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Extraction and evaluation of antioxidant, anti-inflammatory and analgesic effects of *Scabiosa atropurpurea* L. extracts

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DEDICATION

AND

ACKNOWLEDGEMENTS

Dedication

*I dedicate this work to my dear **mother** and **father**, whose prayers have always been with me throughout this work. I ask Allah to bless them with a long life, good health, and endless happiness.*

*To my **sister** and **her daughter** and to my best **brothers***

*to the adorable lab engineers **Intissar**, **Meryem** and **Samira**; for their kindness, support and help.*

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PUBLICATION

AND

COMMUNICATION

Publication

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Communications

SEKHRI ZEGGAR H, KARBAB A, CHAREF N. Reducing power potential of methanolic extract from *Scabiosa atropurpurea.* 3rd International Conference on Innovative Academic Studies, Konya, Turkey. **2023**.

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SEKHRI ZEGGAR H, KARBAB A, CHAREF N. Total phenolic content and antioxidant and hemolytic activities of *Scabiosa atropurpurea.* 3rd Edition of the National Seminar on Interface Sciences: Chemistry-Biology, Souk-Ahras, Algeria. **2023**.

SEKHRI ZEGGAR H, KARBAB A, CHAREF N. Phenolic content and antioxidant activity of sub-fractions from *Scabiosa atropurpurea.* 1st National Seminar on Phyto biotechnology, Taref, Algeria. **2023**.

ABSTRACTS

ملخص:

*Scabiosa atropurpurea*هي عشبة علاجية تنمو في الجزائر معروفة بتركيبها الغني من المستقلبات الثانوية وأنشطتها البيولوجية المتنوعة. هدفت هذه الدراسة إلى تحديد مركباتها النشطة بيولوجيًا وتقييم خصائصها المضادة للأكسدة والمضادة للالتهابات والمضادة للسرطان، بالإضافة إلى دراسة سميتها الحادة. تم الحصول على المستخلص المائي (AqE) والميثانولي الخام (ME) من خلال عمليات الاستخلاص ، حيث بلغت نسبة العائد 9.06% و13.8% على التوالي. أكدت التحاليل الكيميائية النباتية الأولية على وجود البوليفينولات، والتربينويدات، والفلافونويدات، والصابونينات، والتانينات، والأنتراكينونات، والكينونات الحرة، والسكريات المختزلة في كلا المستخلصين، بينما كانت الكومارينات غائبة في ME. كشفت التحاليل الكمية أن ME يحتوي على أعلى مستويات من إجمالي البوليفينولات (114.13±0.92 ميكروغرام مكافىء حمض الغاليك/ميليغرام من المستخلص الجاف) والفلافونويدات (100.57±0.93 ميكروغرام مكافىء الكيرسيتين / ميليغرام من المستخلص الجاف)، في حين سجل AqE أعلى محتوى من العفص المكثف (41.04±0.64 ميكروغرام مكافىء الكاتيشين/ ميليغرام من المستخلص الجاف).

حددت تقنية UPLC- MS/MSوجود 34 مركبًا نشطًا في كلا المستخلصين تحت أوضاع الأيونات السالبة والموجبة. تضمنت المركبات المكتشفة بشكل أساسي الأحماض الفينولية والفلافونويدات، تلتها التربينويدات الثلاثية، التربينويدات الأحادية، التانينات، الصابونينات، الإيريدويدات، الأحماض الدهنية، وغيرها. تم تقييم النشاط المضاد للأكسدة في المختبر باستخدام عدة اختبارات، بما في ذلك مسح جذور DPPH والهيدروكسيل، وقدرة الارتباط بأيونات الحديد الثنائية، واختبار القدرة الاختزالية، وطريقة الفوسفوموليبدات. تم تقييم التأثير المضاد للالتهاب من خلال نموذج تثبيط تحلل البروتين، حيث أظهرت المستخلصات فعالية طردية. أما الدراسة التجريبية المضادة للالتهابات في الجسم الحي، فقد أظهرت انخفاضًا ملحوظًا في الوذمة في الأذن والكف الخلفي بطريقة طردية. بالإضافة إلى ذلك، أظهرت المستخلصات تأثيرًا مسكنًا للألم من خلال تقليل عدد التلويّات في الفئران. أظهرت اختبارات السمية الخلوية ضد خلايا سرطان البروستاتا أن AqE كان له التأثير الأقوى المضاد للتكاثر، مع IC50 بلغ 195.12 ± 3.87 ميكروغرام/مل، يليه ± 8.94 ME (IC50 =441.95ميكروغرام/مل).كما أظهرت دراسة السمية الحادة عدم وجود وفيات او أي علامات تدل على السمية .بشكل عام، تتميز *Scabiosa atropurpurea* بغناها بالمستقلبات الثانوية وتظهر خصائص قوية مضادة للأكسدة، مضادة للالتهابات، ومضادة للأورام، مما يجعلها مرشحًا واعدًا في مجال تطوير الأدوية.

الكلمات المفتاحية : *Scabiosa atropurpurea*, المركبات النشطة بيولوجيًا، مضاد الأكسدة، النشاط المضاد للالتهابات، التأثير المسكن، السمية

Abstract

Scabiosa atropurpurea is a therapeutic herb growing in Algeria, and displayed a variety of biological effects due to its abundance on different secondary metabolites. This investigation focused on the determination of bioactive compounds and evaluation of the antioxidant, anti-inflammatory, anticancer capacities and the acute toxicity of the whole aerial part from *S. atropurpurea*. The extraction procedures conducted to obtain the aqueous (AqE) and methanolic (ME) extracts with yields of 9.06% and 13.8%, respectively. The initial phytochemical analysis confirmed the presence of polyphenols, terpenoids, flavonoids, saponins, tannins, anthraquinones, free quinones and reducing sugars in both extracts, whereas coumarins were absence in the ME. The quantitative analysis showed that ME exhibited the largest total polyphenols with 114.13 ± 0.92 µg gallic acid equivalent /mg dry extract and flavonoids with 100.57 ± 0.93 µg quercetin equivalent /mg dry extract. However, the important condensed tannins tenor was detected in AqE with 41.04 ± 0.64 µg catechin equivalent /mg dry extract. Furthermore, the UPLC-MS/MS analysis characterized 34 bioactive components in both extracts under negative and positive ion modes. The recognized compounds primarily included phenolic acids and flavonoids, followed by triterpenoids, sesquiterpenoids, tannins, saponins, iridoids, fatty acids, and others. The tested extracts presented *in vitro* antioxidant capabilities through different protocols (DPPH and hydroxyl radicals scavenging effects, ferrous iron chelating, reducing power and phosphomolybdate assay). The anti-inflammatory potential was assessed *in vitro* using protein denaturation reduction model and both extracts exhibited a dose related potential. The findings from the *in vivo* anti-inflammatory activity highlighted a significant reduction in ear and hind paw edematous in dose dependent manner. Moreover, the extracts showed a substantial analgesic effect by decreasing writhing in experiments mice. The cytotoxicity was determined against prostate cancer cells and AqE demonstrated the highest cytotoxic and antiproliferative impact on tumor cells with IC_{50} of 195.12 ± 3.87 µg/mL followed by ME with 441.95 ± 8.94 µg/mL. the oral acute toxicity study showed that no death or toxic manifestations were detected. On the whole, *Scabiosa atropurpurea* is characterized by an abundance of secondary metabolites and can serve as a natural antioxidant, anti-inflammatory, and antitumor agent, making it a valuable resource for the drug development field.

Keywords: *Scabiosa atropurpurea,* bioactive compounds, antioxidant, anti-inflammatory activity, analgesic effect, toxicity.

Résumé

Scabiosa atropurpurea est une plante thérapeutique d'Algérie, reconnue pour sa richesse en métabolites secondaires et ses diverses activités biologiques. Cette étude visait à identifier ses composés bioactifs et à évaluer ses propriétés antioxydantes, anti-inflammatoires, anticancéreuses ainsi que sa toxicité aiguë. Les extraits aqueux (AqE) et méthanolique (ME) ont été obtenus par des procédures d'extraction, avec des rendements de 9,06 % et 13,8 %, respectivement. L'analyse phytochimique préliminaire a confirmé la présence de polyphénols, terpénoïdes, flavonoïdes, saponines, tanins, anthraquinones, quinones libres et sucres réducteurs dans les deux extraits, tandis que les coumarines étaient absentes dans le ME. L'analyse quantitative a révélé que le ME contenait la plus grande quantité de polyphénols totaux (114,13 ± 0,92 µg équivalent d'acide gallique /mg extrait sec) et de flavonoïdes (100,57 ± 0,93 µg équivalent quercétine/mg extrait sec). En revanche, l'AqE présentait la plus forte teneur en tanins condensés (41,04 ± 0,64 µg équivalent catéchine /mg extrait sec). L'analyse UPLC-MS/MS a permis d'identifier 34 composants bioactifs dans les deux extraits sous les modes ioniques négatif et positif. Les composés détectés comprenaient principalement des acides phénoliques et des flavonoïdes, suivis de triterpénoïdes, sesquiterpénoïdes, tanins, saponines, iridoïdes, acides gras et autres. L'activité antioxydante in vitro a été évaluée à l'aide de plusieurs tests, notamment la capture des radicaux DPPH et hydroxyles, la chélation du fer ferreux, le pouvoir réducteur et la méthode au phosphomolybdate. Le potentiel anti-inflammatoire a été évalué par un modèle d'inhibition de la dénaturation des protéines, où les deux extraits ont montré une efficacité dépendante de la dose. L'activité anti-inflammatoire in vivo a révélé une réduction significative des œdèmes de l'oreille et de la patte de manière dose-dépendante. De plus, les extraits ont montré un effet analgésique notable en réduisant les contorsions chez les souris. Les tests de cytotoxicité sur les cellules du cancer de la prostate ont montré que l'AqE avait l'effet antiprolifératif le plus puissant, avec un IC50 de 195,12 ± 3,87 µg/mL, suivi du ME (IC50 = 441,95 ± 8,94 µg/mL). L'étude de toxicité aiguë orale n'a révélé aucun signe de toxicité ou de mortalité. Dans l'ensemble, *Scabiosa atropurpurea* est riche en métabolites secondaires et présente des propriétés antioxydantes, anti-inflammatoires et antitumorales significatives, en faisant une candidate prometteuse pour le développement de médicaments.

Mots-clés : *Scabiosa atropurpurea,* composés bioactifs, antioxydant, activité anti-inflammatoire, effet analgésique, toxicité.

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ABBREVIATIONS

ABBREVIATIONS

AA	Arachidonic Acid
AqE	Aqueous Extract
Bax	Bcl-2-Associated X Protein
Bcl-2	B-cell Lymphoma 2
BHT	Butylated Hydroxytoluene
Caco-2	Human Epithelial Colorectal Adenocarcinoma
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
ME	Methanolic Extract
CT	Condensed Tannins
CTC	Condensed Tannins Content
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EDTA	Ethylenediaminetetraacetic Acid
EAA	Equivalent Acid Ascorbic
G2/M	Gap 2/Mitosis
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic Acid
IC_{50}	50% Inhibitory Concentration
IKK	κ Kinase Inhibitor
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
INF-γ	Interferon-Gamma
iNOS	Inducible Nitric Oxide Synthase
JNK	c-Jun N-terminal Kinase
LD_{50}	50% Lethal Dose
MAPK	Mitogen-Activated Protein Kinase
MDA-MB-231	Human Breast Cancer Cell Line
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NF-κB	Nuclear Factor Kappa-B
NSAIDs	Nonsteroidal Anti-Inflammatory Drugs
OECD	Organization for Economic Co-operation and Development
PC-3	Prostate Cancer Cell Line
PGE2	Prostaglandin E2
PLA2	Phospholipase A2
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPMI-1640	Roswell Park Memorial Institute-1640
SD	Standard Deviation
SEM	Standard Error of Mean

TAE	Tannic Acid Equivalent
TCA	Trichloroacetic Acid
TFC	Total Flavonoids Content
TNF	Tumor Necrosis Factor
TPC	Total Phenolic Content
U266	Human Multiple Myeloma Cell Line
UPLC-ESI-MS/MS	Ultra Performance Liquid Chromatography – Electrospray Ionization – Tandem Mass Spectrometry
UPLC-MS/MS	Ultra Performance Liquid Chromatography – Tandem Mass Spectrometry

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RESULTS AND DISCUSSION

INTRODUCTION

Introduction

Maintaining good health depends on balanced metabolic processes, stable homeostasis, and efficient cellular repair mechanisms. Free oxygen radicals are essential for physiological processes but may also induce oxidative stress, associated with inflammation and other illnesses. Recent research demonstrates that oxidative stress is vital in the initiation and persistence of inflammatory reaction, hence leading to the pathogenesis of various chronic diseases, including cardiovascular disorders, diabetes, and cancer. Oxidants influence all phases of the inflammatory process, encompassing the secretion of chemicals that function as innate danger sign and the stimulation of signaling systems that initiate the adaptive cell's response to these signs.

Synthetic drugs, while effective, come with drawbacks such as hypersensitivity, and immunosuppression. Additionally, synthetic antioxidants have been associated with liver damage and potential cancer risks. The prolonged use of synthetic anti-inflammatory medicines can also cause harmful effects like ulcer and renal disorders. Also, the usage of chemotherapeutic agents for treating cancer showed a harmful consequences and high toxicity. Given these concerns, there is a growing need for safer, natural alternatives to minimize adverse health effects while maintaining therapeutic efficacy.

Medicinal plants possess a rich historical significance in conventional healthcare as sources of therapeutic agents. They have been essential in the identification and development of bioactive chemicals exhibiting diverse pharmaceutical benefits, comprising anti-inflammatory, anticancer, antioxidant and antibacterial. In addition to their considerable therapeutic potential, bioactive substances derived from medicinal herbs are frequently favored for their natural origin, which is regarded as more secure and ecologically sustainable than synthetic pharmaceuticals. Furthermore, the ancient traditions and practices

linked to medicinal plants have played a crucial role in the discovering of innovative bioactive compounds, thereby establishing a valuable basis for contemporary drug discovery and development.

Scabiosa is a part of the Caprifoliaceae family with approximately 100 species. The vast majority of Scabiosa genera exist in the Mediterranean region, comprising twelve species growing in Algeria. The richness of Scabiosa species in secondary metabolites such as iridoids, phenolic acids, flavonoids, saponins and triterpenoids giving it a pharmacological effectiveness. *Scabiosa atropurpurea* L. is an annual plant grows in Asia, Europe and southern Africa, known for its bluish-lilac flowers which enhance its appeal as an ornamental plant. However, it is recognized by its traditional use for treating several health problems and have been studied for multiple biological activities.

Herein, the current investigation seeks to determinate the phytochemical profiling and to evaluate the biological capabilities as well as the toxicity of *Scabiosa atropurpurea* aerial part; a medicinal herb from the Algerian flora. The current study was carried out with the followed targets:

- ✓ Extraction procedures, qualitative and quantitative determination of phytochemicals and UPLC-MS/MS analysis.
- ✓ In vitro evaluation of antioxidant and anti-arthritic activities.
- ✓ In vivo evaluation of anti-inflammatory and analgesic potentials.
- ✓ In vitro evaluation of cytotoxicity against cancer cell lines
- ✓ In vivo evaluation of acute oral toxicity

REVIEW

OF

LITERATURE

1. Herbal products

1.1. Definition

A remedial herb is any plant that possesses therapeutic compounds inside its structure, which are beneficial for medicinal purposes. Medicinal plants contain multiple active compounds that assist in addressing diverse health issues. Plants synthesize phytochemicals for their survival and defense; some encourage growth and development, while others serve as poisons to defend against parasites and pathogens (Preethi *et al*., 2011). Herbal compounds are divided as primary and secondary constituents. Primary compounds, including chlorophyll, lipids, carbohydrates, proteins, and nucleotides, are essential for processes such as photosynthesis, inheritance, and growth. Secondary compounds include terpenoids, glycosides, alkaloids and phenols, safeguard plants against herbivores, microbes, environmental extremes, and physiological stress (Begum, 2017).

1.2. Herbal products and synthetics drugs

Herbal medications are increasingly favored over synthetic drugs owing to their accessibility, cost-effectiveness, and reduced adverse effects on individuals and the environment. In contrast to synthetic medications that depend on singular chemical formulations, herbal remedies leverage the synergistic effects of many biochemical substances, so augmenting their efficacy (Okigbo *et al*., 2009). The increasing global population, insufficient health hygiene, and the emergence of resistant pathogens have made current medications inadequate in numerous situations. A variety of herbal medicines are employed to address infectious disorders, and even toxic herbs can offer advantages when utilized cautiously under particular circumstances. The rising demand for herbal pharmaceuticals has intensified the necessity to identify plants with distinctive therapeutic characteristics (Mann *et al*., 2008).

1.3. Phenolic compounds

Phenolic compounds comprise more than 8,000 identified chemicals, and this library is continuously expanding. They are categorized based on the chemical skeleton into phenolic acids, flavonoids, tannins and stilbenes (Alu'datt *et al*., 2017). These molecules generally possess two fundamental structures: the C6–C3–C6 cycle form, observed in condensed tannins, some phenolic acids and flavonoids; and the C6–C1 format, found in hydrolyzed tannins and specific phenolic acids (Zhang *et al*., 2022).

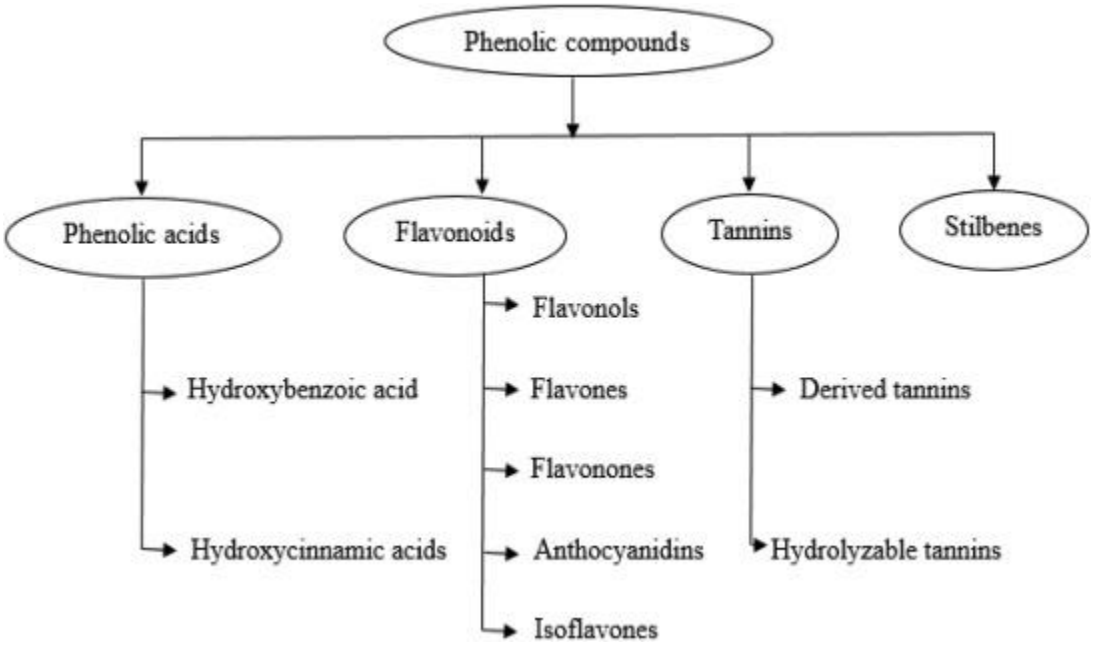

Figure 1: Main classes of phenolic compounds (Alara *et al*., 2021).

1.3.1. Phenolic acids

Phenolic acids constitute a significant category of phenolic chemicals in plants, existing in both free and bonded structures. Phenolic acids categorized into 2 classes: hydroxybenzoic acids, characterized by a C6–C1 form, which comprise vanillic, gallic, syringic and protocatechuic acids and Hydroxycinnamic acids which are aromatic molecules characterized by a 3-carbon lateral chain (C6–C3), which includes coumaric, ferulic, sinapic and caffeic acids (Zhang et al., 2016).

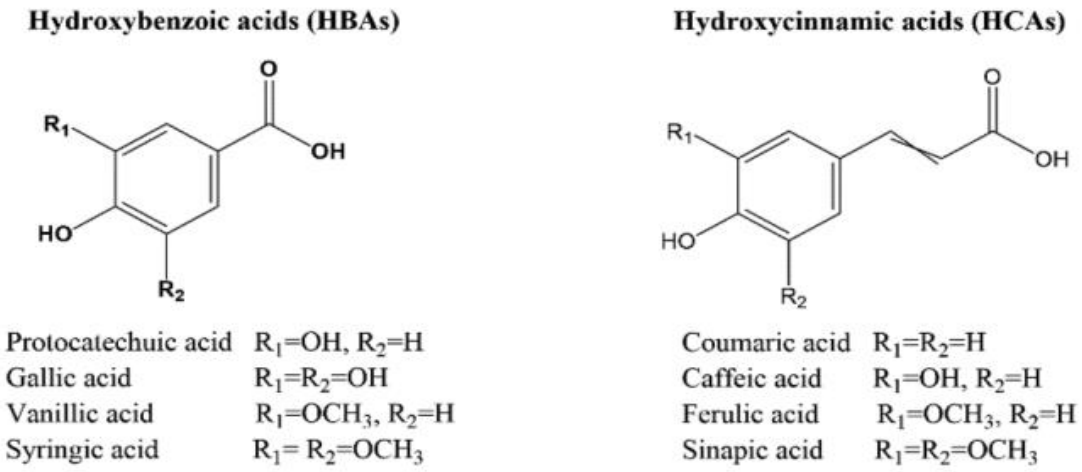

Figure 2: Main structure of phenolic acids groups (Zhang *et al*., 2022).

