly accountable for the reactivity of reactive oxygen species (ROS) with biological molecules. Inhibiting this radical is essential for preventing disorders associated with oxidative stress (Gardès-Albert *et al*., 2003).

The results indicate that both *S.minor* L. extracts requester the hydroxyl radical in a concentration-dependent fashion. The SMAE extract has an IC_{50} of 28±0.00 µg/mL, indicating

superior scavenging efficacy relative to the SMCE extract (IC_{50}=63.93±0.008 µg/mL), and ascorbic acid (IC_{50}=66 ± 0.002 µg/mL), which serves as a reference antioxidant.

The anti-radical characteristics of the two examined extracts are mostly attributable to the presence of polyphenols; chemicals recognized their capacity to sequester radical species and ROS. It has been demonstrated that methyl gallate, the principal constituents in both extracts, together with caffeic acid, ferulic acid, and chlorogenic acid, phenolic acids abundant in our plant exhibit significant anti-radical activity against hydroxyl radicals (Arrua *et al*., 2010; Yun *et al*., 2012; Liang *et al.,* 2023).

1.3.1.1. β-Carotene bleaching activity

The β-carotene bleaching test involves the oxidation of linoleic acid, which produces free radicals through the removal of a hydrogen atom from the methylene groups of linoleic acid. Then the free radical will oxidize the highly unsaturated β-carotene. The presence of the antioxidant in the extract reduce the oxidation of β-carotene by neutralizing hydroperoxides.

The efficacy of *S. minor* L. extracts in decelerating β-carotene oxidation was assessed by quantifying the variation in absorbance over time. The bleaching kinetics of β-carotene in the presence of *S. minor* L. extracts and BHT indicate that both substances considerably impede the combined oxidation of linoleic acid/ β-carotene relative to the negative control **(Figure 16**). The variation in absorbance over various time intervals indicates that SMCE demonstrates inhibitory effects approximatery 86% superior to those of SAME extract, which exhibits 60% inhibition. The efficacy of the extracts may stem from their capacity to reduce lipid peroxidation and/or capture linoleic acid radicals (Krimat *et al*., 2017). Polyphenols possess the capacity to sequester free radicals, thereby postponing the auto-oxidation of lipids (Ayala *et al.,* 2014). This is aligns with the findings of (Pandey *et al.,* 2018), who indicated that phenolic compounds are capable of scavenging free radicals produced during the β-carotene bleaching assay. In a comparable research by Sevgi *et al.,* (2015), kaempferol, caffeic acid and ferulic acid were identified as effective reducing agents for neutralizing free radical-mediated β-carotene/linoleic acid oxidation. The structure and lipophilic characteristics of polyphenols have been shown to enhance antioxidant effects, likely by aiding the integration of these chemicals into the lipid phase of the membrane (Amessis-Ouchemoukh *et al.*, 2014).

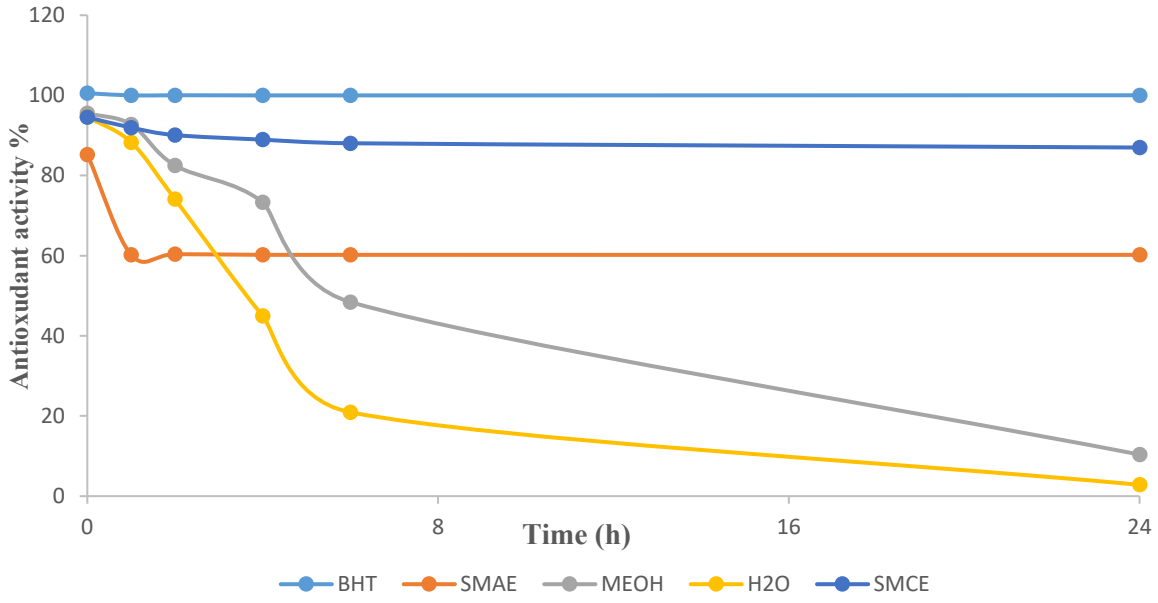

Figure 16: The β-carotene bleaching in the presence of aqueous extract (SAME), ethanolic extract (SMCE), water (H2O), methanol (MeOH) and BHT during 24h.

1.3.1.2. Metal chelating activity

Figure 17 presents the findings on the metal chelating impact of *S. minor* L. Both extracts exhibited a significant difference $p<0.001$) when compared to EDTA, utilized as a positive control. SMCE demonstrated significant iron-chelating action compared to the SMAE. The exceptional ferrous chelating ability of SMCE is likely attributed to the presence of particular phenolic chemicals. Literature indicates that many studies demonstrate that polyphenols and flavonoids can serve as effective chelators for iron and copper due to their specific structure and functional groups (Halliwell, 2007). Moreover, it was found that compounds featuring two or more of the following functional groups: -OH, -SH, -COOH, -PO3H2, -C=O, -NR2, -S-, and –O- have significant metal chelating activity (Biçek *et al.,* 2023). Scarano *et al*. (2023) demonstrated that caffeic acid had significant efficacy in sequestering ferrous ions and suppressing the Fenton process, in conjunction with chlorogenic acid.

A comparison of our findings with those derived from other extracts of the same species , indicates similarity to the results reported by Haouam *et al*. (2023), which recorded 102±0.98 µg/ml for the aqueous extract and an IC_{50} of 19.48±0.88 µg/ml for the ethyl acetate extract , thereby affirming the correlation between polyphenol concentration and the antioxidant activity of the extracts.

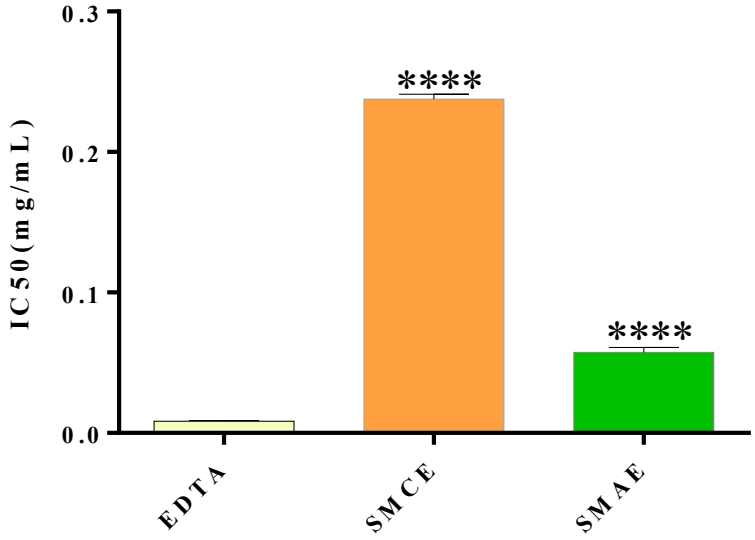

Figure 17: Iron chelating assay of ethanolic extract (SMCE) and aqueous extract (SAME) from *S.minor* L.Value are mean of three parallel measurements ± SD (n=3). ***p<0.001.

1.3.1.3. Ferric reducing capacity

The antioxidant capacity of *S. minor* aerial part was evaluated using aqueous and ethanolic extracts, which exhibited peroxyl and hydroxyl radical scavenging abilities, along with ferric-reducing antioxidant power (Romojaro *et al*., 2013; Vanzani *et al*., 2011).

This rapid, reproducible and straightforward approach assesses antioxidant activity by quantifying the reduction of ferric ion (Fe^{3+}) in the $K_3Fe(CN)6$ complex to ferrous ion (Fe^{2+}).

Figure 3 demonstrates that both extracts exhibit significant concentration-dependent reduction power. In this test, BHT served as a reference. SMAE displayed stronger lowering capability with 55.63± 0.002 µg/ml than SMCE with 65.46±0.008 µg/ml. Our findings align with those reported for the same species by Byun *et al*. (2021). This potent action is attributable to the concentration of flavonoids in the SAME. A significant connection between antioxidant activity and total flavonoid content of *S.minor* L. aerial parts extracts has been discovered (Gawron-Gzella *et al.*, 2016) . The diminished reducing power is likely attributable to the presence of hydroxyl groups in phenolic compounds, which function as electron donors. Antioxidants are regarded as oxidant and inactivators (Bammou *et al*., 2022). The reduction of phenolic acids and flavonoids activity, found in *S. minor* L. extracts, is mostly contingent upon

the quantity, placement and substitution of hydroxyl groups within the molecule (Karkanis *et al*., 2019; Finimundy *et al.,* 2020).

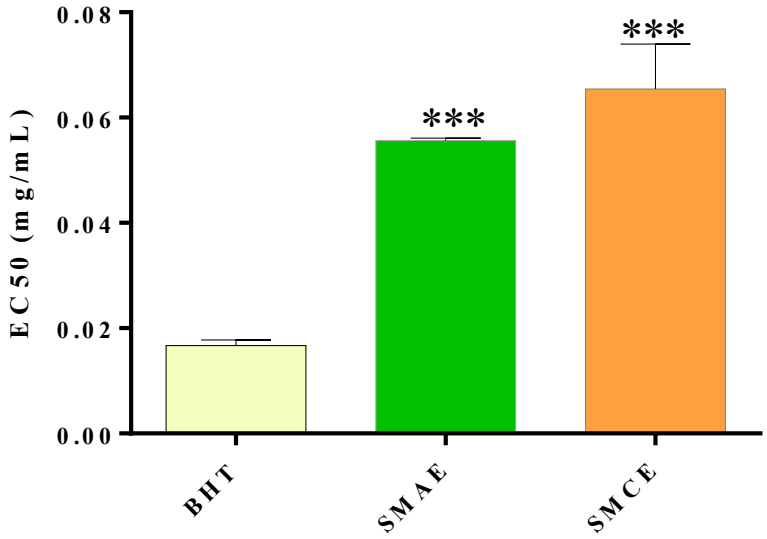

Figure 18: Reducing power assay of aqueous extract (SAME) and ethanolic extract (SMCE) from *S. minor* L. Outcomes are reported as mean± SD (n=3). ***p<0.001.

1.3.1.4. Phosphomolybdate activity

The total antioxidants capacity was assessed via the phosphomolybdate method, which relies on the reduction of molybdate^{+6} to molybdenum^{+5} in the presence of antioxidants, resulting in the production of a green phosphate/Mo^{+5} complex measurable at 695 nm (Jain *et al*., 2024). The results of this test (**Table 4**) unequivocally demonstrate that SMCE extract possesses superior antioxidant potential compared to SMAE extract. A comparison of our results with those obtained by Byun *et al*. (2021), using various extracts from the same species , indicates that their findings are much superior to ours, with IC_{50}= 160.34 ± 2.00 µg/ml for the aqueous extract and IC_{50}=280.04 ± 0.39 µg/ml for the ethanolic extract. The robust antioxidant capacity is ascribed to phenolic chemicals, especially flavonoids, recognized for their capability to sequester free radicals due to their hydroxyl group (Sharififar *et al.,* 2009). Furthermore, antioxidant capacity is influenced not only by the quantity of phenolic compounds but also by the chemical composition, the total number of hydroxyl groups, and their positioning on the aromatic ring (Shi *et al.,* 2022).

1.3.1.5. Nitric oxide assay

Nitric oxide radical can readily permeate cell membranes and interact with other reactive oxygen species. Specifically, it rapidly interacts with oxygen to generate peroxynitrite (ONOO), a potent oxidizing agent (Lee *et al.,* 2006). Mass-generated nitric oxide (NO) may additionally augment peroxynitrite synthesis, resulting in cellular and tissue damage, which contributes to aging and age-related illnesses (Jo *et al.,* 2008). The NO trapping activity of *S. minor* L. extrats is contingent upon concentration. SMCE and SMAE had distinct trapping capacities, with IC_{50} value of 40.31±0.002 µg/ml and 50.00±0.001 µg/ml respectively. Both extracts exhibit a diminished potential for scavenging nitric oxide radicals compared to ascorbic acid, which serves as a reference antioxidant (IC_{50}=6.37±0.008 µg/ml). The antioxidant ability, through the sequestration of nitric oxide radicals, seems to be associated with the presence of flavonoids in these extracts. Flavonoids are significant phytochemicals capable of sequestering radical species (Cheurfa *et al*., 2016).

The substantial positive correlations between the total phenolic content and antioxidant activity of *S. minor* L. extracts observed in this investigation (Table 2) substantiate the idea that phenolic chemicals greatly enhance the overall antioxidant capacity of plant species (Saeed *et al*., 2012; Finimundy *et al.,* 2020).

A significant connection between antioxidant activity and total flavonoids concentration in extracts of *S. minor* aerial parts has been discovered (Gawron-Gzella *et al.*, 2016). We noticed an inverse connection between the antioxidant activity and the total flavonoids content in our extracts. The SMAE extract exhibited elevated total flavonoid content but diminished antioxidant activity. Consequently, we posit that flavonoids in our examined *S. minor* L. extracts do not significantly contribute to the antioxidant activity.

