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Phytochemical study and biological activities of crude
polysaccharides extracted from seeds of (Larta) *Calligonum
polygonoides subsp. comosum* (L'Hér.) Soskov. collected from
Oued Souf region

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DEDICATION

يَرْفَعُ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ

الْحَمْدُ لِلَّهِ الَّذِي بِنِعْمَتِهِ تَتِمُّ الصَّالِحَاتُ، وَبِفَضْلِهِ وَتَوْفِيقِهِ تُجْنَى ثِمَارُ السَّعْيِ وَالْكَفَاحِ، وَالصَّلَاةُ وَالسَّلَامُ عَلَى نَبِيِّ الْعِلْمِ وَالْمَعْرِفَةِ مُحَمَّدٍ وَعَلَى آلِهِ وَصَحْبِهِ أَجْمَعِينَ.

أَهْدِي هَذَا النَّجَاحَ لِنَفْسِي أَوَّلًا.. لِشَعْفِهَا الَّذِي لَمْ يَنْطَفِئْ، لِصُمُودِهَا فِي وَجْهِ كُلِّ لَحْظَةٍ تَعَبٍ أَوْ سَهَرٍ قَضَيْتُهَا بَيْنَ أَوْرَاقِ الْعِلْمِ وَعَتَادِ الْمُخْبَرِ، وَلِإِيمَانِهَا بِأَنَّ الْوُصُولَ مَحْثُومٌ لِمَنْ اسْتَعَانَ بِاللَّهِ. إِلَى حِصْنِي الْمَنِيعِ وَمَنْ كَلَّلَهُ اللَّهُ بِالْهَيْبَةِ وَالْوَقَارِ.. إِلَى مَنْ أَحْمَلُ اسْمَهُ بِفَخْرِ يُطَاوِلُ السَّمَاءَ، الرَّجُلِ الْعَظِيمِ الَّذِي كَانَ يَسْتَمِدُّ مِنْ طُمُوحِي فَرَحَهُ، وَبَدَلَ كُلِّ غَالٍ لِيَرَانِي فِي هَذِهِ الْمَرْتَبَةِ.. أَبِي الْعَالِي، مَنَّكَ اللَّهُ بِالصِّحَّةِ وَالْعَافِيَةِ وَأَطَالَ فِي عُمْرِكَ.

إِلَى أَيَّامِي الْمُلَوَّنَةِ بِنُورِ دُعَائِهَا، إِلَى نَبْعِ الْحَنَانِ وَالتَّقَانِي.. بِسَمَةِ الْحَيَاةِ وَسِرِّ صُمُودِي، مَنْ كَانَتْ دَعَوَاتُهَا فِي جَوْفِ اللَّيْلِ تَمْسُحُ عَنْ قُلُوبِي كُلِّ رَهَقٍ، وَمِنْ حَنَانِهَا أَسْتَمِدُّ الْقُوَّةَ لِلْأَبَدِ مِنْ جَدِيدٍ.. أُمِّي الْحَبِيبَةِ، حَفِظَكَ اللَّهُ لِي جَنَّةً فِي الدُّنْيَا.

إِلَى السَّنَدِ الَّذِي لَا يَمِيلُ، وَالْأَعْمَدَةِ الثَّابِتَةِ فِي مَسِيرَةِ حَيَاتِي.. أَرْضِي الصَّلْبَةَ الَّتِي أَقْفُ عَلَيْهَا بَعْدَ كُلِّ عَائِرَةٍ، الَّذِينَ شَدَّ اللَّهُ بِهِمْ عَضْدِي فَكَانُوا لِي خَيْرَ مُعِينٍ وَخَيْرَ دَافِعٍ.. إِخْوَتِي وَأَخَوَاتِي. إِلَى رَفِيقَةِ الدَّرْبِ وَالْمُخْبَرِ.. الَّتِي تَقَاسَمْتُ مَعَهَا عَنَاءَ السَّاعَاتِ الطَّوِيلَةِ.. وَشَغَفَ النَّحْوِ الْعِلْمِيِّ وَتَقَاصِيلَهُ، كُنْتُ نِعَمَ الشَّرِيكَةِ وَالزَّمِيلَةِ فِي هَذَا الْإِنْجَازِ.

يَسِّرُنِي وَيُسِّرْفُنِي أَنْ أَتَقَدَّمَ بِأَسْمَى عِبَارَاتِ الشُّكْرِ وَأَجْزُلِ آيَاتِ الْإِمْتِنَانِ وَالتَّقْدِيرِ، إِلَى الْأَسْتَاذِ الْفَاضِلِ وَالْمُشْرِفِ الْقَدِيرِ "شُوَيْخِ عَاطِفٍ"، الَّذِي غَمَرَنِي بِنَبِيلِ أَخْلَاقِهِ، وَلَمْ يَخِلْ عَلَيَّ يَوْمًا بِعِلْمِهِ الْوَافِرِ وَتَوْجِيهَاتِهِ النَّبِيرَةِ طَوَالَ فِتْرَةِ إِعْدَادِ هَذَا الْإِنْجَازِ.. إِلَى الْأَسْتَاذَةِ الْفَاضِلَةِ خَوْلَةَ سَقْنِي..

نَتَقَدَّمُ لَكَ بِخَالصِ الشُّكْرِ وَالْإِمْتِنَانِ عَلَى جَهْدِكَ الْمُبَارَكَةِ وَدَعْمِكَ الْمُسْتَمِرِّ لَنَا دَاخِلَ الْمُخْتَبَرِ. لَقَدْ كُنْتُ خَيْرَ عَوْنٍ وَمَوْجِهٍ، وَبِصِمَتِكَ الْعِلْمِيَّةِ وَالتَّوْجِيهِيَّةِ كَانَتْ أَسَاسًا لِنَجَاحِ هَذَا الْعَمَلِ. جَزَاكَ اللَّهُ عَنَّا خَيْرَ الْجَزَاءِ..

By : Maannser Nawel

DEDICATION

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ) النمل: 19

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By : Chaouki Ourida

ABSTRACT

The present study investigated the phytochemical composition, antioxidant potential, biological activities, acute toxicity, and gastroprotective effects of polysaccharide-rich extracts obtained from *Calligonum comosum* seeds. Quantitative phytochemical analyses revealed a total phenolic content (TPC) of 61.37 mg GAE/g extract, a total flavonoid content (TFC) of 16.09 ± 0.22 mg QE/g extract, and a condensed tannin content (CTC) of 194.27 mg CE/g extract, indicating that the extract is particularly rich in polyphenolic constituents associated with antioxidant and protective biological activities.

Evaluation of antioxidant activity using the DPPH radical scavenging assay demonstrated a concentration-dependent response, with inhibition percentages increasing from 2.71% at 0.1 mg/mL to 11.65% at 0.6 mg/mL. The calculated IC_{50} value was 3.79 mg/mL, confirming moderate but biologically significant antioxidant activity of the polysaccharide fraction. In vitro photoprotective analysis revealed exceptionally high sun protection factor (SPF) values of 94.76 ± 10.34 and 113.85 ± 48.30 , suggesting strong UVB-absorbing capacity attributable to phenolic–polysaccharide conjugates.

Biological investigations further demonstrated marked antitussive and gastroprotective activities. In the HCl-induced gastric ulcer model, the untreated ulcerated group exhibited an ulcer area of 125 mm², whereas treatment with the extract significantly reduced lesion severity. The most effective treatment group (A2) produced an ulcer area of only 22 mm² with an inhibition rate of approximately 82.4%, surpassing the positive control treatment. Acute toxicity assessment showed no evidence of severe organ toxicity, indicating a favorable short-term safety profile of the extract. Overall, the findings demonstrate that *Calligonum comosum* seed polysaccharides possess promising antioxidant, photoprotective, antitussive, and gastroprotective properties with potential pharmaceutical applications.

Keywords

Calligonum comosum; polysaccharides; antioxidant activity; DPPH; phenolic compounds; flavonoids; gastroprotective activity; gastric ulcer; SPF activity; phytochemicals

RÉSUMÉ

La présente étude a porté sur l'évaluation de la composition phytochimique, du potentiel antioxydant, des activités biologiques, de la toxicité aiguë ainsi que des effets gastroprotecteurs des extraits riches en polysaccharides obtenus à partir des graines de *Calligonum comosum*. Les analyses phytochimiques quantitatives ont révélé une teneur totale en composés phénoliques (TPC) de 61,37 mg EAG/g d'extrait, une teneur totale en flavonoïdes (TFC) de $16,09 \pm 0,22$ mg EQ/g d'extrait et une teneur en tanins condensés (CTC) de 194,27 mg EC/g d'extrait, indiquant une richesse importante en composés polyphénoliques associés aux activités antioxydantes et protectrices. L'évaluation de l'activité antioxydante par le test de piégeage du radical libre DPPH a montré une réponse dépendante de la concentration, avec des pourcentages d'inhibition augmentant de 2,71 % à 0,1 mg/mL jusqu'à 11,65 % à 0,6 mg/mL. La valeur d'IC₅₀ calculée était de 3,79 mg/mL, confirmant une activité antioxydante modérée mais biologiquement significative de la fraction polysaccharidique. L'analyse photoprotectrice in vitro a révélé des valeurs élevées du facteur de protection solaire (SPF), atteignant $94,76 \pm 10,34$ et $113,85 \pm 48,30$, suggérant une forte capacité d'absorption des rayons UVB attribuée aux conjugaisons phénols-polysaccharides. Les investigations biologiques ont également mis en évidence des activités antitussives et gastroprotectrices remarquables. Dans le modèle d'ulcère gastrique induit par HCl, le groupe ulcéré non traité présentait une surface ulcéreuse de 125 mm², tandis que le traitement par l'extrait a significativement réduit la sévérité des lésions. Le groupe traité le plus efficace (A2) a montré une surface ulcéreuse de seulement 22 mm² avec un taux d'inhibition d'environ 82,4 %, dépassant même le traitement de référence. L'étude de toxicité aiguë n'a révélé aucun signe de toxicité sévère au niveau des organes, indiquant un profil de sécurité satisfaisant à court terme. Dans l'ensemble, les résultats démontrent que les polysaccharides des graines de *Calligonum comosum* possèdent des propriétés antioxydantes, photoprotectrices, antitussives et gastroprotectrices prometteuses, pouvant ouvrir des perspectives intéressantes dans les applications pharmaceutiques et nutraceutiques.

Mots-clés

Calligonum comosum ; polysaccharides ; activité antioxydante ; DPPH ; composés phénoliques ; flavonoïdes ; activité gastroprotectrice ; ulcère gastrique ; activité SPF ; phytochimie

ملخص

هدفت هذه الدراسة إلى تقييم التركيب الكيميائي النباتي، والنشاط المضاد للأكسدة، والأنشطة البيولوجية، والسمية الحادة، إضافة إلى التأثيرات الواقية للمعدة لمستخلصات غنية بالسكريات المتعددة المستخلصة من بذور نبات *Calligonum comosum*. أظهرت التحاليل الكيميائية النباتية الكمية أن المحتوى الكلي للمركبات الفينولية بلغ 61.37 ملغ مكافئ حمض الغاليك/غ من المستخلص، بينما بلغ المحتوى الكلي للفلافونويدات 16.09 ± 0.22 ملغ مكافئ الكيرسيتين/غ من المستخلص، كما سجلت التانينات المكثفة قيمة 194.27 ملغ مكافئ الكاتيشين/غ من المستخلص، مما يدل على غنى المستخلص بالمركبات متعددة الفينول ذات الخصائص الحيوية المضادة للأكسدة والحامية للخلايا .

أظهر تقييم النشاط المضاد للأكسدة باستخدام اختبار DPPH قدرة تثبيطية متزايدة تبعاً للتركيز، حيث ارتفعت نسبة التثبيط من 2.71٪ عند تركيز 0.1 ملغ/مل إلى 11.65٪ عند 0.6 ملغ/مل، بينما بلغت قيمة IC_{50} حوالي 3.79 ملغ/مل، مما يؤكد امتلاك الجزء الغني بالسكريات المتعددة نشاطاً مضاداً للأكسدة ذا أهمية بيولوجية. كما كشفت الدراسة الخاصة بالنشاط الواقى من الأشعة فوق البنفسجية عن قيم مرتفعة جداً لعامل الحماية الشمسية (SPF) بلغت 10.34 ± 94.76 و 48.30 ± 113.85 ، مما يشير إلى قدرة قوية على امتصاص الأشعة فوق البنفسجية UVB.

وأظهرت الاختبارات البيولوجية كذلك نشاطاً ملحوظاً مضاداً للسعال وفعالية واقية للمعدة. ففي نموذج القرحة المعدية المستحثة بحمض الهيدروكلوريك، بلغت مساحة القرحة لدى المجموعة غير المعالجة 125 مم²، في حين أدى العلاج بالمستخلص إلى انخفاض واضح في شدة الآفات المعدية. وقد حققت المجموعة العلاجية الأكثر فعالية (A2) مساحة قرحية بلغت 22 مم² فقط مع نسبة تثبيط تقارب 82.4٪، متفوقة بذلك على العلاج المرجعي. كما بينت دراسة السمية الحادة عدم وجود علامات سمية شديدة على الأعضاء الحيوية، مما يدل على سلامة المستخلص على المدى القصير. وبصفة عامة، تؤكد النتائج أن السكريات المتعددة المستخلصة من بذور *Calligonum comosum* تمتلك خصائص مضادة للأكسدة وواقية للمعدة ومضادة للسعال وذات قدرة وقائية ضوئية واعدة، مما يفتح آفاقاً لاستخدامها في المجالات الصيدلانية والغذائية العلاجية.