1.3.2. Flavonoids

Flavonoids are the biggest category of phenolic compounds, comprising over 6000 types. In higher plants, flavonoids participate in floral coloration, ultraviolet filtration, symbiotic interactions with prokaryotes, defense against pathogenic infections and insect herbivory (Galeotti *et al*., 2008). They are benzopyran derivatives, comprising a 15-carbon fundamental structure that includes two 6-carbon rings connected by a 3-carbon structure; functioning as the third ring. Changes to this essential flavonoid architecture provide several kinds of flavonoids, including flavones, flavonols, isoflavones, flavanones, flavan-3-ol and anthocyanidins (Dimitrios, 2006).

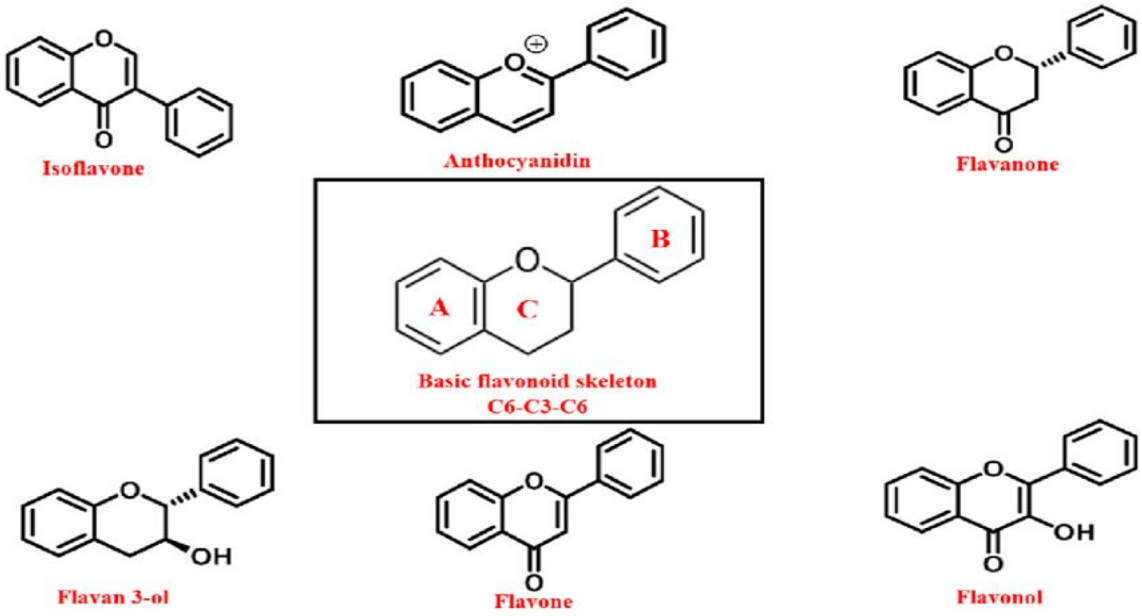

Figure 3: Basic chemical architecture of flavonoids and their sub-division (Bag *et al*., 2023).

1.3.3. Tannins

Tannins are a category of phenolic chemicals generally exist in plants. Tannins are classified into two categories: hydrolysable and condensed. Hydrolysable tannins are diversified polymers composed of phenolic acids, particularly simple sugars and gallic acid. While, condensed tannins are substances created by an association of flavonoid units. They are prevalent components of arboreal flora. Condensed tannins are frequently degraded to anthocyanidins through pretreatment using powerful acids, so referred to as "proanthocyanidins". (Chai *et al*., 2018).

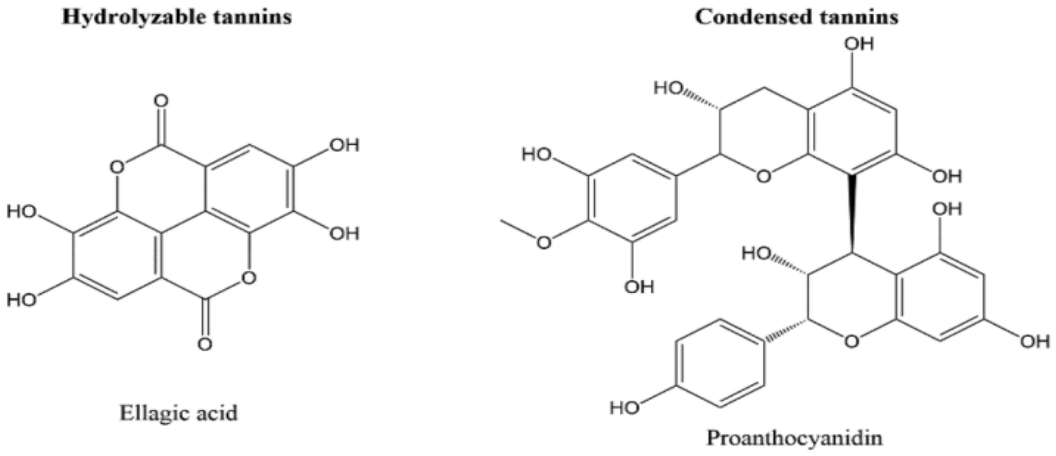

Figure 4: Representative structures of tannins classes (Zhang *et al*., 2022).

2. Phenolics and biological activities

Phenolic substances are recognized for their broad range of biological effects, including antibacterial, anti-hepatotoxic, anti-mutagenic, antiviral, anti-cancer, anti-inflammatory, anti-ulcer, antioxidant abilities and immune system stimulation (Ali-Shtayeh and Jamous, 2011).

2.1. Antioxidant activity

2.1.1. Free radicals and oxidative stress

Free radicals are unstable and reactive ions or particles possessing a number of uncoupled valency electrons in their exterior orbits, existing for just a brief moment (Liu, 2019). They play a vital role in biological reactions, involving in multiple physiological conditions, and include reactive oxygen and nitrogen species (ROS/RNS) that can be both beneficial and harmful (Hassan *et al.,* 2024). Free radicals accumulate in the body due to metabolic processes and external sources like x-rays, air pollution, chemicals, pesticides, radiation, and tobacco. Internal sources include peroxisomes, mitochondria, and the endoplasmic reticulum (Tumilaar *et al.,* 2024). ROS, often linked to tissue injury, disrupt the balance between their production and antioxidant defenses during infections, leading to oxidative stress (Hassan *et al.,* 2024). Oxidative stress is represented as the inequality between cellular antioxidants and

the reactive free radicals. Excessive ROS create oxidative stress as cellular antioxidant systems are insufficient to neutralize them. This stress may trigger molecular harm to nucleic acids, proteins and lipids, causing detrimental health consequences (Briyal *et al*., 2023). Cellular damage from oxidative stress can result in apoptosis or necrosis and it is linked with several illnesses (Teleanu *et al.*, 2022).

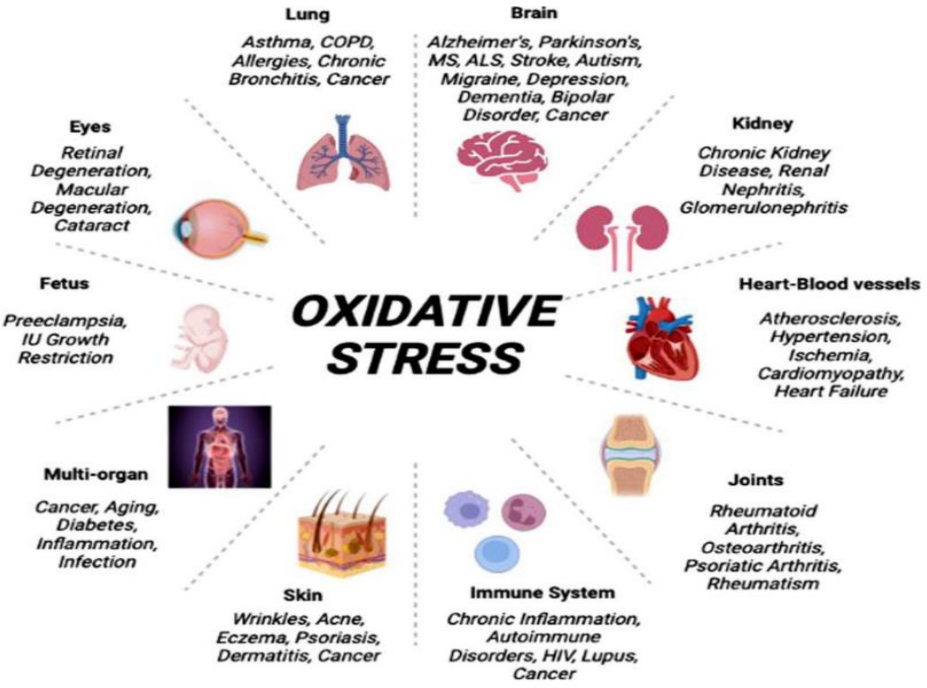

Figure 5: Oxidative stress related diseases (Hassan *et al*., 2024).

2.1.2. Antioxidants

Antioxidants are chemical compounds that can retard or avoid oxidation, often present in lowest concentrations. The human organism has evolved intricate antioxidant mechanisms that may function cooperatively to safeguard cells and organs against free radicals, needing a rapid response to prevent damage to biomolecules (El-Lateef *et al*., 2023; Hunyadi, 2019). Endogenous antioxidants are categorized as enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (glutamate, Coenzyme Q10, uric acid and others) molecules (Ighodaro and Akinloye, 2018). Since endogenous antioxidants

incapable to protect the human organism from ROS, exogenous antioxidants are necessary. The predominant external antioxidants are from natural origin, that includes herbs (phenolic acids, flavonoids, coumarins, and vitamins) and mineral (Zn, Mn, and Se) (Flieger *et al.,* 2021).

In most cases, antioxidant defense includes various mechanisms such as the delay or inhibition of free radical production, scavenging free radicals or converting them into less toxic molecules, postponing the creation of secondary toxic substances, obstructing chain propagation reactions, chelating metal ions, and enhancing the endogenous antioxidant protective process via synergistic interactions between different antioxidants (Losada-Barreiro *et al.,* 2022).

2.1.3. Polyphenols as antioxidants

Natural antioxidants boost endogenous antioxidant defenses versus ROS and preserve the appropriate balancing via removing these reactive species. Polyphenols are known as potent antioxidants; they can modulate internal processes and defend the body against diseases associated with oxidative damage. The detection of bioactivity should be completely evaluated, as antioxidant capacity is regulated by chemical structures notably the quantity and the positioning of the hydroxyl groups, stability, and bioavailability (Adwas *et al*., 2019).

The antioxidant properties of polyphenols are linked to multiple actions: hydrogen atom transfer, wherein Polyphenols neutralize free radicals by cleaving the O–H link to facilitate hydrogen transfer; single electron transfer, where polyphenols serve as donors of electrons to free radicals; and transition metal chelation, in which they bind with metal ions to form stable complexes. Furthermore, polyphenols can serve as a substrate for radicals, including hydroxyl and superoxide. These pathways constitute the ability of polyphenols to safeguard the human organism against free radical assaults (Lang *et al*., 2024; Adwas *et al.,* 2019). The antioxidant capabilities of flavonoids are affected by the quantity and the placement of

hydroxy radicals of catechol B-ring and their arrangement on the pyran C-ring (D'Amelia *et al.,* 2018). The mechanisms involve the chelation of trace metals (like quercetin, which possesses iron-chelating and regulating characteristics), or the blocking enzymes which contribute to free radical generation, including NADH oxidase, xanthine oxidase, glutathione S-transferase and mitochondrial succinoxidase. Additionally, they can upregulate antioxidant enzymes with radical scavenging capabilities (Dias *et al.,* 2021).

2.2. Anti-inflammatory activity

2.2.1. Inflammation

Inflammation is an organism's innate reaction to tissue damage resulting from injury, infection, or contaminants. It induces cellular alterations and immunological responses that facilitate tissue regeneration and stimulate growth at the injured site. Inflammation is categorized as acute and chronic forms. Acute inflammation arises initially and disappears upon the removal of the causative factor. When the causative factor tolerates or regulatory processes falter, acute inflammation advances to chronic inflammation. Chronic inflammation is a critical contributor to the creation of numerous inflammatory disorders (Singh *et al*., 2019). The physiological mechanisms underlying the inflammatory response are universal with typical indications of inflammation manifest as augmented blood flow, vasodilation, increased cellular metabolism, fluid extravasation, secretion of soluble mediators and cellular infiltration. In responding to the inflammatory substances, cellular membranes stimulate phospholipase A2, leading to the liberation of arachidonic acid and inflammatory chemicals, including serotonin, cytokines, prostaglandins, leukotrienes and histamine, which enhance permeability of vessels and promote the flow of leukocyte to the inflammation place (Sá *et al*., 2013). The arachidonic acid pathway is crucial in inducing and propagating inflammation in the respiratory, cardiovascular, and nephro systems,

particularly its metabolites which are linked to various health conditions (Figure 7) (Aradhyula *et al.,* 2024).

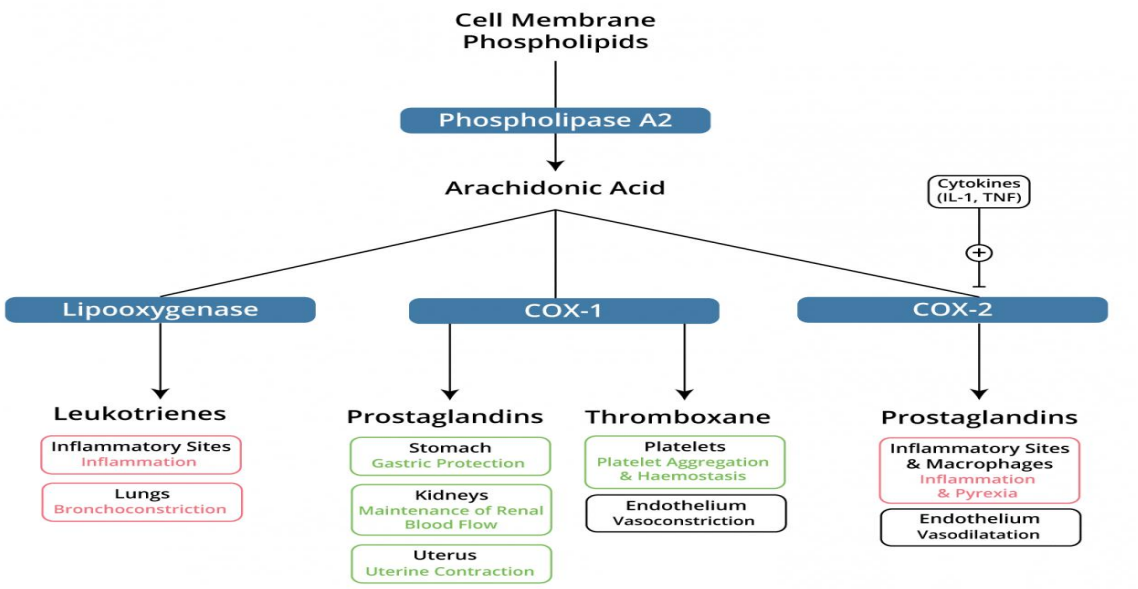

Figure 6: Overview of the arachidonic acid pathway

2.2.2. Anti-inflammatory substances

The mostly common treatments for inflammatory diseases are nonsteroidal anti-inflammatory medications (NSAIDs) and corticosteroids. Corticosteroids are extensively utilized for autoimmune, allergy, and chronic inflammatory disorders. They function by attenuating inflammation through processes such as limiting leukocyte migration, regulating cellular activities, and decreasing humoral substances including prostaglandins and cytokines (Lim and Bolster, 2019). NSAIDs relieve inflammation, temperature and pain through inhibition of COX-1 and COX-2 enzymes within the arachidonic acid metabolic process. NSAIDs may induce adverse consequences, including ulcers, renal complications, and cardiovascular concerns. However, corticosteroids cause lipodystrophy characterized by weight gain, neuropsychiatric effects, dermatological issues, and glucocorticoid-induced osteoporosis (Burayk *et al*., 2022).

Wherefore, the increased harmful impacts of synthetic medicines require their replacement with plant-based treatments that provide minimal risks. Medicinal herbs' extracts are considered globally as effective conventional treatments for anti-inflammatory compounds (Gonfa *et al*., 2023). They serve as abundant sources of phytochemicals utilized in herbal therapy and provide an ideal basis for the development of novel anti-inflammatory medications. Additionally, isolated chemicals from medicinal plant extracts have been documented to possess efficacious anti-inflammatory applications (Yang *et al*., 2019).

2.2.3. Polyphenols as anti-inflammatory molecules

Polyphenols are recognized to have anti-inflammatory benefits. The alleviation of inflammatory stress through the augmentation of systemic mediators, chemokines, and cytokines, promoting infiltration to resolve inflammation and restore tissue coherence is regarded as a mode of polyphenols activity (Dragos *et al.,* 2017, Gonfa *et al.,* 2023).

Flavonoids can inhibit the function of regulating enzymes (phosphodiesterase and protein kinases), transcription elements linked to the regulation of substances involved in inflammation, and control the activities of immune cells (restricting cell stimulation, maturing, signaling transduction, and secreting systems) during an inflammatory process (Dias *et al*., 2021). Table 1 establishes the anti-inflammatory processes of some selected phytochemicals (Jamtsho *et al.,* 2024, Gonfa *et al.,* 2024).

Table 1: Anti-inflammatory mechanisms of some selected bioactive compounds.

Class of compound	Isolated compound	Anti-inflammatory activity
Phenolic	Eugenol and 2-Methoxy-4-vinylphenol	Reducing prostaglandins' formation
Tannins	Tannic acid	Inhibition of formation of the proinflammatory cytokines (IL-6, IL-1, IL-18, PGE2, COX-2, and TNF) and enhance their mRNA transcription.
Stilbene	Resveratrol	Inhibition of the production and liberation of pro-inflammatory elements, changing of eicosanoid creation, and restricting activated immune cells.
Glycoside	Quercimeritrin	Suppressing of the formation of iNOS and NO in lipopolysaccharide-stimulated RAW 264.7 cells.
Flavonoids	Hispidulin	Suppressing the formation of PGE2 and expressions of COX-2 and iNOS by restricting NF-κB DNA-binding action and JNK path.
	Apigenin	Inhibition of TNF, IL-1β and IL-6 synthesis in lipopolysaccharide triggered murine BV2 microglia cells.
	Quercetin	Inhibition of eicosanoids enzymes like 5-LOX and COX-2 from arachidonic acid.

IL-6: Interleukin-6, IL-1β: Interleukin-1 beta, INF-γ: Interferon-gamma, TNF: Tumor necrosis factor, COX-2: cyclooxygenase-2, PGE2: prostaglandin E2, NO: Nitric oxide, iNOS: inducible nitric oxide synthase and JNK: the c-Jun N-terminal Kinase.

3. Scabiosa genus

Scabiosa, named after its historical application in treating scabies, is currently classified under the Caprifoliaceae family (Manning *et al.,* 2014). The genus Scabiosa L. is regarded as an extensive and taxonomically intricate group, comprising over one hundred species globally (Carlson *et al*., 2012). Scabiosa species are utilized in the food sector, cosmetics, and mostly for medicinal applications. From an ethnomedicinal perspective, these plants are utilized to cure various symptoms such as respiratory problems, digestive issues and reproductive disorders (Hlila *et al.,*2013). Scabiosa genera serve as herbal remedies, and phytochemical researches demonstrated their capacity to generate noteworthy bioactive substances, certain of them have shown value as agents of therapy (Pinto *et al*.,2018).

3.1. *Scabiosa atropurpurea* L

Scabiosa atropurpurea L is an annual herb found in Asia, Europe, southern Africa and the Mediterranean (Hrichi *et al*., 2020). This genus is referred to as Ambarina in North Peru, Escabiosa on the Iberian Peninsula and Mor uyuzotu or Seytanotu in Turkey (Erarslan and Yasil, 2018). *S. atropurpurea* produces basal leaf clusters and possesses stems with leaves, reaching a height of 20-60 cm and can be identifiable by its distinctive bluish-lilac flowers and unusual fruit morphology (Bussmann and Glenn, 2010) (Figure 1).

Figure7: *Scabiosa atropurpurea* L. appearance.