3. *In vitro* anti-inflammatory activity

Protein denaturation is a process whereby a protein loses its tertiary and/or secondary structure due to exposure to heat, an infectious agent, or a chemical, resulting in the disruption of the electrostatic, hydrogen, hydrophobic, and disulfide bonds that preserve the protein's three-dimensional configuration (Kpemissi *et al*., 2023). The majority of proteins forfeit their biological activity at denaturation (Marliyah *et al*., 2015; Gueboudji *et al.,* 2022). Protein denaturation is a recognized contributor to inflammation and may result in the formation of autoantigens in specific autoimmune disorders, including arthritis (Aidoo *et al*., 2021; Cherbal *et al*., 2023). Several anti-inflammatory medications may decrease thermaly induced protein denaturation (Aidoo *et al.,* 2021).

The protective effect of *S. minor* L. extracts against the heat denaturation of bovine serum albumin was examined, and the results are depicted in (**Figure 19).** SMAE exhibited the highest inhibitory capacity at two tested concentrations (0.5 and 0.05 mg/mL), achieving 84.98% at 0.5 mg/mL, but SMCE displayed a lower efficacy of 81.11% at the same concentration. The anti-inflammatory impact was assessed in comparison to aspirin as the standard revealing a substantial difference ($p< 0.001$). The two excerpts demonstrated a dose-dependent efficacy in diminishing protein denaturation, attributable to the presence of phytochemicals such as phenols and flavonoids, which many contribute to the anti-arthritic action of the medicinal plant (Saleem *et al.,* 2019). According to the study conducted by Vachali *et al*. (2016) indicates that human serum albumin exhibits a considerable affinity for quercetin and luteolin, potentially elucidating the protective role of polyphenols against the heat denaturation of albumin. Several investigations have demonstrated the anti-inflammatory capabilities of *S. minor* L. plant, *in vitro* through various methodologies. For instance, Yu *et al. (*2011) showed that the ethanol extract of *S. officinalis* effectively inhibited the production of key inflammatory mediators , namely nitric oxide (NO) and prostaglandin E2(PGE2) , at the transcriptional level *in vitro* medel of RAW264.7cells activated with 1 µg/ml lipopolysaccharide (LPS). Another study conducted by Seo *et al. (*2016) demonstrated that ellagic acid, a key component of *S. officinalis*, markedly reduced the production of pro-inflammatory cytokines TNF- α and IL-6 in LPS stimulated RAW264.7 cells *in vitro.*

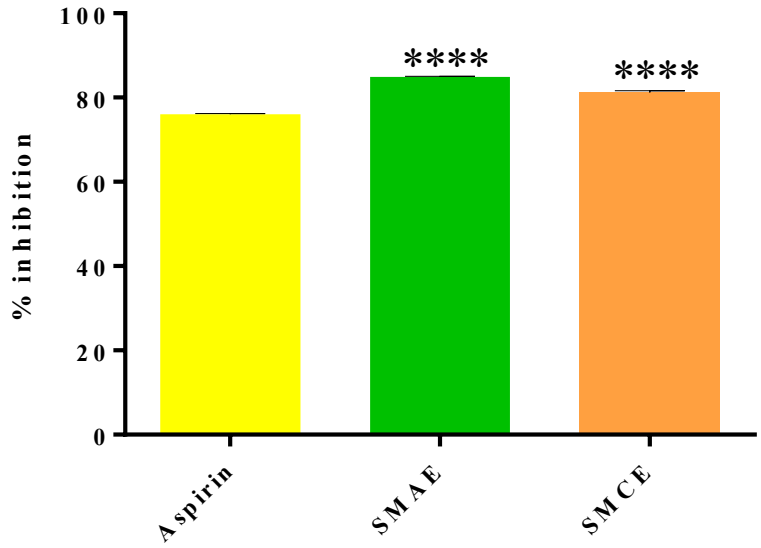

Figure 19: Inhibition percent of protein denaturation by SMCE and SMAE from *S. minor* L. at dose 0.5 mg/mL. Value as mean±SD. ***P<0.01.SMAE: aqueous extract, SMCE: ethanolic extract.

3.1. *In vivo* topical anti-inflammatory activity

3.1.1. Xylene induced ear edema

The topical ant-inflammatory effect was evaluated using xylene-induced ear edema in mice, with the results illustrated in **Figure 20**. The two extracts, SMCE and SMAE, exhibited significantly high inhibitory percentages of 91.28±0.96% and 93.93±0.95% respectively. The simultaneous anti-inflammatory effect of diclofenac, the positive control, was determined to be 89.67±0.99%. Xylene-induced ear edema in mice serves as a widely utilized acute inflammation model, characterized by its reproducibility and strong predictive validity for evaluating anti-inflammatory medicines (Zhao *et al*., 2018; Torres-Rêgo *et al.,* 2016). After the topical administration of xylene, indicators of acute inflammation comprise pronounced vasodilation, dermal edema ,and infiltration of inflammatory cells (Akhtar *et al*., 2022). In added this model is frequently employed to evaluate the effectiveness of $NSAID_S$, which achieve their anti-inflammatory effects through the inhibition of cyclooxygenase and the generation of prostaglandins (Sobeh *et al*., 2020).

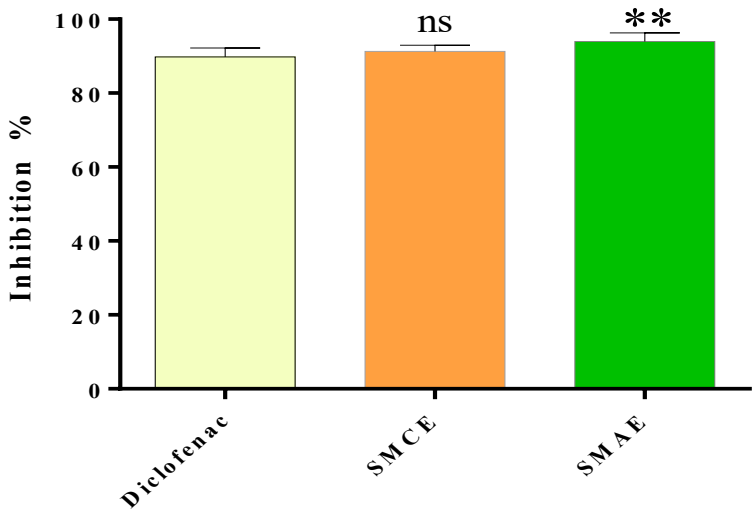

Figure 20: Inhibition percent of xylene-induced ear edematous of both extracts of *S.minor* L of 2mg/ear values are presented as the mean ± SEM(n=6).ns:no significant difference **P<0.01. SMAE:aqueous extract, SMCE: ethanolic extract.

3.1.2. Croton oil induced edematous

Croton oil-induced edema serves as a prevalent experimental model evaluating synthetic or natural anti-inflammatory agents that function at various phases of inflammation. The

phlogogenic activity of croton oil mostly results from its active component, 12-O-tetradecanoylphorbol-13-acetate (TPA). TPA elicits an inflammatory response marked by substancial synthesis of pro-inflammatory mediators , heightened vascular permeability and edema (Mao *et al.,* 2024).

Treatment of mice with 2mg/ear of SMCE and SMAE resulted in a statistically significant ($P<0.001$) decrease in edema, with inhibition rates of 87.32±1.88% and 83.09±1.09% respectively **(Figure 21).** The inhibition percentages exceed those achieved with indomethacin 61.02±1.39%, which serves as a benchmark anti-inflammatory medication.

The pronounced anti-inflammatory activity of the examined extracts is likely attributable to the presence of lipophilic compounds with anti-inflammatory characteristics capable of penetrating the epidermal barrier (Okoli *et al*., 2007). The anti-inflammatory function is associated with the chemical composition of these extracts, which are notably abundant in bioactive chemicals, primarily phenolic acids and flavonoids. Several phenolic acids found in this study, including coumaric acid, ferulic acid, caffeic acid , chlorogenic acid, and ellagic acid , exhibit substantial anti-inflammatory properties (Oliveira *et al.*, 2017 ; Fu *et al.,* 2020). Flavonoids identified in the plant, including quercetin and rutin , markedly inhibit the onset of ear edema induced by croton oil in mice (Choi *et al*., 2021; Sosa *et al*., 2007). The inflammatory response is initialed by the activation of protein kinase C (PKC) ,which facilitates an elevation in phospholipase A_2(PLA_2) (Rahman *et al.,* 2011). This enzyme facilitates the breakdown of membrane phospholipids into arachidonic acid. The enzymatic oxidation of the latter by cyclooxygenases (COX) or lipoxygenases (LOX) result in the synthesis of eicosanoids, prostaglandins, leukotrienes and cytokines (Oliveira *et al.,* 2013). Numerous polyphenols substances possess anti-inflammatory affects through the inhibition of inflammatory mediators. Additionally several investigation have demonstrated that gallic acid efficiently inhibited PGE-2 synthesis and LPS-induced nitric oxide (NO) and interleukin-6 (IL-6) release, without displaying cytotoxic effects (Kulesza *et al.,* 2023). Indeed Chibli *et al.* (2014) argue that quercetin, luteolin and rutin exhibit inhibitory effect against PLA-2, COX, and LOX. Flavonoids and tannins demonstrate anti-inflammatory properties by neutralizing free radicals, hence blocking pro-inflammatory enzymes (Gonfa *et al.,* 2023).

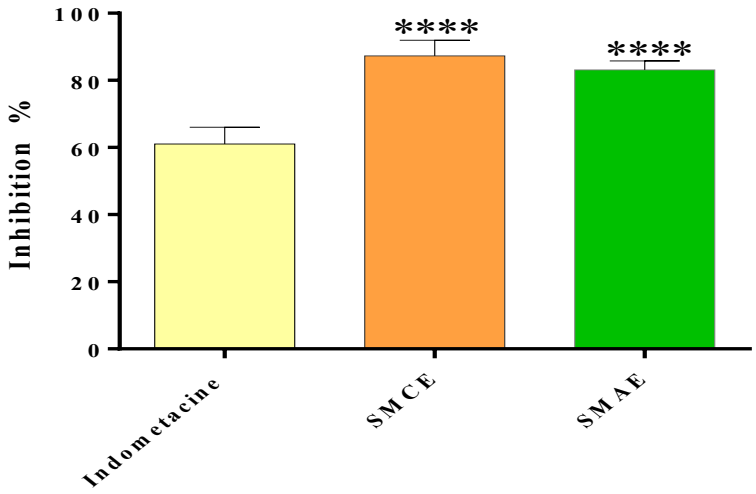

Figure 21: Inhibition percent of croton oil-induced ear edematous of extracts of *S. minor* L. of 2 mg/ear values are illustrated as the mean ± SEM(n=6). ***P<0.001.SMAE: aqueous extract, SMCE: ethanolic extract.

3.1.3. Oral anti-inflammatory activity

3.1.3.1. Xylene and croton-oil ear edema

The results of the oral anti-inflammatory assay utilizing xylene-induced ear edema in mice are presented in **Figure 22**. The SMCE and SMAE exhibited a significant anti-edematous effect at the two doses (150 and 300 mg/kg). We observed that inhibition escalates over time for both extracts, and the positive control as indomethacin. The SMAE extract exhibit robust inhibition at a dosage of 150mg/kg, with an inhibition percentage of 90.45 ± 1.33%, while the SMCE extract demonstrates significant inhibition at dosages of 300 mg/kg, with a value of 99.32± 0.43%.

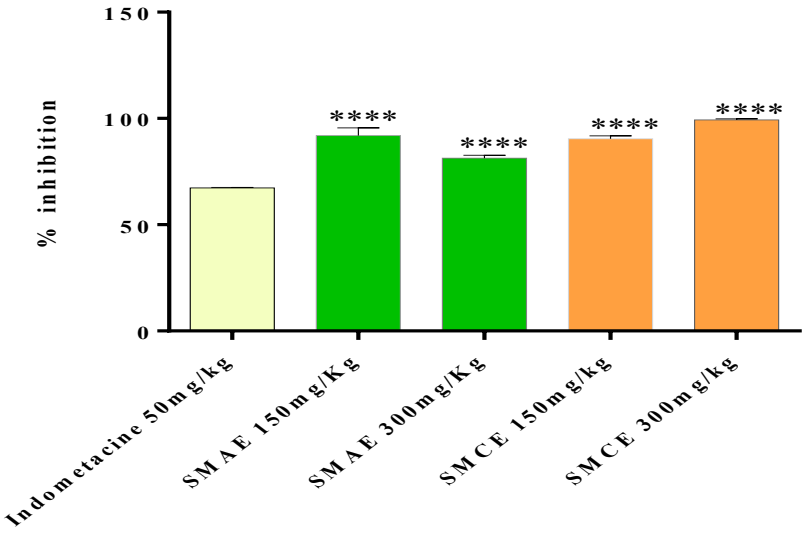

Figure 22: Inhibition percent of xylene-induced ear edema of *S. minor* L. at two doses. Values are demonstrated as mean ± SEM (n=6). ***: P<0.001. SMAE: aqueous extract, SMCE: ethanolic extract.

The efficacy of the oral anti-inflammatory agent was evaluated using the croton oil-induced ear edema paradigm in mice, with results illustrated in **Figure 23**. SMAE and SMCE diminished edema by 85.75% and 88% at a dosage of 150 mg/kg, respectively, conversely at a dosage of 300 mg/kg, edema was reduced by 82.85% and 88.57%. Both extracts demonstrate significant inhibition relative to indomethacin, with a value of 90%, which serves as a positive control. SMAE and SMCE possess anti-inflammatory activities, likely due to the presence of polyphenols and flavonoids. The phytochemical profile of *S. minor* L. revealed a substantial quantity of gallic acid. In addition, it may impede the onset of the inflammatory process by neutralizing superoxide anions, inhibiting the production and function of myeloperoxidase, and potentially affecting the buildup of active NASPH oxidase (Mucha *et al.,* 2021).

Kim *et al*. (2006) demonstrated that gallic acid and its derivatives impede the binding of Kappa-B nuclear factor (NF-$_k$B), which is essential for the generation of pro-inflammatory cytokines.

Additionally, flavonoids can influence the expression of pro-inflammatory cytokines by inhibiting NF-$_K$B transcription (González-Gallego *et al.,* 2007).