الكلمات المفتاحية

Calligonum comosum؛ السكريات المتعددة؛ النشاط المضاد للأكسدة؛ DPPH؛ المركبات الفينولية؛ الفلافونويدات؛

النشاط الواقى للمعدة؛ القرحة المعدية؛ عامل الحماية الشمسية؛ الكيمياء النباتية

LIST OF ABBREVIATION

Abbreviation	Full Term
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BHT	Butylated Hydroxytoluene
CAT	Catalase
CE	Catechin Equivalent
CTC	Condensed Tannin Content
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic Acid
EC₅₀	Effective Concentration 50%
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
HCl	Hydrochloric Acid
HDL	High-Density Lipoprotein
IC₅₀	Inhibitory Concentration 50%
LDL	Low-Density Lipoprotein
MDA	Malondialdehyde
mg	Milligram
mL	Milliliter
nm	Nanometer
OECD	Organisation for Economic Co-operation and Development
PBS	Phosphate Buffered Saline
pH	Potential of Hydrogen

LIST OF ABBREVIATION

QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
RNA	Ribonucleic Acid
rpm	Revolutions Per Minute
SD	Standard Deviation
SPF	Sun Protection Factor
SOD	Superoxide Dismutase
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
WHO	World Health Organization
μg	Microgram
μL	Microliter

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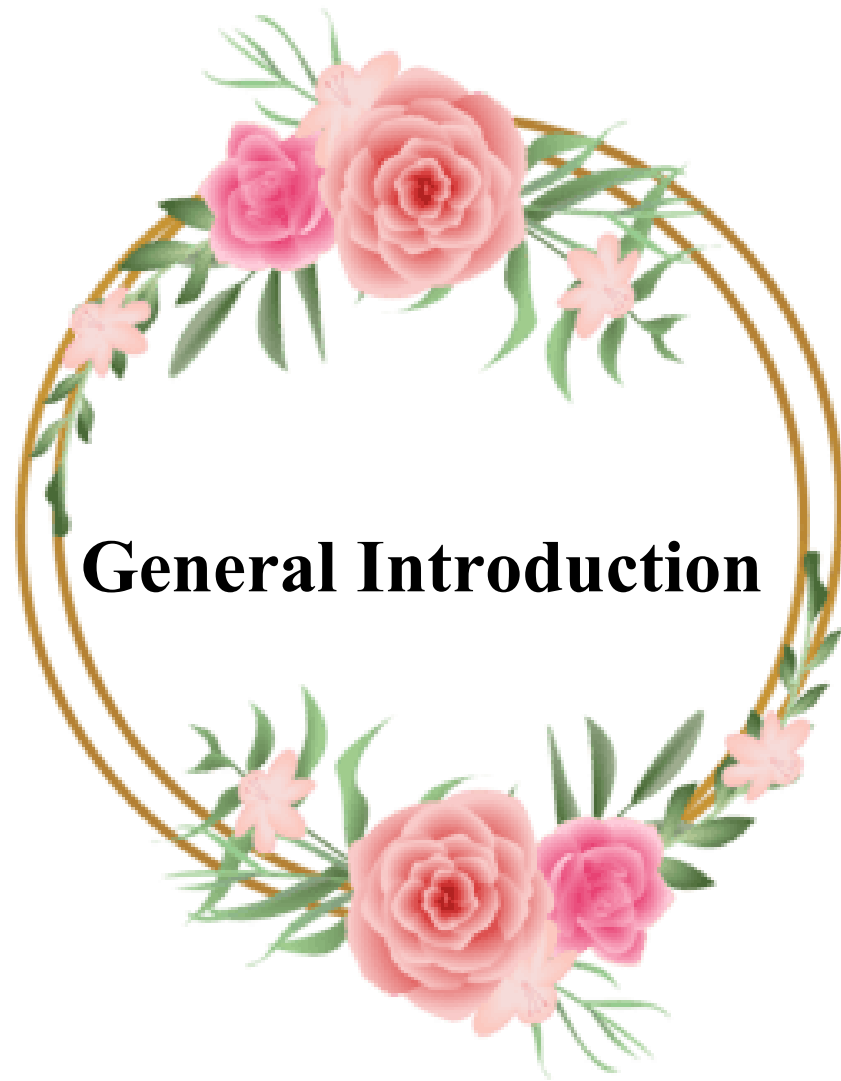
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Medicinal plants have served as the fundamental basis of healthcare across human civilizations, and the **World Health Organization (WHO)** estimates that approximately 80% of the population in developing countries still relies on plant-based remedies for their primary healthcare needs. These plants are distinguished by their content of **secondary metabolites**, such as alkaloids, phenolic compounds, and polysaccharides, which serve ecological functions in the plant and exhibit significant pharmacological activities in humans. In regions like the **Algerian Sahara**, species adapted to extreme arid conditions often accumulate higher concentrations of these metabolites as a protective response to environmental stress, potentially enhancing their therapeutic value. Despite the pharmaceutical importance of natural products, a significant portion of plant biodiversity in underrepresented biomes like desert ecosystems remains pharmacologically unexplored.

Calligonum comosum L'Hér., locally known as Arta, is an ecologically and ethnomedically prominent perennial shrub found across the Saharo-Sindian floristic region, including the sandy ergs of the Algerian Sahara. This species plays a critical ecological role as a sand-binding pioneer and has a rich history of **traditional medicinal use** among Bedouin and Touareg communities. Traditionally, decoctions and infusions of its various parts are used to treat **gastrointestinal disorders** such as gastric ulcers and diarrhea, as well as skin infections and respiratory complaints. Phytochemical characterization of *C. comosum* has revealed a matrix rich in **polyphenols** (including tannins and flavonoids) and **polysaccharides**, which are believed to act synergistically to produce these biological effects.

Scientific interest in these compounds is largely driven by their **antioxidant potential**, which allows them to counteract **oxidative stress**—a state of physiological imbalance linked to cellular damage, aging, and various chronic diseases. While low-molecular-weight phenolics are well-known antioxidants, **plant polysaccharides** are increasingly recognized for their biocompatibility and their ability to enhance endogenous antioxidant enzyme activity. Furthermore, recent investigations suggest that **phenolic-polysaccharide conjugates**—complexes where phenolic constituents are associated with the polysaccharide backbone—may be the primary structural determinants of the antioxidant and protective activities observed in crude plant extracts.

The central problem statement of this thesis is as follows: Although *Calligonum comosum* is widely used in traditional Saharan medicine to treat gastric and respiratory ailments, there is a lack of rigorous scientific data regarding the specific **biological profile and**

pharmaceutical potential of its seed-derived polysaccharides. This study, therefore, seeks to quantitatively evaluate the **phytochemical composition, antioxidant capacity, sun protection factor (SPF), and gastroprotective efficacy** of these extracts to validate their traditional use and assess their safety for potential pharmaceutical and nutraceutical applications.

The primary objective of this thesis is to evaluate the phytochemical profile, antioxidant capacity, and pharmacological potential of polysaccharide-rich extracts derived from the seeds of *Calligonum comosum* collected from the Oued Souf region.

Main Objectives

- To extract and chemically characterize the polysaccharide fractions, quantifying total phenolic, flavonoid, and condensed tannin contents.
- To assess the in vitro antioxidant activity (DPPH and FRAP assays) and photoprotective potential (SPF).
- To evaluate the in vivo pharmacological efficacy of the extracts, specifically focusing on their antitussive and gastroprotective properties using standard murine models.
- To determine the acute toxicity and short-term safety profile of the extracts in Wistar rats.

Part 1

**Bibliographic Section:" Medicinal Plants and Natural
Products "**

1.1 Importance of Medicinal Plants

Medicinal plants have constituted the foundation of healthcare practices in virtually every human civilisation, and they continue to serve as the primary source of medicine for a substantial proportion of the global population. The World Health Organization (WHO) estimates that approximately 80% of the population in developing countries relies on traditional plant-based remedies as the principal component of primary healthcare (World Health Organization [WHO], 2019). This reliance is not merely a reflection of limited access to modern pharmaceuticals; it also speaks to centuries of empirical knowledge accumulated through systematic observation of plant effects on human health.

Medicinal plants are distinguished from food plants primarily by their content of secondary metabolites — compounds that, unlike primary metabolites (proteins, lipids, carbohydrates), are not directly involved in growth and reproduction but serve ecological functions such as defence against herbivores, pathogens, and UV radiation (Croteau et al., 2000). These secondary metabolites include alkaloids, terpenoids, phenolic compounds, and polysaccharides, many of which exhibit pharmacological activities in human systems. The structural diversity of plant secondary metabolites is extraordinary, with estimates suggesting that the plant kingdom produces upwards of 200,000 distinct natural compounds (Hartmann, 2007).

In Algeria and across the broader Saharan region, a rich ethnobotanical tradition has documented the therapeutic uses of numerous desert plants. Species adapted to extreme arid conditions often accumulate higher concentrations of secondary metabolites as a protective response to oxidative and environmental stress, potentially enhancing their pharmacological value (Ramadan et al., 2012). *Calligonum comosum* exemplifies this pattern, as discussed in Section 4 below.

1.2. Their Role in Drug Discovery

Natural products derived from plants have made an enormous contribution to the modern pharmaceutical arsenal. Newman and Cragg (2020) conducted a comprehensive analysis of new drugs approved between 1981 and 2019 and found that approximately 64% of all small-molecule drugs approved during that period were natural products, natural product derivatives, or compounds pharmacologically inspired by natural products. Landmark medicines such as aspirin (derived from salicin in willow bark), morphine (from *Papaver somniferum*), taxol (from *Taxus brevifolia*), and artemisinin (from *Artemisia annua*) all trace their origins to the plant kingdom.

The process of drug discovery from medicinal plants typically follows a phytochemical-guided pipeline: ethnopharmacological reports guide plant selection, followed by extraction,

bioassay-directed fractionation, compound isolation, structural characterisation, and ultimately pharmacological and toxicological evaluation (Fabricant & Farnsworth, 2001). Advances in high-throughput screening and structural biology have accelerated this process considerably. More recently, computational docking approaches have enabled researchers to rapidly model the interactions between plant-derived molecules and therapeutic targets, further streamlining the path from plant to drug candidate (Harvey et al., 2015).

Despite these advances, a significant proportion of plant biodiversity remains pharmacologically unexplored. This is particularly true of plants from underrepresented biomes, including desert ecosystems such as the Algerian Sahara. The phytochemical investigation of plants like *Calligonum comosum* therefore represents not merely an academic exercise, but a potentially valuable contribution to the global search for novel bioactive compounds, particularly antioxidants and anti-inflammatory agents with relevance to chronic non-communicable diseases (Cragg & Newman, 2013).

1.3. Polysaccharides

1.3.1. Definition and Structural Characteristics

Polysaccharides are high-molecular-weight carbohydrate polymers composed of monosaccharide units linked by glycosidic bonds. They represent the most abundant class of biopolymers on Earth and are integral to the structural integrity, energy storage, and intercellular communication of living organisms (Varki et al., 2015). The general formula for polysaccharides may be expressed as $(C_6H_{10}O_5)_n$, where n represents the degree of polymerisation, which can range from several hundred to several hundred thousand monosaccharide residues depending on the source and type.

The structural complexity of polysaccharides arises from several factors: (1) the type and configuration (D or L) of the constituent monosaccharides, which include hexoses (glucose, galactose, mannose, fructose), pentoses (arabinose, xylose), and uronic acids (glucuronic acid, galacturonic acid); (2) the position and anomeric configuration (alpha or beta) of the glycosidic bond; and (3) the extent and type of branching, as well as the presence of non-carbohydrate substituents such as sulphate, phosphate, acetyl, or methyl groups (Mao & Franks, 2015). These variables collectively determine the three-dimensional conformation of the polymer and, consequently, its physicochemical and biological properties.

Polysaccharides can adopt various secondary structures, including helical (as in starch's amylose component), extended ribbon-like (as in cellulose), and highly branched globular

conformations. The rigidity or flexibility of the chain backbone influences solubility, viscosity, and interactions with receptors and enzymes. For example, beta-1,3-glucans form triple helices that are critical to their immunostimulatory activity, while the sulphated polysaccharides from marine algae adopt extended conformations that facilitate interactions with growth factor receptors (Zhu et al., 2021).