3.1.1. Taxonomy

Scabiosa atropurpurea is classified as follow:

- ✓ **Kingdom:** Plantae
  - ✓ **Phylum:** Streptophyta
    - ✓ **Class:** Equisetopsida
      - ✓**Subclass:** Magnoliidae
        - ✓ **Order:** Dipsacales
          - ✓ **Family:** Caprifoliaceae
            - ✓ **Genus:** Scabiosa
              - ✓ **Species:** *Scabiosa atropurpurea* L

3.1.2. Traditional usage and biological activities

S. atropurpurea L. is an herbal remedy recognized for its therapeutic effects related to its substantial phytochemical profile (Ben jalloul *et al*., 2022). In north Peru, the aerial components were employed for menstruation management, while in Iberia, they serve as a veterinarian's diuretics (Hrichi *et al*., 2020). An infusion of *S. atropurpurea* L. flowers is

applied topically for acne therapy and consumed for rubeola, measles, and scarlet fever in the Iberian Peninsula (Bonat *et al*.,1999, Polat *et al*., 2010).

Biological investigations on *S. atropurpurea* extracts revealed numerous pharmacological effects, including antioxidants, hepatoprotective, hypoglycemic (Elhawary *et al*., 2011), antipyretic, anti-inflammatory, antibacterial and analgesic (Marhuenda-Requena *et al*., 1987; Saenz-Rodrigues *et al*.,1987). Anti-microbial and antifungal activities were also demonstrated (Pinto *et al*., 2018).

3.1.3. Chemical composition

Previous phytochemical analyses of *S. atropurpurea* identified the existence of secondary metabolites, predominantly terpenoids, iridoids, phenolic acids and flavonoids. Table 2 summarized some outcomes of the different studies.

Table 2: Some secondary metabolites identified from *S. atropurpurea.*

N^0	Compound Name	Identification	References
1	Maslinic acid-pentosyl-rhamnosyl-glucoside	LC-MS/MS	(Ben toumia *et al*., 2020)
2	Oleanolic acid-pentosyl-rhamnosyl-glucosyl-glucosyl-di-glucoside	LC-MS/MS	(Ben toumia *et al*., 2020)
3	Oleanolic acid-pentosyl-rhamnosyl-pentosyl-glucosyl-di-glucoside	LC-MS/MS	(Ben toumia *et al*., 2020)
4	Atropurpurin A	LC-MS	(Kilinc *et al*., 2023)
5	Atropurpurin B	LC-MS	(Kilinc *et al.,* 2023)
6	Loganic acid	NMR and HR-ESI-MS	(Polat *et al*., 2010)
7	Loganin	NMR and HR-ESI-MS	(Polat *et al*., 2010)
8	3-O-caffeoylquinic acid methyl ester	UV, EI/MS, ^{1}H and ^{13}C NMR	(Elhawary *et al*., 2011)
9	Caffeic acid	HPLC-PDA-ESI/MS	(Hrichi *et al.,* 2020)
10	Chlorogenic acid	HPLC-PDA-ESI/MS	(Hrichi *et al.,* 2020)
11	Luteolin 7-O-β-D-glucoside	HPLC-PDA-ESI/MS	(Hrichi *et al*., 2020)
12	Quercimeritrin	HPLC-PDA-ESI/MS	(Hrichi *et al*., 2020)

LC-MS/MS: Liquid Chromatography – Tandem Mass Spectrometry, NMR: Nuclear Magnetic Resonance, HPLC-PDA-ESI/MS: High-Performance Liquid Chromatography -Photodiode Array Detector
Electrospray Ionization – Mass Spectrometry, HR-ESI-MS: High-Resolution Electrospray Ionization Mass Spectrometry.

MATERIALS

AND

METHODS

1. Materials

1.1. Plant material collection

The plant specimens obtained from the Chaabia district of Ouricia, Setif, which is situated in the north-east of Algeria (36° 17' 1.14" N and 5° 24' 34.43"E), within the flowering and fruiting months of May to July 2021. The plant identified by Pr. Laouer Hocine., a botanist in the Laboratory of Valorization of Natural Biological Resources, University Ferhat Abbas Setif 1, Algeria, and recognized using a voucher specimen labeled as (020/DBEV/UFA/22). The complete aerial pieces (leaves, stems, flowers and fruits) were thoroughly cleaned, drained in a shady area, pulverized, and afterwards preserved to be examined.

Figure 8: *Scabiosa atropurpurea* aerial part from Setif region- Algeria. A: Fruits/B: Flower.

1.2. Animals

Female albino mice ranging around 25 and 30 g were tested. The mice bought from the 'Institute Pasteur of Algeria'. Prior to the start of the research, the mice were placed in caged and maintained under norms over one week. Mice received water and food (ad libitum) and kept in compliance with the guidelines specified in the Animals By-Laws N° 425–2008. The experiments were authorized under the approval No. 88– 08/1988 by the 'Algerian Association of Sciences in Animal Experimentation'.

1.3. Mammalian cell lines

PC-3 cells, derived from human prostate carcinoma, were sourced from the American Type Culture Collection (ATCC, Rockville, MD). They were propagated in RPMI-1640 medium enriched with 10% heat-inactivated fetal calf serum and 50 µg/mL gentamycin. Cultivation was carried out at 37°C under a humidified 5% CO_2 environment, with routine subculturing.

1.4. Reagents and chemicals

The utilized chemicals and reagents were acquired from Sigma (Germany), Sigma (St. Louis, Mo., USA), Prolab, Fluka and Biochem.

Fetal Bovine serum, RPMI-1640, L-glutamine, HEPES buffer solution, 0.25% Trypsin-EDTA and gentamycin were procured from Lonza (Belgium).

2. Methods

2.1. Extraction procedures

2.1.1. Preparation of the decocted extract

The aqueous extract (AqE) was made according to Karbab et al. (2020a) by decoction of 100g of desiccated above ground section in one liter of purified water for 20 minutes. Afterwards, the solution was passed through muslin towels and then underwent centrifugation at 3000 rpm for 15 minutes. The mixture was evaporated until it became completely dry. The collected dry extract was subsequently preserved at 4°C.

2.1.2. Preparation of the methanolic extract

The methanolic extract (ME) was prepared as described by Karbab et al. (2021); 4kg of aerial portion was defatted using 15L of petroleum ether. Following that, the plant was dehydrated and were subjected to maceration utilizing 15 liters of pure methanol during seven days and the methanolic extract was evaporated. The dry extract was collected and preserved at 4°C.

2.2. Qualitative phytochemical screening

The extracts were analyzed for bioactive chemicals utilizing the subsequent procedures mentioned in Table 3 (Amari *et al.,* 2023a, Karbab, 2020).

Table 3: Qualitative phytochemical procedures.

Bioactive compounds	**Procedures**	**Expected Results**
Alkaloids	Two drops of Dragendorff reagent + 50 mg/3mL of extracts/ethanol-water (60:40 v/v).	Reddish-brown or orange-red precipitate.
Terpenoids	5ml of extract + 3ml of concentrated H_2SO_4.	Brown red color
Saponins	60mg of extracts in 2ml of distilled water were intensely shaken during 15 min**.**	Foam formation
Polyphenols	2% alcoholic solution of ferric chloride + 2 mL of extracts.	Green or darkish blue color
Flavonoids	Extracts + concentrated HCL + Mg turning.	Orange or red color
Tannin	2ml of extracts + 2% ferric chloride ($FeCl_3$).	Green dark color
Coumarins	Boiling 2 mL of sample + 0.5 mL of 10% NaOH in a water bath. Following cooling, 4 mL of distillate water are introduced.	Fluorescence color
Anthraquinones	10 mL of the extract + 5 mL of a solution of ammonium hydroxide (10%).	Red ring appearance
Reducing sugars	Boiling 1ml of extracts + 1ml of Fehling's reagent in water bath.	Brick red precipitate

2.3. Quantitative phytochemical investigation

2.3.1. Estimation of total phenolic content

A polyphenol analysis was carried out applying the Folin-Ciocalteu process (Karbab *et al.,* 2020a). A total of 0.1 mL of each extract and 0.5 mL of Folin-Ciocalteu reagent were combined. Next, 0.4 mL of a solution containing 7.5% sodium carbonate was poured. After a two-hour incubation period, the spectrophotometric reading was recorded at 765 nm and the TPC was shown as micrograms of gallic acid equivalents per milligram of extract (µg GAE/mg DE). A calibrated curve of gallic acid was utilized to give the polyphenols tenor.

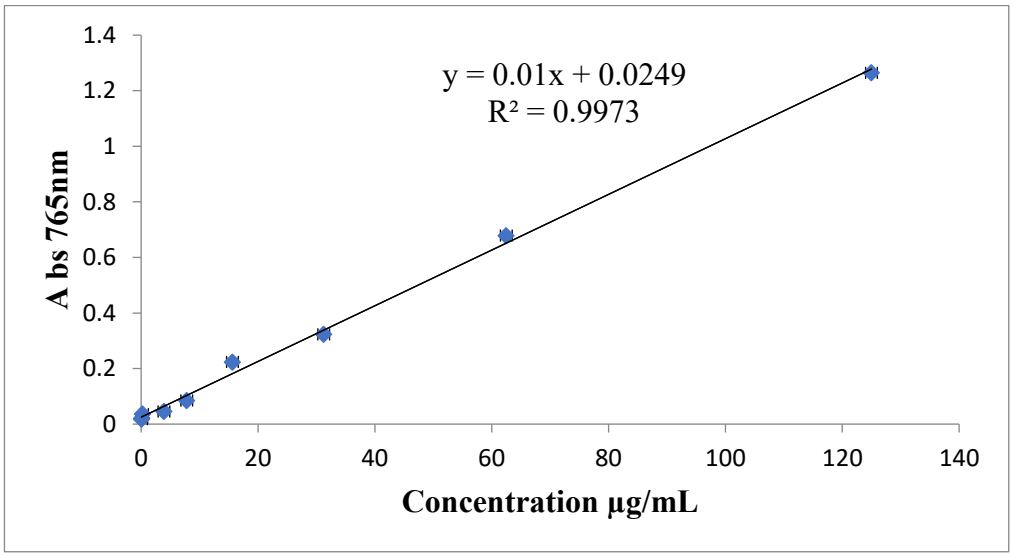

Figure 9: Calibration curve of gallic acid for setting total polyphenols in *S. atropurpurea.* Absorbances are shown as mean ± SD (n=3).

2.3.2. Quantification of total flavonoids content

Measuring the overall quantity of flavonoids was done using aluminum chloride reagent (ACR) (Amari *et al.,* 2023a). After mixing 0.5 mL of samples with 0.5 mL of 2% ACR, the produced mix was left for 10 minutes. Spectrophotometry was performed at 430 nm. The data were presented as milligrams of quercetin equivalent (µg QE/mg DE) for each milligram of dried extract (mg DE). The dose of flavonoids was determined by calibration curve of quercetin.

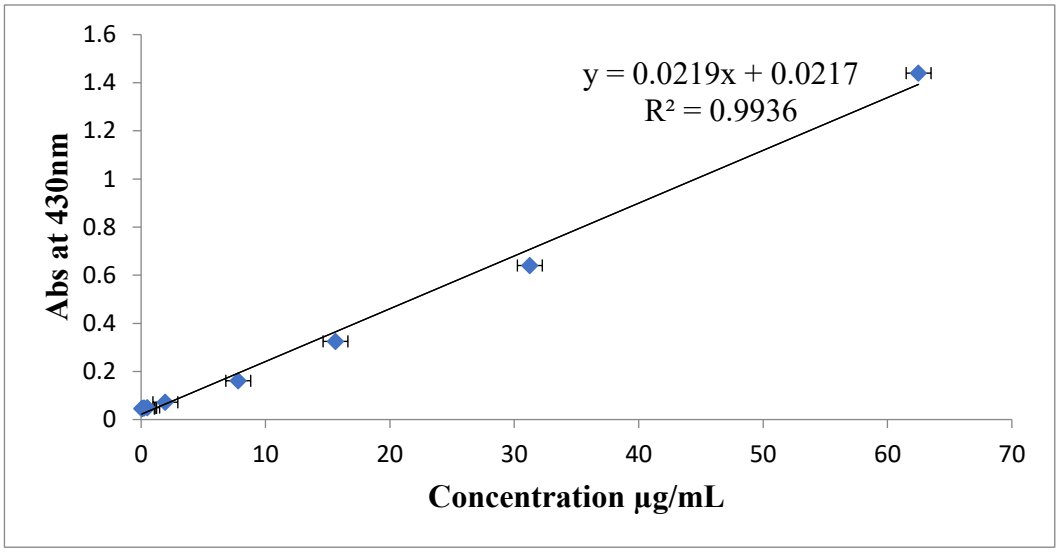

Figure 10: Calibration curve of quercetin for setting total flavonoids in *S. atropurpurea.* Absorbances are shown as mean ± SD (n=3).

2.3.3. Estimation of condensed tannins content

A modifying vanillin approach was used to perform the condensed tannins tenor (Karbab *et al.,* 2020b). 375 µL of vanillin reagent (4%) was mixed with 250 µL of tested samples. Following that, 187.5 µL of hydrochloric acid was added. After twenty minutes, a spectrophotometric analysis was carried out at 550 nm. The CTC was reported as micrograms of catechin equivalent per milligram of dry extract (µg CE/mg DE). The dosage of condensed tannins was measured by calibration curve of catechin.

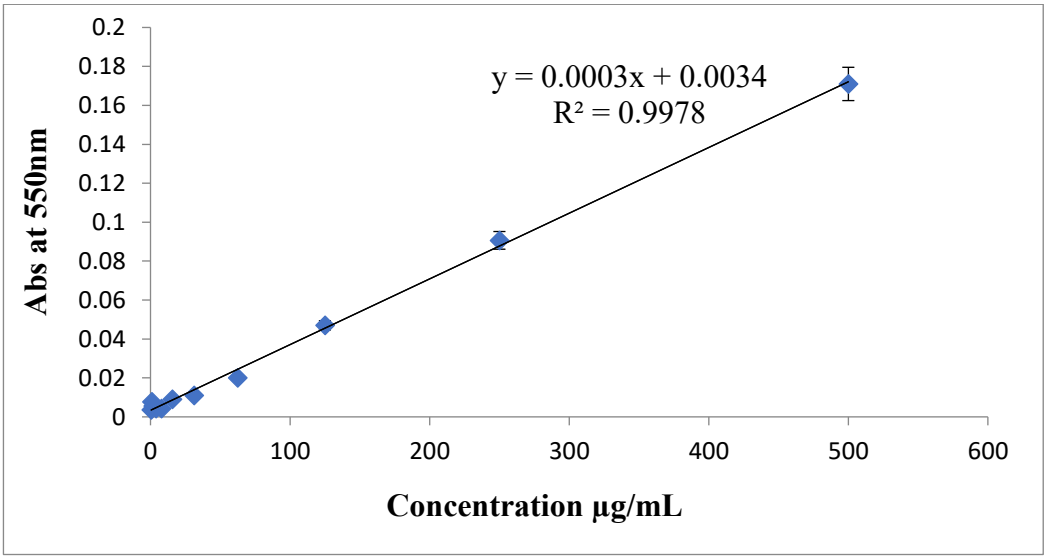

Figure 11: Calibration curve of catechin for setting total condensed tannins in *S. atropurpurea.* Absorbances are shown as mean ± SD (n=3).

2.4. UPLC-ESI-MS/MS analysis

UPLC-ESI-MS/MS (Ultra Performance Liquid Chromatography – Electrospray Ionization - Tandem Mass Spectrometry) was performed according to Elhawary et al. (2021), in positive and negative ion acquisition methods. The material was subjected to chromatographic separation by injecting 10 µL (100 µg/mL in HPLC grade methanol) into a UPLC device supplied with a reverse phase C-18 column. The sample was subjected to filtration and degassing prior to the injection. A gradient mobile phase consisting of two eluents: eluent A water/eluent B methanol; both are acidified with 0.1% formic acid. Elution was conducted at a flow rate of 0.2 mL/min. The procedure was conducted under particular conditions: source temperature 150°C, capillary voltage 3 kV, cone voltage 30 eV, cone gas flow 50 L/h, desolvation temperature 440°C, and desolvation gas flow 900 L/h.

Mass spectra were obtained using Electrospray ionization (ESI) (m/z 100–1000). MassLynx 4.1 software was used for data processing, and compounds were tentatively identified by comparing retention times, mass spectra, and fragmentation patterns with previously reported data.

2.5. Biological activities

2.5.1. *In vitro* antioxidant potentials

2.5.1.1. DPPH radical scavenging assay

The free radical elimination efficacy of the AqE and ME was conducted by measuring the reduction of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in absorption at 517 nm (Charef *et al.,* 2015). In summary, 50 µL of varying dosages of the extracts and reference were combined with 1.25 mL of a 0.004% of DPPH. The extracts' absorption was taken at 517 nm followed a thirty-minutes incubating in dark. Butylated hydroxytoluene

(BHT) served as the positive control. The outcomes were given as an IC_{50}; which indicate the dose that inhibits a 50% of the free radical in the solution.

2.5.1.2. Hydroxyl radical scavenging activity

The hydroxyl radical elimination capability was estimated accordingly with Laroui et al. (2023). 150 µL of extracts at varied doses mixed with 300 µL of a stock mixture consist of 9 mM $FeSO_4$ and 0.3 % hydrogen peroxide (H_2O_2), then incubated at 32 °C during fifteen min. After, 75 µL of salicylic acid (20 mM) was pipetted and the reaction solution was let for fifteen minutes at 32 °C. The absorption was detected at 562 nm spectrophotometrically, and BHT served as reference. The results were given as an IC_{50}; which indicate the concentration that inhibits a 50% of the free radicals in the test solution.

2.5.1.3. Iron chelating activity

The assessment of ferrous iron chelation of samples has been performed through a spectrophotometric test (Karbab *et al.,* 2019). The approach relies on the prevention of the Fe2+-ferrozine complex creation following the addition of samples with Fe2+ ions. 450 µL of methanol and 50 µL of $FeCl_2$ (0.6 mM) were combined with 250 µL of extracts at varied dosages. 50 µL of ferrozine (5 mM) was pipetted after five minutes, and the resulting mixture was let to interreact for ten minutes. A reading of results was estimated at 562 nm. EDTA was used as reference chelator. An IC_{50} value was calculated, which is the concentration of products that produce 50% of the maximal neutralizing efficiency.

2.5.1.4. Reducing power activity

The test serves to assess the samples' reduction capacity of potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}); that interact with ferric chloride to form ferric ferrous complex exhibiting a maximum absorption at 700nm (Amari *et al.,* 2023a). 400 µL of either extract or ascorbic acid was blended with a similar amount of potassium ferricyanide (1%)

and phosphate buffer (0.2 M, pH = 6.6) and rest for twenty minutes at 50 °C. A 400 µL of 10% TCA was introduced and the produced mix was subjected to centrifugation at 3000 rpm during ten minutes, then 400 µL from the upper layer was combined with purified water (400 µL) and 80 µL of 0.1% ferric chloride. Following ten minutes of incubating, the formed product was observed at 700 nm.

2.5.1.5. Phosphomolybdate assay

This method is used to evaluate the total antioxidant effect according to Jain et al. (2013). One milliliter of combination consists of (4 mM ammonium molybdate, 0.6 M H_2SO_4 and 28 mM sodium phosphate) has been blended with 100 µL of samples. The resulting mixture rest during 90 minutes at 95 °C. Next, the results were determined at 695 nm. The outcomes were determined as $A_{0.5}$ (mg/mL), which corresponds to the absorption at 0.5nm.

2.5.2. Anti-inflammatory activity

2.5.2.1. *In-vitro* anti-inflammatory potential

The evaluation of the anti-arthritic impact of the samples was performed using the bovine serum albumin denaturation method established by Mouffouk et al. (2018). A mixture was generated by combining 500 µL of 0.2 % BSA suspension (in Tris-HCl buffer; 20 mM, pH 6.4) with 500 µL of varying dosages of each sample or diclofenac (as a reference). The resultant solution was left for fifteen minutes at 37°C, subsequent by ten minutes at 70°C. The denaturation was detected at 660 nm. The inhibition percent of denaturation of protein was obtained via this formula:

$$I\% = 100 \times (A_c - A_t)/A_c$$

Where A_c and A_t represent the absorbances of the negative control and the tested extracts.

2.5.2.2. *In-vivo* topical anti-inflammatory activity

2.5.2.2.1. Xylene-induced edematous

The topical anti-inflammatory capability was studied in mice, as described in reference (Amari *et al*., 2023b). The mice were allocated into sets of six. In parallel, the examined samples (2 mg/ear) or indomethacin (0.5 mg/ear) and 30 µL of xylene were locally given to mice onto the area of the right ears. The negative control was given xylene. By employing a digital caliper, the swelling of ear was calculated prior and 2 hours later to the procedure. The percentage of inhibition (I) of ear edema was found applying the given equation:

$$I\ (\%) = 100 \times (\Delta n - \Delta t)/\Delta n$$

Where Δ_n and Δ represent the variation in the thickness of ear oedema of the negative and treated groups.

2.5.2.2.2. Croton oil-induced edematous

Following the protocol laid out in (Karbab *et al.,* 2019), irritation was produced locally in mice by croton oil. Mice were allocated into six-animal groups. 15 µL of croton oil was applied onto the surface of the mice ears in parallel with 15 µL of various samples (2 mg/ear) or indomethacin (0.5 mg/ear). The negative control group received just croton oil. The thickness of the ear was recorded prior to application and 6 hours after inducing inflammation and the inhibition percent of ear edematous was calculated as describe previously.