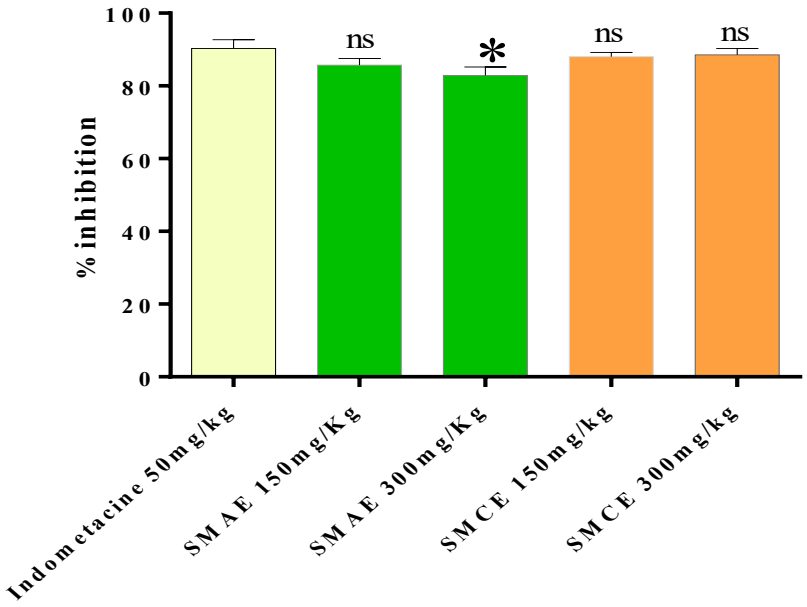

Figure 23: Inhibition percent of croton oil-induced ear edema of *S. minor* L. at 150 and 300 mg/kg doses. Values are demonstrated as mean ±SEM(n=6). ns: no significant difference *: P<0.1. SMAE: aqueous extract, SMCE: ethanolic extract.

3.1.4. Carrageenan induced-paw edema activity

Table 5 illustrates that the anti-edematous effect of both extract exhibited a dose-dependent and time dependent response at dosages of 150 and 300 mg/kg. The oral treatment of SMCE and SMAE at 150 mg/kg resulted in the most significant reduction in paw edema 4hours post-carrageenan injection, exhibiting maximum inhibitory activities of 87.23% and 76.64% respectively. The administration of SMCE and SMAE at a dosage of 300 mg/kg resulted in a reduction in paw edema inhibition after 2 hours post carrageenan injection, with values of 38.95% and 34.33% respectively. Arıhan *et al*. (2015) observed nearly identical results for the aqueous extract of *Sanguisorba minor* Scop at dosages of 25, 50 and 100 mg/kg with inhibition percentages of 41.9%, 76.4% and 83.4% respectively.

Table 5 : Effect of both extracts of *S. minor* L. on carrageenan-induced paw edema in rats

Groups	**Paw edema inhibition (%)**			
	1 h	**2 h**	**3 h**	**4 h**
PG	64.29 ± 1.32	32.56 ± 2.33	33.33 ± 0.98	65.48 ± 0.85
SMCE D1	24.52 ± 0.32***	47.09 ± 0.21**	65.7 ± 0.35***	87.23 ± 0.38***

SMCE D2	$23.96 \pm 0.20^{***}$	$38.95 \pm 0.26^{*}$	$56.13 \pm 0.23^{***}$	$57.65 \pm 0.42^{*}$
SMAE D1	$85.01 \pm 0.88^{***}$	$49.61 \pm 0.87^{***}$	$42.26 \pm 0.72^{**}$	$76.64 \pm 0.54^{**}$
SMAE D2	$43.48 \pm 0.26^{***}$	34.33 ± 0.31^{ns}	33.12 ± 0.21^{ns}	$70.77 \pm 0.38^{*}$

Results are reported as mean ± SEM (n=6), ns: no significant difference, *: $p<0.05$, **: $p<0.01$,***: $p<0.001$.PG: positive control , D1:dose 150 mg/kg, D2:dose 300 mg/kg. SMAE: aqueous extract, SMCE: ethanolic extract.

Carrageenan-induced paw edema serves as a prevalent initial assay for the examination of novel anti-inflammatory drugs and is considered to have biphasic characteristics (Ben Khedir *et al.,* 2016). This inflammation is characterized by the sequential release of several inflammatory mediators. The initial phase lasts up to one-hour post-carrageenan injection and is characterized by the release of histamine, serotonin, and bradykinin (Hmamou *et al*., 2023). The latter phase (3-6hours) encompasses prostaglandins (Zhou *et al*., 2008). A case study of same space demonstrated that the ethanolic extract of *Sanguisorba officinalis* inhibits prostaglandin E (PGE) production ,indicating significant anti-inflammatory efficacy through the inhibitory characteristics of Nuclear factor Kappa-B (NF-$_k$B) and Activator protein1(AP-1), (Yu *et al*., 2011). The anti-inflammatory efficacy of both extracts may derive from the synergistic action of several phenolic chemicals. Research has demonstrated a correlation between the flavonoids content of the *S. minor* L. and its anti-inflammatory properties (Kim *et al.,* 2008; Yu *et al* 2011; Chen *et al*., 2022).

4. Analgesic effect

The analgesic effects of SMCE and SMAE at two doses (150 and 300 mg/kg), along to the aspirin (100 mg/kg), on acetic acid-induced writhing in mice were assessed, with results illustrated in **Figure 24.** The reduction in writhing at 150 mg/kg of SMCE and SMAE was ($p>0.05$) considerably comparable to that of aspirin at 100 mg/kg, achieving 84.30%, 83.40% and 81.02% respectively. The analgesic impact is typically assessed using the acetic acid test, which is well-established for its capacity to detect both peripheral and central molecules. This action was emphasized by the abdominal pains assessment. The onset of pain is triggered by tissue injury that leads to the production of chemical mediators such as histamine and serotonin in the intraperitoneal fluid, which activate nociceptive receptors located in the peritoneum (Hamoudi *et al*., 2021). Contraction induced by acetic acid are also attributed to the production and release of algogenic mediators through cyclooxygenases (COX) and the manufacture of prostaglandins, especialy PG2 produced by COX1 (Ashagrie *et al*., 2023). These then activate pain nerve terminals, as abdominal contraction is linked to the sensitization of nociceptive

receptors to prostaglandins (Jang *et al*., 2020). The analgesic mechanism of action may include the inhibition of lipo- and cyclooxygenases ,along with the generation of inflammatory mediators and prostaglandins(PGE2 and PG2α), (Dou *et al*., 2004).

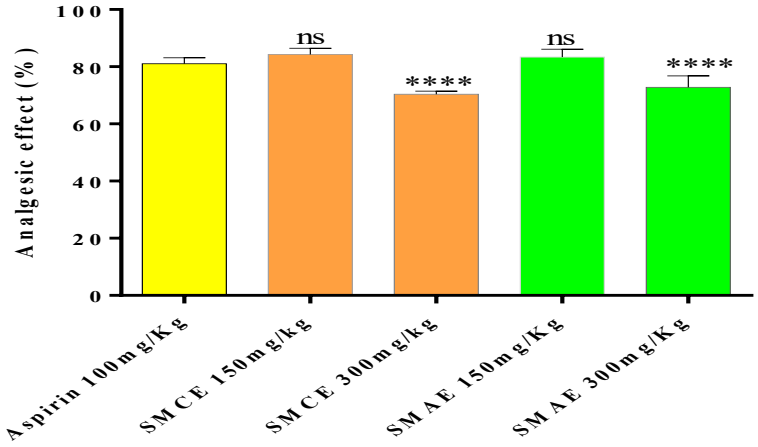

Figure 24: Effects of SMCE and SMAE extract of *S. minor* L. on mice (n=6) peripheral nociception. Mean± SEM is used to express the results, and p<0.001 when compared with aspirin as standard.

SMCE and SMAE effectively alleviated painful feelings in mice and had an anti-nociceptive effects comparable to that of aspirin, a recognized COX inhibitor (Voilley *et al*., 2001). The presence of phenolic chemicals in both extracts may account for the analgesic effects, particularly given the analogous tendency observed with aspirin at a dosage of 150 mg/kg. Polyphenols such as caffeic, gallic and ferulis acids shown anti-nociceptive effects in visceral inflammatory pain models by inhibiting COX, hence reducing prostaglandin synthesis and inflammatory cytokines (Khan *et al*., 2016; Kasik *et al*., 2019; Yang *et al.,* 2021). Furthermore, it was confirmed that rutin possesses both central and peripheral antinociceptive effects (Ghedjati *et al.,* 2025). Consequently, the findings indicate that the identified anti-nociceptive action is attributed to the natural components present in both extracts.

5. Acute toxicity

The examination of the acute toxicity of an extract or molecule is crucial for determining the LD_{50}, which facilitates the adjustment of treatment parameters within safe limits. Our work adhered to OECD Guideline 425 for the evaluation of toxicity in animals.

5.1. Observation

The acute toxicity assessment revealed that the aqueous extract at varying dosages (2000 and 5000 mg/kg) did not induce toxic symptoms or mortality, and no significant behavior alterations were noted in any of the mice. Therefore, these findings suggest that a single

administration doses not lead to any negative outcomes, indicating that the median lethal dose (LD_{50}) exceeds 5000 mg/kg for mice. The plant extract is deemed non-toxic based on the acute toxicity classification technique (Duan *et al*., 2011).

Clinical indications manifest within the initial four hours post-administration, including hypoactivity, tremors, heightened hunger, and increased respiration. Nevertheless, 24 hours post-administration and throughout the duration of the experiment, the treated mice exhibited hyperactivity in comparison to the control animals. Moreover, behavioral alterations and diarrhea were noted in mice at a dosage of 5g/kg of the aqueous extract. No fatalities were recorded during our experiment. The manifestation of symptoms noted within the initial hours of therapy in mice administered the aqueous extract of *Sanguisorba minor* L. may result from handling settings that influence the animal's natural state under stress. Subsequently, following a phase of stability, the mice revert to their customary routine (Amida *et al*., 2007). Our findings align with the research conducted by Tocai *et al*., (2023), which indicated that SMAE of *S. minor* does not induce mortality in mice and relatively non-toxic when delivered at doses of 300, 2000 and 3000 mg/kg, indicating its tolerance in mice. Additionally the research by Ansari *et al*. (2021) demonstrated that the LD_{50} of the *S. minor* L. in mice might surpass 3000 mg/kg confirming its non toxicological effects.

The relative organ weights are displayed in **Table 6.** The findings indicate that the aqueous extract of *S. minor* exerts little influence on the majority of the assessed organs (kidneys, lungs, heart, spleen and stomach). The liver weight of mice administered the 5g/kg dose exhibits a substantial increase ($p<0.001$) relative to the control group. The augmentation in liver relative weight may result from an enhanced metabolic response to extract, perhaps elevating hepatic activity (Aouachria *et al*., 2017). Raina *et al*. (2015) assert that organ weight serves as a significant indicator of the physiological and pathological health of animals. The liver and kidneys are organs sensitive to poisoning due to their essential functions in toxin elimination (Unuofin *et al*., 2018).

Table 6. mice treated with aqueous extract from *S. minor* L. (2 and 5 g/Kg) and their relative organ weights during acute toxicity. The means ± SEM (n=4) is used to express the values). ns: ns there was no discernible difference in relative organ weights between the control and treated groups .(**:p<0.01,***:p<0.001).

Organ	Control group	2g /kg	5g/kg
Liver	4.868 ± 0.483	5.438 ± 0.473***	5.860 ± 1.004***
Kidney	1.376 ± 0.039	1.219 ± 0.043 ^{ns}	1.272 ± 0.156 ^{ns}
Lung	0.650 ± 0.071	0.832 ± 0.260 ^{ns}	0.806 ± 0.181 ^{ns}
Heart	0.464 ± 0.042	0.486 ± 0.041^{ns}	0.534 ± 0.128^{ns}
Spleen	0.690 ± 0.234	0.421 ± 0.131^{ns}	0.642 ± 0.095^{ns}

Stomach	0.662 ± 0.315	0.774 ± 0.082 ^{ns}	1.069 ± 0.258 **

The oral administration of SMAE derived from the aerial portion of *S. minor* L. to mice results in minimal disruption in body weight over the initial five days (**Figure 25)**

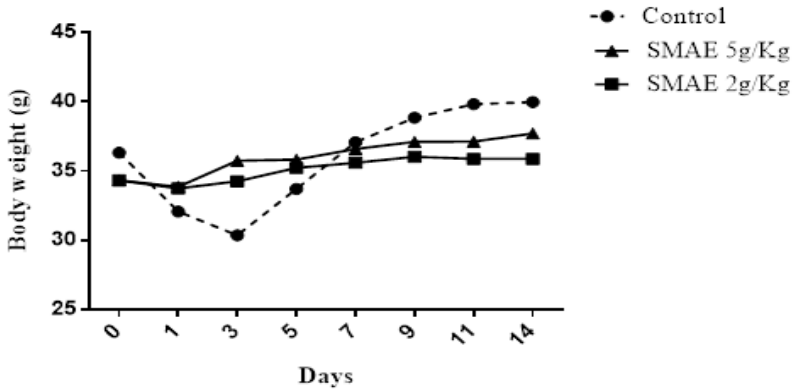

Figure 25: Changes in body weight in mice given an aqueous extract of *S. minor* L. during acute toxicity. The means ± SEM (n=4) is used to express the values .ns there was no discernible difference between the control and treated groups

There is no significant change compared to the control group. Furthermore, these variations can be correlated with the physiological state of the mice due to increased appetite or anxiety and stress. Numerous studies have demonstrated the impact of orally administered medicinal plant extracts on the body weight of animals (Rhiouani *et al*., 2008).

The biochemical analysis of serum from male mice administered SMAE at varying doses (2000 and 5000 mg/kg) is detailed in **Table 7.**

Table 2: Biochemical characteristics of male mice administered with SMAE (2000, 5000 mg/Kg) throughout the 14-day course of treatment.