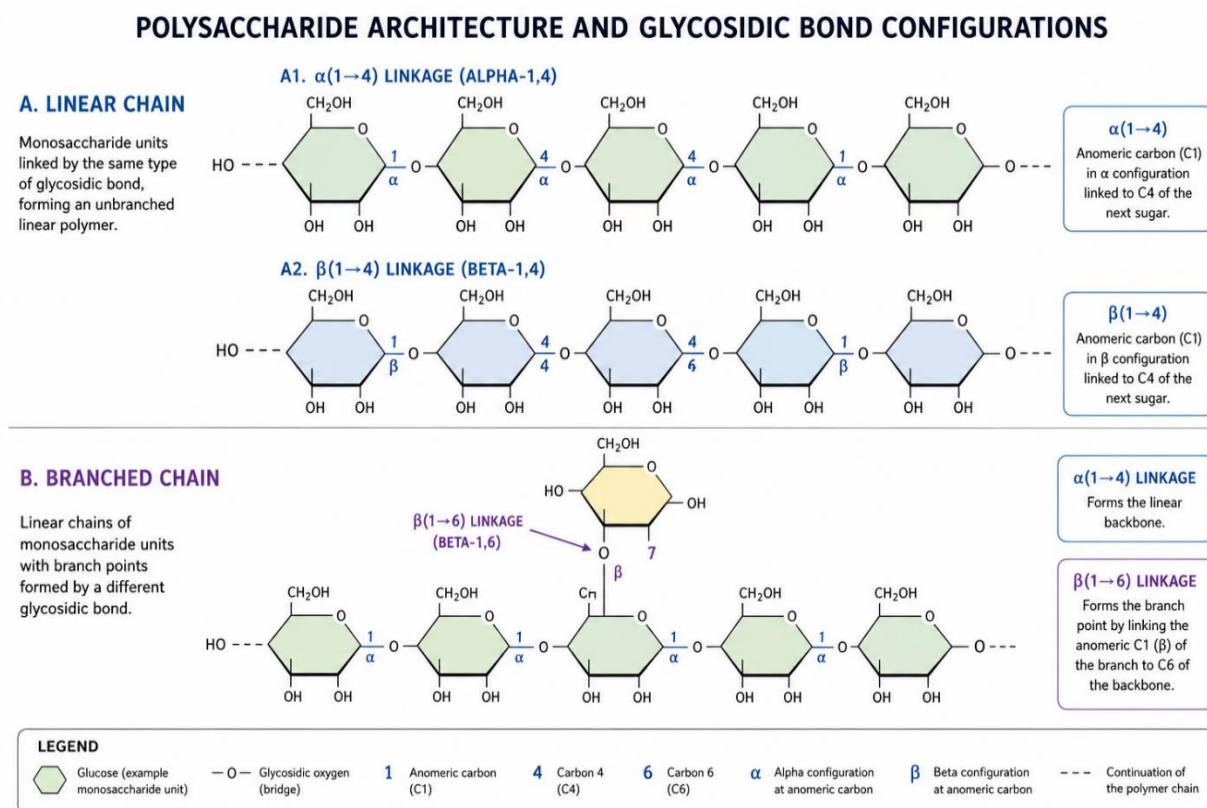


Figure 1. Structural representation of a polysaccharide chain showing alpha- and beta-glycosidic linkages. Source: Adapted from Varki et al. (2015). *Essentials of Glycobiology* (3rd ed.). Cold Spring Harbor Laboratory Press

1.3.2. Biosynthesis of Polysaccharides

The biosynthesis of polysaccharides in plants is a highly regulated process involving multiple enzymatic steps. The pathway begins with the activation of monosaccharides into nucleotide sugar precursors — the so-called 'sugar nucleotides' — most commonly in the form of UDP-glucose, GDP-glucose, or ADP-glucose, depending on the polysaccharide being synthesised (Seifert, 2004). These nucleotide sugars serve as glycosyl donors in transglycosylation reactions catalysed by glycosyltransferases (GTs), a superfamily of enzymes that transfer glycosyl groups to acceptor molecules with high stereospecificity.

In plants, cell wall polysaccharides — including cellulose, hemicelluloses, and pectins — are synthesised by distinct sets of glycosyltransferases localised to the plasma membrane (for cellulose) or the Golgi apparatus (for hemicelluloses and pectins). Cellulose synthase complexes (CSCs) at the plasma membrane polymerise UDP-glucose into beta-1,4-glucan chains, which are then extruded directly into the apoplast (Somerville, 2006). Hemicellulosic polysaccharides such as xyloglucans, glucomannans, and arabinoxylans are assembled in the Golgi and then transported to the cell wall via vesicle fusion.

Storage polysaccharides, principally starch (in plants) and glycogen (in animals and fungi), are synthesised differently. Starch biosynthesis in plastids involves ADP-glucose pyrophosphorylase (AGPase), which produces ADP-glucose from glucose-1-phosphate and ATP. Starch synthases then catalyse the elongation of the alpha-1,4-glucan chain, while branching enzymes introduce alpha-1,6 branch points to form the amylopectin component (James et al., 2003). The regulation of these pathways responds to environmental signals, developmental stage, and metabolic status, ensuring that polysaccharide composition is dynamically adjusted to the plant's needs.

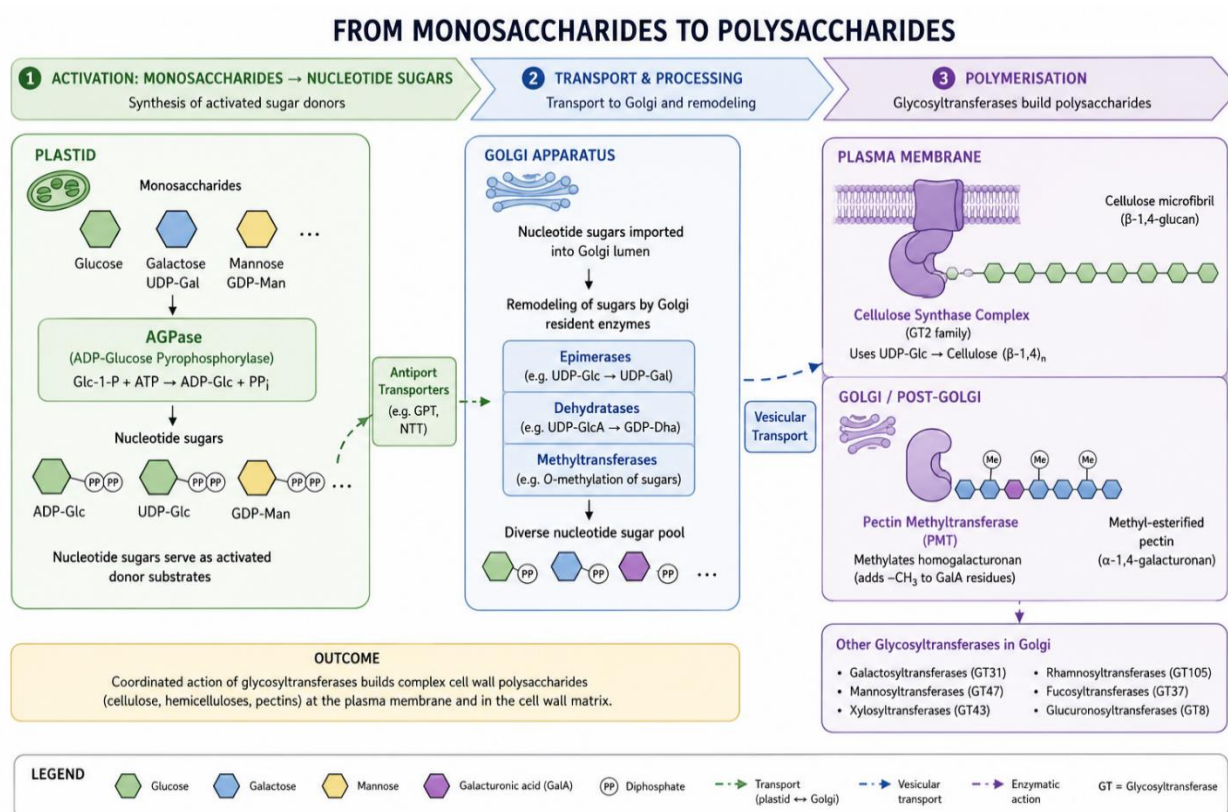


Figure 2. Biosynthetic pathway of polysaccharides via nucleotide sugar precursors. Source: Adapted from Seifert (2004). *Current Opinion in Plant Biology*, 7(3), 277–284

1.3.3. Classification

Polysaccharides are classified according to several criteria, the most fundamental being their origin, monosaccharide composition, and function. Based on origin, they may be divided into plant polysaccharides (e.g., cellulose, starch, pectin, guar gum), animal polysaccharides (e.g., glycogen, chitin, hyaluronic acid, heparin), microbial polysaccharides (e.g., dextran, xanthan, pullulan), and algal polysaccharides (e.g., carrageenan, agar, fucoidan, laminarin).

Based on monosaccharide composition, polysaccharides are described as homopolysaccharides when composed of a single type of monosaccharide — such as cellulose (glucose), chitin (N-acetylglucosamine), and inulin (fructose) — or heteropolysaccharides when containing two or more different types, such as pectins (galacturonic acid, rhamnose, arabinose, galactose) and hyaluronic acid (glucuronic acid and N-acetylglucosamine) (Rehm, 2010).

Functionally, polysaccharides are classified as structural (cell wall components such as cellulose, pectin, and hemicelluloses), storage (starch, inulin, glycogen), and signalling or bioactive (sulphated polysaccharides, beta-glucans, arabinogalactans). This functional classification is particularly relevant to pharmacological research, as bioactive polysaccharides from plants and fungi have attracted considerable interest for their immunomodulatory, antioxidant, antitumour, and prebiotic activities (Liu et al., 2020).

Table 1. Classification of polysaccharides based on origin, monomeric composition, and biological function

Origin	Composition Type	Example	Biological Function
Plant polysaccharides	Homopolysaccharide	Cellulose	Structural (cell wall)
Plant polysaccharides	Homopolysaccharide	Starch (amylose/amylopectin)	Storage (plastids)
Plant polysaccharides	Heteropolysaccharide	Pectin (GalA, Rha, Ara, Gal)	Structural / gel-forming
Animal polysaccharides	Homopolysaccharide	Glycogen	Storage (liver, muscle)
Animal polysaccharides	Heteropolysaccharide	Hyaluronic acid (GlcA, GlcNAc)	Structural / lubricant
Animal polysaccharides	Heteropolysaccharide	Heparin (GlcA, GlcNS)	Anticoagulant / signalling

Microbial polysaccharides	Homopolysaccharide	Dextran (alpha-1,6-glucan)	Biofilm / food additive
Microbial polysaccharides	Heteropolysaccharide	Xanthan gum	Industrial / thickening
Algal polysaccharides	Heteropolysaccharide	Carrageenan (sulphated galactans)	Structural / bioactive
Algal polysaccharides	Heteropolysaccharide	Fucoidan (sulphated fucose)	Antioxidant / antiviral

Source: Compiled from Rehm (2010) and Liu et al. (2020)

1.3.4. Biological Activities

The biological activities of plant polysaccharides have been the subject of intensive research over the past two decades, with particular attention to their immunomodulatory, antioxidant, antitumour, antiviral, and hypoglycaemic effects. These activities are generally attributed to the structural features of the polysaccharide — including its molecular weight, degree of branching, type of glycosidic linkage, and the presence of sulphate or other substituent groups — rather than to any single chemical characteristic.

Immunomodulatory activity has been documented for a wide range of plant polysaccharides, including those derived from *Astragalus membranaceus*, *Panax ginseng*, and *Lycium barbarum*. These polysaccharides appear to activate macrophages, natural killer cells, and T lymphocytes through interaction with pattern recognition receptors such as Toll-like receptors (TLRs) and Dectin-1, stimulating the production of cytokines including IL-6, TNF-alpha, and IFN-gamma (Schepetkin & Quinn, 2006). The beta-1,3-glucans from *Lentinus edodes* (lentinan) and *Ganoderma lucidum* have received regulatory approval as adjuvant antitumour agents in some Asian countries, underscoring the clinical relevance of this activity.

Antioxidant activity in polysaccharides is attributed to several mechanisms: (1) direct scavenging of free radicals through hydroxyl groups on the polysaccharide backbone; (2) chelation of transition metal ions (Fe^{2+} , Cu^{2+}) that catalyse the Fenton reaction; and (3) indirect enhancement of endogenous antioxidant enzyme activity, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Wang et al., 2014). It should be noted that the antioxidant potency of polysaccharides is generally lower than that of low-molecular-weight phenolic compounds on a per-gram basis, but their greater biocompatibility and lower toxicity may confer advantages in therapeutic applications.

Antitumour and anti-inflammatory activities have also been documented, though the mechanisms remain an active area of investigation. Several studies suggest that plant polysaccharides may induce apoptosis in cancer cell lines through modulation of the Bcl-2/Bax ratio and activation of caspase cascades, while their anti-inflammatory effects appear to be mediated through inhibition of NF-kappaB signalling and downregulation of pro-inflammatory mediators (Zhang et al., 2022).

1.4. Phenolic Compounds

1.4.1. Overview

Phenolic compounds constitute the most structurally diverse and numerically abundant class of plant secondary metabolites, with over 10,000 individual structures identified to date (Tsao, 2010). All share the defining feature of one or more aromatic rings bearing at least one hydroxyl substituent, but this common structural motif supports an extraordinary range of molecular architectures, from simple phenolic acids with a single benzene ring to complex polymeric tannins with molecular weights exceeding 20,000 Da. This chemical diversity translates into an equally broad spectrum of biological activities, making phenolic compounds of central interest in nutritional science, pharmacology, and the study of plant defence mechanisms.

In plants, phenolic compounds are primarily synthesised through the shikimate pathway, which provides the aromatic amino acid precursors phenylalanine and tyrosine (Vogt, 2010). The enzyme phenylalanine ammonia lyase (PAL) catalyses the first committed step in phenylpropanoid metabolism by deaminating phenylalanine to trans-cinnamic acid, from which a vast array of phenylpropanoid derivatives is subsequently elaborated through hydroxylation, methylation, glycosylation, and condensation reactions. These biosynthetic pathways are induced by environmental stresses including UV radiation, pathogen attack, wounding, and nutrient deficiency, explaining why stress-adapted desert plants such as *Calligonum comosum* tend to accumulate elevated concentrations of phenolic compounds.