2.5.2.3. *In-vivo* oral anti-inflammatory properties

2.5.2.3.1. Xylene-induced edematous

The oral anti-edematous effect was also investigated employing the protocol given in (Karbab *et al.,* 2020). Mice were allotted to groups of six. After one hour of oral administration of varied dosages of AqE and ME (50, 150, 300mg/kg), indomethacin (50mg/kg) and distilled water (negative control), edematous was locally provoked in mice ears by 30 µL of xylene. A digital caliper was employed to determine the ears' swelling both prior to and 2 hours subsequent to edematous induction.

2.5.2.3.2. Croton oil-induced edematous

The oral anti-edematous impact was investigated employing the protocol as presented by Amari. (2023). The mice were distributed into sets of six. The groups were given indomethacin orally at a concentration of 50mg/kg, distilled water as a negative control, and varying doses (50, 150, 300mg/kg) of the substances under tested. Following a duration of 60 minutes, inflammation was topically provoked in mice ear by applying a solution of croton oil (80µg/15 µL). After a duration of six hours, the ear thickness was detected using a digital caliper. The percent of suppression of inflammation was estimated using the previous cited formula.

2.5.2.4. Formalin-induced paw edematous

In this case, inflammation is induced by the injection of formalin at the level of the plantar arch of the right paw of mice (Arzi *et al.,* 2015, Rahmani *et al.,* 2016). Mice were allocated onto sets of five. Mice received intraperitoneally varied dosages of extracts (25, 50 and 100 mg/kg), the positive and negative controls received diclofenac (25 mg/kg) and 0.9% NaCl (10 mL/ kg), respectively. Oedema was caused on the right hind paw of mice by a subplantar injection of 0.025 mL of formaldehyde (1 %) 30 minutes post-samples reception. The swelling of the paw injected with formaldehyde was evaluated with a caliper over a three-

hour period following the injection. The efficacy of products to inhibit paw edematous was quantified as an inhibition percentage, computed using the subsequent formula:

$$I\% = 100*(V_2-V_1)/V_2$$

Where V_1 and V_2: The mice paw volume before formaldehyde injection and after drugs and formalin injection.

2.5.3. Analgesic activity

The analgesic efficacy of extracts was estimated by induction of abdomen contraction in mice using acetic acid (Yimer *et al.,* 2020). The mice were categorized to sets of five. The negative control was given distilled water, whereas the positive control was administered aspirin (100 mg/kg). Test groups administered extracts at dosages of 50, 150, and 300 mg/kg, respectively. Writhing was caused intraperitoneally by 0.7% acetic acid (10 mL/kg body weight) 60 minutes post-treatment of all groups. The count of writhes was recorded for each group, commencing 5 minutes post-acetic acid injection and continuing for 30 minutes. The % inhibition of the writhing response was determined applying the following equation:

$$\text{Inhibition (\%)} = 100* (Cn–Ct)/Cn$$

where Cn and Ct are the mean of constriction' count in mice in the negative control and the treated groups with different concentrations of extracts or aspirin.

2.5.4. Toxicity study

2.5.4.1. Cytotoxicity evaluation using viability assay

As per Mosmann (1983); PC-3 cells were suspended in media at a dose of 5x10^4 cells / well and thereafter kept for 24 h. The evaluated compounds were subsequently introduced into 96-well plates (three repetitions) to attain six dosages for each component. Each 96-well plate had six vehicle controls with medium or 0.5% DMSO as a control measure. Following

a 48-hour incubation, the quantity of surviving cells was assessed utilizing the MTT method. The media was expunged and substituted with 100 µl of new RPMI 1640 culture media devoid of phenol red, followed by the addition of 10 µl of a 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to all wells, even the non-treated controls and were subsequently maintained at 37°C with 5% CO_2 for four hours. An 85 µl aliquot of the media was extracted from the wells, followed by the addition of 50 µl of DMSO to all wells, which was vigorously stirred using a pipette and kept within 10 minutes at 37°C. The optical density was subsequently detected at 590 nm to ascertain the number of survival cells, and the percent of viability was computed as

$$\% \text{ Viability} = (\Delta t/\Delta) \times 100$$

where Δt is the mean of optical density of treated cells and Δ is the mean of optical density of non-treated wells.

2.5.4.2. Acute oral toxicity

Acute oral toxicity of AqE and ME was tested in mice in accordance with OECD guidelines (Madoui *et al*., 2019). Mice were allocated into groups consisting of five mice each. After 12h of fasting, mice were administered orally with a single dose of the extracts (2 and 5 g/kg) and the control group received just distilled water. Mice were watched for any indication of toxicity during the first hour after treatment and regularly for the following 24h. Then, the observation of any toxic sign was made daily for 14 days.

2.6. Statistical analysis

Statistical analysis applying one-way ANOVA tests, with Dunnett and Tukey tests. The outcomes were represented in triplicate, with the mean value accompanied by the standard deviation (SD) and standard error of the mean (SEM). The study was performed using

GraphPad Prism-8. Variation defined Significant at a p-value < 0.05. A linear regression was utilized to calculate the IC_{50}.

RESULTS

AND

DISCUSSION

1. Extraction yield

For the extraction; two methods were adopted to obtain the methanolic and the aqueous extracts: maceration utilizing absolute methanol and decoction with distilled water. The extraction yield was determined in relation to the weight of the dry powdered plant material, and findings were shown as a percentage (Table 4).

Table 4: Extraction yield, color and consistency of *S. atropurpurea* L extracts. AqE: aqueous extract. ME: methanolic extract.

Extracts	Extraction yield %	Color and consistency
AqE	9.06	Dark brown powder
ME	13.8	Dark green powder

The yield of extraction indicated that ME recorded the highest proportion with 13.8% compare to the AqE with 9.06%. Our results concurred with those of Elhawary et al. (2011), who documented a hydro-ethanolic extraction yield of 14.26% for the aerial parts of *S. atropurpurea* and with those of Ben jalloul et al (2022), who mentioned a median yield of hydro-methanolic extracts from various plant parts varied from 9.32% to 15.7%.

There are different extraction techniques of bioactive components from plant materials, such as solvent extraction and distillation procedure, based on cold or heat extraction. Phytochemical extraction efficiency is affected by variables like the dimensions of the plant product, solvent characteristics, solid-to-solvent proportion, temperature and duration of extraction (Mordeniz, 2019). Solvents with polarity matching that of the solute yield higher extraction efficiency. Elevated temperatures enhance dispersal and solubility but may also lead to the decomposition of thermolabile molecules. Extraction effectiveness improves with prolonged extraction duration (Nortjie *et al*., 2022).

2. Phytochemical investigation

2.1. Qualitative phytochemical screening

A phytochemical study of *Scabiosa atropurpurea* identified various secondary metabolites with recognized biological activities. The extracts include many phytochemical components, as indicated in Table 5. The analysis suggested the existence of polyphenols, flavonoids, saponins, free quinones, tannins, terpenoids, anthraquinones, and reducing sugars in aqueous and methanolic extracts. Nevertheless, the results showed that coumarins are found just in the AqE. This phytochemical investigation demonstrates the prevalence of secondary metabolites in both extracts.

Table 5: Qualitative analysis of secondary metabolites from *S. atropurpurea.* AqE: aqueous extract. ME: methanolic extract.

Phytochemical compounds	**AqE**	**ME**
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Terpenoids	+	+
Free quinones	+	+
Anthraquinones	+	+
Coumarins	+	-
Saponins	+	+
Reducing sugar	+	+

Numerous studies have confirmed the abundance and the availability of a diverse bioactive components in *Scabiosa* different species, which are known to exhibit various beneficial biological impacts. Phenolic acids, flavonoids, terpenoids and iridoid glucosides were detected in *S. atropurpurea*, and the other phytochemicals were found in other scabiosa genus (Pinto *et al.,* 2018).

The determination and profiling of plant constituents in medicinal herbs facilitate the prediction of their possible pharmacological effects and provide evidence for validating the therapeutic benefits against different illnesses (Shaikh and Patil, 2020).

Phytochemical extraction from plant is determined by various pre-extraction elements, such as the plant part, particle size, its origin, drying technique, and processing level. Additionally, extraction-specific parameters, including the method employed, solvent type and extraction duration (Shaikh and Patil, 2020).

2.2. Determination of total phenolic (TPC), flavonoids (TFC) and condensed tannins (CTC) content

For the quantified phytochemical analysis; the overall phenolic, flavonoids, and condensed tannins amount are represented in Table 6. The outcomes revealed that ME displayed a substantial dose of TPC with 114.13 ± 0.92 µg GAE/mg DE and TFC with 100.57 ± 0.93 µg QE/mg DE. On the other hand, AqE polyphenols and flavonoids tenors were 81.6 ± 1.6 µg GAE/mg DE and 45.33 ± 0.36 µg QE/mg DE, respectively. However, the biggest condensed tannins level was observed in AqE reaching 41.04 ± 0.64 µg CE/mg DE.

Table 6: Total phenolic, flavonoids and condensed tannins content of *S. atropurpurea* extracts.

Extracts	TPC (µg GAE/mg DE) [a]	TFC (µg QE/mg DE) [b]	CTC (µg CE/mg DE) [c]
AqE	81.6 ± 1.6	45.33 ± 0.36	41.04 ± 0.64
ME	114.13 ± 0.92	100.57 ± 0.93	26.22 ± 1.11

Outcomes are represented as mean ±SD (n=3). [a] Gallic acid equivalent/mg of dried extract, [b] Quercetin equivalent/mg of dried extract, [c] Catechin equivalent/mg of dried extract.

TPC of different parts of *S. maritima* during the flowering and fruiting stage was ranged from 26.62 to 142.85 mg GAE/g DE and TFC was ranging from 17.31 to 88.50 mg QE/g E (Ben jalloul *et al.*, 2022). Total phenolic tenor of aerial part of hydro-ethanolic extract from the Egyptian *S. atropurpurea* was 0.424% (Elhawary *et al*., 2011), and the TPC and TFC of

methanolic extract from the Algerian *S. atropurpurea sub. maritima* leaves were 1.303 mg GAE/g DE and 0.613 mg QE/g DE respectively (Ali-rachedi *et al*., 2018), those outcomes are lower than our results.

Furthermore, an investigation conducted by Niama et al (2024) on *S. artropurperea var. maritima* aqueous extract of the whole plant during the flowering stage, revealed a low total phenolic and flavonoids content with 47.9 mg GAE/g DE and 25.8 mg QE/g DE, respectively compared with our extracts.

Condensed tannins content of *S. maritima's* different part at flowering and fruiting period was recorded in Ben jalloul et al (2022) research to reach between 3.44 and 13.49 mg CE/g E and Ali-rachedi et al (2018) estimated the CTC of leaves' methanolic extract and found 0.560 mg CE/g DE. Additionally, the aqueous extract from *S. artropurperea var. maritima* show a minor condensed tannins tenor with 9.8 mg TAE/g DE (Niama *et al.,* 2024). The results from different studies are lower than ours.

In general, the difference in the phytochemical amount can be attributed to various elements like plant part, the used solvent, collection stage and the geographical factors (Ben jalloul *et al.,* 2022).

In addition, numerous factors related to the environment (atmosphere, soil, altitude, water, precipitation and temperature), and genetic diversity amongst plant species or among individuals of the same species significantly affect the production and the content of secondary metabolites and their bioactive capabilities (Bibi *et al.,* 2022).

2.3. UPLC-ESI-MS/MS profile

LC-ESI-MS/MS is crucial for the metabolomic analysis of plant extracts, facilitating both qualitative and quantitative comparisons among various extracts. It facilitates the identification and quantification of diverse components and their daughter fragments,

assisting in the profiling and fingerprinting of plant species and closely related variations based on their chemical makeup (Elhawary *et al.,* 2021).

In this study, both extracts were examined by UPLC/MS in ESI positive and negative ion modes for identifying their active metabolites. Thirty-four compounds were tentatively identified (Table 7), with the predominant groups being phenolic acids and flavonoids, following by triterpenoids, sesquiterpenoids, tannins, saponins, iridoids, fatty acids, and others.

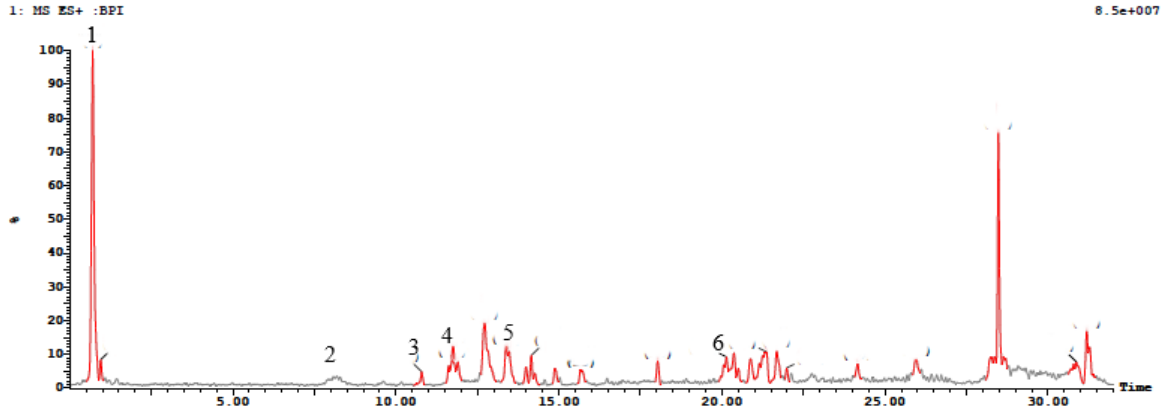

Figure12: Chromatogram of UPLC-MS/MS profile of aqueous extract (AqE) in the negative mode.

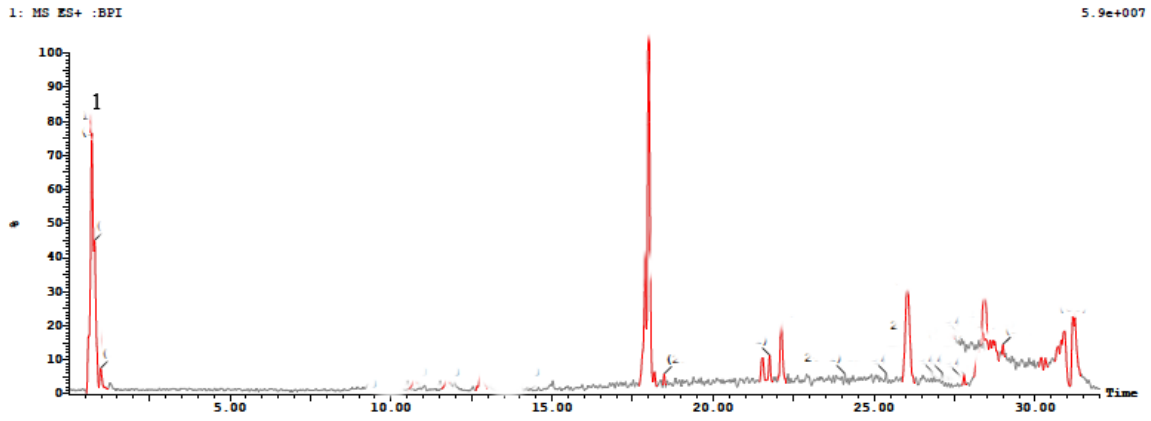

Figure13: Chromatogram of UPLC-MS/MS profile of aqueous extract (AqE) in the positive mode.

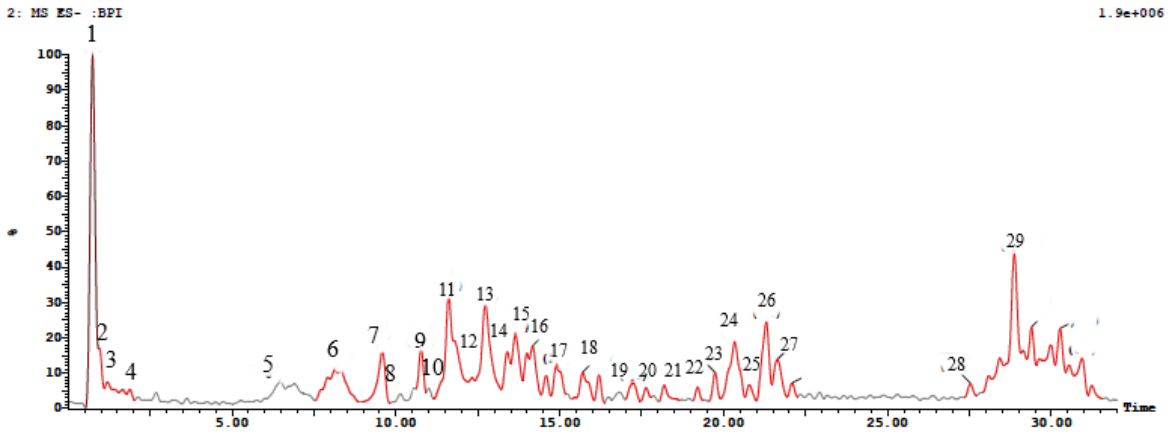

Figure14: Chromatogram of UPLC-MS/MS profile of methanolic extract (ME) in the negative mode.

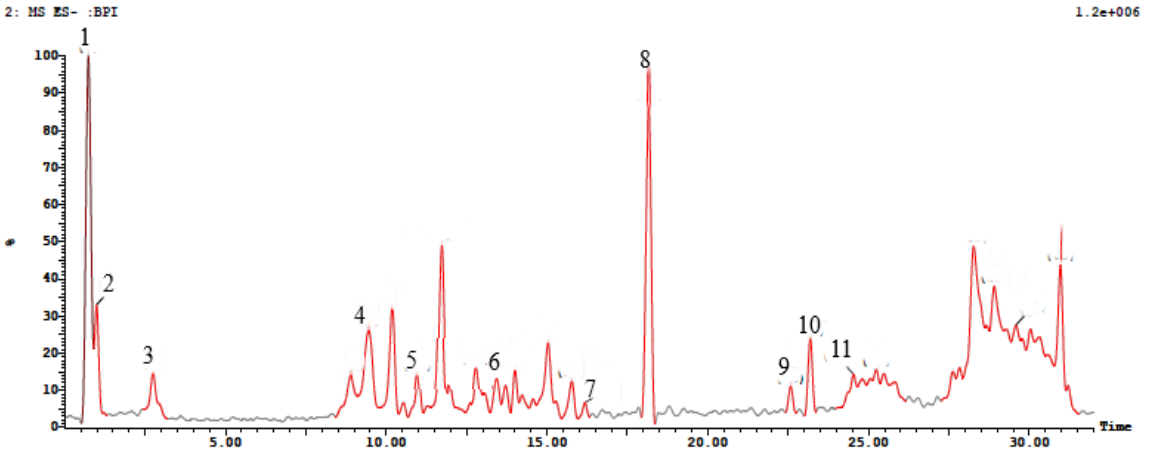

Figure15: Chromatogram of UPLC-MS/MS profile of methanolic extract (ME) in the positive mode.

As shown in Table 7, compounds 2, 6, 16, 22, 24, 30, 31, 32 and 34 represented the major part of the phenolic acids. Chlorogenic acid, its derivatives and its dimer were the most abundant components where chlorogenic acid itself showed a deprotonated peak at *m/z* 353 with fragments at *m/z* 339, 294, 265, 239, 191, 179 where the characteristic quinic acid peak was seen at *m/z* 191(Kilinc *et al.,* 2023). Moreover, *di*-caffeoyl quinic acid and its isomer presented the same deprotonated value at *m/z* 515 due to positional isomerism (Kilinc, 2021, Ben toumia *et al.,* 2020) . Similarly, caffeoyl quinic acid dimer appeared at *m/z* 707(732)

with two main fragments at *m/z* 353 for the caffeoyl quinic acid and at *m/z* 191 for quinic acid which were defining for this compound (Morsi *et al.,* 2022).

One iridoid and one secoiridoid, both reported before for genus *Scabiosa*, were tentatively identified as loganic acid and eustomoside, respectively. Loganic acid showed a deprotonated peak at *m/z* 377(381) with fragments at *m/z* 353, 341, 265, 191 (Akar, 2021) while eustomoside had its deprotonated peak at *m/z* 389(401) with fragments at *m/z* 353, 233, 225, 179 (Kilinc *et al.,* 2023), (Table 7).

Three flavonoid glycosides were denoted as major components in this study namely; luteolin-hexoside, apigenin-hexoside and myricetin-(galloyl)-pentoside with deprotonated peaks at *m/z* 447(449), 431(433) and 601, respectively.

In addition to that, several other metabolites were tentatively identified (Table 7) including; tannins (*tri*-galloyl-hexose, *m/z* 635(637) (Nilofar *et al.,* 2024, Mekam *et al.,* 2019), glycosilated dimeric proanthocyanidin, *m/z* 739 (Maldini *et al.,* 2009). Moreover, one sesquiterpenoid peak appeared at *m/z* 295 $[M-H]^-$ and *m/z* 298 $[M+2H]^+$ which was assigned to *alpha*-artemisic acid (Yagi *et al.,* 2024). Compounds 9 and 13 were tentatively defined as a fragment of ursolic acid (Mostafa *et al.,* 2023) and ursolic acid derivative (Nilofar *et al.,* 2024, Abdelazizi *et al.,* 2020) as they presented deprotonated peaks at *m/z* 363 and *m/z* 547, respectively with the characteristic fragmentation patterns at *m/z* 353, 336, 308, 191, 179 and *m/z* 447, 417, 395, 300, 291, 253, 241, 149, respectively where the fragments at *m/z* 411 and 393 are characteristic for ursolic acid according to literature reports (Novotni *et al.,* 2003), it is also worthy noted that ursolic acid itself is reported for genus *Scabiosa* (Khamees, 2022). Hederagenin, a saponin previously reported from genus *Scabiosa*, was observed at *m/z* 471(476) (Skala and szopa, 2023).