Parameters	Control	2g/kg	5g/kg
ALT(U/L)	25.43± 2.75	22.9± 1.74^{ns}	21.25± 1.41^{ns}
AST(U/L)	103.70± 3.23	92.6± 10.33^{ns}	75± 8.02*
Gly(g/L)	1.57± 0.07	1.51± 0.09^{ns}	1.37± 0.09^{ns}
ALP (U/L)	105.50± 9.27	84.0± 8.03^{ns}	70.5± 8.70*
Chol(g/L)	0.97± 0.07	0.88± 0.04^{ns}	0.89± 0.07^{ns}
Trig(g/L)	1.83± 0.06	2,16± 0,37^{ns}	1.90± 0.26^{ns}
Urea(g/L)	0.40± 0.01	0.52± 0.05^{ns}	0.43± 0.05^{ns}

Créat(mg/L)	6.54± 0.13	3.37± 0.05***	3.25± 0.18***

Value are expressed as mean ± SEM (ns: non significant p>0.05, *: p<0.05, ***: p<0.001

In male mice, following the administration of the maximum dose of 5000 mg/kg SMAE of *S. minor* L. triglyceride (TRIG) and urea levels exhibits a modest rise (p<0.05). Blood glucose (BG), ALT (alanine aminotransferase), and CHOL (cholesterol) levels in male mice exhibited a considerable decrease. The parameters exhibited no significant alterations (p>0.05) in the treated male animals relative to the control group, as illustrated in **Table 7.** Consequently**,** the forced administration of SAME did not result in any notable alterations in urea, ALAT, TRG, CHOL, GLY, or blood glucose levels.

The assessment of liver function can be conducted by glucose metabolism, protein synthesis, bile secretion, or by analyzing aberrant protein levels in the blood to determine potential liver cell injury (Peng *et al.,* 2016). Specific enzymes and proteins, including as ALT, AST, gamma-glutamyltransferase, and bilirubin, are recognized as sensitive indicators of hepatic function (Traesel *et al*., 2014). In instances of hepatic injury, serum levels of AST and ALT elevate (Ozer *et al*., 2008). This study indicates that AST levels drastically dropped at a dosage of 5000 mg/kg (p<0.05), implying that elevated doses of SAME may result in hepatic tissue damage and injury. These findings correspond with the histological examination of the liver, as illustrated in **(Figure 26).**

The SMAE results in a reduction of creatinine levels (3.37 and 3.25 mg/L) at doses of 2 g/kg and 5 g/kg respectively, and diminishes protein catabolism, hence impairing the renal filtration mechanism. Urea and creatinine serve as semiological indicators for diagnosing renal conditions (El Hilaly *et al.,* 2004). Furthermore, oral treatment of SMAE resulted in a significant reduction (p<0.05) in alkaline phosphatase (ALP) levels, measuring 70.5 U/L at the 5 g/kg dosage. This measure is a significant indicator of renal impairment. This study demonstrated that mice administered doses of 2 g/kg and 5 g/kg exhibited markedly reduced levels of alkaline phosphatase (ALP), creatinine (CREA), and aspartate aminotransferase (AST) relative to the control group. In contrast, the research by Ansari *et al*. (2021) revealed that the oral administration of the hydroethanolic extract of *S. minor* L. did not elevate any biochemical parameters at the highest dosage of 3 g/kg. This requires a sub-acute and chronic examination of this plant.

5.2. Histological studies

Histological assessment of essential organs: liver and kidneys as seen in **Figure 26.** Histological examination of the liver and kidneys revealed no significant microscopic or histological alterations in the control group. The liver exhibited normal architecture without indications of injury, while the kidney displayed sufficient glomeruli in mice with doses (2000 and 5000 mg/kg) of *S. minor* L. Histological analysis of the kidneys reveals vascular congestion and hepatic necrosis at the maximum dosage **(Figure 26).** These findings correspond with the biochemical analysis of creatinine and alkaline phosphatase. The vascular congestion observed in kidney sections may result from the vasoconstrictive effect of the extract (SMAE) on blood vessel walls (Ashley, 2004; Grande *et al*., 2017). Tissue damage may occur due to the generation of reactive metabolites, including free radicals, which are formed following the activation by hazardous chemicals introduced into the body.

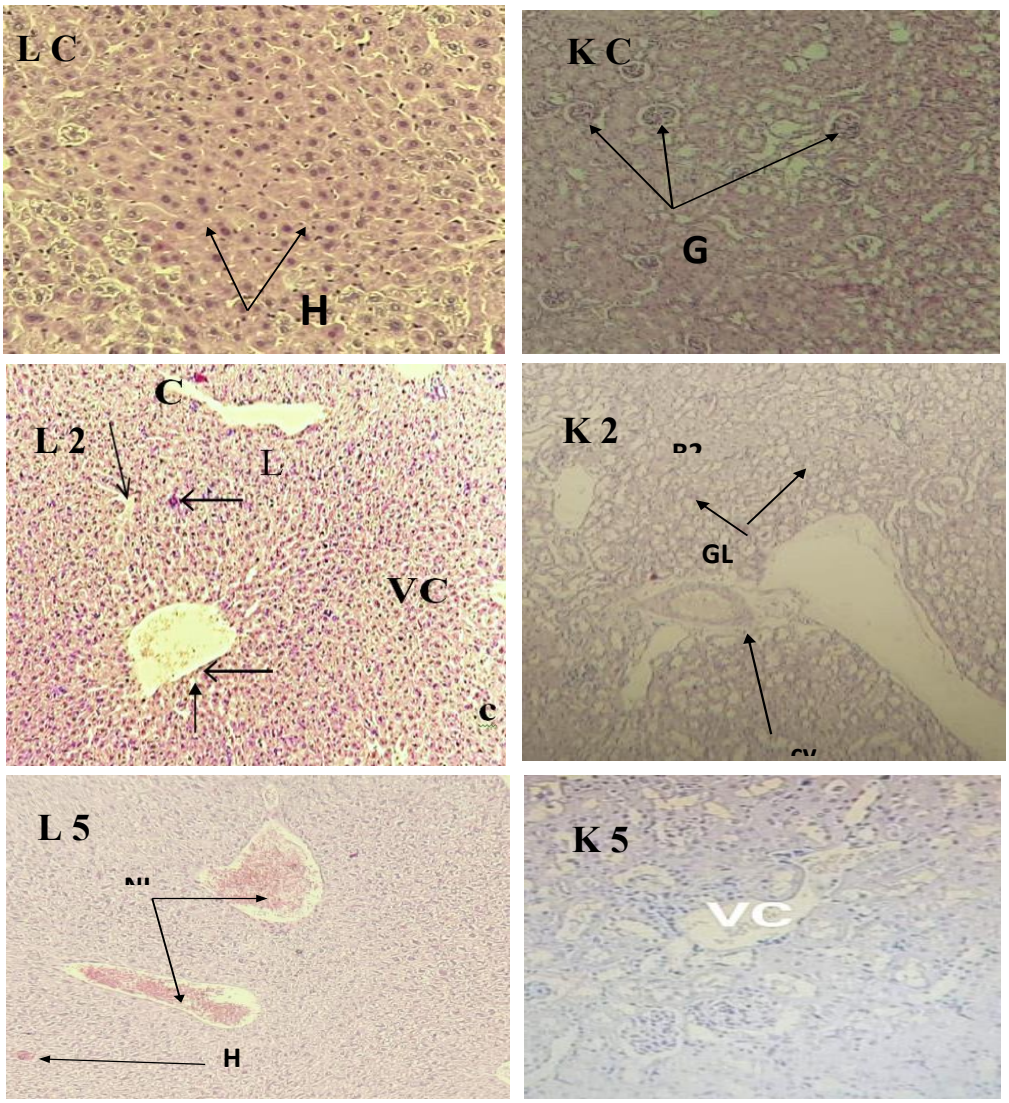

Figure 26: Histological section of mice liver and kidney of control and treated with SMAE (2 g/kg, 5 g/kg) in acute toxicity. VC: vascular congestion. LC: liver control, L2: liver at dose 2 g/kg, L5: liver at dose 5 g/kg, KC: kidney control, K2: kidney (2 g/kg),

6. Gastric ulcer

Intragastric injection of 100% ethanol to the untreated group of rats (negative control) resulted in extensive band-like hemorrhagic erosions in the glandular stomach (34.23± 3.69%), (**Figure 27).** Pretreatment with SMAE at the administered doses (100,250, and 500 mg/kg) provided varying levels of mucosal protection against ethanol-induced injury (20.94± 2.20%, 18.32±1.55%, and 1.16±0.19%, respectively; $p<0.01$ to $p<0.0001$). In comparison to the negative control, all administered dosages of SMCE (100, 250, and 500 mg/kg) significantly and dose-dependently decreased stomach ulceration (23.78±2.45%,21.17±2.16%,and 3.13±0.21%) respectively; ($p<0.01$ to $p<0.0001$). SMAE at a dosage of 250 mg/kg exhibited an impact comparable to that of the positive control, omeprazole (10± 0.46%);($p>0.05$). The maximum dosage of SMCE and SMAE demonstrated a comparable gastric morphology to that of the normal control (1.09 ± 0.90%) $p>0.05$, and a superior reduction in gastric ulcers compared to the positive control ($p< 0.05$).

Gastric ulcers are generally understood to arise from an imbalance between aggressive and defensive elements. The primary aggressive factors consist of acid, pepsin, helicobacter pylori, NSAIDs, ethanol, and reactive oxygen species, while the principal defensive factors include mucin, bicarbonate, nitic oxide, and growth factors. Excessive alcohol intake can lead to gastritis, hemorrhagic stomach erosions,mucosal edema, cellular exfoliation, and inflammatory cell infiltration (Hussain *et al*., 2015). A gastric ulcer is a benign lesion of the mucosal epithelium that arises in the stomach due to excessive acid exposure (Sabiu *et al*., 2015).

The rat model of ethanol-induced gastric mucosal injury is frequently employed in research to evaluate medicines with potential anti-ulcer properties, as ethanol is the primary contributor to stomach ulcers observe in humans (Sistani Karampour *et al.,* 2019; Beiranvand *et al*., 2022).

Ethanol induces disarray in mucosal microcirculation and ischemia, generates free radicals, and disrupts the endothelium, leading to its ulcerogenic effects (Prazeres *et al.,* 2019).

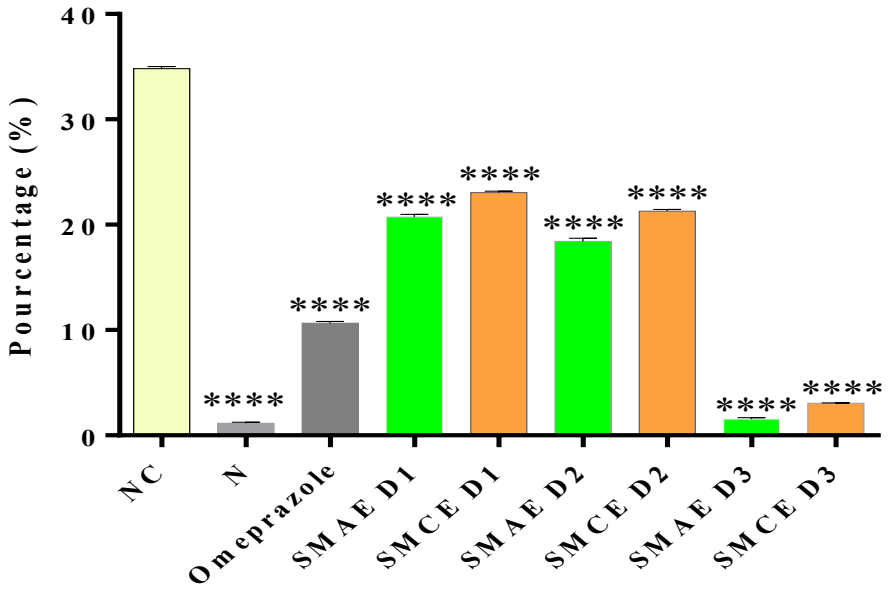

Figure 27: Effects of SMAE and SMCE at different doses (100, 250, 500 mg/kg) on gastric ulcer in rats. SMCE :ethanolic extract, SMAE :aqueous extract. NC: negative control, N: native Bars represent means±SEM (n=8).****P<0.0001.

The significant increase in ulcer index after oral ethanol therapy in ulcerated rats may be attributed to either free radical generation or inhibition of prostaglandin synthesis (Kouitcheu *et al*., 2017). Numerous studies have associated the prevention of stomach ulcers with the presence of phenolic acids and flavornoids in plant extract (Amaral *et al.,* 2013). The initial phytochemical investigation of SMAE and SMCE indicated the presence of flavonoids. Flavonoids have been documented to provide antiulcer action in multiple experimental paradigms (Shahzad *et al*., 2024). Various methods have been suggested to elucidate their biological effects, including the augmentation of mucosal prostaglandin levels, reduction of histamine release from mast cells, suppression of acid production, and inhibition of Heliobacter pylori proliferation (Gürbüz *et al*., 2005). Because of their anti-inflammatory and antioxidant properties, flavonoids are widely known to have anti-ulcerogenic properties. Fruits and vegetables that include quercetin and catechins have been shown to have the capacity to reduce inflammation and oxidative stress in the stomach mucosa (Serafim *et al*., 2020; Shahzad *et al*., 2024). Hussain *et al*. (2009) elucidated the significant gastropretective effects of rutin, ascribed to its anti-inflammatory properties, vasoactive characteristics, and capacity to scavenge reactive oxygen species (ROS) generated during ethanol-induced lesions. Moreover, kaempferol has demonstrated preventive properties against ethanol-induced stomach ulcers by diminishing

oxidative stress and augmenting the activity of antioxidant enzymes (de Lira Mota *et al*., 2009). Polyphenols, which effectively scavenge superoxide anion, hydroxyl, peroxyl, and alcoxyl radicals, have demonstrated antioxidant and gastroprotective activities in prior research (Amaral *et al*., 2013). Chlorogenic acids are recognized for their antioxidant and their capacity to alleviate stomach damage caused by acidified ethanol or NSAIDs. The protective impact is due to the reduction of leukocyte migration, enhancement of antioxidant enzyme activity, including the restoration of CAT, SOD, and GSH levels, and the suppression of TNF-α (Krawisz *et al*., 1984). The presence of beneficial substances (polyphenols and flavonoids) clarifies the preventive properties of *S. minor* L. against ethanol-induced stomach damage. Moreover, many extracts obtained from *S. minor* have been demonstrated in previous research to dramatically diminish damages caused by ethanol in rat models (Gürbüz *et al*., 2005).