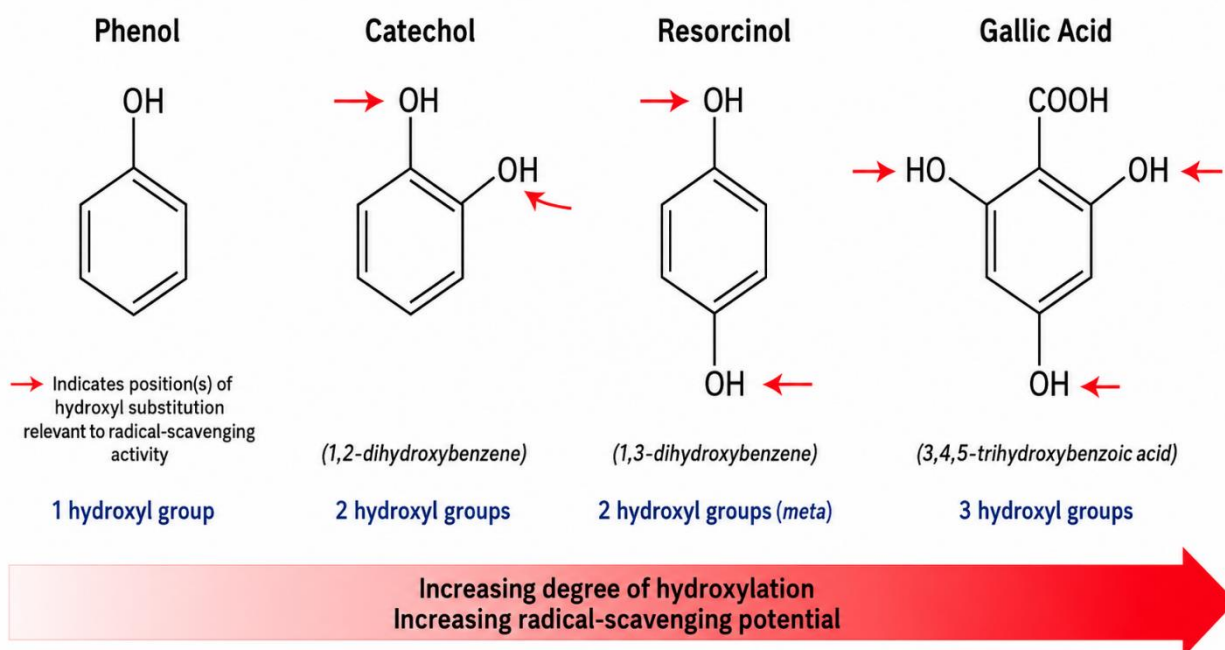


Figure 3. Basic phenolic ring structure and representative hydroxylation patterns of simple phenols.

Source: Adapted from Tsao (2010). Advances in Food and Nutrition Research, 61, 1–35

1.4.2. Phenols

Simple phenols and phenolic acids represent the most basic subgroup of the phenolic compound family. They are characterised by a benzene ring bearing one or more hydroxyl groups, often with additional carboxyl, methoxyl, or glycosyl substituents. Phenolic acids are generally divided into two structural families: hydroxybenzoic acids (C6–C1 skeleton), including gallic acid, protocatechuic acid, and ellagic acid; and hydroxycinnamic acids (C6–C3 skeleton), including p-coumaric acid, caffeic acid, ferulic acid, and sinapic acid (Robbins, 2013).

Both hydroxybenzoic and hydroxycinnamic acids are widely distributed in plants, typically occurring as esters, glycosides, or amides rather than in free form. Chlorogenic acid — an ester of caffeic acid and quinic acid — is among the most abundant dietary phenolic acids, present at high concentrations in coffee, artichoke, and numerous medicinal plants. Caffeic acid itself has demonstrated antioxidant, anti-inflammatory, and antiproliferative activities in multiple *in vitro* and *in vivo* models (Sova, 2012). The antioxidant activity of phenolic acids correlates strongly with the number and position of hydroxyl groups on the aromatic ring, as well as the presence of an α,β -unsaturated carboxyl group, which extends conjugation and enhances electron-donating capacity.

Gallic acid and its derivatives (gallotannins and ellagitannins) deserve special mention given their frequent occurrence in *Calligonum* species. Gallic acid (3,4,5-trihydroxybenzoic acid) is a

potent antioxidant and has been shown to exert cytotoxic effects against several cancer cell lines, anti-inflammatory activity through inhibition of NF-kappaB, and antimicrobial effects against a broad spectrum of bacteria and fungi (Kahkonen & Heinonen, 2023).

1.4.3. Flavonoids

Flavonoids constitute the largest and most studied subgroup of phenolic compounds, with over 6,000 naturally occurring structures identified. All flavonoids share a basic C6–C3–C6 skeleton consisting of two aromatic rings (A and B) connected by a three-carbon bridge that typically forms a central pyran ring (ring C). Variation in the oxidation state and substitution pattern of ring C provides the basis for classification into six major subclasses: flavones, flavonols, flavanones, flavanols (catechins), isoflavones, and anthocyanidins (Harborne & Williams, 2019).

Flavonols (e.g., quercetin, kaempferol, myricetin) and flavones (e.g., apigenin, luteolin) are among the most widely distributed flavonoids in the plant kingdom and among the most extensively characterised pharmacologically. Quercetin, in particular, has been the subject of thousands of scientific studies demonstrating antioxidant, anti-inflammatory, antiviral, and neuroprotective activities. Its antioxidant mechanism involves direct radical scavenging through hydrogen atom donation, metal chelation, and induction of endogenous antioxidant enzymes (Rice-Evans et al., 1996). The structural features that confer high antioxidant activity to quercetin — the 3',4'-catechol group on the B ring, the 3-OH and 4-oxo groups on ring C, and the 2,3-double bond — are regarded as a benchmark for evaluating the antioxidant potential of other flavonoids.

Isoflavones (e.g., genistein, daidzein), predominantly found in legumes, have attracted attention for their oestrogenic activity and potential role in hormone-dependent cancer prevention. Anthocyanidins are responsible for the red, blue, and purple pigmentation of many fruits and flowers and have demonstrated particularly potent antioxidant and anti-inflammatory properties, largely attributed to their positively charged flavylum structure and extensive hydroxylation (Pietta, 2020). The biological activities of flavonoids are often enhanced by glycosylation, which increases water solubility and may alter bioavailability, though the aglycon forms are generally more bioactive in vitro.

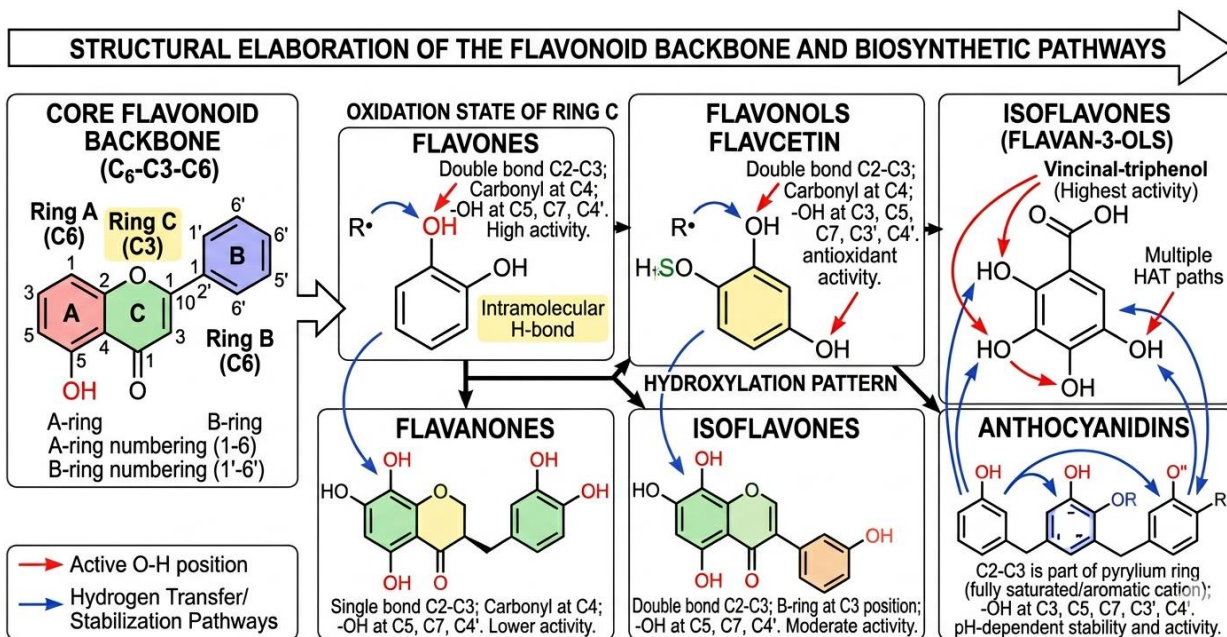
STRUCTURAL DIAGRAMS: THE C₆-C₃-C₆ FLAVONOID BACKBONE AND DERIVED SUBCLASSES


Figure 4. General flavonoid skeleton and classification of major subgroups. Source: Adapted from Harborne & Williams (2000). *Phytochemistry*, 55(6), 481–504

Table 2. Major classes of phenolic compounds and their structural features

Class	Carbon Skeleton	Examples	Key Activities
Simple phenols	C ₆	Catechol, resorcinol, phloroglucinol	Antimicrobial, antioxidant
Hydroxybenzoic acids	C ₆ -C ₁	Gallic acid, protocatechuic acid, ellagic acid	Antioxidant, antitumour, chelating
Hydroxycinnamic acids	C ₆ -C ₃	Caffeic, ferulic, p-coumaric, sinapic acids	Antioxidant, anti-inflammatory
Flavones	C ₆ -C ₃ -C ₆	Apigenin, luteolin	Anti-inflammatory, antioxidant
Flavonols	C ₆ -C ₃ -C ₆	Quercetin, kaempferol, myricetin	Strong antioxidant, metal chelation
Flavanones	C ₆ -C ₃ -C ₆	Naringenin, hesperidin	Anti-inflammatory, antiviral
Flavanols (catechins)	C ₆ -C ₃ -C ₆	Catechin, epicatechin, EGCG	Antioxidant, antiproliferative

Isoflavones	C6-C3-C6	Genistein, daidzein	Oestrogenic, antitumour
Anthocyanidins	C6-C3-C6	Cyanidin, delphinidin, malvidin	Antioxidant, anti-inflammatory
Hydrolysable tannins	Polymer	Gallotannins, ellagitannins	Astringent, antioxidant, antitumour
Condensed tannins	Polymer	Proanthocyanidins (catechin oligomers)	Antioxidant, antimicrobial, astringent

Source: Compiled from Tsao (2010) and Harborne & Williams (2000).

1.4.4. Tannins

Tannins are a structurally heterogeneous group of high-molecular-weight polyphenolic compounds characterised by their capacity to precipitate proteins and form stable complexes with metal ions. They are broadly classified into two major categories: hydrolysable tannins and condensed tannins (proanthocyanidins), which differ fundamentally in their chemical structure and biosynthetic origin (Haslam, 2007).

Hydrolysable tannins consist of a polyol core (most commonly glucose) esterified with gallic acid (gallotannins) or ellagic acid (ellagitannins). Upon hydrolysis by acid, base, or tannase enzymes, they release the polyol core and the constituent phenolic acids. Ellagitannins are of particular pharmacological interest because their hydrolysis products — ellagic acid and urolithins generated by gut microbiota — exhibit significant antioxidant and antiproliferative activities (Landete, 2011). Several *Calligonum* species have been reported to contain substantial amounts of gallotannins and ellagitannins, potentially contributing to their antioxidant activity.

Condensed tannins (proanthocyanidins) are polymers of flavan-3-ol units (catechin, epicatechin) linked primarily through C4–C8 or C4–C6 bonds, and they constitute the most widespread form of tannins in higher plants. They are not hydrolysable under normal conditions, though oxidative degradation yields anthocyanidins — hence the alternate name proanthocyanidins. Their biological activities include antioxidant, antimicrobial, antidiarrhoeal, and anti-inflammatory effects, mediated largely through their protein-binding capacity and radical-scavenging ability (Ferreira et al., 2011).

TWO-PANEL DIAGRAM: GALLOTANNIN (LEFT) AND CONDENSED TANNIN (RIGHT) STRUCTURES

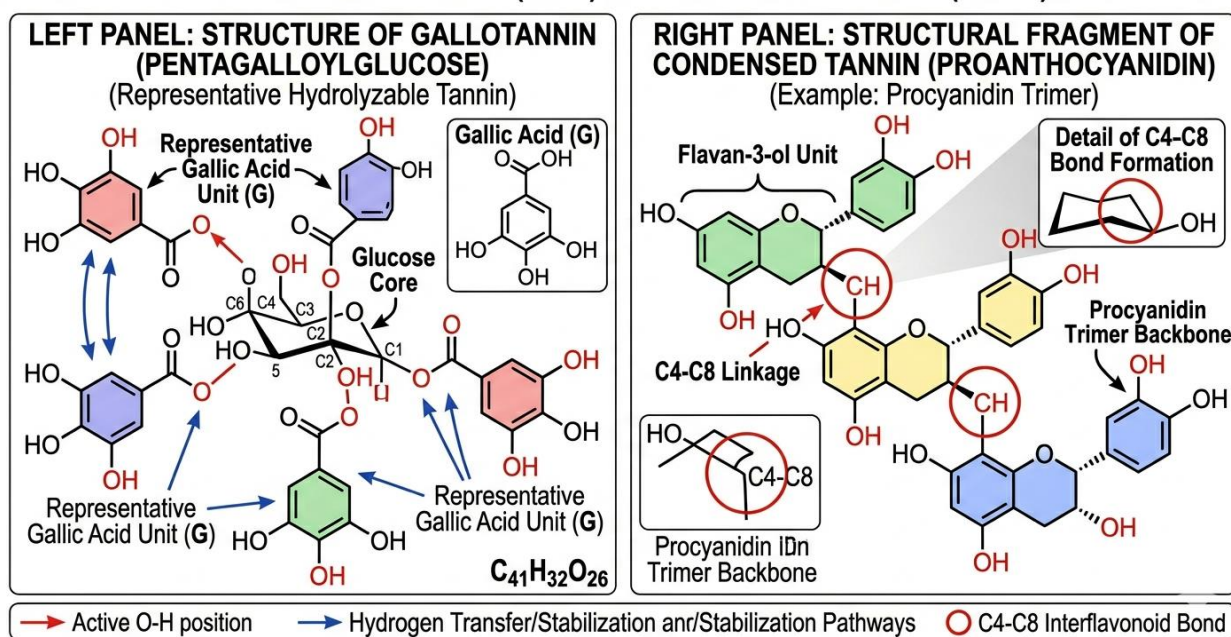


Figure 5. Structural diversity of tannins: hydrolysable versus condensed tannins. Source: Adapted from Haslam (2007). *Phytochemistry*, 68(22–24), 2713–2721

1.4.5. Biological Importance

The biological importance of phenolic compounds extends across multiple domains, from plant ecology to human health. In plants, phenolics serve as UV screens, antifeedants, antimicrobial agents, and signals in plant-microbe interactions. In the context of human health, they have attracted intense research interest for their potential roles in the prevention and management of cardiovascular disease, neurodegenerative disorders, diabetes, cancer, and infectious diseases (Scalbert et al., 2005).