Table 7: Tentatively identified secondary metabolites from ME and AqE of *S. atropurpurea* aerial part.

NO	Compound name	Chemical Class	Molecular Formula	R_t (min)	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	% Composition		MS/MS fragments	Ref.
							ME.	AqE.		
1	**Loganic acid***	Iridoid	$C_{16}H_{24}O_{10}$	0.73	377	381	**13.29 (28.33)**	**5.30 (18.22)**	353, 341, 265, 191	(Akar, 2021)
2	**Chlorogenic acid***	Phenolic acid	$C_{16}H_{18}O_9$	1.00	353	-	0.79	**4.27**	339, 294, 265, 239, 191, 179	(Kilinc *et al.*, 2023)
3	**Luteolin-hexoside***	Flavonoid	$C_{27}H_{30}O_{16}$	1.19	447	449	0.62	**4.39**	405, 390, 379, 315, 285, 279, 243	(Koradi *et al.,* 2016)
4	Catechin hexoside	Flavonoid	$C_{21}H_{24}O_{11}$	1.60	451	-	1.06	-	405, 375, 353, 339, 305, 247, 181	(Nilofar *et al.,* 2024, Kang *et al.,* 2016)
5	Catechin hexoside isomer	Flavonoid	$C_{21}H_{24}O_{11}$	5.96	451	-	0.21	-	405, 389, 353, 325, 243, 191	(Nilofar *et al.,* 2024, Kang *et al.*, 2016)
6	**Caffeoyl quinic acid dimer**	Phenolic acid	$C_{32}H_{36}O_{18}$	7.94	707	732	**8.54 (5.62)**	-	353, 191	(Morsi *et al*., 2022)
7	**Eustomoside***	Secoiridoid	$C_{16}H_{22}O_{11}$	9.40	389	401	**5.76**	-	353, 233, 225, 179	(Kilinc *et al.*, 2023)
8	**Apigenin-hexoside***	Flavonoid	$C_{21}H_{20}O_{10}$	9.95	431	433	0.79	**4.67**	405, 377, 363, 295, 269, 231, 202	(Coradi *et al.,* 2016)
9	Fragment of ursolic acid*	Triterpenoid	-----	10.33	363	-	2.35	-	393, 336, 308, 191, 179	(Mostafa *et al.,* 2023)
10	Phloridzin	Dihydrochalcone	$C_{21}H_{24}O_{10}$	10.57	435	447	1.19 (1.12)	-	403, 381, 377, 353, 335, 281, 246, 195	(Kilinc *et al.,* 2023, Nilofar *et al.,* 2024, Hossain *et al.,* 2010)
11	Tetrahydroxy trimethoxy dihydroxyfla-vone	Flavonoid	$C_{19}H_{18}O_{10}$	10.79	377	-	0.52	-	374, 336, 305, 275, 199, 179	(Elhawary *et al.,* 2021, Nilofar *et al.,* 2024)

Table 7: Tentatively identified secondary metabolites from ME and AqE of *S. atropurpurea* aerial part.

NO	Compound name	Chemical Class	Molecular Formula	R_t (min)	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	% Composition		MS/MS fragments	Ref.
							ME.	AqE.		
12	**Derivative of syringaldehyde**	Miscellaneous	-----	11.42	403	449	**7.92 (5.03)**	**5.03**	391, 361, 309, 283, 265, 235, 179	(Nilofar *et al.,* 2024, Abdelaziz *et al*., 2020)
13	**Ursolic acid derivative***	Triterpenoid	-----	12.64	547	-	**4.50**	-	447, 411, 393, 300, 291, 253, 241, 149	(Nilofar *et al.,* 2024, Chandradevan *et al.,* 2020)
14	*di*-Caffeoyl quinic acid*	Phenolic acid	$C_{25}H_{24}O_{12}$	13.19	515	-	2.21	-	447, 431, 353, 337, 265, 231, 201, 169	(Kilinc *et al.,* 2021, Ben toumia *et al.,* 2020)
15	**Myricetin-(galloyl)-pentoside**	Flavonoid	$C_{28}H_{24}O_{17}$	13.32	601	-	-	**4.48**	525, 483, 447, 431, 379, 265, 225, 189, 179	(Elhawary *et al.,* 2021, Nilofar *et al*., 2024, Saldanha *et al.,* 2013)
16	***di*-Caffeoylquinic acid isomer***	Phenolic acid	$C_{25}H_{24}O_{12}$	13.56	515	-	**7.18**	-	489, 447, 353, 301, 265, 193, 187, 179, 161	(Kilinc *et al.,* 2021, Ben toumia *et al.,* 2020)
17	**Unknown saponin**	Saponin	-----	13.88	789	808	**4.45** (1.93)	-	706, 653, 631, 584, 525, 461, 372, 331, 265, 225, 199, 181, 161	-
18	Caffeoyl-succinyl-[(hydroxyphenyl)-acetyl)]-methyl quinate	Phenolic acid	-----	14.79	629	-	1.52	-	587, 520, 458, 421, 373, 285, 201, 187, 179, 161	(Nilofar *et al.,* 2024, Brito *et al.,* 2014)
19	Diosmetin-hexoside*	Flavonoid	$C_{22}H_{22}O_{11}$	16.05	461	-	0.30	-	440, 433, 389, 330, 285, 253, 212, 191, 179	(Ben toumia *et al.,* 2020)

Table 7: Tentatively identified secondary metabolites from ME and AqE of *S. atropurpurea* aerial part.

NO	Compound name	Chemical Class	Molecular Formula	R_t (min)	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	% Composition		MS/MS fragments	Ref.
							ME.	AqE.		
20	*tri*-Galloyl-hexose	Tannin	$C_{27}H_{24}O_{18}$	16.66	635	637	0.45	-	537, 465, 419, 368, 341, 309, 280, 269, 181	(Nilofar *et al.,* 2024, Mekam *et al.,* 2019)
21	Glycosilated dimeric proanthocyanidin	Tannin	-----	17.12	739	-	2.50	-	538, 467, 426, 393, 313, 289, 213, 183, 163	(Maldini *et al.,* 2009)
22	**Protocatechuic acid-hexoside***	Phenolic acid	*$C_{13}H_{16}O_9$*	17.17	315	-	-	**4.52**	293, 271, 219, 212, 179, 153	(Akar *et al.,* 2021)
23	Hypolaetin-allosyl-[acetyl]-hexoside	Flavonoid	-----	17.59	667	-	0.55	-	537, 489, 375, 344, 309, 285, 185, 179	(Nilofar *et al.,* 2024, Petreska *et al.,* 2011)
24	**Caftaric acid**	Phenolic acid	$C_{13}H_{12}O_9$	18.40	311	-	0.61	**4.69**	262, 257, 197, 191, 157	(Yagi *et al.,* 2024, Attia *et al.,* 2022)
25	Cirsimaritin*	Dimethoxyflavone	$C_{17}H_{14}O_6$	19.05	313	-	0.44	-	297, 249, 229, 177, 175	(Nilofar *et al.,* 2024, Gkioni *et al.,* 2022)
26	Hederagenin*	Saponin	$C_{30}H_{48}O_4$	19.99	471	476	0.42	-	457, 434, 325, 293, 249, 190	(Skala and szopa, 2023)
27	**Hydroxy-octadecatrienoic acid**	Fatty acid	$C_{18}H_{30}O_3$	20.24	293	305	**9.01 (7.95)**	**4.70**	275, 255, 215, 179, 161	(Elgendi *et al.,* 2023)
28	Apigenin-rutinoside	Flavonoid	$C_{27}H_{30}O_{14}$	20.70	577	-	0.06	-	564, 476, 448, 339, 295, 291, 217, 191, 181	(Nilofar *et al.,* 2024, Gkioni *et al.,* 2022)
29	***alpha*-Artemisic acid**	Sesquiterpenoid	$C_{18}H_{32}O_3$	21.19	295	298	**6.36**	-	246, 223, 205, 199, 177, 167, 127	(Kilinc *et al.,* 2023)
30	**Feruloyl-caffeoyl-quinic acid derivative**	Phenolic acid	-----	22.30	265	-	**-**	**32.81**	243, 196, 154, 116	(Kilinc *et al.,* 2023, Simirgiotis *et al.,* 2015)

Table 7: Tentatively identified secondary metabolites from ME and AqE of *S. atropurpurea* aerial part.

NO	Compound name	Chemical Class	Molecular Formula	R_t (min)	$[M-H]^-$ *m/z*	$[M+H]^+$ *m/z*	% Composition		MS/MS fragments	Ref.
							ME.	AqE.		
31	**Feruloyl-caffeoyl-quinic acid derivative isomer**	Phenolic acid	-----	22.57	265	-	**-**	**4.71**	249, 203, 194, 178, 156, 123, 117	(Kilinc *et al.,* 2023, Simirgiotis *et al.,* 2015)
32	***p*-Coumaric acid-hexoside***	Phenolic acid	$C_{15}H_{18}O_8$	25.82	325	-	**-**	**8.67**	311, 293, 271, 265, 235, 145	(Coradi *et al.,* 2016)
33	Caffeoyl-2-hydroxyethane-1,1,2-tricarboxylic acid	Phenolic acid	$C_{14}H_{12}O_{10}$	27.39	339	-	0.41	-	326, 299, 265, 186, 174	(Yagi *et al.,* 2024, Ben said *et al.,* 2017, Ye *et al*., 2005)
34	**Caffeic acid-hexouronide**	Phenolic acid	$C_{15}H_{16}O_{10}$	28.78	355	-	**7.21**	-	339, 311, 300, 221, 197, 161, 145	(Yagi *et al.,* 2024, Zhong *et al.*, 2020)
		% Identification								
		ESI –ve mode					**91.22%**	**88.24%**		
		ESI +ve mode					**49.98%**	**18.22%**		

Compounds in bold are the major components. R_t; retention time, Ref.; reference(s). * For compounds previously identified from genus *Scabiosa.*

3. *In vitro* antioxidant potentials

The antioxidant capacity can be assessed using numerous assays employing distinct modes of action, such as single electron transfer, hydrogen atom transfer, and focused scavenging capabilities. These approaches differ regarding the antioxidant action, substrate nature, oxidation catalyst, resultant expression, and operational simplicity. The choice of a suitable approach or combination of tests is crucial for the accurate evaluation of antioxidant capacity (Christodoulou *et al.,* 2022).

The various antioxidants techniques are classified into three primary categories: chromatography, spectrometry and electrochemical approaches. Amongst these tests, spectrophotometric techniques are regarded as the predominant analytical approach for assessing antioxidant potential owing to sensitiveness, repeatability, speed, and Low-cost advantage (Christodoulou *et al.,* 2022).

In this study, the antioxidant activity of *Scabiosa atropurpurea* was determined by applying different tests including DPPH and hydroxyl radical scavenging assay, ferrous ion chelating, reducing power and total antioxidant activity using phosphomolybdate method.

3.1. DPPH radical scavenging assay

The IC_{50} (inhibitory concentration) was employed to estimate the DPPH elimination ability of extracts derived from *S. atropurpurea*, and the findings are reported in Figure 16. Both extracts demonstrated a significant scavenging activity; AqE with 0.06 ± 0.00 mg/mL and ME with 0.06 ± 0.02 mg/mL and no significant difference recorded by the two extracts ($p>0.05$). The test was in comparison with BHT (butylated hydroxy toluene) as a standard.

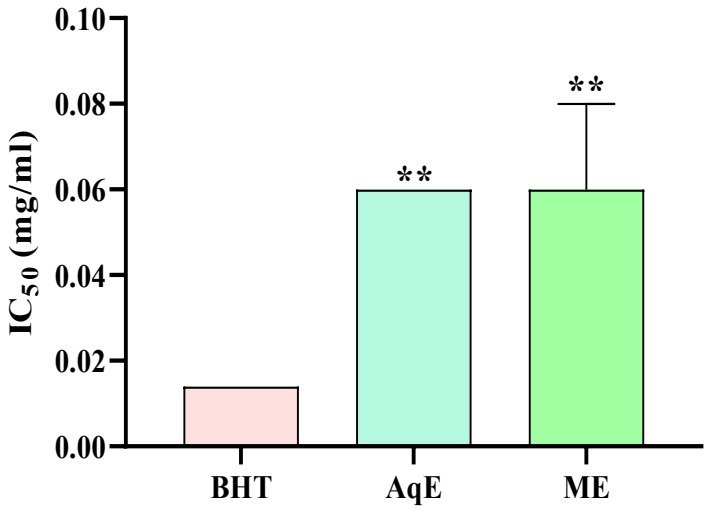

Figure16: DPPH radical scavenging effect of aqueous extract (AqE) and methanolic extract (ME) from *S. atropurpurea* L. Outcomes are shown as mean ± SD (n=3). **: P<0.01.

DPPH is a conventional test and widely utilized for assessing antioxidant capacity, initially designed to identify hydrogen donors from natural sources. It is also the preferred way measuring the ability to neutralize free radicals of innovative drugs owing to its easy usage, effectiveness and quickness (Jain *et al*., 2024). The mechanism involves antioxidants donating hydrogen, resulting in the conversion of DPPH• to non-radical DPPH-H, which causes a color change from purple to yellow and decreases in absorbance at 517 nm (lang *et al.,* 2024).

In this study, AqE and ME from *S. atropurpurea* presents a substantial effect in eliminating free radicals, which was superior to the ethanolic extract from *S. atropurpurea* stem with IC_{50} 0.1383 ± 0.0789 mg/mL (Hrichi *et al*., 2020). The findings obtained by Ben jalloul et al (2022) from different parts of *S. atropurpurea* ranged from 237.95 to 31.74 µg/mL. Compared with other scabiosa species like *S. stellata* (IC_{50} 86 µg/mL) and *S. arenaria* (IC_{50} 87.25 µg/mL) (Ben jalloul *et al.,* 2022); our plant exhibited the highest activity.

Actually, the anti-radical effect of our extracts is owing to the polyphenols, which are known to have antioxidant effects by means of multiple processes, notably the neutralization of free radicals by hydrogen atom transfer; so, they deactivate those radicals (DPPH) by breaking the O–H link (Lang *et al.,* 2024).

Additionally, the free radical neutralization capacity of flavonoids is extensively established in numerous research, and they are known fer their substantial free radical effect. Their effectiveness as antioxidants is influenced by their structure, particularly the quantity and positioning of hydroxyl groups and the aromatic ring (Saleem *et al.,* 2023).

Thus, the non-significant difference between the two extracts, despite ME's higher content of polyphenols and flavonoids compared to AqE, suggests that DPPH radical scavenging may not solely rely on the quantity of phenolic compounds but also on their composition (Ben jalloul *et al.,* 2022).

3.2. Hydroxyl radical scavenging activity

The effect of hydroxyl radical scavenging ability of *S. atropurpurea* was measured as an IC_{50} value and the findings are shown in Figure 17. Regardless the effect of BHT (0.22 ± 0.00 mg/mL) as a reference; both extracts exhibited a moderate scavenging effect. However, AqE showed a higher IC_{50} with 6.1 ± 0.34 mg/mL than ME with 9.64 ± 0.74 mg/mL.

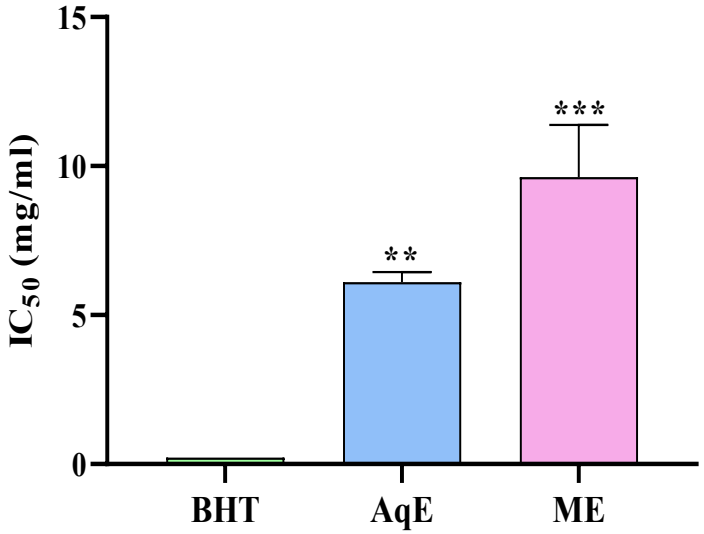

Figure 17: Hydroxyl radical scavenging effect of aqueous extract (AqE) and methanolic extract (ME) from *S. atropurpurea* L. Outcomes are given as mean ± SD (n=3). $^{**}p<0.01$, $^{***}p<0.001$.

The hydroxyl radical is a highly reactive oxygen species in biological systems that causes cellular damage by interacting with the polyunsaturated fatty acid component of

phospholipids in cell membranes. The hydroxyl radical eliminating test is a widely utilized technique to assess the antioxidant ability of bioactive compounds found in plants towards hydroxyl radicals, which are generated by the breaking of hydrophilic bonds (Bibi *et al.,* 2022). Moreover, the antioxidant efficacy of an extract is directly proportional to its capacity to neutralize hydroxyl radicals (Jain *et al.,* 2024).

Natural polyphenols have demonstrated efficacy in safeguarding biological systems from damage triggered by hydroxyl radicals. The elimination of hydroxyl radicals may result from the existence of hydrogen-donating phenolic and flavonoids substances in our extracts. These polyphenols are known to have the capacity of scavenging reactive species of oxygen, such as hydroxyl radicals, so aiding in the prevention or reduction of illnesses associated with oxidative harm. Consequently, polyphenols are extensively acknowledged as potent antioxidants (Lang *et al.,* 2024).

Otherwise, flavonoids substances, specifically quercetin and genistein, have been identified as excellent scavengers of hydroxyl radicals due to the incorporation of hydroxyl groups into the double bonds of their aromatic rings. Only polyphenolic substances with accessible ortho positions in their phenolic rings can efficiently neutralize hydroxyl radicals (Lipinski, 2011). The chemical makeup of tannins significantly affects their antioxidant activity; through a high level of polymerization which enhances their radical scavenging ability (Serrano *et al.,* 2011).

3.3. Iron chelating activity

The findings of metal chelating effect of *S. atropurpurea* are reported in figure 18. Both extracts demonstrated a substantial difference ($p < 0.001$) in comparison to EDTA, which used as a positive reference. Despite the effect of EDTA (0.01 ± 0.00 mg/mL), AqE exhibited a high iron-chelating activity, attaining 0.16 ± 0.03 mg/mL. Conversely, ME showed the minimal IC_{50} with 0.64 ± 0.01mg/mL.

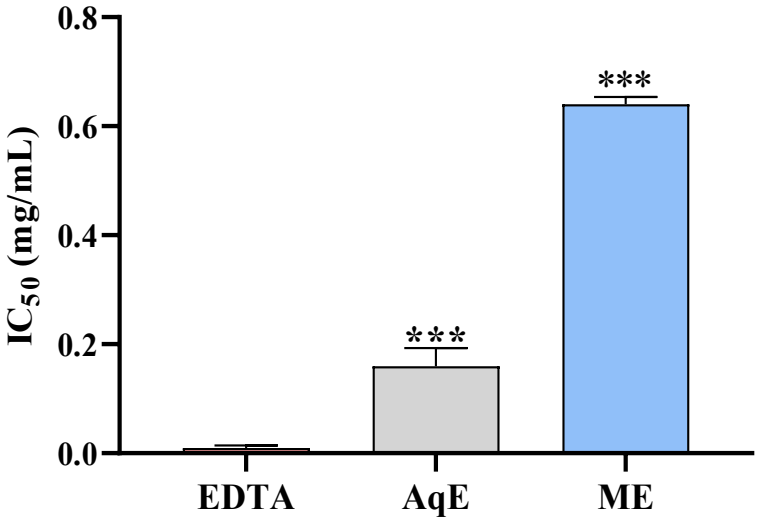

Figure 18: Iron chelating impact of aqueous extract (AqE) and methanolic extract (ME) from *S. atropurpurea* L. Outcomes are reported as mean ± SD (n=3). $^{***}p<0.001$.

Metal chelation is typically regarded as the most prevalent antioxidant mechanism, serving as an indicator of antioxidant capacity and it has been examined with other antioxidant assays for diverse natural components and extracts. This activity involves measuring the absorbance of the iron (Fe^{2+})–ferrozine complex at 562 nm; thus, chelating agents can bind with ferrous ions prior to their interaction with ferrozine (Gulcin and Alwasel, 2022).

Mouffouk et al (2018) study conducted on *S. stellata* ferrous ion chelating effect revealed a moderate to weak activity compared to our results.