6.1. Macroscopic examination

The assay demonstrated the impact of ethanol gastric tissues in conjunction with SAME or SMCE extracts at varying dosages, as illustrated in **Figure 28.** Absolute ethanol induced significant evident hemorrhagic necrosis of the stomach mucosa in the negative group **(Figure 28 C-).** The positive control group administered omeprazole at a dosage of 100 mg/kg exhibited less severe damage to the gastric mucosa compared to the negative control group (**Figure 28 C+**). Oral treatment of SMAE **(Figure 28AD**) and SMCE **(Figure 28 ED**) substantially mitigated ethanol-induced stomach damage in a dose-dependant manner, significantly reducing the gastric ulcer area. The protective efficacy of both SMAE and SMCE at their maximum concentrations seemed to surpass that the positive control group.

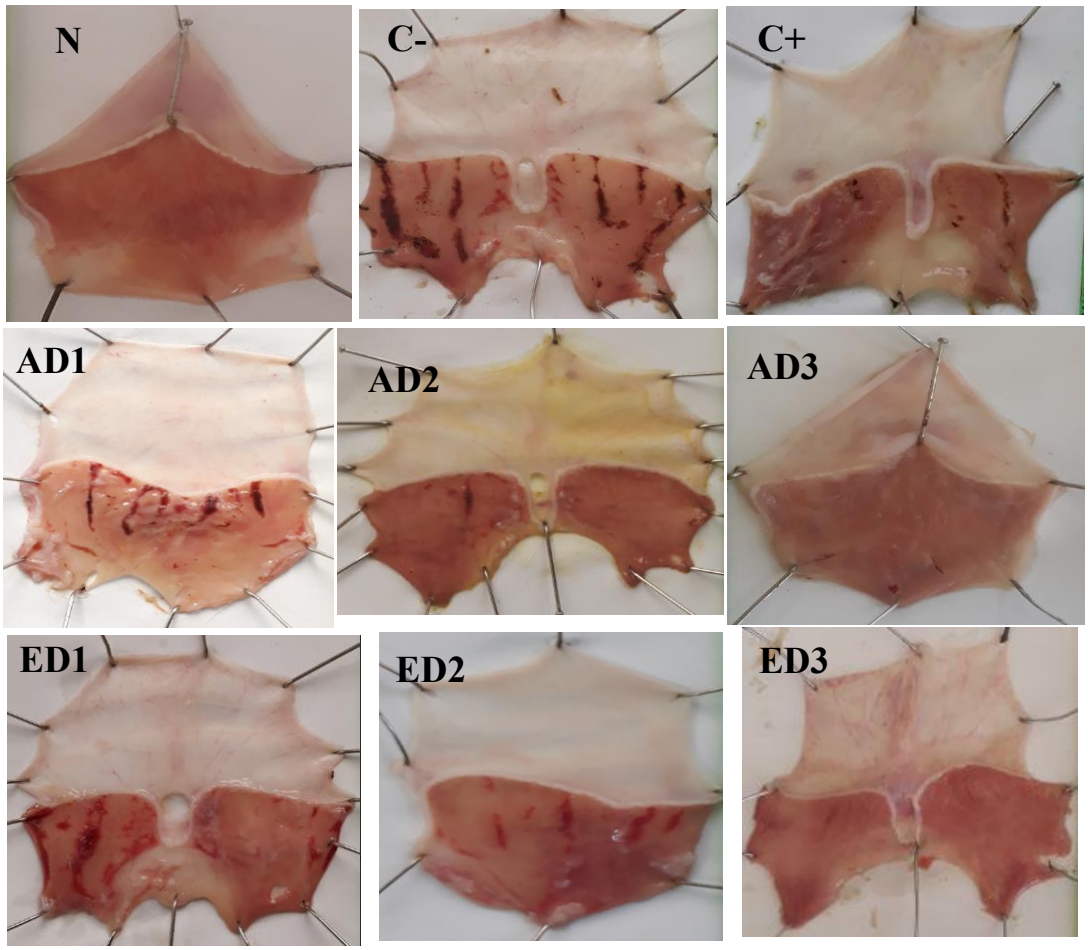

Figure 28: Effects of SMAE and SMCE on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats. (N) Normal control group. (C+) The group pre-treated with omeprazole (100 mg/kg) as positive control.(C-) The group pre-tre

6.2. Histological analysis

Figure 29 illustrates the histological examination of ethanol gastric mucosal injury in rats, alongside the administration of omeprazole, SMAE, and SMCE at varying dosages. Stomach of typical rat (**Figure 29 N**) exhibiting the standard histological architecture of the mucosa. The stomach of the group administrated pure ethanol as a negative control (**Figure 29 C-)** exhibits necrosis in the superficiel mucosal epithelium (red arrow), accompanied by oedema, infiltration of inflammatory cells (black arrow), and stomach invasion by polynuclear cells (white arrow). The stomach of the omeprazole treated rat in the positive control group **(Figure 29 C+)** has an intact histological structure of the mucosa, characterized by mild oedema and infiltration of inflammatory cells (green arrow)

Rats pretreated with SMAE (Figure 29 AD) and SMCE(Figure 29ED) exhibit comparatively superior protection in a dose-dependant manner, as evidenced by the

preservation of gastric epithelium and reduction or complete absence of eodema and leukocyte infiltration(blue arrow).

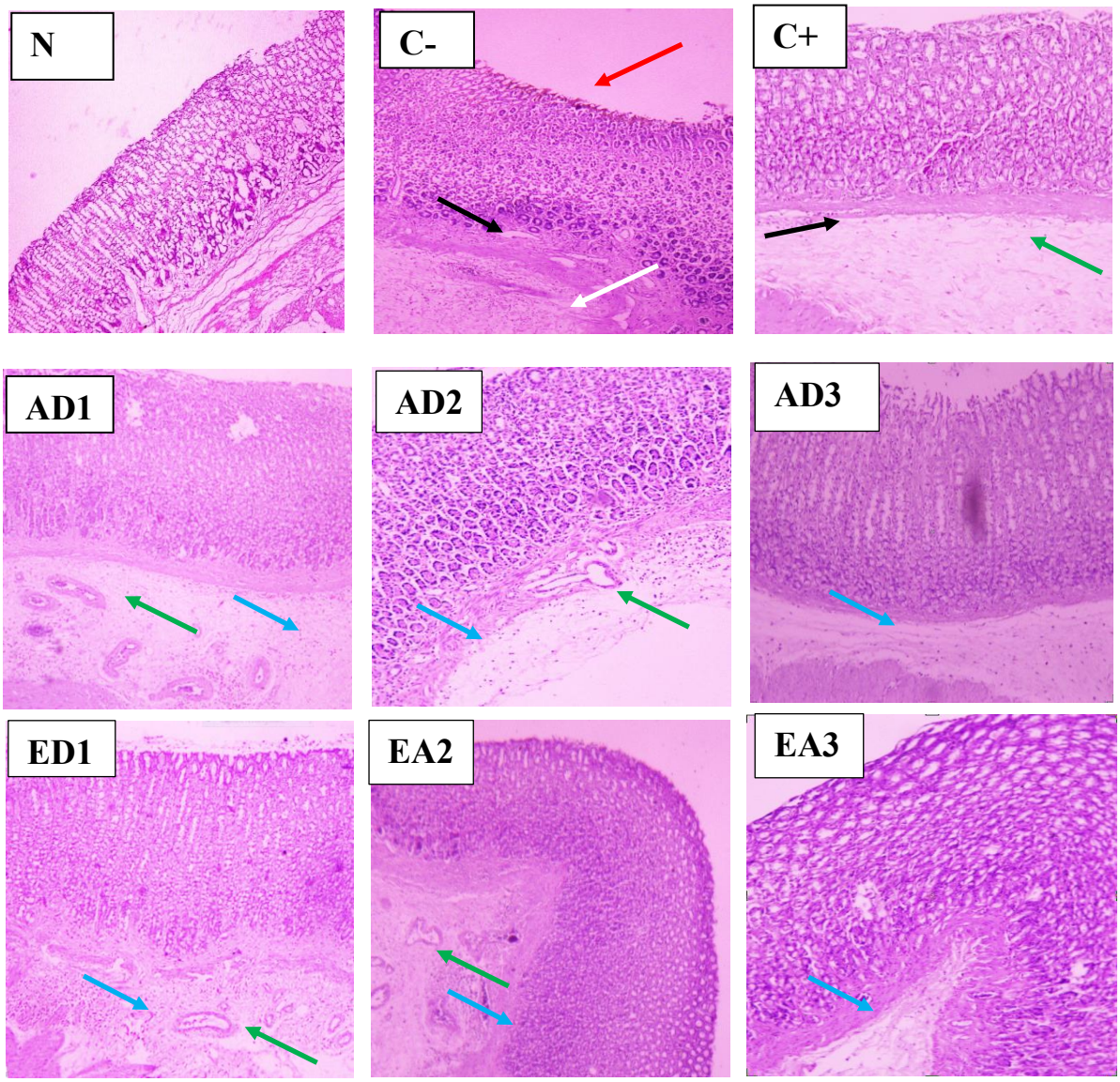

Figure 29: Histological examination for the protective effect of SMAE and SMCE against ethanol-induced gastric damage in rat stomach tissue; (N) Normal control group. (C+) The group pre-treated with omeprazole (100 mg/kg) as positive control.(C-) The group pre-treated.

Conclusion

This thesis sought to substantiate the conventional application of *Sanguisorba minor* L. in relation to various diseases and complications through scientific evidence, including *in vivo* and *in vitro* evaluations of its biological activities including antioxidant, anti-inflammatory, and anti-ulcerogenic properties, alongside a safety assessment of its secondary metabolite compounds.

The current investigation revealed that *S. minor* extracts are abundant in phenolic compounds, with markedly elevated concentrations in the ethanolic extract relative to the aqueous extract. HPLC-DAD qualitative and quantitative tests indicated the predominant existence of flavonols, including kaempferol, and phenolics acids, including gallic, chlorogenic, caffeic and ellagic acids. Several studies based on iron chelating, FRAP, and radical scavenging were carried to indicate antioxidant capacities. Both extracts exhibited strong antioxidant activity *in vitro.* In contrast to the ethanolic extract, which shown significant activity in the majority of experiments, the aqueous extract demonstrated great activity against the hydroxyl radical.

The bulk of the antioxidants under study showed a strong association with the total phenolics content, which may suggest that these chemicals are most likely the cause of these activities.

The model employed in this study, bovine serum albumin heat denaturation, exhibited the anti-inflammatory effects of the plant extracts even at low concentrations. Inflammatory disorders associated with protein denaturation can be fatal. Carrageenan, xylene, and croton oil were used to create edema models *in vivo*. The finding showed that the SMAE and SMCE, when taken at a dose of 150 mg/kg, had substantial anti-edematous efficacy. Additionally, at the same dosage as before, anti-nociceptive action was seen. The safety of these plants was confirmed by the absence of harmful effects in mice at 5000 mg/kg. Rat stomachs were shielded from ethanol-induced gastric ulcers by both SMAE and SMCE extracts. Reduction of the stress on the stomach mucosa may be part of the protective effect.

Additionally, the results demonstrate *S. minor'*s L. potential as a natural anti-inflammatory, antioxidant and anti-ulcerogenic indicating that it may find use as a functional food ingredient and in pharmaceutical applications.

Perspectives

Additional investigation is required to ascertain the active principles of this plant:

- ✓ To ascertain the precise mechanism behind the reported activities, the active molecules essential for the exceptional antioxidant and anti-inflammatory properties must be isolated, identified, and characterized.
- ✓ Evaluation *in vivo* antioxidant activity
- ✓ To confirm their safety profile, the toxicity of biologically active chemicals (purified from this plant) should be evaluated.
- ✓ It is crucial to conduct more research on the process underlying the plants gastroprotective benefits.
- ✓ Comprehensive cytotoxicity testing on a range of cell lines, with a focus on cancer cell lines, are strongly recommended.

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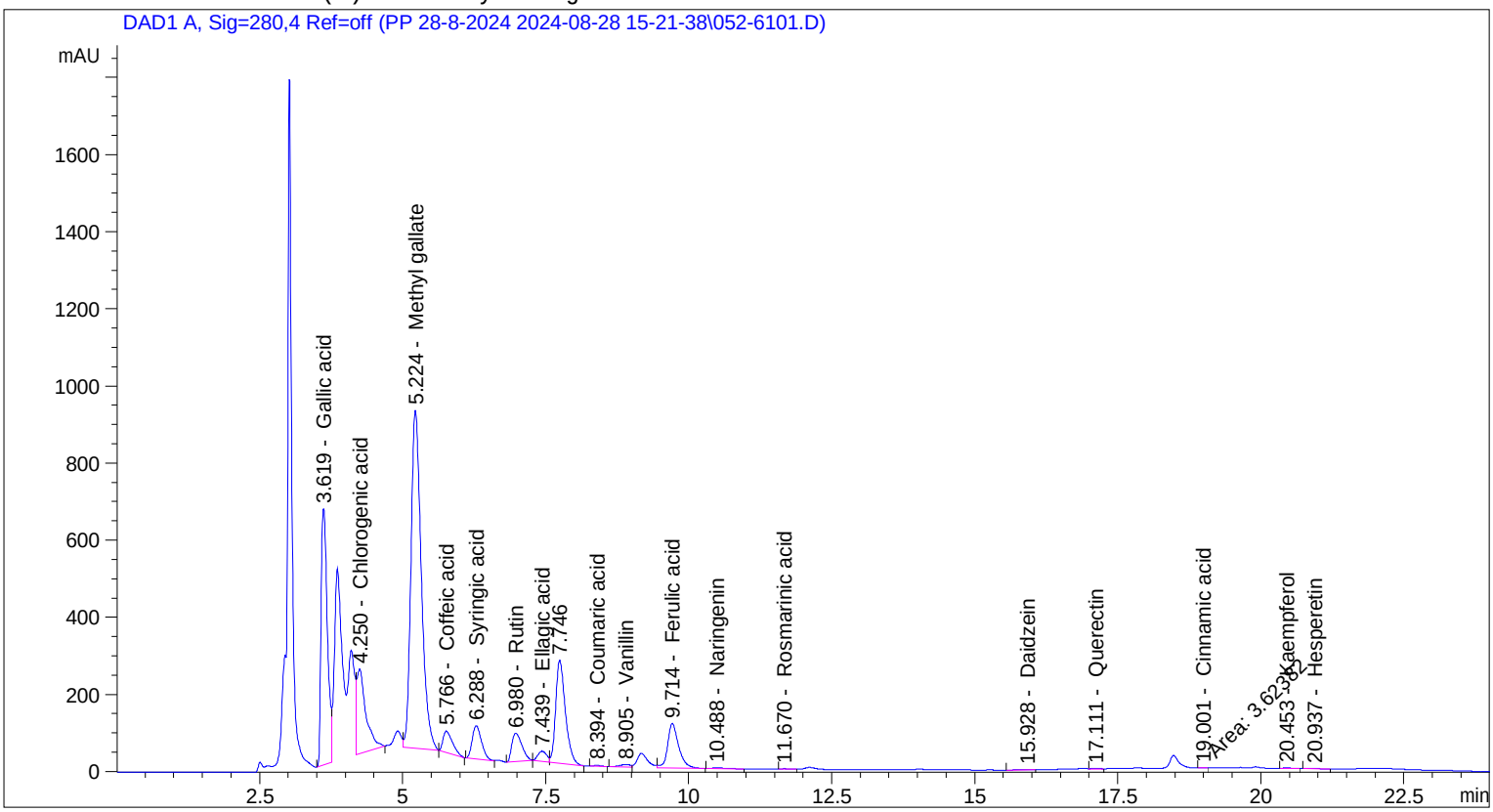