The antioxidant activity of phenolic compounds is their most widely studied biological property and the primary focus of the present thesis. Phenolic antioxidants protect biological macromolecules — proteins, lipids, nucleic acids — from oxidative damage by donating hydrogen atoms or electrons to free radicals, thereby terminating chain reactions. The effectiveness of this mechanism depends on the stability of the resulting phenoxyl radical: a more stable phenoxyl radical is less reactive and less likely to propagate oxidative damage (Bors et al., 1990).

In addition to direct radical scavenging, phenolic compounds exhibit antioxidant activity through metal chelation (forming stable complexes with Fe²⁺ and Cu²⁺ that prevent their participation in Fenton-type reactions), inhibition of pro-oxidant enzymes (such as xanthine oxidase and lipoxygenase), and upregulation of endogenous antioxidant defences through activation of the Nrf2-ARE (Nuclear Factor Erythroid 2-Related Factor 2 — Antioxidant Response

Element) signalling pathway (Dai & Mumper, 2010). The clinical relevance of these activities is supported by epidemiological evidence linking high polyphenol intake to reduced risk of cardiovascular disease and certain cancers, as well as by numerous preclinical studies demonstrating efficacy in models of oxidative stress-related pathology.

1.5. *Calligonum comosum* L'Hér. (Arta)

1.5.1. Botanical Description

Calligonum comosum L'Hér. is a perennial shrub belonging to the family Polygonaceae, order Caryophyllales. The genus *Calligonum* comprises approximately 80 species, distributed primarily across the arid and semi-arid regions of North Africa, the Middle East, Central Asia, and the Mediterranean basin (Huang et al., 2013). Within this genus, *Calligonum comosum* is one of the most ecologically and ethnomedically prominent species, particularly in the Algerian and Libyan Sahara.

Morphologically, *Calligonum comosum* is a multi-stemmed shrub reaching 1–1.5 metres in height, with a deep and extensive root system that enables it to anchor in shifting sand dunes and access deep water tables. The stems are articulated and green, serving a photosynthetic function in the near-absence of leaves — an adaptation to the extreme aridity of its natural habitat. The leaves, when present, are reduced to tiny, ephemeral scales at the nodes. Flowers are small, bisexual, and white to pale pink, borne in axillary clusters. The fruit is a distinctive achene enclosed in a papery perianth bearing branched, hair-like projections ('comose' bristles), which facilitate wind dispersal — a morphological feature that gives the species its name (Pottier-Alapetite, 1981).

The plant's phenology is closely tied to soil moisture: brief vegetative and reproductive bursts follow episodic rainfall events, after which the plant enters a state of reduced metabolic activity. This highly responsive growth strategy, combined with its capacity for deep rooting and its photosynthetic stems, makes *Calligonum comosum* a keystone species in many Saharan plant communities (Chehma et al., 2018).

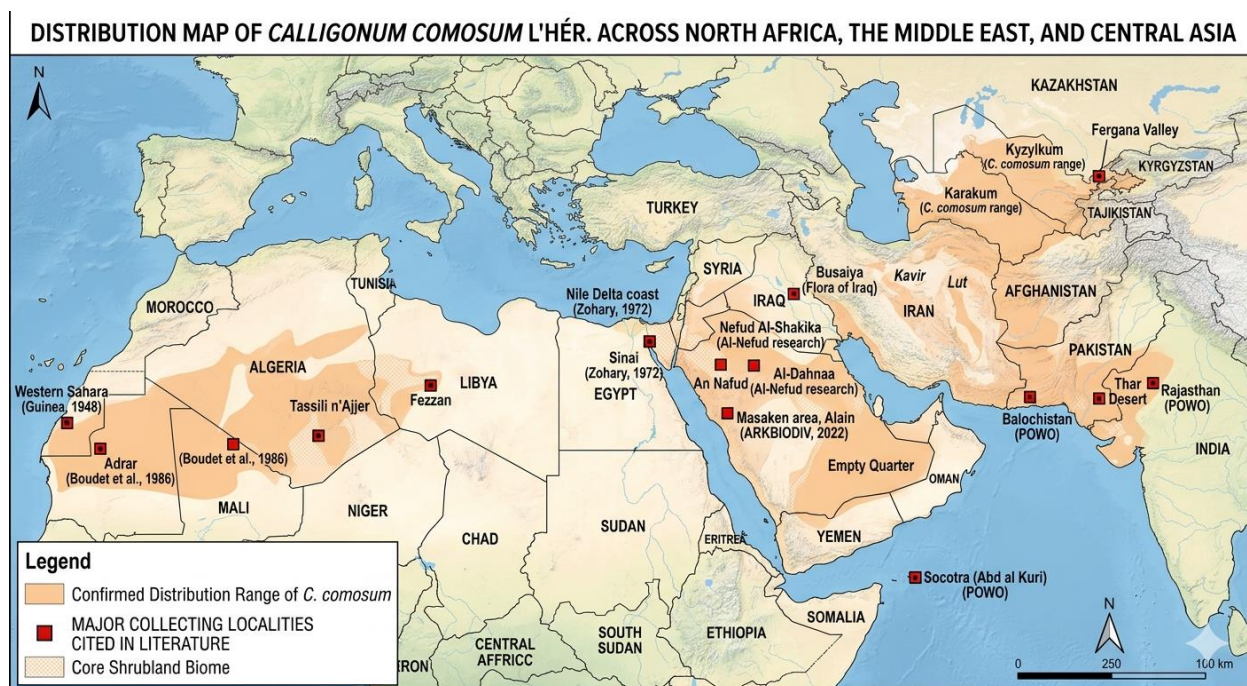


Figure 6. Geographical distribution map of *Calligonum comosum* across Saharan and semi-arid regions. Source: Adapted from Huang et al. (2003). *Flora of China*, Vol. 5. Missouri Botanical Garden Press

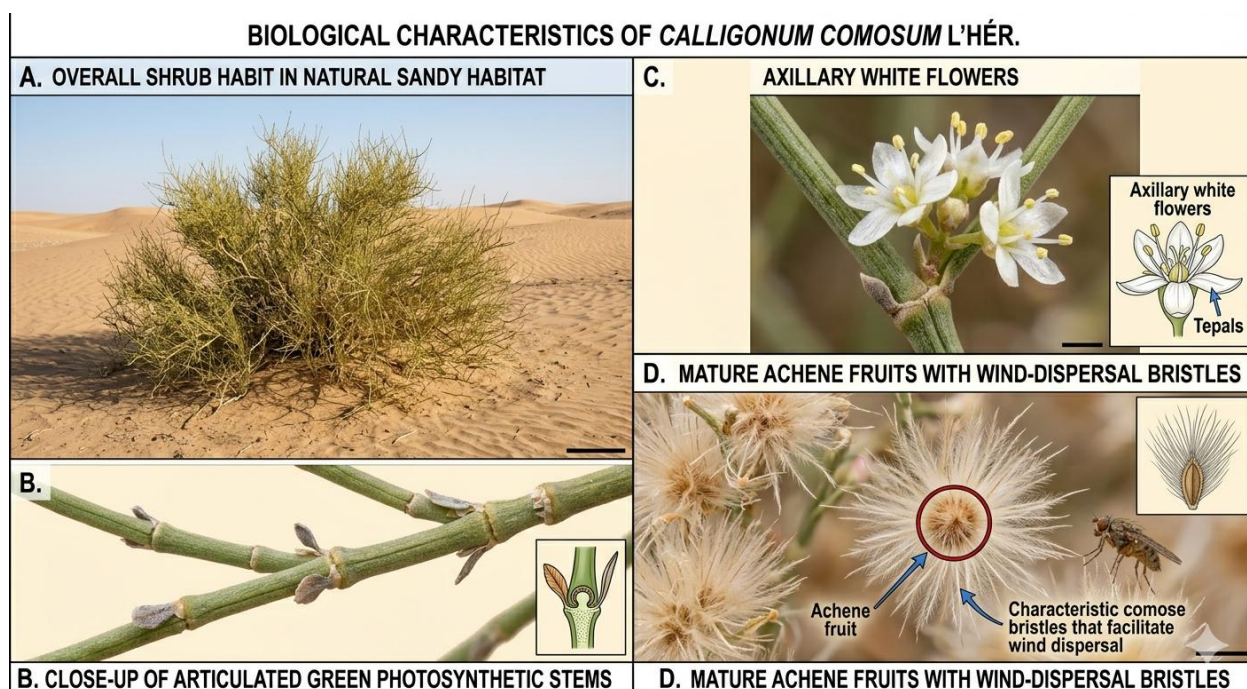


Figure 7. Morphological features of *Calligonum comosum*: habit, stem, flowers, and fruits. Source: Source: Own photographic documentation or adapted from Chehma et al. (2008). *Phytothérapie*, 6(1), 22–29

1.5.2. Geographical Distribution

The geographical range of *Calligonum comosum* spans the Saharo-Sindian floristic region, one of the world's most extensive and climatically extreme phytogeographical zones. In North Africa, the species is prevalent in the sandy erg formations of the Algerian Sahara (particularly the Grand Erg Oriental and Occidental), as well as in Tunisia, Libya, Egypt (Sinai and the Western Desert), Morocco, and the northern Saharan fringes of Mauritania (Ozenda, 1991). Further east, it extends through the Arabian Peninsula — including Saudi Arabia, Yemen, and the UAE — and into parts of Iran, Afghanistan, and Central Asia.

In Algeria, *Calligonum comosum* is among the most characteristic psammo-halophytic shrubs of the erg ecosystem. It plays a critical ecological role as a sand-binding pioneer species that stabilises dune margins and facilitates the establishment of other plant species in its wake. It also constitutes an important browse plant for camels and other desert-adapted livestock during periods when other fodder is scarce, reflecting its nutritional and pastoral significance to nomadic and semi-nomadic communities (Chehma et al., 2018).

The plant thrives in areas receiving less than 100 mm of annual rainfall, at altitudes ranging from sea level to approximately 1,200 metres, on sandy soils with poor organic matter content but sometimes elevated salinity. Its tolerance of wind, heat, and drought far exceeds that of most other shrub species in its range, making it a valuable subject for studies of stress-adaptive phytochemistry (Ozenda, 1991).

1.5.3. Traditional Uses

Calligonum comosum has accumulated a rich body of traditional medicinal knowledge across its distribution range, reflecting centuries of empirical observation by Bedouin, Touareg, and Berber communities. Ethnopharmacological surveys conducted in Algeria, Libya, Egypt, and Saudi Arabia consistently document the use of various plant parts — particularly the aerial shoots, flowers, and fruits — for the treatment of a wide range of conditions (Al-Snafi, 2020).

In Algeria and Libya, decoctions and infusions of Arta stems and flowers are traditionally administered orally for the treatment of gastrointestinal disorders, including diarrhoea, dysentery, and gastric ulcers — applications that may reflect the astringent properties of tannins and the antimicrobial activity of phenolic compounds. The plant is also used as a wound-healing agent, applied topically as a poultice, and as a treatment for skin infections and inflammatory conditions. In Saudi Arabia, the plant has been used in traditional medicine for the management of diabetes and liver complaints, potentially pointing to hypoglycaemic and hepatoprotective activities (Ali et al., 2024).

Among Touareg communities in southern Algeria and Mali, *Calligonum comosum* is reportedly used to relieve fever and as a general tonic for debility and fatigue, suggesting possible antipyretic and adaptogenic properties. The fruits are consumed directly in some areas for their mild sweetness and nutritional value. The plant also has non-medicinal traditional uses: its woody stems serve as firewood and for the construction of temporary shelters, while its extensive root system has traditionally been exploited for desert road stabilisation and anti-desertification plantings (Chehma et al., 2018).

1.5.4. Phytochemical Composition

The phytochemical characterisation of *Calligonum comosum* has been the subject of a growing body of research, particularly over the past two decades. The picture that emerges from the literature is of a plant rich in polyphenolic compounds — including tannins, flavonoids, and phenolic acids — as well as in polysaccharides, sterols, and terpenoids. The specific composition varies depending on the plant part examined (aerial parts, roots, flowers, fruits), the geographic origin of the material, and the extraction and analytical methods employed.

Among phenolic compounds, gallic acid and its derivatives (including gallotannins and ellagitannins) have been consistently identified as major constituents of *Calligonum comosum* extracts (El-Hawary et al., 2012). Flavonoids including quercetin, kaempferol, and their glycosides have also been reported, along with hydroxycinnamic acid derivatives such as caffeic acid and ferulic acid. Condensed tannins (proanthocyanidins) contribute significantly to the total polyphenol content, consistent with the traditional use of the plant as an astringent agent. An HPLC-DAD study by Al-Snafi (2016) identified at least 15 individual phenolic compounds in methanolic extracts of the aerial parts, with gallic acid, quercetin-3-glucoside, and ellagic acid among the most abundant.