In fact, the ferrous ion chelating activity of AqE was higher than ME with significant difference ($p<0.001$), this result suggests that the chelating ability of an extract is not necessarily linked to TPC and the TFC high content; these results align with those reported by Karbab. (2020).

The ferrous ion chelating effect of *S. atropurpurea* extracts may be ascribed to the existence of polyphenols, which include several hydroxyl groups that allow them to create stable chelates with transition metal ions, therefore diminishing the production of free radicals

facilitated by these metals. For example, curcumin and quercetin can chelate ferrous ions (Yahfoufi *et al.,* 2018).

Moreover, flavonoids employ the transition metal chelation pathway to carry out their antioxidant properties. They possess chelating capabilities that bind to metal ions in structural cells and inhibit oxidation. Certain flavonoids can bind to heavy or transition metal ions, like iron and copper, which are crucial in free radical production and oxidation processes. Nonetheless, the structural characteristics of flavonoids may significantly influence their iron chelation capacity (Tumilaar *et al.,* 2024). Condensed tannins are known by their antioxidant activity and chelate metal ions through the same flavonoid' mechanism (Soldado *et al.,* 2021).

Chelation of metal ions is crucial to prevent the creation of reactive oxygen species and radical generation, which can cause harm to biomolecules. Moreover, natural metal chelating substances such as flavonoids and phenolics are preferred over manufactured chelating agents due to their related toxicity issues (Gulcin and Alwasel, 2022).

3.4. Reducing power activity

The reducing power of *S. atropurpurea* was estimated by measuring $A_{0.5}$ (mg/mL), which represents the dose correlating with the absorption at 0.50 nm (Figure 19). In this test, vitamin C served as a standard. AqE exhibited higher reducing capacity with 0.08 ± 0.00 mg/mL than ME with 0.13 ± 0.01 mg/mL.

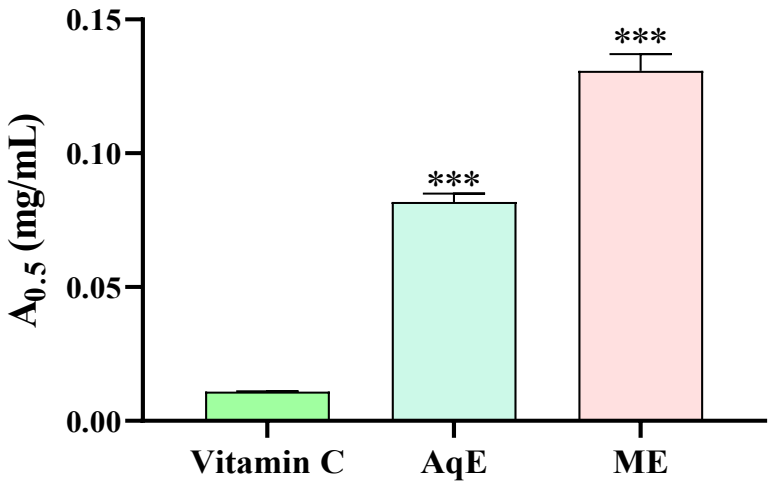

Figure 19: Reducing power activity of aqueous extract (AqE) and methanolic extract (ME) from *S. atropurpurea* L. Outcomes are given as mean ± SD (n=3). $^{***}p<0.001$.

The reduction power test is a commonly utilized and reliable technique for evaluating antioxidant capability. Moreover, substances having reduction potential react with potassium ferricyanide to yield potassium ferrocyanide. This molecule subsequently reacts with $FeCl_3$, leading to the formation of ferric ferrocyanide a dark blue complex with a maximum absorption at 700 nm (Xiao *et al.,* 2020). This method quantifies the electron-donating ability of a compound, which is a crucial indicator of its antioxidant ability (Alavi *et al.,* 2018).

Different studies on reducing power of scabiosa genus reducing power were conducted. The results on *S. stellata* aerial part*, S. arenaria* flowers different fractions and ethanolic extract from *S, tschilliensis* were higher than ours (Mouffouk, 2019, Hlila *et al*., 2013). However, studies on *S. comosa*, *S. arenaria* fruits fractions and *S. stellata* aerial part (another study) showed a reducing power impact and confirm the ferric reduction potential of scabiosa species, noting that, our extracts demonstrated a high effect comparing with the last-mentioned species (Mouffouk, 2019, skala and szopa, 2023).

The reducing power is typically associated with substances that act as electron donors, and the reducing capacities of the extracts in this study can be elucidated by their total phenolic

and flavonoid content, which are identified as potent antioxidants capable of preventing or aiding in the management of oxidative diseases. Flavonoids primarily recognized for their antioxidant properties, are efficient in rapidly transferring electrons mechanism (Djeussy *et al.,* 2022).

Nonetheless, the characteristics of the flavonoid structure may significantly influence its reducing power and chelating ability (Tumilaar *et al.,* 2024).

Actually, the efficacy of the various extracts can be ascribed to the presence of phenolic chemicals in our plant. However, the concentration of polyphenols and flavonoids is not the only determinant associated with this activity, the potential synergy between polyphenols and other constituents in the extracts may account for this effect (Mouffouk, 2019).

3.5. Phosphomolybdate assay

The phosphomolybdate test was assessed to estimate the total antioxidant effect of *S. atropurpurea,* and the findings were given as $A_{0.5}$ mg/ml (Figure 20). Regarding the capacity of vitamin C (0.09 ± 0.00 mg/mL), the extracts showed a substantial effect. ME registered the highest activity; reaching 0.18 ± 0.01 mg/mL compared to AqE with 0.23 ± 0.01 mg/mL. A significant difference recorded among the two extracts ($p < 0.001$).

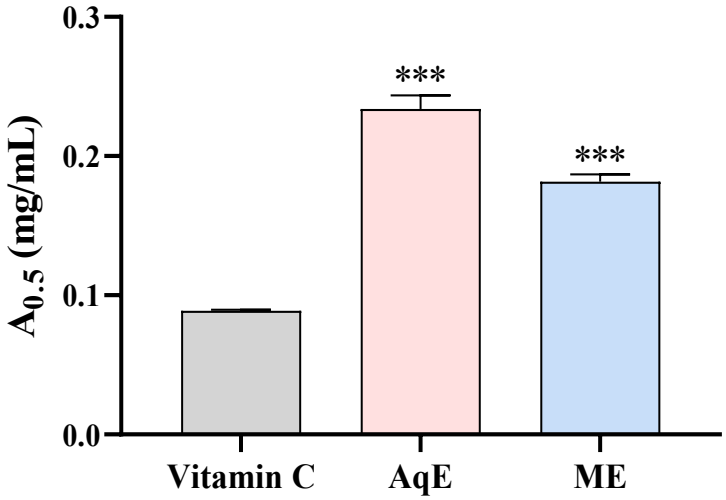

Figure 20: Total antioxidant activity of aqueous extract (AqE) and methanolic extract (ME) from *S. atropurpurea* L. Findings are given as mean ± SD (n=3). ***p<0.001.

The phosphomolybdate assay has been identified as an excellent technique for assessing the overall antioxidant capacities of diverse plant extracts (Mbinda and Musangi, 2019). This spectrophotometric technique quantifies antioxidant capacity by measuring the sample analyte's ability to reduce $Molybdenum^{6+}$ to $Molybdenum^{5+}$, which subsequently forms a green phosphate-$Molybdenum^{5+}$ complex in acidic conditions, establishing the assessment of antioxidant capacity (Jain *et al.,* 2024).

Our analysis revealed that both AqE and ME demonstrated a reduction impact on molybdenum (VI), showing significant antioxidant capacity. The total antioxidant capacity of *S. atropurpurea* may be associated with the levels of flavonoids and phenolic acids in the extracts.

Studies on different parts of *S. atropurpurea sub. maritima* at flowering and fruiting period revealed a total antioxidant activity ranged from 62.14 to 160.39 mg EAA/g Ext (Ben jalloul *et al.,* 2022). Furthermore, *S. stellata* showed a reducing activity on molybdenum attaining 0.03 to 0.0885 µg EAA/mg Ext (Mouffouk *et al.,* 2018). These findings confirm the total antioxidant potential of scabiosa genus.

In addition, the overall antioxidant capability of the AqE and ME can result from the existence of non-phenolic chemicals that can interact with buffered reaction solution and tolerate incubation conditions (Mouffouk, 2019).

4. Anti-inflammatory activity

4.1. *In-vitro* anti-inflammatory potential

The *in vitro* anti-inflammatory capability of *S. atropurpurea* was examined by employing protein denaturation inhibition procedure. Findings are reported in Figure 21. Both extracts revealed a dose-dependent activity on reducing protein denaturation. AqE showed the greatest inhibiting ability across all tested doses (0.25, 0.5 and 1 mg/ml) attaining 61.97% at 1mg/ml, while ME demonstrated a lower value of 33.6% at the same dose. The anti-

inflammatory effect was evaluated relative to diclofenac, a NSAID, with a significant difference ($p < 0.001$).

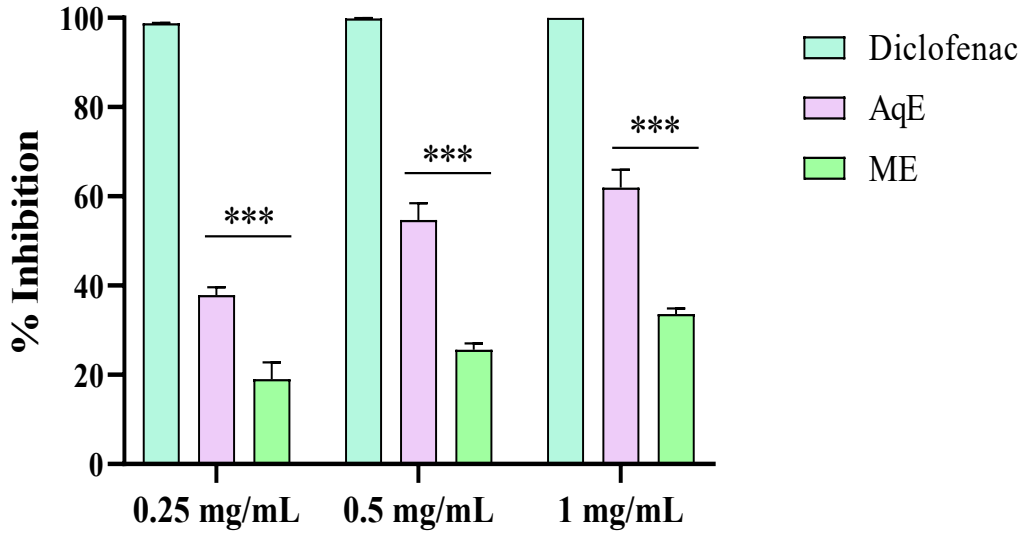

Figure 21: Inhibition percent of protein denaturation by aqueous extract (AqE) and methanolic extract (ME) from *S. atropurpurea* L at different doses. Data are given as mean ± SD (n=3). $^{***}p < 0.001$.

Protein denaturation functioned as an *in vitro* pharmacological technique to assess the anti-inflammatory properties of plant extracts (Akhtar, 2022). It is a significant contributor to inflammation and arthritic conditions, closely associated with several inflammatory factors and having substantial implications for inflammatory responses. The denaturation of proteins is essential to the inflammatory process, encompassing a sequence of molecular processes (Anyasor *et al.,* 2019). Furthermore, numerous inflammatory diseases, including rheumatoid arthritis and serum sickness, are typically linked to the denaturation of proteins' tissue. In arthritis, it induces the production of autoantigens that negatively impact the synovial membrane and cartilage of the joints (Saleem *et al.,* 2019; Aidoo *et al.,* 2021).

Both extracts exhibit a dose-dependent anti-protein denaturation effect, which can be attributed to the existence of phytochemicals like alkaloids, phenols and flavonoids and could represent one of the processes implicated in the anti-arthritic activity of the medicinal

plant (Saleem *et al.,* 2019). Furthermore, the plants' extracts have been discovered for their ability to inhibit denaturation of proteins to a certain level (Gonfa *et al*., 2023).

Numerous nonsteroidal anti-inflammatory medications (NSAIDs) are recognized for their intrinsic capacity to maintain or inhibit the denaturation of heat-treated albumin (Aidoo *et al.,* 2021), which explain the high effect recorded by diclofenac with inhibition value reaching 100% at a concentration of 1mg/mL; the same potential was recorded by ibuprofen at the same dose and test model (Mouffouk *et al.,* 2018).

The inhibitory impact of *S. atropurpurea* on denatured proteins may be linked to the extension and contact with membrane's proteins, suggesting anti-rheumatoid characteristics (Aidoo *et al.,* 2021).

On the other hand, Mouffouk et al (2018) suggested that the anti-denaturation capacity of extracts may be through the prevention of the hydrogen, hydrophobic, electrostatic and disulfide bonds; consequently, preserving the three-dimensional form of proteins and regulating self-antigen production in rheumatoid arthritis.

4.2. In-*vivo* topical anti-inflammatory activity

4.2.1. Xylene-induced edematous

The topical ant-inflammatory impact was examined utilizing xylene-induced ear edematous in mice and the Data are represented in Figure 22. ME exhibited a substantial activity (73.77%) comparable to indomethacin (81.48%); an anti-inflammatory medicine ($p > 0.05$). In addition, AqE displayed significantly a high inhibition percent reaching 62.96%.

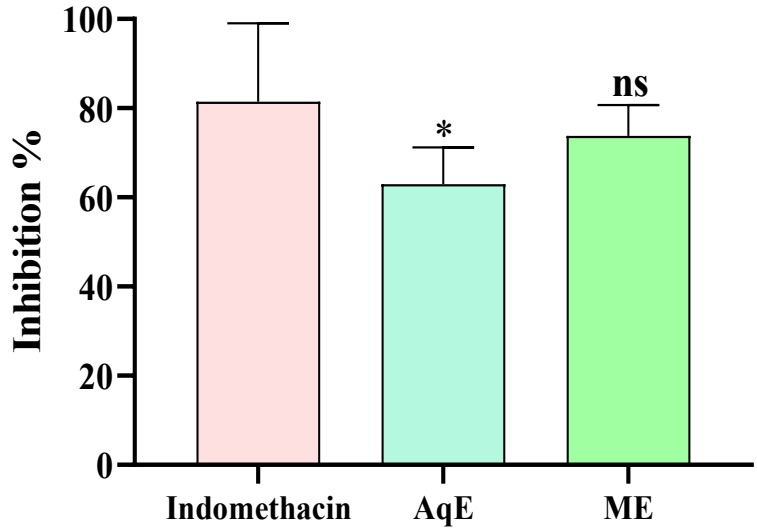

Figure 22: Inhibition percent of xylene-induced ear edematous of *S. atropurpurea*, at a concentration of 2mg/ ear. Values are illustrated as the mean ± SEM (n = 6). ns: no significant difference, *: P<0.05. AqE: aqueous extract, ME: methanolic extract.

The xylene-induced mouse ear edematous method is widely used to evaluate the anti-inflammatory activity of novel compounds. Studies confirm that xylene application triggers acute inflammation, resulting in ear swelling. Measuring these changes provides a reliable method for assessing the effectiveness of new anti-inflammatory agents (Huang *et al.,* 2024). Topical use of xylene provokes acute epidermal ear inflammation, marked by secretion of pro-inflammatory substances, vascular dilation, cells infiltration, and oedema development (Akhtar, 2022).

4.2.2. Croton oil-induced edematous

Croton oil-induced ear edema in mice model was applying to test the anti-inflammatory potential of *S. atropurpurea.* At dose of 2mg/ear*,* AqE and ME demonstrated an inhibitory percent with 36.41% and 44.62%, respectively (Figure 23), while Indomethacin served as a reference drug.

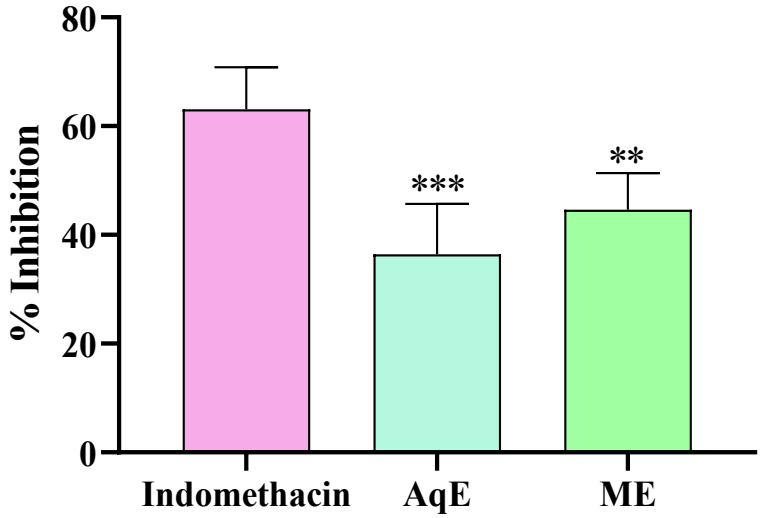

Figure 23: Inhibition percent of croton oil-induced ear edematous of *S. atropurpurea*, at a concentration of 2mg/ ear. Values are illustrated as the mean ± SEM (n = 6). **P<0.01, ***P<0.001. AqE: aqueous extract, ME: methanolic extract.

Croton oil is extensively utilized in inflammatory experimental in mice models, particularly in the ear oedema test, primarily to assess novel anti-inflammatory pharmacological agents. Croton oil comprises a collection of phorbol-12, 13-diesters and 12-o-tetracanoyl forbol 13-acetate (TPA); that activate protein kinase C and several inflammatory mediators, inducing irritating and dermal manifestations comparable to psoriasis (Mao *et al*., 2024).

The topical administration of croton oil provokes cutaneous inflammation by activating an enzymatic cascade that includes phospholipase A2 (PLA2), subsequently leading to the release of platelet-activating agent, arachidonic acid (AA) and prostaglandins, resulting in oedema, increased vascular permeability, leukocyte infiltration, eicosanoid production by the enzymes cyclooxygenase (COX) and lipoxygenase (5-LOX), and the release of histamine and serotonin (Ferreira *et al.,* 2023).

In this study, *S. atropurpurea* demonstrated considerable anti-inflammatory efficacy through topical applications. These extracts may have inhibited the release of pro-inflammatory mediators linked to acute inflammation, such as histamine and prostaglandin. Consequently, the extracts could exert an anti-inflammatory effect by blocking prostaglandin synthesis,

identical to the mechanism of non-steroidal anti-inflammatory medication (Okoli *et al.,* 2006). This effect could result from the various bioactive chemicals in plants, such as saponins, tannins, flavonoids, and reducing sugars (Puspawati and Rita 2019). Furthermore, flavonoids can suppress certain inflammatory mediators, while terpenoids possess anti-inflammatory characteristics. In fact, this effect has been associated with the ability to inhibit phospholipase A2, which in turn prevents the metabolism of arachidonic acid (Annana *et al.,* 2022).

4.3. Oral anti-inflammatory activity

4.3.1. Xylene-induced edematous

Findings from the oral anti-inflammatory test using xylene-induced ear oedema in mice are cited in Figure 24. Both extracts showed a substantial and dose-dependent anti-edematous response at the administered doses (50, 150, 300 mg/kg). ME and AqE registered a comparable effect to indomethacin, which served as reference drug ($p > 0.05$), with reducing edema at 300 mg/kg reaches 81.48% and 77.37%, respectively, while indomethacin effect at dose of 50 mg/kg was 82.51%.

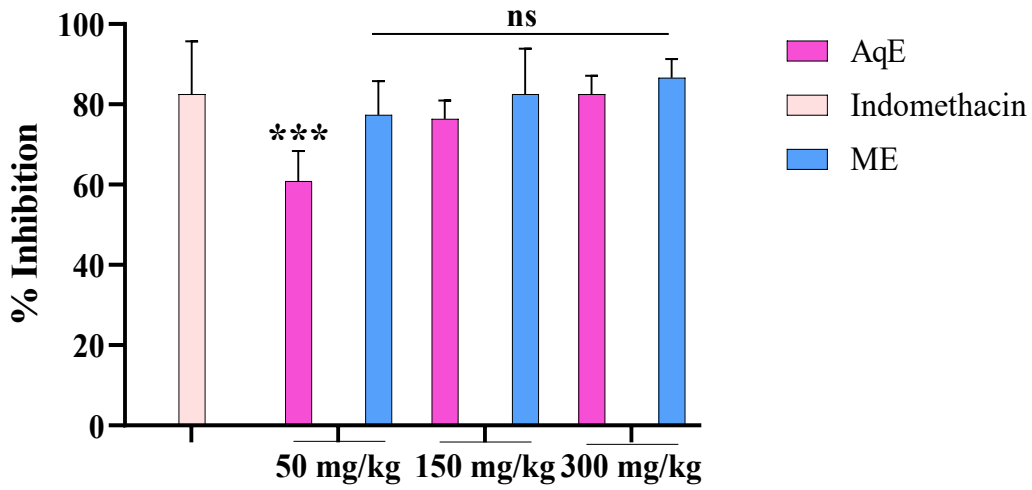

Figure 24: Inhibition percent of xylene-induced ear edema of *S. atropurpurea*, at different concentrations. Values are represented as mean ± SEM (n = 6). ns: no significant difference, ***P<0.001. AqE: aqueous extract, ME: methanolic extract.