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Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


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   1   3.619 BV      0.1190 5071.57178  19.3374 Gallic acid
   2   4.250 VB      0.1558 2442.17969   9.3118 Chlorogenic acid
   3   4.438         0.0000    0.00000   0.0000 Catechin
   4   5.224 VB      0.1931 1.10498e4   42.1316 Methyl gallate
   5   5.766 BB      0.1769  633.98108   2.4173 Coffeic acid
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Data File C:\CHEM32\1\DATA\PP 28-8-2024 2024-08-28 15-21-38\052-6101.D
Sample Name: 14

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
6	6.288	BV	0.1763	981.07178	3.7407	Syringic acid
7	6.980	BB	0.1942	889.85809	3.3929	Rutin
8	7.439	BV	0.1718	272.95889	1.0408	Ellagic acid
9	7.746	VB	0.1791	3138.85059	11.9681	?
10	8.394	BB	0.1461	17.67960	0.0674	Coumaric acid
11	8.905	BV	0.1918	78.37183	0.2988	Vanillin
12	9.714	VB	0.2010	1549.91101	5.9097	Ferulic acid
13	10.488	BB	0.2820	44.55376	0.1699	Naringenin
14	11.670	VB	0.1679	14.12192	0.0538	Rosmarinic acid
15	15.928	BB	0.1958	5.28269	0.0201	Daidzein
16	17.111	VV	0.1492	11.70069	0.0446	Querectin
17	19.001	MM	0.1151	3.62382	0.0138	Cinnamic acid
18	20.453	VB	0.1469	15.89222	0.0606	Kaempferol
19	20.937	BB	0.2114	5.40545	0.0206	Hesperetin

Totals : 2.62268e4

19 Warnings or Errors (10 first messages follow) :

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Warning : Calibrated compound(s) not found
Warning : Invalid calibration curve, (Gallic acid)
Warning : Invalid calibration curve, (Chlorogenic acid)
Warning : Invalid calibration curve, (Methyl gallate)
Warning : Invalid calibration curve, (Coffeic acid)
Warning : Invalid calibration curve, (Syringic acid)
Warning : Invalid calibration curve, (Rutin)
Warning : Invalid calibration curve, (Ellagic acid)
Warning : Invalid calibration curve, (Coumaric acid)

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*** End of Report ***

Data File C:\CHEM32\1\DATA\PP 30-12-2024 2024-12-05 14-36-47\009-1001.D
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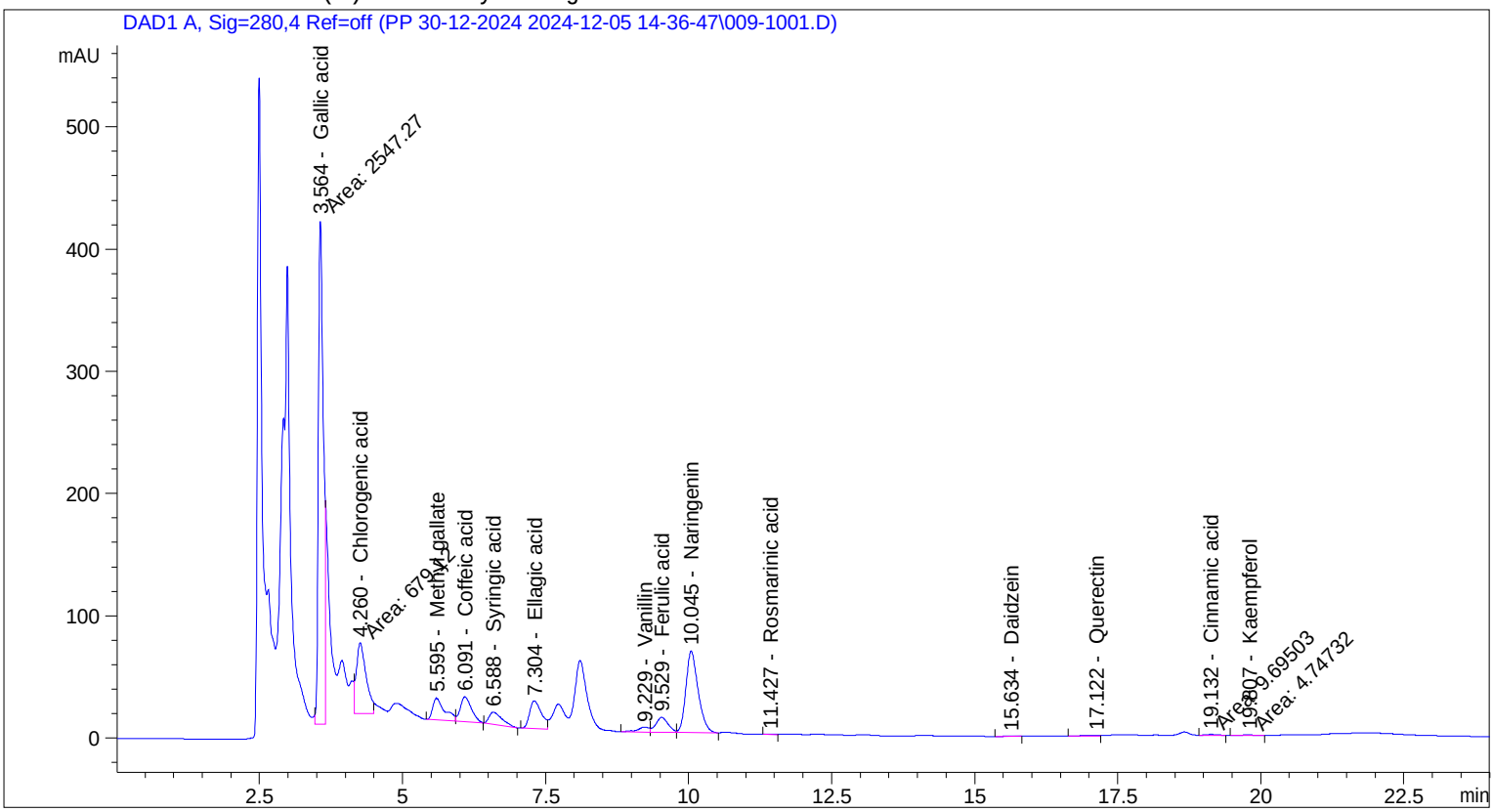


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==========================================================================
                          Area Percent Report
==========================================================================

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Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=280,4 Ref=off