Polysaccharide fractions have been extracted from the plant and characterised by GC-MS and NMR spectroscopy as arabinogalactans and glucomannans, with molecular weights in the range of 15–80 kDa. These polysaccharides have been shown to exhibit immunostimulatory and antioxidant activities in preliminary assays, suggesting that they may contribute to the overall bioactivity of the plant (Liu et al., 2020). Other documented phytochemicals include beta-sitosterol and stigmasterol (phytosterols), oleanolic acid and ursolic acid (pentacyclic triterpenoids), and several volatile terpenoids identified in essential oil fractions.

Table 3. Principal phytochemical constituents reported in *Calligonum comosum*.

Compound Class	Plant Part	Key Identified Compounds	Reported Activities
Phenolic acids	Aerial parts, fruits	Gallic acid, ellagic acid, caffeic acid, ferulic acid, protocatechuic acid	Antioxidant, antimicrobial, anti-inflammatory
Flavonoids	Aerial parts, flowers	Quercetin, kaempferol, quercetin-3-glucoside, rutin	Antioxidant, anti-inflammatory, antiproliferative
Tannins	Bark, aerial parts	Gallotannins, ellagitannins, proanthocyanidins	Astringent, antioxidant, antimicrobial
Polysaccharides	Aerial parts, roots	Arabinogalactans, glucomannans	Immunomodulatory, antioxidant
Phytosterols	Aerial parts, seeds	beta-Sitosterol, stigmasterol	Anti-inflammatory, hypocholesterolaemic
Triterpenoids	Aerial parts	Oleanolic acid, ursolic acid	Anti-inflammatory, hepatoprotective, antitumour
Volatile terpenoids	Aerial parts (EO)	Various monoterpenes and sesquiterpenes	Antimicrobial, aromatic

Source: Compiled from El-Hawary et al. (2012), Al-Snafi (2016), and Ali et al. (2014)

1.5.5. Pharmaceutical Properties

The pharmaceutical properties of *Calligonum comosum* documented in the scientific literature are notably diverse, encompassing antioxidant, antimicrobial, anti-inflammatory, antidiabetic, cytotoxic, and hepatoprotective activities. These activities are likely attributable to the synergistic interaction of the plant's rich phytochemical matrix, rather than to any single constituent.

Antioxidant activity has been the most thoroughly investigated property. Multiple studies employing in vitro assays — including DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) assay, FRAP (ferric reducing antioxidant power), and beta-carotene bleaching — have reported significant antioxidant capacity for *Calligonum comosum* extracts, with the methanolic and aqueous fractions consistently

performing most strongly (Al-Snafi, 2016). Positive correlations between total polyphenol content and antioxidant capacity have been observed across studies, implicating the phenolic fraction as the primary contributor to this activity. It should be noted, however, that polysaccharide fractions also contribute measurable antioxidant activity through the mechanisms described in Section 2.4.

Antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as pathogenic fungi, has been reported for ethanol and methanol extracts of *Calligonum comosum* in several disc diffusion and minimum inhibitory concentration (MIC) studies. The tannin and phenolic acid fractions appear to be primarily responsible for this activity, consistent with the known protein-precipitating and membrane-disrupting effects of these compound classes (Ali et al., 2014). Anti-inflammatory activity has been demonstrated in both in vitro (inhibition of COX-1 and COX-2) and in vivo (carrageenan-induced oedema model) studies, supporting the traditional use of the plant for inflammatory conditions.

Cytotoxic activity against cancer cell lines — including HeLa (cervical), MCF-7 (breast), and HepG2 (hepatocellular) — has been documented for *Calligonum comosum* extracts, with ellagic acid and quercetin proposed as key active constituents (El-Hawary et al., 2012). Antidiabetic activity (alpha-glucosidase and alpha-amylase inhibition) has been demonstrated in vitro, suggesting potential for the management of postprandial hyperglycaemia. Hepatoprotective activity has been reported in a carbon tetrachloride-induced liver injury model in rats, with treated animals showing attenuation of elevated liver enzymes (ALT, AST) and preservation of hepatic architecture — an effect attributed to the antioxidant and anti-inflammatory properties of the plant's phytochemical constituents.

1.6. Oxidative Stress and Antioxidant Systems

1.6.1. Free Radicals and Reactive Oxygen Species (ROS)

Free radicals are chemical species containing one or more unpaired electrons in their outer orbital, rendering them highly reactive and capable of initiating chain reactions with biological macromolecules. Reactive oxygen species (ROS) constitute a subgroup of free radicals and related oxidising molecules derived from molecular oxygen. They include the superoxide anion radical ($\text{O}_2^{\bullet-}$), the hydroxyl radical ($\bullet\text{OH}$), the peroxy radical ($\text{ROO}^\bullet$), and the non-radical species hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$) (Halliwell & Gutteridge, 2015).

ROS are generated continuously in aerobic organisms as natural by-products of metabolic processes. The mitochondrial electron transport chain (ETC) is the principal endogenous source, contributing approximately 1–3% of consumed oxygen as superoxide through single-electron

leakage at Complexes I and III (Turrens, 2003). Other important endogenous sources include NADPH oxidase complexes (NOX) in immune cells and vascular endothelium, xanthine oxidase during purine catabolism, cytochrome P450 enzymes in the liver, and peroxisomal oxidases. Exogenous sources of ROS include ionising radiation, UV light, cigarette smoke, air pollutants, heavy metals, and certain drugs and pesticides.

The superoxide anion ($O_2^{\bullet-}$), produced by one-electron reduction of O_2 , is the primary ROS generated in cells. Although $O_2^{\bullet-}$ itself has limited reactivity with most biological molecules, it serves as a precursor to more damaging species. Dismutation of $O_2^{\bullet-}$, either spontaneously or catalysed by SOD, produces H_2O_2 , which, in the presence of reduced transition metals (Fe^{2+} , Cu^{2+}), undergoes the Fenton reaction to generate the highly reactive hydroxyl radical ($\bullet OH$). $\bullet OH$ is arguably the most damaging ROS in biological systems, reacting at near-diffusion-limited rates with proteins, lipids, DNA, and carbohydrates in its immediate vicinity (Halliwell & Gutteridge, 2015).

Table 4. Key reactive oxygen species (ROS), their sources, and primary biological targets

ROS	Primary Source	Reactivity	Main Biological Targets	Key Antioxidant Defense
Superoxide anion ($O_2^{\bullet-}$)	Mitochondrial ETC (Complex I & III), NADPH oxidase, xanthine oxidase	Low direct reactivity; precursor to H_2O_2 and $\bullet OH$	Lipids, [4Fe-4S] clusters	SOD
Hydrogen peroxide (H_2O_2)	Dismutation of $O_2^{\bullet-}$, peroxisomal oxidases, beta-oxidation	Moderate; cell-permeable; Fenton substrate	DNA, proteins (thiol groups)	CAT, GPx
Hydroxyl radical ($\bullet OH$)	Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow \bullet OH + OH^- + Fe^{3+}$	Extremely high; reacts at diffusion limit	DNA, proteins, lipids	No specific enzyme; preventive

Singlet oxygen (1O₂)	Photosensitisation, peroxidase reactions	High; reacts with electron-rich molecules	Lipids, proteins (Trp, His)	Carotenoids, tocopherols
Peroxyl radical (ROO•)	Lipid peroxidation chain propagation	Moderate; propagates lipid peroxidation	Membrane lipids (PUFAs)	Vitamin E (tocopherol)

Source: Compiled from Halliwell & Gutteridge (2015) and Turrens (2003).

1.6.2. Mechanisms of Oxidative Stress

Oxidative stress is defined as a disruption in the balance between the production of ROS and the capacity of biological systems to detoxify reactive intermediates or repair the resulting damage (Sies, 2015). This imbalance arises either from excessive ROS generation, impaired antioxidant defences, or both, and it may be acute (as in ischaemia-reperfusion injury) or chronic (as in ageing, diabetes, and neurodegenerative disease).

At the molecular level, oxidative stress inflicts damage through several parallel mechanisms. Lipid peroxidation — a self-propagating chain reaction initiated by radical attack on polyunsaturated fatty acid (PUFA) side chains in membrane phospholipids — produces lipid hydroperoxides (LOOH) and secondary breakdown products including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which are widely used as biomarkers of oxidative stress and can further damage proteins and DNA (Esterbauer et al., 1991).

Oxidative protein damage occurs through carbonylation of amino acid side chains (particularly lysine, arginine, proline, and threonine), nitration of tyrosine residues, and formation of disulphide cross-links. These modifications can alter protein conformation, inhibit enzymatic activity, and impair cell signalling. Proteins damaged by oxidative stress are typically targeted for degradation by the proteasome, but under severe or chronic oxidative stress, the accumulation of oxidised proteins may overwhelm degradative capacity, contributing to cellular dysfunction (Davies, 2016).

Oxidative DNA damage produces a wide array of base modifications — most notably 8-oxo-2'-deoxyguanosine (8-OHdG), the most abundant oxidative base lesion — as well as single- and double-strand breaks, DNA-protein crosslinks, and abasic sites. These lesions, if not repaired by base excision repair (BER) or other repair mechanisms, may result in mutations, genomic

instability, and apoptosis. 8-OHdG is widely used as a biomarker of oxidative DNA damage and is elevated in tissues subject to chronic oxidative stress (Cooke et al., 2003).

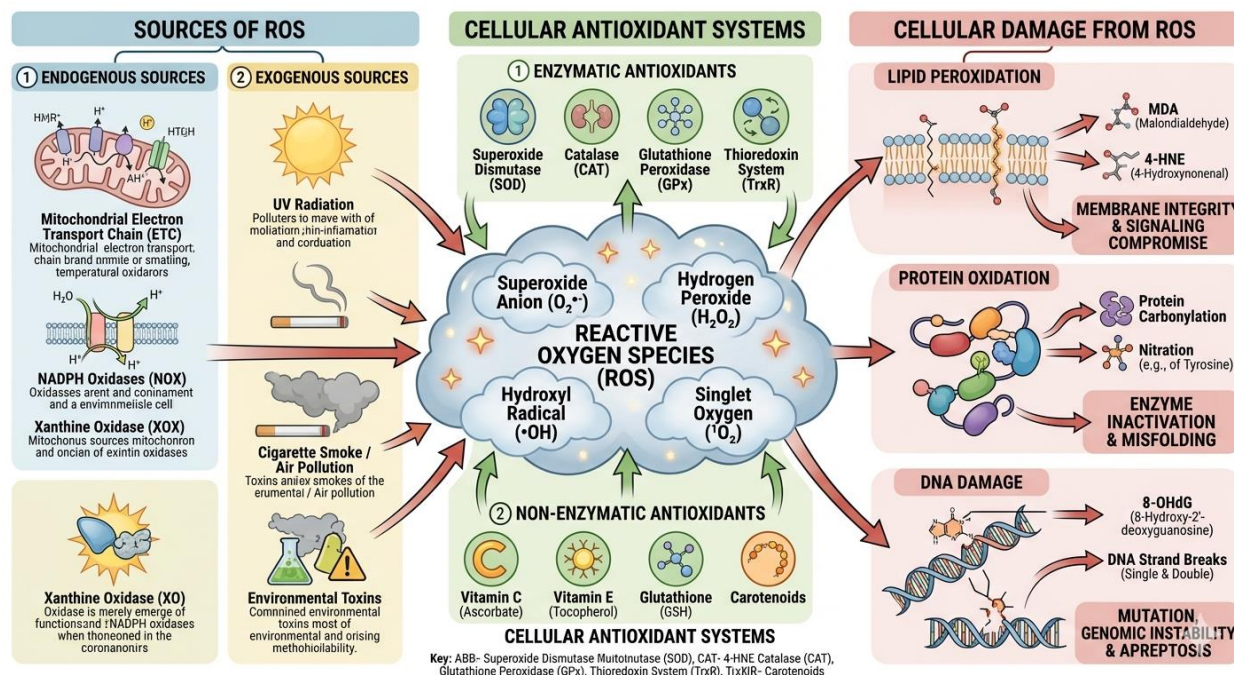


Figure 8. Schematic overview of oxidative stress: sources of ROS and cellular damage pathways.

Source: Adapted from Halliwell & Gutteridge (2015). *Free Radicals in Biology and Medicine* (5th ed.).

Oxford University Press

1.6.3. Antioxidant Defense Systems

Aerobic organisms have evolved a multilayered antioxidant defense system to counteract the continuous challenge of ROS. This system comprises enzymatic antioxidants, low-molecular-weight non-enzymatic antioxidants, and repair systems for damaged macromolecules. The coordinated action of these components maintains ROS at concentrations that are tolerable and even functionally necessary for cell signalling, while preventing the accumulation of damaging species.

The enzymatic antioxidant defense includes three primary enzymes. Superoxide dismutase (SOD) catalyses the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 at a rate approximately 10,000-fold faster than the uncatalysed reaction. Three isoforms exist in mammals: cytosolic Cu/Zn-SOD (SOD1), mitochondrial Mn-SOD (SOD2), and extracellular Cu/Zn-SOD (SOD3). Catalase (CAT) degrades H_2O_2 to water and oxygen, predominantly in peroxisomes, and is one of the most efficient enzymes known, operating at a turnover rate of approximately 6 million molecules of H_2O_2 per minute per enzyme molecule (Halliwell & Gutteridge, 2015). Glutathione peroxidase (GPx) reduces H_2O_2 and lipid hydroperoxides using glutathione (GSH) as a reducing substrate,

regenerating oxidised glutathione (GSSG), which is subsequently reduced back to GSH by glutathione reductase (GR) using NADPH as the ultimate electron donor.