4.3.2. Croton oil-induced edematous

The oral anti-inflammatory activity was tested using croton oil-induced ear edema model in mice, and the outcomes are illustrated in Figure 25. Both extracts decreased the edema response by dosage-dependent manner. At doses of 150 and 300 mg/kg; the effect was statistically equivalent to that of indomethacin (NSAID) ($p > 0.05$). AqE and ME reduced oedema by 58.97% and 61.03% at 300 mg/kg, respectively. Conversely, indomethacin achieved a reducing edema of 63.08% at a concentration of 50 mg/kg.

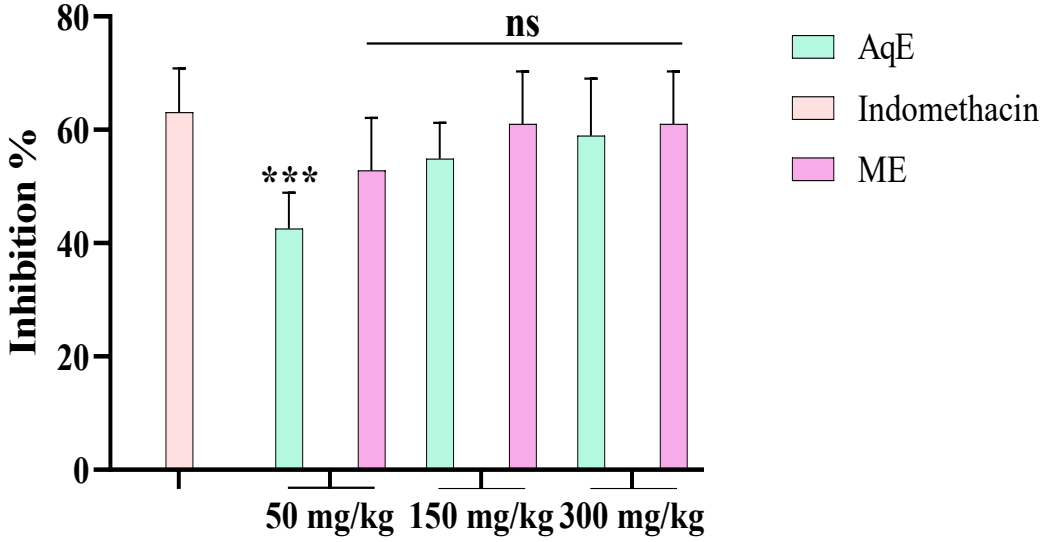

Figure 25: Inhibition percent of croton oil-induced ear edema of *S. atropurpurea*, at different concentrations. Values are shown as mean ± SEM (n = 6). ns: no significant difference, ***P<0.001. AqE: aqueous extract, ME: methanolic extract.

In this study, AqE and ME from *S. atropurpurea* was tested for the oral ant-inflammatory effect against xylene and croton oil-induced ear oedema. Both extracts recorded a significant anti-edematous activity compared to the drug reference. This potential is attributed to the polyphenolic content of our plant, which is known for its ant-inflammatory impact through several mechanisms as Inhibition of inflammatory markers and modulation of intracellular signals by eliminating NF-κB (nuclear factor κB) stimulation, consequently decreasing inflammatory expression of genes. This occurs via the inhibition of IKK (κ kinase inhibitor), modulation of oxidant levels, or interruption of NF-κB DNA binding (Sun *et al.,* 2024).

Furthermore, polyphenols and saponins regulate the MAPK (mitogen-activated protein kinase) and arachidonic acid signaling pathways. Additionally, they inhibit the release of TNF-α, hence leading to their anti-inflammatory actions. Their synergistic actions minimize the inflammatory response and enhance physical well-being (Sun *et al*., 2024, Chen *et al.,* 2024). Arifin et al. (2015) reported that quercetin significantly inhibits eicosanoid-producing enzymes such as COX-2 and 5-LOX derived from arachidonic acid, while resveratrol modulates the inflammatory response by obstructing the synthesis and release of pro-inflammatory mediators, altering eicosanoid synthesis, and inhibiting activated immune cells (Arifin *et al.,* 2015). Flavonoids and tannins exert anti-inflammatory effects by scavenging free radicals, hence inhibiting pro-inflammatory enzymes (Gonfa *et al*., 2021).

4.4. Formalin-induced paw edematous

S. atropurpurea extracts were tested for the inhibition of the swelling induced by formalin in paw edema in mice. Results are represented in Table 8. Extracts (25, 50, 100 mg/kg) exhibited a significant and dose-dependent reducing impact within 3 hours after inflammation induction with formalin solution (1%) in the right paw of mice, while, diclofenac was used as standard medication at 25 mg/kg. The maximum of inflammation reduction by ME and AqE reached in the third hour with 61.11% and 57.91% respectively at concentration of 100 mg/kg. However, the reference drug inhibited paw swelling with 70.25% after 3 hours.

Table 8: Anti-inflammatory potential of *S. atropurpurea* on formalin-induced paw edema. Values are given as mean ± SEM (n=5). ns: no significant difference, *P<0.05, **P<0.01, ***P<0.001. AqE: aqueous extract, ME: methanolic extract.

Extracts/ Standard	[C] mg/kg	Inhibition %			
		30 min	1h	2h	3h
AqE	25	$27.29 \pm 1.42^{**}$	$29.44 \pm 1.79^{***}$	$30.77 \pm 0.84^{***}$	$32.28 \pm 2.37^{***}$
	50	32.6 ± 2.7^{ns}	37.9 ± 3.12^{ns}	$46.15 \pm 1.66^{**}$	$48.58 \pm 2.46^{***}$
	100	39.22 ± 2.85^{ns}	42.26 ± 4.57^{ns}	52 ± 2.89^{ns}	$57.91 \pm 3.76^{**}$
ME	25	34.64 ± 2.31^{ns}	39.87 ± 0.8^{ns}	$43.46 \pm 1.11^{***}$	$50.65 \pm 3.07^{***}$
	50	37.25 ± 2.29^{ns}	40.2 ± 2.4^{ns}	52.94 ± 2.75^{ns}	$55.88 \pm 2.37^{***}$
	100	42.48 ± 1.74^{ns}	43.79 ± 2.35^{ns}	54.9 ± 2.17^{ns}	$61.11 \pm 1.58^{*}$
Diclofenac	25	39.13 ± 2.04	44.35 ± 3.64	60 ± 2.79	70.25 ± 3.52

Formalin-induced paw oedema is a prevalent technique for evaluating the acute anti-inflammatory properties of medicinal herbs, characterized by a biphasic reaction. Neurogenic pain in the initial phase (0–1 h), then, succeeded by the development of inflammatory reaction due to the secretion of mediators including histamine, serotonin, bradykinin, prostaglandins, cytokines (IL-1β, IL-6, TNF-α) and nitric oxide (NO) in the subsequent phase (2–3 h) (Akhtar, 2022).

Our findings demonstrate that AqE and ME exhibited a potential anti-inflammatory effect in reducing paw oedema induced by formalin. This effect is attributed to the bioactive compounds in the plant like quercetin, which can inhibit the COX-2 and modulate the NF-kB signaling (Alabi *et al.,* 2024). The effect of extracts on the edematous could be explained by the inhibition of the synthesis of pro-inflammatory substances and inhibiting the release of inflammatory mediators, which are the anti-inflammatory mechanisms of polyphenols, flavonoids and saponins.

In general, the anti-inflammatory properties of polyphenols make them prospective therapeutic agents for the treatment and prevention of inflammatory disorders. The anti-inflammatory efficacy of polyphenols may vary based on several parameters, including their chemical composition, dosage, method of administration, and individual variability (Sun *et al.,* 2024).

5. Analgesic activity

Results of acetic acid-induced writhing in mice are cited in Figure 26. The analgesic effect of extracts from *S. atropurpurea* increased in dose-dependent manner in comparison with standard medicine. ME showed the highest activity compared to AqE across all examined doses (50, 150, 300 mg/kg). Writhing reduction at 300 mg/kg of AqE and ME was significantly ($p > 0.05$) comparable to that of aspirin at 100 mg/kg, attaining 65.32%, 67.63% and 72.83% respectively.

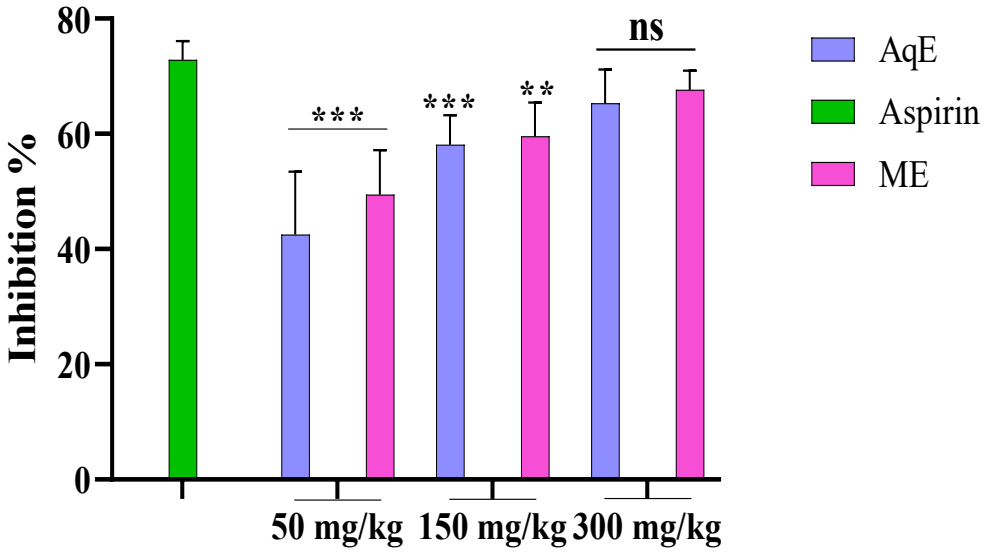

Figure 26: Analgesic impact of *S. atropurpurea*, at different concentrations. Values are represented as mean ± SEM (n = 5). ns: no significant difference, **P<0.01, ***P<0.001. AqE: aqueous extract, ME: methanolic extract.

Acetic acid-induced writhing serves as a model for abdominal inflammatory ache, elicited by the release of endogenous mediators that activate nociceptors (Bighetti *et al.,* 1999; Xie *et al*., 2023). Acetic acid is administered into the peritoneal cavity of the experimental animal, resulting in the secretion of proinflammatory cytokines, including IL-8, TNF-α and IL-1β, by peritoneal mast cells and macrophages. Consequently, an immediate inflammatory reaction occurs in the peritoneal region of the experimental animals, leading to typical writhing behavior. Substances possessing anti-inflammatory properties can reduce the frequency of writhing over time (Akhtar, 2022).

Bioactive substances are recognized for their analgesic abilities through the modulation of pain mechanisms and the alleviation of inflammation-related discomfort, especially phenolic compounds, which have attracted significant interest for their impact on inflammatory signaling systems (Kokabiyan *et al.,* 2023).

The mechanism by which the extract induced peripheral pain relief in this model may involve the inhibition of the production and secretion of several endogenous inflammatory substances, as well as the suppression of sensitivity in peripheral nociceptors within the peritoneal free nerve endings in response to acetic acid-induced pain. These are the processes of any analgesic substance that reduces writhing by decreasing the formation and release of prostaglandins and by obstructing peripheral pain transmission (Yimer *et al.,* 2020).

Furthermore, the bioactive compounds derived from medicinal plants reveal potential efficacy in preventing as well as therapy of neuropathic pain. In fact, Saponins have demonstrated anti-nociceptive effects in many animal studies by inhibiting the release of IL-6, IL-1β, COX-2, and iNOS, reducing neuronal damage and promoting peripheral nerve regeneration and functional restoration (Tan *et al.,* 2022).

6. Toxicity

6.1. Cytotoxicity against prostate carcinoma cell line

The MTT procedure was employed to test the cytotoxic impact of aqueous and methanolic extracts from *S. atropurpurea* on prostate cancer cell lines (PC-3). The inhibitory percent is shown in Table 9. Both extracts displayed a cytotoxic effect on PC-3 in dose dependent manner. AqE eliminated the tumor cells with 85.42% at 0.5 mg/mL, while ME recorded a 60.95% effect at the same dose. Doxorubicin; a chemotherapeutic agent, served as a reference drug.

Table 9: Inhibitory % of *S. atropurpurea* against prostate cancer cell line. Data are shown as mean ± SD (n=3). AqE: aqueous extract, ME: methanolic extract.

[C] µg/mL	**Inhibitory %**		
	Doxorubicin	**AqE**	**ME**
500	99.02 ± 0.06	85.42 ± 1.26	60.95 ± 1.45
250	97.53 ± 0.11	62.85 ± 2.31	13.79 ± 0.87
125	91.85 ± 0.37	33.58 ± 1.64	1.26 ± 0.22
62.5	82.58 ± 0.24	11.35 ± 1.29	0
31.25	70.39 ± 0.96	1.86 ± 0.32	0
15.6	64.92 ± 1.45	0	0

Figure 27 represent the percentage of cell viability after 48 hours of experiment which decreased with the increasing doses. On the other hand, the IC_{50} values were measured and reached a 195.12 ± 3.87 µg/mL and 441.95 ± 8.94 µg/mL for AqE and ME, respectively. Doxorubicin registered a potent cytotoxicity activity with an IC_{50} of 2.34 ± 0.26 µg/mL.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) experiment is a widely utilized method for assessing the metabolic process of viable cells. The assay relies on the enzymatic reduction of a softly colored tetrazolium salt to its intensely purple-blue

formazan derivative, which may be detected by spectrophotometry. Under optimally adapted conditions, the measured absorbance value is directly proportional to the total number of viable cells (Grela *et al.,* 2018).

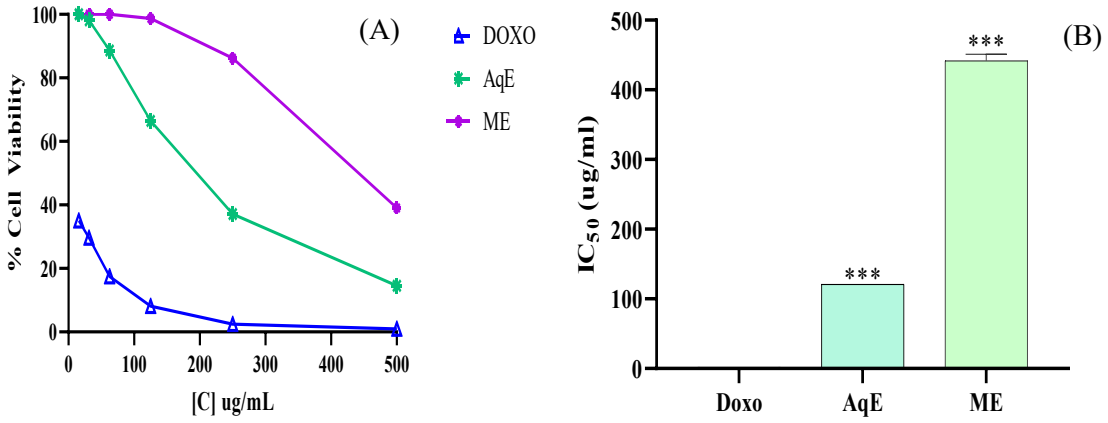

Figure 27: Cytotoxic impact of *S. atropurpurea* against PC-3 cells. (A) % cell viability after treatment with AqE and ME compared with doxorubicin as standard. (B) IC_{50} (µg/mL) of AqE and ME compared with doxorubicin. Results are given as the mean ± SD (n=3). ***P<0.001.

The outcomes revealed the cytotoxic property of *S. atropurpurea* extracts on PC-3 cell lines. Actually, several studies validate the anticancer efficacy of Scabiosa genera on distinct cancerous cells. A silver nanoparticle produced from fruits aqueous extract of *S. atropurpurea subsp. maritima* displayed a substantial anti-tumor efficiency against MDA-MB-231 (human breast cancer cell line) and U266 (Human multiple myeloma cell line) (Ben toumia *et al.,* 2020, Skala and szopa, 2023) and leaves methanolic extract of the same species demonstrated a cytotoxicity contra Caco-2 (Human epithelial colorectal adenocarcinoma) both independently and combined with doxorubicin.

Herein, these cytotoxic impacts are associated with the synergistic combination of particular bioactive components found in the medicinal plants (Ben toumia *et al.,* 2020). Indeed, specific biochemicals, including iridoids, triterpenoid derivatives and phenolic acids derived from Scabiosa, exhibited lethal effects in several cancer cells. Further, several saponins

extracted from the Dispacus genera demonstrated a promising anti-cancer action against various carcinoma cell lines.

The anticancer pharmacotherapy primarily focusses on inhibiting the proliferation of malignant cells and promoting apoptosis. However, the antitumor efficacy of phytochemicals frequently involves a multifaceted mechanism encompassing antioxidant properties, antiproliferative effects, arrest of cell cycles, carcinogen neutralization, angiogenesis blocking, apoptosis and differentiation induction, and the reversal of multidrug resistance (Kowalczyk *et al.,* 2022).

Additionally, Nawab et al. (2012) indicated that an extract rich on polyphenols from *Solanum nigrum* show an anticancer activity on various human prostate cancer cells including PC-3 cells; by causing arrest in the G2/M phase of the cell cycle and inhibits selectively cell growth, decreases cell viability and accelerates apoptosis by the increase of ROS generation, the reduction of mitochondrial membrane capacity, the secretion of cytochrome c, the stimulation of caspase 3, 8 and 9, the elevation of the level of proapoptotic proteins, including Bax, and the decrease of the level of anti-apoptotic proteins, including Bcl-2 (Nawab *et al.,* 2012, Kowalczyk *et al.,* 2022).

Particularly, bioactive compounds from plants are recognized for their ability to cause cellular death in many cancer cell types through the activation of distinct signaling pathways. These variations may arise from the chemical nature of the phyto-components and the particular sensitivity of cancer cells (Nkwe *et al.,* 2021).

6.2. Acute oral toxicity

Medicinal plants should have minimal toxic effects due to their prolonged use in human. Nonetheless, some therapeutic herbs utilized in traditional medicine have been documented to demonstrate harmful consequences (Jatoth *et al*., 2025). Conducting toxicity assessments

in suitable models of animals is essential for guaranteeing drug safety. As per the recommendations of the Organization for Economic Co-operation and Development (OECD), acute toxicity studies represent a sophisticated method for assessing the safety profile of specific drugs through single and multiple-dose evaluations (Majumdar *et al.,* 2021).

The safety of phytopharmaceuticals, including isolated fractions, extracts, and herbs, is extensively established and continues to be a significant concern (Jatoth *et al.,* 2025).

The oral administration of aqueous and methanolic extracts from *S. atropurpurea* did not result in any mortality among the treated mice over the 14-day experimental period. Furthermore, there were no behavioral changes or evident signs of acute poisoning. Consequently, the LD_{50} of both extracts exceeds 5 g/kg of body weight.

A study of acute oral toxicity of *Scabiosa artropurperea var. maritima* aqueous extracts in mice showed that the extract did not cause detrimental effects on body weight, haematological, and reproductive indicators in male mice. In addition, no indications of toxicity were identified in the liver and kidney sections of the treated mice. Consequently, the oral LD_{50} of the plant's aqueous extract is likely over 4 g/kg (Niama *et al.,* 2024).

CONCLUSION AND PERSPECTIVES

Conclusion and perspectives

Currently, the majority of medications have a botanical origin. The natural items represent safety, whereas synthetic substances are regarded as dangerous to individuals and the environment. Approximately eighty percent of the world's inhabitants depends on conventional treatments for basic healthcare. *Scabiosa atropurpurea* L. a medicinal herb that comprises a variety of secondary metabolites known for their antioxidant, antibacterial, anti-inflammatory and anticancer activities. Indeed, *S. atropurpurea* was utilized traditionally to handle several health problems like acne, measles and for menstrual regulation.

Hence, the extraction of polyphenolic components was performed by applying a decoction and maceration methods to obtain the aqueous and methanolic extracts respectively. The yield proportion was affected by several elements such the used solvent and extraction procedure, temperature and duration of extraction.

The qualitative phytocomponents estimation indicated the existence of polyphenols, flavonoids, saponins, free quinones, tannins, terpenoids, anthraquinones, and reducing sugars in both extracts, whereas, coumarins were present only in AqE. Moreover, the quantitative determination demonstrated that ME have the highest polyphenols and flavonoids tenor with 114.13 ± 0.92 µg GAE/mg DE and 100.57 ± 0.93 µg QE/mg DE, respectively, followed by AqE with 81.6 ± 1.6µg GAE/mg DE and 45.33 ± 0.36 µg QE/mg DE, respectively. However, AqE recorded the highest condensed tannins content, which attaining 41.04 ± 0.64 µg CE/mg DE.

In addition, the UPLC-MS/MS analysis identified thirty-four bioactive components in both extracts in the negative and positive ion modes. The defined compounds belonging to phenolic acids and flavonoids, following by triterpenoids, sesquiterpenoids, tannins, saponins, iridoids, fatty acids, and others.