Peak RetTime  Type   Width     Area      Area    Name
  #   [min]          [min]   [mAU*s]       %
----|-------|------|-------|----------|--------|--------------------
   1   3.564 MM      0.1023 2547.26538  46.4006 Gallic acid
   2   4.260 MM      0.1946  679.11987  12.3707 Chlorogenic acid
   3   4.460         0.0000    0.00000   0.0000 Catechin
   4   5.595 BV      0.2096  259.23682   4.7222 Methyl gallate
   5   6.091 VB      0.2044  271.49680   4.9455 Coffeic acid
   6   6.588 BB      0.2428  155.37073   2.8302 Syringic acid
```

Data File C:\CHEM32\1\DATA\PP 30-12-2024 2024-12-05 14-36-47\009-1001.D
Sample Name: 2S

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Area %	Name
7	6.975		0.0000	0.00000	0.0000	Rutin
8	7.304	BV	0.2209	328.89236	5.9910	Ellagic acid
9	8.579		0.0000	0.00000	0.0000	Coumaric acid
10	9.229	BV	0.2137	59.14752	1.0774	Vanillin
11	9.529	VV	0.2250	181.15091	3.2998	Ferulic acid
12	10.045	VB	0.2207	985.83447	17.9578	Naringenin
13	11.427	BV	0.1569	1.94004	0.0353	Rosmarinic acid
14	15.634	BB	0.2126	1.41013	0.0257	Daidzein
15	17.122	BV	0.2142	4.42200	0.0806	Querectin
16	19.132	MM	0.2170	9.69503	0.1766	Cinnamic acid
17	19.807	MM	0.3224	4.74732	0.0865	Kaempferol
18	21.204		0.0000	0.00000	0.0000	Hesperetin

Totals : 5489.72938

16 Warnings or Errors (10 first messages follow) :

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Warning : Invalid calibration curve, (Syringic acid)
Warning : Invalid calibration curve, (Ellagic acid)
Warning : Invalid calibration curve, (Vanillin)
Warning : Invalid calibration curve, (Ferulic acid)

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*** End of Report ***

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HPLC–DAD phytochemical profile, antioxidant, anti–inflammatory and analgesic properties of *Sanguisorba minor* L.: An *in vitro* and *in vivo* assessment

Perfil fitoquímico HPLC–DAD, propiedades antioxidantes, antiinflamatorias y analgésicas de *Sanguisorba minor* L.: Una evaluación *in vitro* e *in vivo*

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ABSTRACT

Sanguisorba minor L. is a plant species of the Rosaceae family to treat several illnesses. The purpose of this investigation is to verify the amount of flavonoids and polyphenols in the extract of the plant *S. minor* and to evaluate its antioxidant, anti–inflammatory and analgesic activities. Phytochemical profile of this plant extract was determined by HPLC–DAD. This latter was used to evaluate polyphenols in the ethanolic extract of *S. minor,* in addition to the previous study, an anti–inflammatory effectiveness and of the extract was evaluated *in vitro* using the bovine serum albumin (BSA) denaturation test. The paw edema induced by carrageenan model and Xylene induced edematous foot model were used to evaluate the *in vivo* anti–inflammatory efficacy. Finally, the extract's analgesic qualities were evaluated using the acetic acid–induced writhing test. The ethanolic extract exhibited a high content of total phenols (623.33 ± 0.01 µg EAG·mg^{-1} extract), but a relatively low concentration of flavonoids (8.71 ± 0.04 µg·mg^{-1} extract). The ethanolic extract's IC_{50} value were 11.00 ± 0.00 µg·mg^{-1} of DPPH radical showed that it was a significant antioxidant; 3 ± 0.00 µg·mg^{-1} for OH radical scavenging ; and 7 ± 0.00 µg·mg^{-1} for FRAP assay. Methyl gallate and other chemicals, including gallic acid, rutin, syringic acid, ferulic acid, caffeic acid were identified by the HPLC–DAD analysis, suggesting that the sample contained a high concentration of phenolics. The extract's powerful ability to scavenge and reduce radicals indicated that it exhibited high antioxidant capacity. At 150 and 300 mg·kg^{-1} the ethanolic extract of the *S. minor* showed notable anti–inflammatory action with inhibition percentages of 87.22 ± 0.38%, and 57.65 ± 0.42, respectively. At a concentration of 150 mg·kg^{-1}, the ethanol extract exhibited the greatest analgesic activity (84.30%), showing a more pronounced inhibitory effect on abdominal cramps compared to the 300 mg·kg^{-1} concentration. The extract of the plant of *S. minor* is rich in polyphenols, demonstrating significant antioxidant, anti–inflammatory, and analgesic properties.

Key words: *Sanguisorba minor* L.; HPLC–DAD; antioxidant activity; anti–inflammatory activity, analgesic test

RESUMEN

Sanguisorba minor L., una especie vegetal de la familia de las rosáceas, se utiliza para el tratamiento de diversas enfermedades. El objetivo de esta investigación es determinar mediante HPLC–DAD, el perfil fitoquímico del extracto de *S. minor* y evaluar sus propiedades antioxidantes, antiinflamatorias y analgésicas La eficacia antiinflamatoria del extracto se evaluó *in vitro* mediante la prueba de desnaturalización de la albúmina sérica bovina (BSA). El modelo de edema de pata inducido por carragenina y el modelo de pie edematoso inducido por xileno, se utilizaron para evaluar la eficacia antiinflamatoria *in vivo.* Finalmente, se evaluaron las propiedades analgésicas del extracto mediante la prueba de retorcimiento inducido por ácido acético. El extracto etanólico exhibió un alto contenido de fenoles totales (623,33 ± 0,01µg EAG·mg^{-1} extracto), pero una concentración relativamente baja de flavonoides (8,71 ± 0,04 µg·mg^{-1} extracto). Los valores de CI_{50} de los extractos etanólicos fueron 11,00 ± 0,00 µg·mg^{-1} de radical DPPH mostraron que era un antioxidante significativo; 3 ± 0,00 µg·mg^{-1} para la eliminación de radicales OH; y 7 ± 0,00 µg·mg^{-1} para el ensayo FRAP. El metil galato y otros químicos, incluyendo ácido gálico, rutina, ácido siríngico, ácido ferúlico y ácido cafeico fueron identificados por el análisis HPLC–DAD, sugiriendo que la muestra contenía una alta concentración de compuestos fenólicos . La potente capacidad del extracto para eliminar y reducir radicales libres indicó que exhibió una alta capacidad antioxidante. A dosis de 150 y 300 mg·kg^{-1}, el extracto etanólico de *S. minor* mostró una notable acción antiinflamatoria, con porcentajes de inhibición de 87,22 ± 0,38 % y del 57,65 ± 0,42%, respectivamente. La concentración de 150 mg·kg^{-1} del extracto etanólico, exhibió mayor acción analgésica (84,30%), observándose mayor efecto inhibidor de los calambres abdominales en comparación con la concentración de 300 mg·kg^{-1}. El extracto de la planta de *S. minor* es rico en polifenoles, lo que demuestra importantes propiedades antioxidantes, antiinflamatorias y analgésicas.

Palabras clave: *Sanguisorba minor*, HPLC–DAD, actividad antioxidante, actividad antiinflamatoria, Parueba analgésica

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INTRODUCTION

The body uses inflammation as a defense against aggression that is chemical, infectious, physical or biological [1]. Because of the pathogen's aggressiveness, persistence, anomalies in the control and generation of inflammatory cells, this defense immunological response can occasionally be detrimental. Numerous diseases in humans, including diabetes, cancer, arthritis, asthma and allergies, are linked to these inflammatory processes [2].

The development and maintenance of inflammation are closely linked to oxidative stress, which can lead to beginning and/or maintenance of variety diseases. Oxidative stress results from an imbalance in redox homeostasis. It results in excessive formation and/or poor elimination of free radicals, which are extremely reactive nitrogen or oxygen molecules, which are highly reactive molecules of either oxygen or nitrogen [3].

This imbalance causes oxidative damage to the cell's different molecules (nucleic acids, proteins, and lipids), resulting in necrosis or apoptosis, which kills cells. Aging and the pathogenesis of numerous illnesses, including heart diseases, cancer and inflammatory diseases, are both influenced by oxidative stress [4]. Using non –steroidal anti–inflammatory drugs can have a number of harmful side effects, including kidney, asthma and gastrointestinal problems [5].

However, teratogenic, mutagenic and carcinogenic effects [6] accompany prolonged usage of artificial antioxidants such as butylated hydroxytoluene in the agri–food, cosmetics and pharmaceutical sectors. To stop these oxidative and inflammatory diseases from developing, it is vital to find novel therapeutic medicines with minimal adverse effects. Plants represent an inexhaustible reservoir of secondary metabolites. These plants are used in traditional medicine without any precise scientific rules. In order to do this, research is concentrated on increasing the usefulness of traditional medicine, confirming the effectiveness of the plants utilized, and developing scientific guidelines for their various applications. The edible perennial herb *Sanguisorba minor*, commonly known as salad, has reddish–green flowers and pinnate leaves [7].

In Asia, North Africa and Europe, *S. minor* is a common herbaceous plant that belongs to the Rosaceae family. It is usually found in semi–dry and dry meadows in Mediterranean areas. This plant is used as culinary additive in fruit juices, cheese, ice drinks and a number of traditional foods [8, 9, 10]. It is known, this plant is widely used in certain illnesses like fever, diarrhea, conjunctivitis, duodenal ulcers, intestinal infections, bleeding, and burns [9, 10, 11, 12].

Plants in the sanguisorba genus [13, 14, 15] expressed numerous pharmacological properties, including hemostatic, antibacterial, anti–inflammatory, antioxidant, hypoglycemic, neuroprotective, anti–alzheimer, and anticancer activities. This is the background to this research, determinate by HPLC–DAD, the phytochemical profile, and antioxidant, anti–inflammatory, and analgesic properties of ethanolic extract of *Sanguisorba minor*.

MATERIALS AND METHODS

Plant materials

In March 2023, aerial portions of *S. minor* were gathered for the Guenzet Setif Mountains (Northern Algeria). Pr. Laouer Hocine of the University Ferhat Abbas Setif 1, El Bez, Algeria's Department of Biology and Vegetal Ecology's Laboratory of Natural Resources Valorization confirmed the species identification. The laboratory received a voucher specimen with the number SNV/0035/2024. For two weeks, the collected samples were allowed to dry outdoors.

Preparation of extract

According to the procedure, outlined by [16] about 50 g of powdered aerial parts of *S. minor* were extracted using pure ethanol (100%). A first filtrate was realized using Wathman number 1 paper, the same maceration was repeated for four weeks, at the end evaporated with a rotavap (Buchi rotavap R-205 Switzeland) at a temperature of 45°C, and subsequently dried at 40°C to obtain *S. minor* crude extract (SMCE).

In vitro studies

Phytochemical analysis, total flavonoids and phenolics contents

The amount of polyphenols was measured using the colorimetric Follin–Ciocateau technique (Shimadzu UV1800 spectophotometer, Japan). About 500 µL of ten–fold diluted Folin–Ciocalteu reagent was combined with 100 µL of either the ethanolic extract or the standard which is gallic acid solution from (Sigma Aldrich), with concentration ranging from 20 $\mu g \cdot mL^{-1}$ to 160 $\mu g \cdot mL^{-1}$. Furthermore, 400 µL of a sodium carbonate (Na_2CO_3) solution containing 75 $g \cdot mL^{-1}$ was added 4 min later. After the reaction mixture was incubated for one hour (h) and 30 min in the stove at 28°C (Memmert UM200, Germany) the absorbance at 765 nm was measured (Shimadzu UV1800 spectrophotometer,Germany).

The findings were presented as equivalent to micrograms of gallic acid per milligrams of dry extract (µg GAE·mg^{-1} DE). In addition, the extract's total flavonoids concentration was ascertained using the aluminum chloride ($AlCl_3$). About 500 µL of aluminum chloride solution (2%) was combined with an aliquot of 500 µL of either the quercetin standard (from Sigma Aldrich) with concentrations ranging from 5 to 50 $\mu g \cdot mL^{-1}$ or diluted extract.

The absorbance at 430 nm was determined after 10 min incubation at room temperature (Shimadzu UV1800 spectrophotometer, Germany) [17]. Utilizing the quercetin curve calibration, the concentrations of flavonoids was determined as equivalent to micrograms of quercetin per milligrams of dry extract (µgQE·mg^{-1} DE).

HPLC conditions

The HPLC (Agilent 1260,USA) was carried out using an Agilent 1260 series ; a Zorbax eclipse plus C8 column (4.6 mm×250 mm i.d.,5 µm) was utilized. The mobile phase consisted of water (A) and 0.05 % tricloroacetic acid in acetonitrile (B) at a flow rate of 0.9 $mL \cdot min^{-1}$ comprising the mobile phase. The mobile phase was coded in a linear gradient as follows: 0-1min (82% A); 1-11 min (75%A); 11-18 min (60% A); 18-22 min (82% A); 22-24 min

(82% A). The multi wavelength detector was spotted at 280nm. Each sample's injection volume is fixed at 5 µL. The differents standards compounds utilized in the HPLC–DAD is: gallic acid, chlorogenic acid, caffeic acid, methyl gallate , syringic acid, rutin, ellagic acid, coumaric acid, ferulic acid, naringenin, rosmarinic acid, daidzein, querctin, kaempferol, hesperetin, catechin and vanillin.

Antioxidants activity

DPPH radical

The antioxidant capacity of the extract was assessed using the 2,2–diphenyl–1–pycrilhydrazil (DPPH) radical experiment. It is a stable free radical that is reduced by antioxidant molecules, by acceptation of an electron or hydrogen. In its original radical state, DPPH exhibits a rich purple hue, transitioning to yellow in its reduced form. About 50 µL of different concentrations of SMCE (0.05 to 1 mg·mL^{-1}) was combined with 1.25 mL of a methanolic mixture of 2,2–diphenyl–1–pycrilhydrazil. Following 30 min the resultants mixture was measured at an absorbance 517 nm [18]. The % inhibition of DPPH free radical is calculated using the formula below:

$$Radical\ \ scavenging\ \ capacity = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Reducing power assay

The technique described was used gauge the action of the decreasing power [19]. The development of this technique aimed to assess the ability of extract to reduce the ferric ion (Fe^{3+}) found in potassium ferricyanide $K_3[Fe.(CN)_6]$ complex (red color) in ferrous ion (Fe^{2+}) which turns blue or green, the color change is proportional to the antioxidant activity. The mixture contain 50 µL of potassium ferrocyanide 10%, 40 µL of sodium phosphate buffer (0.2M, pH:6.6) and 10 µL of various concentrations (0.005 to 1 mg·mL^{-1}) of the SMCE, following a 20 min incubation period at 50°C in a water bath (Memmert WNB22, Germany). Thereafter, 10% of TCA (tricloroacetic acid) was added. Lastly, a volume of 10 µL of ferric chloride ($FeCl_3$, 0.1%) and 40 µL of the upper layer's H_2O were added to the mixture. At 700 nm, the mixture's was measured. The concentration providing 0.5 absorbance was deduced from a linear regression curve of each extract and standard.

Iron–chelating assay

The method used to evaluate the ferrous ion chelating ability of the SMCE is based on the measurement of the pink chromophore resulting from ferrous ion–ferrozine complex formation [20]. A volume of 40 µL of SMCE or Ethylenediaminetetra acetic acid (EDTA) as standard was combined with the same volume 40 µL of methanol and $FeCl_2$ (0.02%). After five minutes 0.08 mL of ferrozine (0.5 mM) was added to beginning the complexion reaction which proceeded for 10 min at room temperature. The absorbance of the complex Fe^{+2}-ferrozine was registred at 562 nm. The following formula was used to quantify the chelating capacity as an inhibition percentage:

$$Chelation\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Phosphomolybdate test

The reduction of Mo (VI) to Mo (V) is the basis of the phosphomolybdenum technique, it used to assesses the antioxidant activity of SMCE by spectrophotometries (Shimadzu UV1800 spectrophotometer, Germany) [21]. This method is quantitative to determine the antioxidant capacity of the extract by the reduction of molybdate ions, and the formation of a phosphate / Mo(V) complex followed by the degradation of the green color. At different concentrations of ethanolic extract and standard as ascorbic acid with various concentration (0.01 to 0.5 mg·mL^{-1}), 100 µL of each concentration was shaken with 1 mL of the reagent solution which contain 0.28 M sodium phosphate, 0.04 M ammonium molybdate and 0.6 M sulfuric acid. The mixtures were incubated to 95°C in a water bath for 90 min. At 765 nm, the sample and standard absorbance was measured.

The radical hydroxyl scavenging