Non-enzymatic antioxidants include both endogenous molecules and dietary compounds. Glutathione (GSH), a tripeptide (gamma-Glu-Cys-Gly), is the most abundant intracellular antioxidant, serving as the substrate for GPx, as a cofactor for glutathione-S-transferases (which detoxify electrophilic compounds), and as a direct scavenger of hydroxyl radicals and singlet oxygen. Uric acid, bilirubin, and albumin also contribute to plasma antioxidant capacity. Vitamins C (ascorbic acid) and E (alpha-tocopherol) are essential dietary antioxidants: ascorbate acts in the aqueous phase to scavenge $O_2^{\bullet-}$, $\bullet OH$, and H_2O_2 , and regenerates tocopherol from the tocopheroxyl radical, while alpha-tocopherol acts within lipid membranes to terminate lipid peroxidation chain reactions (Sies & Stahl, 1995).

Plant-derived antioxidants — particularly phenolic compounds and polysaccharides — may augment endogenous antioxidant defenses through multiple mechanisms, as discussed in Sections 2.4 and 3.5. Importantly, an increasing body of evidence suggests that many plant polyphenols do not act primarily as direct radical scavengers in vivo (where their bioavailability may limit this activity) but rather as inducers of endogenous antioxidant gene expression through the Nrf2-Keap1 pathway, thereby producing a more sustained and amplified antioxidant response (Dinkova-Kostova & Talalay, 2008). This mechanistic distinction has important implications for the interpretation of in vitro antioxidant assay data, which measure direct radical-scavenging capacity and may not fully reflect the in vivo antioxidant potential of plant-derived compounds.

Table 5. Overview of enzymatic and non-enzymatic antioxidant defense mechanisms.

Type	Antioxidant	Mechanism / Reaction	Localisation	Cofactor/Note
Enzymatic	Superoxide dismutase (SOD)	$O_2^{\bullet-} \rightarrow H_2O_2 + O_2$	Cytosol, mitochondria, extracellular	Cu/Zn, Mn (cofactors)
Enzymatic	Catalase (CAT)	$H_2O_2 \rightarrow H_2O + O_2$	Peroxisomes	Haem iron
Enzymatic	Glutathione peroxidase (GPx)	$H_2O_2/LOOH + 2GSH \rightarrow H_2O/LOH + GSSG$	Cytosol, mitochondria	Selenium
Enzymatic	Glutathione reductase (GR)	$GSSG + NADPH \rightarrow 2GSH + NADP^+$	Cytosol, mitochondria	FAD, NADPH

Enzymatic	Glutathione-S-transferase (GST)	Electrophile + GSH -> GS-conjugate	Cytosol, microsomes	GSH
Non-enzymatic	Glutathione (GSH)	Direct scavenger; GPx substrate	Cytosol (mM concentrations)	-
Non-enzymatic	Ascorbic acid (Vitamin C)	Scavenges O ₂ • ⁻ , •OH; regenerates Vit E	Aqueous phase	-
Non-enzymatic	Alpha-tocopherol (Vitamin E)	Terminates lipid peroxidation chain	Lipid membranes	-
Non-enzymatic	Carotenoids (beta-carotene)	Quenches 1O ₂ ; scavenges ROO•	Lipid compartments	-
Non-enzymatic	Uric acid	Scavenges O ₂ • ⁻ , •OH, 1O ₂ in plasma	Plasma	-
Plant-derived (exogenous)	Phenolic compounds (e.g., quercetin)	Radical scavenging, metal chelation, Nrf2 activation	Multiple compartments	-
Plant-derived (exogenous)	Plant polysaccharides	Radical scavenging, metal chelation, SOD/CAT induction	Cellular/systemic	-

Source: Compiled from Halliwell & Gutteridge (2015) and Sies & Stahl (1995).

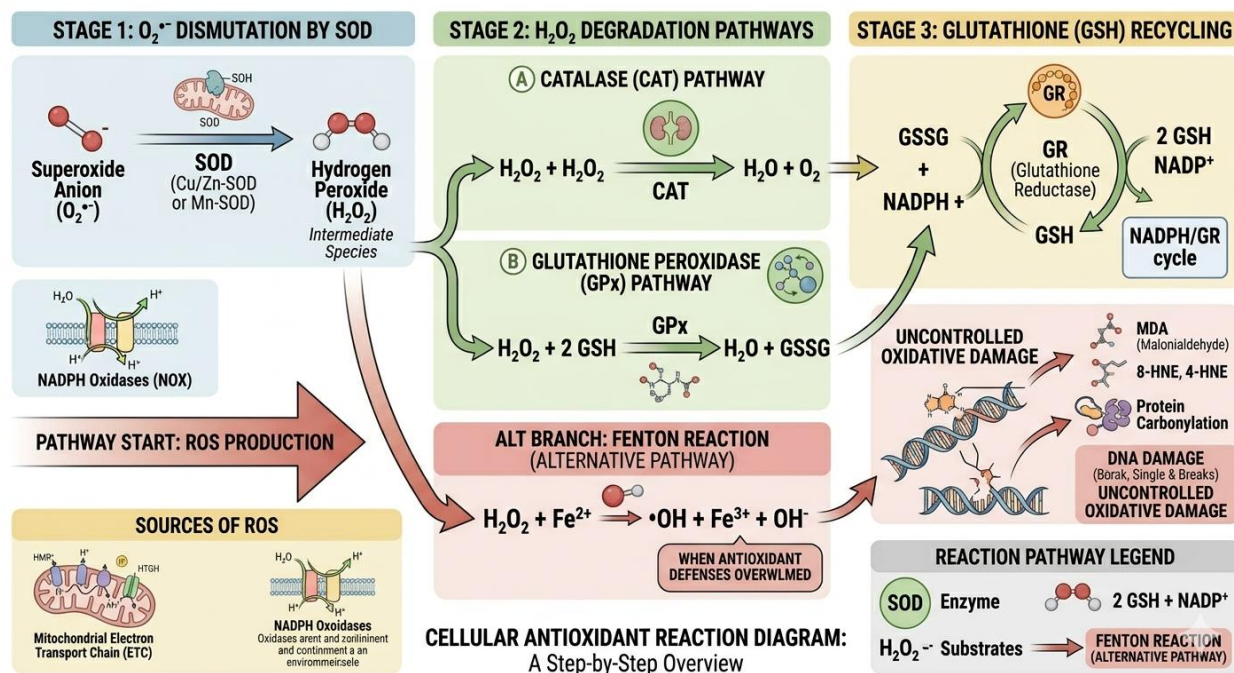


Figure 9. Enzymatic antioxidant cascade: SOD, CAT, and GPx interactions. Source: Adapted from Halliwell & Gutteridge (2015). *Free Radicals in Biology and Medicine* (5th ed.). Oxford University Press

Part 2

Practical Part

Chapter 1

Materials and methods

2.1. Extraction, Isolation, and Purification of Legume Polysaccharides

Legume polysaccharides are major structural constituents of plant cell walls; therefore, their extraction relies on controlled disruption of the cell wall matrix while preserving polymer integrity. The objective is to solubilize polysaccharides under moderate conditions that minimize depolymerization and structural alteration.

2.1.1. Sample Preparation

Dried legume seeds are finely ground and sieved (typically 50-mesh) to obtain a homogeneous powder. Pre-treatment is performed by soaking the powder in 95% ethanol for approximately 3 h to remove low-molecular-weight compounds such as pigments, lipids, monosaccharides, and oligosaccharides. This step enhances the purity of the extracted polysaccharides.

2.1.2. Extraction Procedure (Hot Water Method)

The defatted and decolorized powder is subjected to **hot water extraction at 80°C for exactly 3 h**. This process facilitates the dissolution of water-soluble polysaccharides. The extract is then centrifuged at **3,000–5,000 rpm** to remove insoluble residues, and the supernatant is collected. The resulting extract is subsequently concentrated under reduced pressure and polysaccharides are precipitated by the addition of ethanol. The precipitate is collected, washed, and dried for further characterization.

2.1.3. Precipitation of Crude Polysaccharides

The aqueous extract is concentrated under reduced pressure, followed by the addition of four volumes of absolute ethanol to induce polysaccharide precipitation. The mixture is incubated at 4°C overnight to ensure complete precipitation. The resulting precipitate represents crude polysaccharides.

2.1.4. Purification Steps

The crude polysaccharide fraction is further purified through sequential treatments:

- **Redissolution** in distilled water
- **Decolorization** (e.g., activated charcoal treatment)
- **Dialysis** against distilled water to remove small molecules and salts
- **Freeze-drying (lyophilization)** to obtain purified polysaccharide powder

2.1.5. Alternative Extraction Techniques

In addition to conventional hot water extraction, several advanced methods have been developed to improve yield and efficiency:

- **Acid–base extraction:** enhances solubilization of tightly bound polysaccharides
- **Enzymatic extraction:** uses cellulases or pectinases to degrade cell walls selectively
- **Ultrasonic-assisted extraction:** improves mass transfer via cavitation
- **Microwave-assisted extraction:** accelerates extraction through rapid heating
- **Hot-compressed water extraction:** utilizes high temperature and pressure to increase solvent efficiency
- **Subcritical water extraction:** employs water under subcritical conditions to enhance solubility and diffusion

These techniques can significantly improve extraction efficiency, reduce time, and preserve bioactivity when optimized.

2.1.6. Conclusion

The extraction and purification of legume polysaccharides involve a combination of physical, chemical, and biochemical processes designed to maximize yield while maintaining structural integrity. Selection of the appropriate extraction method depends on the desired purity, functionality, and downstream applications of the polysaccharides.

2.2. Chemical Characterization

2.2.1. Quantitative Determination of Total Phenolic Content (Folin–Ciocalteu Method)

1. Principle

The quantitative assessment of phenolic compounds is based on the reduction of the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, producing a blue chromophore measurable spectrophotometrically at 760–765 nm. The intensity of the color is proportional to the total phenolic content (TPC) in the sample.

2. Reagents and Materials

- Folin–Ciocalteu reagent (FCR), diluted (1:10 v/v)
- Sodium carbonate (Na_2CO_3), 7–10% (w/v)
- Standard phenolic compound: Gallic acid
- Distilled water
- Plant extract (methanolic, ethanolic, or aqueous)
- Spectrophotometer ($\lambda = 765 \text{ nm}$)
- Test tubes, pipettes, vortex mixer

3. Experimental Procedure

a. Preparation of calibration curve

Prepare gallic acid standard solutions (0–200 mg/L).

b. Reaction mixture

- Add 0.5 mL of sample or standard solution into a test tube.
- Add 2.5 mL of diluted Folin–Ciocalteu reagent.
- Mix and allow to react for 5 minutes at room temperature.

c. Alkalinization

- Add 2.0 mL of Na₂CO₃ solution.
- Mix thoroughly.

d. Incubation

- Incubate the mixture for 30–40 minutes in the dark at room temperature.

e. Measurement

- Measure absorbance at 765 nm against a blank (containing all reagents except sample).

4. Calculation

Total phenolic content is expressed as mg gallic acid equivalents (GAE)/g extract using the calibration curve:

$$TPC = \frac{C \times V}{m}$$

Where:

- C = concentration from calibration curve (mg/mL)
- V = volume of extract (mL)
- m = mass of sample (g)

5. Expected Results and Interpretation

A color change from yellow to blue-green indicates the presence of phenolic compounds. Increased absorbance over time (as shown schematically in the notebook) reflects higher phenolic concentration.

6. Notes and Improvements

- Perform all measurements in triplicate for accuracy.
- Protect samples from light to avoid degradation.
- Ensure proper blank correction.
- The method is not entirely specific, as other reducing substances may interfere.

2.2.2. Quantitative Determination of Total Flavonoid Content (Aluminum Chloride Colorimetric Method)

1. Principle

The quantification of flavonoids is based on the formation of a stable complex between flavonoid compounds and Aluminum chloride under acidic conditions. This complex produces a yellow coloration whose intensity is proportional to the flavonoid concentration and can be measured spectrophotometrically at 415–430 nm (commonly 430 nm).

2. Reagents and Materials

- Aluminum chloride solution (AlCl_3 , 2% w/v in methanol or ethanol)
- Methanol or ethanol (analytical grade)
- Standard flavonoid: Quercetin
- Distilled water
- Plant extract (prepared in methanol/ethanol)
- Spectrophotometer ($\lambda = 430 \text{ nm}$)
- Volumetric flasks, test tubes, micropipettes

3. Experimental Procedure

3.1 Preparation of Standard Curve

Prepare a series of quercetin standard solutions (e.g., 0–100 $\mu\text{g/mL}$) in methanol.

3.2 Sample Reaction

1. Pipette 1.0 mL of plant extract (or standard solution) into a test tube.
2. Add 1.0 mL of AlCl_3 solution (2%).
3. Adjust the total volume (if necessary) with methanol to ensure consistent reaction conditions.

3.3 Incubation

- Mix thoroughly and incubate at room temperature for 10–15 minutes in the dark.

3.4 Measurement

- Measure absorbance at 430 nm against a blank (containing all reagents except AlCl_3 or sample).

4. Calculation

Total flavonoid content (TFC) is expressed as mg quercetin equivalents (QE)/g of dry extract:

$$TFC = \frac{C \times V}{m}$$

Where:

- C = concentration from calibration curve (mg/mL)
- V = volume of extract (mL)
- m = mass of plant material (g)

5. Observations and Interpretation

The formation of a yellow complex indicates the presence of flavonoids. Increased absorbance corresponds to higher flavonoid concentration. Results are typically reported as mean $\pm$ standard deviation of triplicate measurements.

6. Notes and Methodological Considerations

- Perform all assays in triplicate to ensure reproducibility.
- Protect solutions from light to avoid flavonoid degradation.
- Ensure proper blank correction to eliminate solvent interference.
- Some phenolic compounds may interfere slightly with the assay, affecting specificity.

2.2.3. Quantitative Determination of Condensed Tannins (Proanthocyanidins) by the Vanillin–HCl Method

1. Principle

Condensed tannins (proanthocyanidins) react with Vanillin in the presence of Hydrochloric acid to form red-colored anthocyanidin-like compounds. The intensity of the color is proportional to the concentration of condensed tannins and is measured spectrophotometrically at approximately 500 nm. Results are expressed relative to a standard such as Catechin.