For the assessment of antioxidant capabilities, several tests were conducted depending on free radicals scavenging, iron chelating and reducing power. Both extracts exhibited a similar PPH free radical scavenging effect with an IC_{50} value of 0.06 mg/mL. AqE demonstrated a substantial hydroxyl scavenging capacity, iron chelating and reducing power impacts, as compared with ME. However, ME showed the highest total antioxidant activity with $A_{0.5}$ value reaching 0.18 ± 0.01mg/mL. Many previous research confirms the relation between the presence of bioactive compounds and the antioxidant activity. Thus, our results indicated that no correlation between the high polyphenolic content and the antioxidant potentials.

The anti-arthritic activity was carried out *in vitro* utilizing protein denaturation inhibition assay. Both extracts showed a dose-dependent effect and the AqE inhibited the protein denaturation with 61.97% at 1mg/ml, whilst ME demonstrated a lower value of 33.6% at the same dose. This test was in comparison with diclofenac, a non-steroidal drug.

The anti-inflammatory activity of *S. atropurpurea* was tested topically and orally by applying different approaches; xylene and croton oil induced ear edematous and formalin induced paw edema in mice. Both extracts displayed an anti-edematous potential in dose-dependent manner. Additionally, acetic acid induced writhing in mice method was employed to evaluate the analgesic effect. Extracts demonstrated a significant pain relief which was dose-related. These anti-inflammatory and analgesic abilities of extracts are attributed to the secondary metabolites, which are known to have multiple mechanisms to deal with inflammation and pain.

S. atropurpurea extracts exerted a cytotoxic impact on prostate cancer cell lines (PC-3), by using the MTT assay. AqE and ME displayed a significant cytotoxicity and antiproliferative effect in dose dependent way, and the test was in comparison with doxorubicin; a chemotherapeutic agent.

Results of the acute oral toxicity revealed the safety of extracts from *S. atropurpurea* and no toxic or harm signs were observed during 14 days. The LD_{50} was determined to be higher than 5 g/kg.

To conclude, the findings of the present study provide important knowledge regarding the biochemical profile of *S. atropurpurea*, and confirm the antioxidant, anti-inflammatory and anticancer potentials, besides its safety. *S. atropurpurea* can serve as a source of phytochemicals and perform as significant health-promoting agent and natural therapeutic substance to deal with health conditions, and can be exploited in the pharmaceutical sector.

More studies and investigation are required to performed by other experiments:

- ✓ Isolation, purification and identification of bioactive compounds and assess their biological potentials to comprehend their molecular mechanisms.
- ✓ *In vivo* antioxidant activity.
- ✓ Assessment of anti-inflammatory activity and determine the biomechanisms of secondary metabolites at the molecular level.
- ✓ A complete and detailed toxicity study.

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RESEARCH ARTICLE

Phytochemical screening and antioxidant activity of aqueous and methanolic extracts from *Scabiosa atropurpurea* L.

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ABSTRACT:
Scabiosa atropurpurea L. is a medicinal herb that has been traditionally employed for the treatment of diverse ailments. This work aimed to determine the phytochemical composition and assess the antioxidant capacity of aqueous and methanolic extracts derived from the aerial part of *S. atropurpurea* L., which grows in Algeria. The content of polyphenols, flavonoids, tannins, and many other bioactive components was measured using colorimetric methods. The antioxidant capabilities were evaluated using the DPPH radical scavenging, iron chelating, reducing power, and phosphomolybdate models. The quantitative phytochemical analysis showed that CrE had a greater amount of polyphenols and flavonoids, with 114.13±0.92µg GAE/mg DE and 100.57±0.93 µg QE/mg DE, respectively. AE had the greatest value of condensed tannins, with 41.04±0.64 µg CE/mg DE. AE exhibited a potential impact on iron chelating and reducing power tests. Nevertheless, AE and CrE exhibited significant efficacy in DPPH scavenging and phosphomolybdate testing. The findings suggest that extracts derived from *S. atropurpurea* have a high quantity of phytochemical components and exhibit notable antioxidant properties, making them a potential source of natural antioxidants.

KEYWORDS: *Scabiosa atropurpurea*, Polyphenols, Phytochemical screening, Extracts, Antioxidant activity.

INTRODUCTION:
The significant role of oxidation in the bodies and in nutrients has been widely acknowledged and it is necessary for cell's functioning. One unintended result of this dependency is the formation of free radicals as well as other reactive oxygen species, causing oxidative alterations[1]. Increasing data suggests that these species have a function in various conventional in vivo regulation systems. Excessive amounts of free radicals may overpower defensive enzymes like catalase, superoxide dismutase and peroxidase. This may result deadly impacts on organisms through oxidizing cellular proteins, membrane lipids, DNA, and enzymes, ultimately halting cell respiration[2].

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Moreover, reactive oxygen species appear to modify cell signaling processes in previously unknown manners. An antioxidant is a substance which, even in little amounts, has the ability to inhibit or significantly slow down the process of oxidation in materials that are subject to oxidation[3]. It can also delay or prevent the oxidation of substances when exist in low quantities comparing to the oxidizable substrate. In addition, antioxidants possess the ability to regulate or counteract the process of lipid or biomolecule oxidation by scavenging reactive oxygen species. The action of different antioxidants serves as a means of protection against the harmful consequences of excessive oxidation, and the need of assessing antioxidant activity is widely acknowledged[4,5].

A natural state of balanced dynamic presents between the creation of free radicals in our bodies and the existence of antioxidants that shield the organism from detrimental effects. The quantity of antioxidant compounds available under typical physiological conditions is often insufficient to counteract the effects of free radicals. Consequently, there is an increasing fascination in the food industry and in preventive

medicine regarding the creation of "natural antioxidants" derived from plant[6]. As a result, plants having antioxidant capabilities are becoming increasingly popular across the world.

Scabiosa is a taxonomically intricate genus consisting of several species that are distributed over the Mediterranean Basin, southern Africa and Asia[7]. Scabiosa genera are recognized for their medical properties, and studies on their phytochemical composition have shown the presence of intriguing secondary metabolites, among them have demonstrated interest as medical treatments[8]. Numerous reports have shown that Scabiosa species in the Mediterranean area possess a wide range of biopotential and therapeutic benefits. These species have demonstrated many biological benefits, notably antioxidant, anti-inflammatory and antibacterial activities[9]. *Scabiosa atropurpurea* is a yearly plant that forms clusters of leaves at the base and has stems with leaves, attaining an altitude of 20-60 cm. This genus may be identified by its unique bluish-lilac blossoms with peculiar fruit form[10,11].

To our understanding, the antioxidant properties of the above-ground portion of *S. atropurpurea* have not been investigated utilizing a comparison between aqueous and methanolic extracts. Hence, this research investigation seeks to analyze the antioxidant efficacy of aqueous and methanolic extracts from *S. atropurpurea*, a plant species found in the Algerian flora, utilizing different techniques and assays.

MATERIALS AND METHODS:

Plant species selection

The plant samples were gathered locally in Chaabia region of Ouricia, Setif, located in northeastern Algeria during the blossom and fruiting season of may to july (2021). The plant was identified under voucher specimen (020/DBEV/UFA/22). The entire aerial portion was completely cleansed, dehydrated in a shaded area, powdered, and thereafter kept for testing purposes.

Extractions procedures:

Preparation of the decocted extract:

The aqueous extract (AE) was obtained by boiled 100g of dehydrated aerial part in one liter of purified water for a duration of 20 minutes[12]. Subsequently, the mixture underwent filtration using muslin cloth and was subjected to centrifugation at 3000 rpm for a duration of 15 minutes. The extract's solution was gathered and evaporated to dryness. The resulting dry extract was then kept at a temperature of 4°C.

Preparation of the crude methanolic extract:

The complete above-ground portion of *scabiosa atropurpurea* weighing 4kg was defatted by 15L of petroleum ether. Subsequently, the plant was subjected to air drying and then underwent maceration using 15 liters of 100% methanol. The crude methanolic extract (CrE) was evaporated and the resultant concentrated extract was preserved at 4°C[13].

Qualitative phytochemical screening:

Analysis of plant compounds using qualitative methods to identify the presence or absence of certain chemical constituents. The samples were analyzed to determine the presence of bioactive components including polyphenols, flavonoids, tannins, coumarins, quinones, terpenoids, and anthraquinones adopting the procedures described by[14,15]

Quantification phytochemical estimation:

Total phenolic content:

Polyphenol tenor was conducted employing Folin-Ciocalteu (FC) procedure[16,17]. 0.1 mL of samples was combined with 0.5 mL (FC) reagent. 0.4 mL of sodium carbonate (7.5%) was then introduced. Following two hours of incubation, the spectrophotometric measurement was estimated at 765 nm and the findings were represented as ug of gallic acid equivalents per mg of extract (ug GAE/mg DE).

Overall flavonoid tenor:

Aluminum chloride reagent (ACR) was utilized to calculate the total flavonoid amount[12]. 0.5 mL of samples was combined with 0.5 mL ACR (2%), and the resulting combination was allowed to incubate for ten minutes. The spectrophotometric analysis conducted at 430 nm. The findings were expressed as ug of quercetin equivalent per mg of dried extract (µg QE/mg DE).

Condensed tannins content:

The condensed tannins tenor has been performed using a modifying vanillin procedure[14]. 250 µL of samples was combined with 375 µL of vanillin reagent (4%). Later, 187.5 µL of HCl was added. The resulting blend has been incubated for twenty minutes. The spectrophotometric measurement was conducted at 550 nm. The obtained data were reported in ug of catechin equivalent per mg of dry extract (µg CE/mg DE).

Evaluation of the anti-oxidant property:

DPPH radical scavenging activity:

Examining samples' capacity to remove free radicals has been achieved via measuring a decreased absorption (at 517 nm) of a DPPH reagent[18]. 50 µL of samples' varied doses or butylated hydroxytoluene (BHT) as a reference were blended with 1.25 mL of a DPPH reagent (0.004 %). The absorption was recorded at 517 nm over 30 min of incubating in the absence of light.

Iron chelating assay:
The assessment of ferrous iron chelation of samples has been performed through a spectrophotometric test[19]. 50 µL of Fecl2 (0.6 mM) and 450 µL of methanol were combined with 250 µL of extracts at various concentrations. 50 µL of ferrozine (5 mM) was introduced after five minutes, and the mixture was let to interreact for ten minutes. A reading of results was estimated at 562 nm. An IC50 value was calculated, which is the amount of products that produce 50% of the maximal neutralizing efficiency.

Reducing power assay:
This assay is employed to assess the reduction capacity of samples[19]. 400 µL of either extract or ascorbic acid was blended with a similar amount of phosphate buffer (0.2 M, pH = 6.6) and potassium ferricyanide (1%) and rest for 20 minutes at 50 °C. A 400 µL of 10% TCA was introduced and the produced mix was subjected to centrifugation at 3000 rpm during ten minutes, then combined with purified water (400 µL) and 80 µL of 0.1% ferric chloride. Following ten minutes of incubating, the formed product was observed at 700 nm.

Phosphomolybdate assay:
This method is used to evaluate the total antioxidant effect[20,21]. One milliliter of combination consists of (28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M H2SO4) has been blended with 100 µL of samples. The resulting mixture rest during 90 minutes at 95 °C. Next, the results were determined at 695 nm. The outcomes were determined as µg equivalents of ascorbic acid per mg of extract (µg EAA/mg extract).

Statistical analysis:
Statistical analysis implied Student's and one-way ANOVA tests, coupled with Dunnett and Tukey tests. The results were provided in triplicate, with the mean value accompanied by the standard deviation (SD). The study was conducted using GraphPad Prism-8. Variation defined Significant at a p-value less than 0.05. An IC_{50} was calculated by linear regression.

RESULTS:

Phytochemical screening:
The extracts of *S. atropurpurea* comprised various phytochemical compounds, as documented in Table 1. The outcomes demonstrated the existence of polyphenols, flavonoids, free quinones, tannins, saponins, terpenoids, anthraquinones, coumarins and reducing sugars in aqueous extract. However, CrE contains all phytochemical compounds except coumarins. This phytochemical analysis reveals the abundance of secondary metabolites in both extracts.

Table 1: Phytochemical screening of AE and CrE extracts from *S. atropurpurea*.

Phytochemical compounds	AE	CrE
Polyphenols	+	+
Flavonoids	+	+
Tannins	+	+
Terpenoids	+	+
Free quinones	+	+
Anthraquinones	+	+
Coumarins	+	-
Saponins	+	+
Reducing sugar	+	+

Extraction yields and determination of bioactive compounds:
The outcomes of extraction are illustrated in Table 2. The yield percent of CrE (13.8%) was higher than AE (9.06%). The overall phenolic amount, flavonoids, and condensed tannins are presented in Table 2. The findings of the quantified phytochemical assessment suggest that CrE exhibited a significant level of TPC and TFC with 114.13±0.92µg GAE/mg DE and 100.57±0.93 µg QE/mg DE, respectively. However, the highest CT content was recorded by AE with 41.04±0.64 µg CE/mg DE.

Table 2: Extraction yields and total phenolic, flavonoids and condensed tannins content of *S. atropurpurea* extracts.

Extracts	Yields %	TPC (µg GAE/ mg DE) [a]	TFC (µg QE/ mg DE)[b]	CT (µg CE/ mg DE) [c]
AE	9.06	81.6±1.6	45.33±0.36	41.04±0.64
CrE	13.8	114.13±0.92	100.57±0.93	26.22±1.11

Data are represented as mean ±SD (n=3). [a] Gallic acid equivalent/mg of dried extract, [b] Quercetin equivalent/mg of dried extract, [c] Catechin equivalent/mg of dried extract.

***In vitro* antioxidant capacities:**
The DPPH scavenging activity of the AE and CrE produced from *S. atropurpurea* was expressed using the inhibitory concentration (IC50), and the results appear in Table 3. Both extracts demonstrated a high eliminating efficacy compared to BHT. It is noteworthy that the significant chelator effect of AE (0.16±0.03mg/ml) compared to the EDTA as a reference (0.01 ± 0.00 mg/ml), whereas CrE exhibited the lowest effect (0.64 ± 0.01 mg/ml). The extracts' reducing power effect was evaluated by measuring A0.5 (mg/mL), a dose corresponds to the absorbance at 0.50 nm. Despite the ferrous reducing capacity of vitamin C (0.01 ± 0.00 mg/mL), AE and CrE demonstrated significant ability. Furthermore, it is essential to mention the superior reducing ability of AE compared to CrE (0.08 ± 0.003 mg/mL) (Table 3). The total antioxidant ability was assessed employing the phosphomolybdate test. AE and CrE showed almost a similar potency with 0.2±0.01 and 0.19±0.01 µg EAA/mg E, respectively.

Table 3: Antioxidant capacities of *S. atropurpurea*.

Extracts/ standards (mg/mL)	IC_{50} (mg/ mL)		$A_{0.5}$ (mg/ mL)	µg EAA/ mg E
	DPPH	Chelating assay	Reducing power assay	Phosphomoly-bdate assay
AE	0.06±0.00**	0.16±0.03***	0.08 ±0.00***	0.2±0.01 ns
CrE	0.06 ± 0.02**	0.64 ± 0.01***	0.13 ± 0.01***	0.19±0.01ns
BHT	0.014 ± 0.00	--	--	--
EDTA	--	0.01 ± 0.00	--	--
Ascorbic acid	--	--	0.01 ± 0.00	--

Data are represented as mean ±SD of three measurements compared to standards. ns: no significant difference, ** p<0.01, *** p<0.001. BHT: Butylated hydroxytoluene, EDTA: Ethylenediaminetetraacetic acid

DISCUSSION:

For millennia, traditional medicine has utilized herbs to cure various ailments among different communities worldwide. The medical effectiveness can be related to the abundant collection of diverse bioactive components found in plants[22]. This research examines the phytochemical composition and antioxidant properties of *S. atropurpurea* utilizing several experimental techniques. The results indicated that the yields of the extracts varied, and this variation may be explained by taking into account the extraction technique used, the selection of solvents, and the particular circumstances within the extraction process[23]. The phytochemical screening of *S. atropurpurea* revealed the existence of several secondary metabolites with established biological activity, involving flavonoids, polyphenols, free quinones, tannins, terpenoids, anthraquinones, saponins, coumarins, and reducing sugars. Multiple investigations have verified the abundance of phytochemical substances in scabiosa species, which have advantageous biological effects[24]. Multiple studies on scabiosa species have conclusively demonstrated the presence of diverse bioactive chemicals *S. atropurpurea*[25], and *S. comosa*[26]. Flavonoids were

isolated from *S. caucasica*[27], *S. stellata*[28] and *S. atropurpurea*[29]. Terpenoids and irridoid glucosides were detected in *S. stellata*[30], *S. tschilliensis*[31], *S. atropurpurea subsp. Maritima*[32,33] and *S. arenaria*[34]. Coumarins were identified in *S. hymettia*[35]. The amount of polyphenols, flavonoids, and condensed tannins was measured using spectrophotometry. The findings demonstrated a high level of these compounds, particularly when compared to other scabiosa species such as *S. arenaria*[34] and *S. stellata*[36]. The choice of solvent for extraction and its polarity can have an impact on the phenolics content and the characteristics of the extracted molecules[21].

Antioxidants are highly regarded for their significant health benefits and crucial role in preventing against diseases and the evaluation of antioxidant capacity should be conducted using multiple approaches[37]. This study utilizes different in vitro techniques to assess the antioxidant properties of S. atropurpurea, including scavenging of free radicals, ferric reducing power, metal chelating, and the phosphomolybdate method. DPPH is a highly stable free radical that is commonly used to study the capability of natural products to scavenge free radicals. It is also the favored approach assessing the ability to remove free radicals of novel medications because of its simplicity and effectiveness and rapidity[38]. The scavenging effect seen in AE and CrE was superior to that of the ethanolic extract of *S. atropurpurea* (IC50 0.1383 ± 0.0789 mg mL-1)24 and the methanolic extract of *S. stellata* (IC50=86 µg mL-1)[36]. The antiradical action of *S. atropurpurea* extracts is attributed to their high content of polyphenols, which are well-known for their capacity to eliminate free radicals by donating hydrogen. Also, the most significant mechanisms of polyphenols are estimated to be free radical scavenging, in which they can interrupt the free radical chain reaction, and eradication of free radical formation through regulating enzyme activity[39]. The scavenging effect is influenced by the presence and positioning of hydroxyl and carboxyl groups on the molecules of polyphenols. In addition, the metal chelation characteristic is used as a sign of antioxidant potential. It has been studied in conjunction with other antioxidant tests for various antioxidants and extracts[40]. Antioxidants are shown to effectively bind iron due to their functional groups that work as metal binders[41]. The chelating effect values of S. stellata were inferior to our results[36]. Moreover, the presence of polyphenols in extracts enables them to bind to metal ions, such as iron, thereby increasing their antioxidant properties by inhibiting redox-active transition metals from supporting free radical formation of free radicals[42]. Furthermore, one of the main characteristics of flavonoids is their antioxidant activity, plus their capability to chelate transition metal ions[43]. Conversely, the reduction of antioxidant power assay is a widely used and dependable method for assessing antioxidant capacity[44]. Furthermore, compounds possessing reduction potential react with potassium ferricyanide to produce potassium ferrocyanide. This compound then interacts with FeCl3, resulting in the creation of a highly concentrated blue complex with a maximum absorbance at 700 nm[45]. Among extracts, AE revealed the greater reducing effect with 0.08 ± 0.00 mg mL^{-1}, this activity was higher than those of n-butanol fraction of flowers, stem and leaves from *S. arenaria* and lower than the fruits' butanolic fraction[34]. In fact, the features of components that reduce ferric ions are attributed to their ability to transport electrons, which is an important characteristic indicating

their antioxidant capacity[46]. A spectrophotometric test was devised to accurately measure the total antioxidant capacity by utilizing the phosphomolybdenum method. This approach relies on the conversion of molybdenum (IV) to molybdenum (V) by the plant samples, causing the creation of a phosphate/molybdenum (V) complex under acidic circumstances. This complex exhibits a green color its highest absorbance at 695 nm[21]. The phosphomolybdate assay has been documented as a reliable technique for assessing the overall antioxidant capabilities of different plant extracts[47]. Our investigation found that both AE and CrE exhibited a suppressive effect on the molybdenum (IV), indicating a high antioxidant capability. The overall antioxidant capacity of *S. atropurpurea* may be related to the existence of flavonoids and phenolics in the plant's extracts.

The findings of this work confirm the conventional use of S. atropurpurea for treating various ailments and show that different extracts containing distinct secondary metabolites possess strong antioxidant properties and benefits. Typically, the antioxidant properties of plants are mainly attributed to the existence of polyphenols and may be influenced by other substances such as coumarins and flavonoids[48]. Numerous investigations have demonstrated that plant polyphenols can serve as antioxidants, safeguarding against diseases caused by oxidative stress[49]. Current studies have demonstrated that plants contain numerous bioactive chemicals that exhibit potent antioxidant actions. The antioxidant properties exhibited by our plant extracts can be related to the existence of polyphenols, flavonoids and tannins. The extracts exhibit properties such as free radical inhibition, chelation of ferrous iron, high reducing power, and total antioxidant capacities. Therefore, it can be utilized as a natural antioxidant and applied for dealing with illnesses linked to oxidative stress.

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