Following the method outlined, the radical hydroxyl scavenging was evaluated in the SMCE by generating hydroxyl radicals in a Fenton reaction [22]. A solution containing 1 mL of 0.2 M of sodium salicylate, 1 mL of 0.15 M of $FeSO_4$, 0.7 mL of 0.6 M of hydrogen peroxide was added in each different concentration of SMCE. After 1 h of incubation at 37°C in the stove (Memmert UM200, Germany), the absorbance was measured at 562 nm. The % inhibition of the OH scavenging capacity was calculated using this formula:

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

In vitro anti–inflammatory activity

The anti–inflammatory effect was assessed [23] with minor adjustments. 0.1 mL of aspirin and SMCE at various concentrations (0.025 to 0.5 mg·mL^{-1}) were combined with 1 mL of BSA (bovine serum albumin 0.2%) solution prepared in Tris–HCl (pH 6.6). The mixture was incubated for 15 min at 37°C, and later was heated at 72°C in water bath for 5 min. After cooling , the supernatant was aspirated and the absorbance was read at 660 nm .

The *in vivo* studies

Animals

Mice (*Mus musculus*) and rats (*Rattus norvegicus*) aged two months and weighing (PS600.R2, Germany) between (25–30 and 150–200 g respectively) were given from the Institute Pasteur in Algiers. Before the research started, they were acclimated in an animal facility for two weeks under typical lab settings (25 ± 2°C, 12–hour dark/light cycle). The animals had access to tap water and were fed a typical commercial meal.

In vivo anti–inflammatory investigation

A rat model of carrageenan–induced paw edema was used to evaluate the inflammation inhibition properties of SMCE [24]. In this research, 24 female rats split up into four equal groups: the 1st group was used as the positive control and was given 20 mg·kg^{-1} of the reference medication indomethacin. The 2nd group was used as the negative control and was given tap water. The test groups receiving (150–300 mg·kg^{-1}) of SMCE are represented by the 3rd and 4th groups. In this research, animals had been fasting for 16 hours.

Xylene–induced edematous

The ear edema induced by Xylene method was used to assess topical anti–inflammatory properties. About 18 female mice split up into three equal groups. All three groups received topical administration. The 1st group received 0.5 mL distilled water as the negative control, the 2sd group received the 0.5 mg indomethacin as the positive control and the 3rd group received 2 mg of the ethanolic extract. Edema was induced in all mice by applying 0.03 mL of pure Xylene per ear. The same ear area was then treated with the same volume (0.03 mL) of indomethacin and SMCE, and then applied to the same ear surface, while the control group was given only Xylene.

In the oral anti–inflammatory activity method [25], the 24 female mice were split up into four groups, each of which had 6 mice. The group 1 received distilled water as considered negative control, the group 2 received the positive control (Indometacin, 50 mg) and the 3 and 4 groups received the 2 doses of SMCE (150–300 mg·kg^{-1}). After 1 h of treatment with positive and negative controls with both doses of SMCE, we applied 30 µL of Xylene to induce edema in all mice. After 1 h , 1 h:30 min and 2 h a digital caliper was used to measure the thickness of the ear for all groups, utilizing this formula:

$$I\,(\%) = \frac{(\Delta T - \Delta E)}{\Delta T} \times 100$$

ΔT represents a difference in ear edema thickness in the negative control, ΔE corresponds to the difference in the positive control or SMCE.

Evaluation of analgesic activity

The analgesic efficacy of the SMCE was assessed using the method outlined by [26] against pain induced by acetic acid. For this test, all groups received ethanolic extract and the standard orally: the positive control was given aspirin at concentration of 100 mg·kg^{-1}, the negative control was given distilled water, and both the 150-300 mg·kg^{-1} of SMCE were given as a single dose to the experimental group. After one hour, 10 mL·kg^{-1} of 0.6 % acetic acid was injected intraperitoneal to each mouse . In a cage take each mouse individually and count the number of twitches from time 0 to 30 min at 5 min intervals. The following formula was used to determine the pain inhibition percentage:

$$I\,(\%) = \frac{(C_{nc} - C_{tr})}{C_{nc}} \times 100$$

C_{nc} noted the negative control group's average and C_{tr} noted the mean number of twitches among groups that received different doses of aspirin and SMCE.

Statistical analysis

The results from the *in vitro* experiments were displayed as mean ± standard error of the mean, while the data from the *in vivo* trials were displayed as mean ± standard. The GraphPad Prism version 6 was utilized to assess the results using Dunnett's test and One–Way analysis.

RESULTS AND DISCUSSION

Total flavonoids and phenolics contents

Since ancient times, humans have utilized medicinal plants to treat various ailments, including inflammatory conditions. These plant's complex combinations of secondary metabolites, such as flavonoids and phenolic chemicals, are frequently responsible for these positive benefits [27]. A key challenge for scientists lies in identifying the specific chemical compounds responsible for the observed pharmacological activities [28]. In this investigation the quantitative analysis revealed that the ethanolic extract of the plant *S. minor* had high levels of total phenolics compounds, 623.33 ± 0.01 µgGAE·mg^{-1} extract and the total flavonoids present in the SMCE were 8.71 ± 0.04 µgQE·mg^{-1} of extract, which may justify about diversity in phenolic compounds characterized in the HPLC–DAD profile.

HPLC–DAD phytochemical analysis

HPLC–DAD phytochemical analysis showed that the ethanolic extract contains 17 different phenolic compounds (TABLE I and FIG. 1). The major compound present in the SMCE is methyl gallate with a concentration of 24574.54 µg·g^{-1}, followed by gallic acid 16778.74 µg·g^{-1}

Following HPLC–DAD, it is found the existence of seventeen phenolic compounds in *S. minor* aerial part amongst, the phenolic acids such as methyl gallate as a major compound, gallic acid, syringic acid, caffeic acid, ellagic acid, chlorogenic acid, ferulic acid and rutin.

TABLE I
HPLC–DAD phytochemical analysis of *Sanguisorba minor* ethanolic extract

	Area	Conc (µg·mL^{-1})	Conc (µg·g^{-1})
Gallic acid	5071.57	335.57	16778.74
Chlorogenic acid	2442.18	280.38	14018.97
Catechin	/	/	/
Methyl gallate	11049.80	491.49	24574.54
Caffeic acid	633.98	42.61	2130.44
Syringic acid	981.07	55.81	2790.34
Rutin	889.86	121.10	6055.03
Ellagic acid	272.96	18.79	939.44
Coumaric acid	17.68	0.52	25.94
Vanillin	78.37	2.46	122.98
Ferulic acid	1549.91	74.20	3709.97
Naringenin	44.55	3.46	172.86
Rosmarinic acid	14.12	1.25	62.37
Daidzein	5.28	0.28	13.96
Quercetin	11.70	0.78	39.11
Cinnamic acid	3.62	0.06	2.77
Kaempferol	15.89	0.94	46.84
Hesperetin	5.41	0.22	11.04

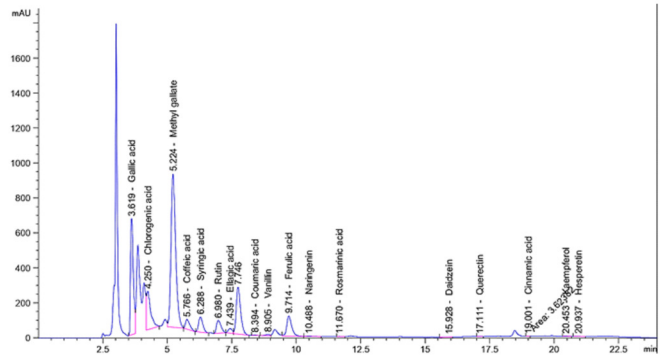

FIGURE 1. Chromatogram of *Sanguisorba minor* aerial part extract

Evaluation of antioxidant activity

As there is a multitude of factors that may influence the antioxidant activity of an extract, it is important to test more than one method to assess the extract antioxidant potential, to consider the different antioxidant pathways involved.

In this study antioxidant properties were assessed using several techniques, including 2,2–diphenyl–1–pycrilhydrazil (DPPH) assay, iron–chelating test, phosphomolybdate assay, and OH assay. Results shown in TABLE II were expressed as inhibition concentration IC_{50} and as $A_{0.50}$ for ferric reducing antioxidant power expressing the concentration that records an absorbance equal to 0.5.

Given the fact that the extract is rich in terms of phenolic compounds, its antioxidant ability was investigated in vitro as a first step towards understanding the eventual antioxidant, anti–inflammatory relationship of the plant. The findings showed a significant antioxidant activity with IC_{50} values varying in an interval from 11 ± 0.00 to 71 ± 0.00 µg·mL^{-1}, in different in vitro systems, including the DPPH assay, Iron–chelating activity, phosphomolybdate assay, OH assay and reducing power assay. This high antioxidant activity may be due to the existence of significant amounts of powerful phenolics compounds in the SMCE. Indeed, methyl gallate is a polyphenol naturally occurring secondary metabolite in many medicinal herbs and pharmacologically bioactive as a potent antioxidant compound [29] was the major compound in the extract. Another antioxidant phenolic acid characterized in the extract is chlorogenic acids (CGA), are common caffeoylquinic acid isomers (3–, 4–, and 5–CQA) that are well known because of its powerful antioxidant and capacity to scavenge radicals [30]. Regarding the phytochemical profile of the *S. minor* plant in previous study [31] they found fewer phenolic compounds than the results obtained in this new study ,which may be due to changes in climates or soil.

In vitro anti–inflammatory activity

The in vitro inhibition activity of SMCE was assessed using the bovine serum albumin product; the findings are shown in FIG. 2. At a concentration of 500 µg·mL^{-1} SMCE could prevent BSA denaturation with a maximum inhibition rate of 81.11%.

At 500 µg·mL^{-1}, acetylsalicylic acid is an nonsteroidal anti–inflammatory drug (NSAID), widely available and affordable globally, making it an excellent standard for comparison in experimental studies. It was utilized as positive control, reduced inflammation by 76.04%. The SMCE demonstrated a strong inhibitory effect of BSA denaturation.

The second phase in this study, was the evaluation of the anti–inflammatory properties of *S. minor* ethanolic extract. The anti–inflammatory impact of the SMCE was assessed using bovine serum albumin denaturation method. The results showed that the SMCE might decrease bovine serum albumin denaturation in a dose–dependent manner. The highly nominated bioactive compounds extracted from *S. minor*, phenols and flavonoids have a great anti–denaturant activity. This activity observed by SMCE was

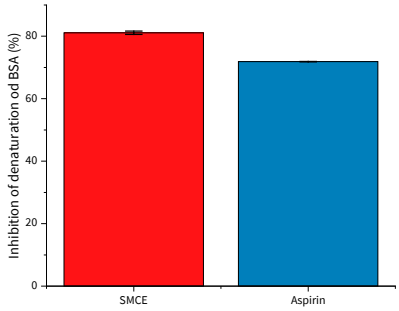

FIGURE 2. The *in vitro* inflammation inhibition activity of ethanolic extract of *Sanguisorba minor*. The data is shown as mean± standard deviation (n=3). ***P<0.001

TABLE II
In vitro* antioxidant activity of ethanolic extract of *Sanguisorba minor

	***In vitro* antioxidant estimation**				
Extract / standard	**DPPH assay**	**Iron–chelating assay**	**Phosphomolybdate**	**OH assay**	**FRAP assay**
			IC_{50} (µg·mL^{-1})		
SMCE	11.03 ± 0.00	23.77 ± 0.00	46.83 ± 0.00	63.93 ± 0.00	71.82 ± 0.00
BHT	8.81 ± 0.00	NE	NE	NE	16.67 ± 0.00
EDTA	NE	84.50 ± 0.00	NE	NE	NE
Ascorbic acid	NE	NE	21.20 ± 0.00	66.55 ± 0.00	NE

IC_{50}: sample concentration that inhibited 50% of the activity of free radicals, SMCE: ethanolic extract of *Sanguisorba minor,* BHT: butylated hydroxytoluene, EDTA: ethylene diamine tetra acetic, NE: not evaluated. Values are the mean of triplicates ± SD

highly to ferulic acid, caffeic acid, vanillin binding ability to humain serum albumin [32]. However, Liu *et al.* [33] showed that rutin and quercetin have a binding affinity towards bovine serum albumin.

In vivo anti–inflammatory activity

According to present research, SMCE significantly reduces inflammation at 150 mg·kg^{-1} in both carrageenan paw edema (TABLE III) and Xylene induced ear edema (FIG. 3). According to the data SMCE significantly and dose–dependently decreased the ear edema reaction caused by Xylene. The results showed that SMCE, at 150 mg·kg^{-1} decreased the carrageenan–induced paw edema impact by 87.23%.

TABLE III
Effect of ethanolic extract of *Sanguisorba minor* at different doses with indomethacin as positive control on paw edema induced by carrageenan

Groups	Inhibition (%) of inflammation			
	1h	2h	3h	4h
G	64.29	32.56	33.33	65.48
D1 G	24.52***	47.09**	65.7***	87.23***
D2 G	23.96***	38.95*	56.13***	57.65*

The results are expressed using the average of 6 rats ± SEM: ***P<0.001, **P<0.01, *P>0.05 compare with PG: positive group; D1 G: 150 mg·kg^{-1}; D2 G: 300 mg·kg^{-1}

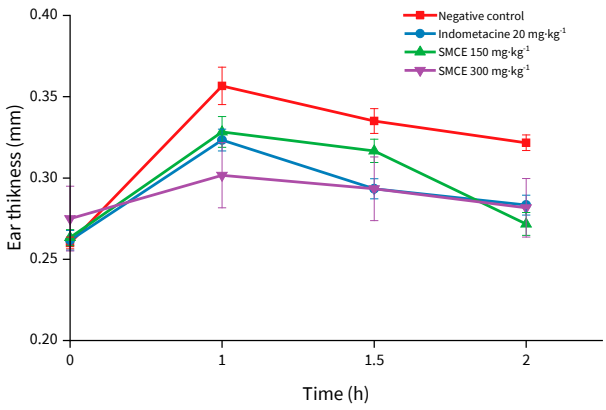

FIGURE 3. Investigation of anti–inflammatory properties by gavage of SMCE with ear edema induced by Xylene in albino mouse (n=6). SMCE was highly active P<0.05

The *in vivo* anti–inflammatory effectiveness of SMCE was investigated using two well–established models: ear edema induced by Xylene and paw edema induced by carrageenan [34]. During the first hour histamine and 5–hydroxytryptamine mediate the rat paw edema brought on by carrageenan, followed by sustained vascular permeability due to kinin release in the subsequent hour. Leukocyte migration into the inflamed site between 2 and 4 h is accompanied by the release of prostaglandins, which are believed to be the primary mediators during this phase. Prostaglandins are essential in fostering inflammation by inducing the formation of inflammatory exudates. The findings demonstrate that SMCE exhibited significant anti–inflammatory effects during the prostaglandin–mediated phase in both models, comparable to indomethacin.

Phlogistic agents that cause ear edema for example Xylene are susceptible to cyclooxygenase inhibitors. This model is commonly used to assess the efficacy of NSAIDs, which exert their anti–inflammatory effects by inhibiting cyclooxygenase and prostaglandin synthesis [35, 36]. Recently [37] summarized the multiple therapeutic functions of gallic acid using both in vivo and in vitro systems. Human cells are protected from the acute and chronic oxidative stress by gallic acid, which is known for its potent antioxidant and free radical scavenging properties , and is an excellent option as a therapeutic agent or dietary supplements [38, 39]. By neutralizing superoxide anion, preventing myeloperoxidase release and activity, and maybe influencing the accumulation of active NADH oxidase, gallic acid may delay the development of inflammation [40]. According to certain research gallic acid efficiently inhibits the synthesis of PGG–2, LPS–induced NO and interleukin–6(IL–6) release without having any harmful effects [41, 42].

Analgesic activity

The ethanolic extract of *Sanguisorba minor,* demonstrated a considerable dose–dependent analgesic capability against acetic acid induced abdominal construction in mice. The extract significantly (P<0.05) decreased abdominal constriction by 84.30% at 150 mg·kg^{-1} compared with acetylsalicylic acid as the control positive with value of 81.02% (FIG. 4).

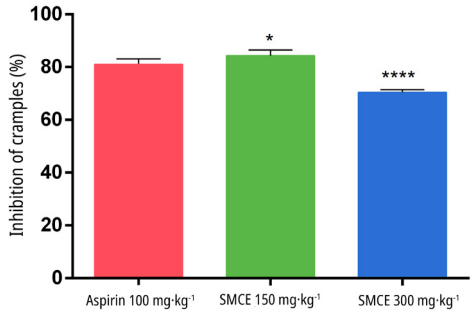

FIGURE 4. Effects of SMCE extract of *Sanguisorba minor* on mice (n=6) peripheral nociception. Mean ± SEM is used to express the results, and P<0.001 when compared with acetylsalicylic acid as standard

During the experiment the mice's acetic acid–induced abdominal contractions were considerably (P<0.001) and dose–dependently decreased by SMCE. These results suggests that the SMCE anti nociceptive action either directly suppresses the receptors for inflammatory chemicals or stops their endogenous manufacture. Swiss albino mice were used in a hot plate test to evaluate the analgesic impact of rutin, and the results confirmed that it had an analgesic effect [43]. Additionally it was verified that rutin has both central and peripheral antinociceptive properties, in Peripheral Nervous System, by reducing prostaglandin effects released in response to inflammation and in central nervous system, by directly stimulating opioid receptors at various CNS levels [44, 45]. These findings show that *S. minor* has potential as a source of anti–inflammatory chemicals and merit more research.

CONCLUSIONS

In present study, based on the results, the aerial part of *Sanguisorba minor* L. have proven to have antioxidant and anti–inflammatory activity. The antioxidant properties of the extract were demonstrated by different techniques such as DPPH, total antioxidant capacity, hydroxyl radical scavenging assay, ferrous ion chelating activity and ferric reducing antioxidant power. The extract showed a strong capacity to scavenge free radicals due to its high content of phenolic compounds. The traditional use of the plant *S. minor* to treat inflammatory diseases is proven by the results obtained in this study. This study brings attention to the beneficial substances found in *S. minor* plant, which possess antioxidant, anti–inflammatory, and analgesic activities. These compounds make *S. minor* a potential candidate for inclusion in functional food or pharmaceutical industries.

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