2. Reagents and Materials

- Vanillin solution (1% w/v in methanol)
- Concentrated hydrochloric acid (HCl)
- Methanol (analytical grade)
- Catechin standard solutions (e.g., 0–500 $\mu\text{g/mL}$)
- Plant extract (methanolic or ethanolic)
- Distilled water
- Spectrophotometer ($\lambda = 500 \text{ nm}$)
- Test tubes, pipettes, vortex mixer

3. Preparation of Solutions

3.1 Vanillin Reagent

Prepare fresh 1% vanillin solution in methanol before use.

3.2 Acid Solution

Use concentrated HCl directly or prepare a suitable dilution if required.

3.3 Standard Solutions

Prepare catechin solutions at different concentrations for calibration curve construction.

4. Experimental Procedure

1. Pipette 0.5 mL of plant extract into a test tube.
2. Add 3.0 mL of vanillin solution (1% in methanol).
3. Add 1.5 mL of concentrated HCl.
4. Mix thoroughly using a vortex mixer.
5. Incubate the reaction mixture at room temperature for 15 minutes.
6. Measure absorbance at 500 nm against a blank (prepared with methanol instead of vanillin or sample).

5. Blank and Control

- **Blank:** Methanol + vanillin + HCl (without sample)
- **Standard:** Catechin solutions treated under identical conditions

6. Calculation

Condensed tannin content (CTC) is expressed as mg catechin equivalents (CE)/g of dry extract:

$$CTC = \frac{C \times V}{m}$$

Where:

- C = concentration obtained from calibration curve (mg/mL)
- V = volume of extract (mL)
- m = mass of sample (g)

7. Observations and Interpretation

The development of a red coloration indicates the presence of condensed tannins. The absorbance increases proportionally with tannin concentration. Measurements should be performed in triplicate, and results expressed as mean $\pm$ standard deviation.

8. Notes and Methodological Considerations

- The vanillin reagent must be freshly prepared to ensure sensitivity.

- The reaction is highly dependent on acidic conditions and timing; therefore, consistency is essential.
- Protect reaction mixtures from light when possible.

The method is selective for flavan-3-ol type tannins but may show variability depending on tannin structure.

2.3. In vitro Biological Activities

2.3.1. Evaluation of Antioxidant Activity by DPPH Free Radical Scavenging Assay

1. Principle

The antioxidant capacity of plant extracts is commonly evaluated using the DPPH assay. DPPH is a stable free radical characterized by a deep violet color with strong absorbance at 517 nm. Upon reaction with an antioxidant (hydrogen or electron donor), DPPH is reduced to a yellow-colored diphenylpicrylhydrazine. The decrease in absorbance reflects the radical scavenging ability of the tested sample.

2. Reagents and Materials

- DPPH solution (0.1 mM in methanol or ethanol)
- Methanol or ethanol (analytical grade)
- Standard antioxidant: Ascorbic acid
- Plant extract (prepared in methanol/ethanol)
- Spectrophotometer ($\lambda = 517$ nm)
- Test tubes, pipettes, vortex mixer
- Aluminum foil (to protect from light)

3. Preparation of Solutions

3.1 DPPH Solution

Prepare a 0.1 mM DPPH solution by dissolving approximately 3.94 mg of DPPH in 100 mL of methanol. Store in the dark.

3.2 Extract Solution

Dissolve the plant extract to obtain a concentration of 1 mg/mL (e.g., 3 mg in 3 mL methanol/ethanol).

3.3 Standard Solution

Prepare ascorbic acid solutions at different concentrations (e.g., 10–100 µg/mL) for calibration or comparison.

4. Experimental Procedure

1. Pipette 2.0 mL of DPPH solution into a test tube.
2. Add 0.5–1.0 mL of plant extract solution.
3. For control, mix DPPH solution with solvent only.
4. For standard, replace extract with ascorbic acid solution.
5. Mix thoroughly and incubate in the dark at room temperature for 30 minutes.
6. Measure absorbance at 517 nm against a blank (methanol/ethanol).

5. Calculation of Radical Scavenging Activity

The percentage inhibition of DPPH radical is calculated as:

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

Where:

- A_{control} = absorbance of DPPH solution without sample
- A_{sample} = absorbance of DPPH with extract or standard

Optionally, IC₅₀ (the concentration required to inhibit 50% of DPPH radicals) can be determined from the inhibition curve.

6. Observations and Interpretation

A visible color change from deep violet to pale yellow indicates free radical scavenging activity. Greater discoloration corresponds to higher antioxidant capacity. The schematic in the original notes (T₀ vs. T₃₀ min) reflects this transition.

7. Notes and Methodological Considerations

- Perform all assays in triplicate to ensure reproducibility.
- Protect DPPH solution from light to prevent degradation.
- Ensure consistent incubation time and temperature.
- The assay is sensitive to solvent type; methanol is most commonly used.

2.3.2 Determination of Reducing Power by the FRAP Assay

Principle of the Method

The Ferric Reducing Antioxidant Power (FRAP) assay is a spectrophotometric method widely used to evaluate the antioxidant capacity of plant extracts and bioactive compounds. The assay is based on the ability of antioxidant molecules to donate electrons, reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). In this procedure, potassium ferricyanide is reduced to potassium ferrocyanide, which subsequently reacts with ferric chloride (FeCl_3) to form Perl's Prussian blue complex. The intensity of the resulting blue-green coloration is proportional to the reducing power of the tested sample and is measured spectrophotometrically at 700 nm. Higher absorbance values indicate stronger reducing and antioxidant capacities. (Oyaizu, 1986).

Experimental Protocol

1. Preparation of Plant Extract Solutions

A concentration gradient was prepared in order to evaluate the dose-dependent reducing activity of the plant extract.

Tested concentrations

- 400 $\mu\text{g/mL}$
- 600 $\mu\text{g/mL}$
- 800 $\mu\text{g/mL}$
- 1200 $\mu\text{g/mL}$
- 1400 $\mu\text{g/mL}$
- 1600 $\mu\text{g/mL}$

Preparation of stock solution

A stock solution was prepared by dissolving 600 mg of the dry extract in 6 mL of distilled water to obtain a concentrated solution suitable for serial dilution.

2. Preparation of Reagents

Potassium ferricyanide solution (1%)

Dissolve 1 g of potassium ferricyanide in 100 mL of distilled water.

Trichloroacetic acid solution (10%)

Dissolve 10 g of trichloroacetic acid (TCA) in 100 mL of distilled water.

Ferric chloride solution

Prepare a fresh ferric chloride ($FeCl_3$) solution according to standard laboratory procedures.

3. Experimental Procedure

The reducing power assay was carried out according to the method described by Makoto Oyaizu with slight modifications.

Step 1: Preparation of the reaction mixture

Transfer 1 mL of the extract solution at the desired concentration into a clean test tube.

Step 2: Addition of potassium ferricyanide

Add 1 mL of 1% potassium ferricyanide solution.

Step 3: Incubation

Incubate the reaction mixture at 50°C for 20 minutes to promote the reduction of ferricyanide ions.

Step 4: Termination of the reaction

Add 1 mL of 10% trichloroacetic acid (TCA) to terminate the reaction.

Step 5: Formation of Perl's Prussian blue complex

Transfer 1 mL of the resulting mixture or supernatant into a new test tube, then add:

- 1 mL of distilled water

- 250 μ L of ferric chloride ($FeCl_3$)

The appearance of a greenish-blue coloration indicates the presence of reducing compounds capable of converting ferric ions into ferrous ions.

4. Spectrophotometric Measurement

The absorbance of the final reaction mixture was measured at 700 nm using a UV-Visible spectrophotometer against a suitable blank.

Interpretation of results

An increase in absorbance at 700 nm reflects an increase in reducing power and therefore a stronger antioxidant activity of the tested extract.

5. Scientific Significance of the FRAP Assay

The reducing power assay is considered an important indicator of antioxidant potential because compounds with reducing properties act as electron donors and can terminate free radical chain reactions. Plant extracts rich in polyphenols, flavonoids, and tannins generally exhibit strong reducing capacities due to the presence of hydroxyl groups capable of electron donation. Therefore, the FRAP assay provides valuable information concerning the potential biological and pharmacological properties of medicinal plants. (Prior et al., 2005)

2.3.3 Determination of Sun Protection Factor (SPF) Activity

Principle of the Method

The Sun Protection Factor (SPF) assay is a spectrophotometric method used to evaluate the photoprotective potential of plant extracts and cosmetic formulations against ultraviolet (UV) radiation, particularly within the UV-B region (290–320 nm). The method estimates the ability of the tested sample to absorb or block ultraviolet radiation responsible for erythema, photoaging, and oxidative skin damage. The SPF value is calculated from the absorbance of the sample measured at specific wavelengths using the mathematical equation proposed by Mansur. Higher SPF values indicate greater photoprotective activity. (Mansur et al., 1986).

Experimental Protocol

1. Preparation of Samples

Two different samples were prepared for comparative evaluation:

Plant Extract Solution

- Weigh 0.5 g of the plant extract.
- Dissolve the extract in 1 mL of distilled water.
- Mix thoroughly until a homogeneous solution is obtained.

Commercial Sunscreen Solution

- Weigh 0.5 g of Eucerin sunscreen cream.
- Dissolve in 1 mL of distilled water.
- Homogenize the mixture completely before analysis.

2. Spectrophotometric Measurement

The absorbance of each prepared solution was measured using a UV-Visible spectrophotometer.

Measurement Conditions

- Wavelength range: 290–320 nm
- Interval between measurements: 5 nm
- Blank solution: Distilled water

Absorbance readings were recorded at the following wavelengths:

- 290 nm
- 295 nm
- 300 nm
- 305 nm
- 310 nm
- 315 nm
- 320 nm

3. Calculation of SPF

The SPF value was calculated according to the Mansur equation:

Where:

- CF = Correction factor (commonly equal to 10)
- $EE(\lambda)$ = Erythemal effect spectrum
- $I(\lambda)$ = Solar intensity spectrum
- $Abs(\lambda)$ = Absorbance of the sample at wavelength λ

The values of $EE(\lambda) \times I(\lambda)$ are constants established by Mansur et al. and are used for SPF estimation.

4. Scientific Interpretation

An increase in absorbance within the UV-B range indicates greater ultraviolet absorption capacity and therefore stronger photoprotective activity. Plant extracts rich in polyphenols, flavonoids, and tannins frequently exhibit sunscreen properties due to their ability to absorb UV radiation and neutralize reactive oxygen species generated by solar exposure. (Saewan & Jimtaisong, 2013).

The comparison between the plant extract and the commercial sunscreen formulation allows evaluation of the natural extract's effectiveness as a potential photoprotective agent. If the extract demonstrates elevated SPF values, it may represent a promising natural alternative for cosmetic and pharmaceutical sunscreen formulations.

Importance of Polyphenols and Flavonoids in Photoprotection

Polyphenolic compounds and flavonoids contribute significantly to UV protection because of:

- their strong UV-absorbing capacity;
- antioxidant activity;
- free radical scavenging effects;
- and inhibition of oxidative stress induced by ultraviolet radiation.

These compounds may reduce skin inflammation, lipid peroxidation, and cellular damage associated with prolonged UV exposure. (Nichols & Katiyar, 2010).

2.3.4. In Vitro Evaluation of the α -Amylase Inhibitory Activity of a Plant Polysaccharide Using the Microplate Starch–Iodine Colorimetric Assay

The α -amylase inhibitory activity of a plant-derived polysaccharide (designated as PS) was evaluated *in vitro* using a modified starch–iodine colorimetric assay performed in a 96-well microplate format. This assay is based on the principle of enzymatic hydrolysis of starch into oligosaccharides and simple sugars by α -amylase, resulting in a decrease in the intensity of the blue starch–iodine complex, which is quantifiable via spectrophotometry at 630 nm. The retention of the blue coloration indicates the presence of residual, unhydrolyzed starch, thereby reflecting the inhibitory capacity of the tested compound against enzymatic activity. This approach serves as a widely utilized preliminary *in vitro* model for screening bioactive plant compounds with potential antidiabetic properties through the inhibition of carbohydrate-digesting enzymes.

The starch substrate solution was prepared by dissolving 200 mg of soluble starch in 25 mL of 0.4 M NaOH, followed by heating at 100 °C for 5 minutes to ensure complete solubilization of the starch granules. After cooling to room temperature, the pH was adjusted to 7.0, and the final volume was brought to 100 mL with distilled water, yielding a homogeneous and stable substrate solution optimized for the enzymatic reaction.

The polysaccharide PS was solubilized in distilled water containing 1% dimethyl sulfoxide (DMSO) to enhance solubility and ensure a homogeneous dispersion of the compound. A stock solution of 40 mg/mL was prepared, from which a series of successive two-fold (1:2) dilutions were performed to obtain the following final concentrations: 40, 20, 10, 5, and 2.5 mg/mL, thereby enabling the evaluation of the inhibitory effect in a dose-dependent manner.



Figure 10. Experimental setup of the *in vitro* α -amylase inhibitory assay: 96-well microplate prepared with polysaccharide extract (PS) serial dilutions, alongside starch substrate solution (Starch), TRIS-HCl buffer, and reaction tubes, prior to enzymatic incubation at 37 °C.

For each reaction well, 40 μ L of the starch solution was mixed with 20 μ L of the PS solution at the designated concentration. Acarbose was employed as a positive control, serving as the standard reference α -amylase inhibitor, whereas the negative control consisted of the enzymatic reaction mixture without any inhibitory compound. The mixtures were pre-incubated at 37 °C for 3 minutes to facilitate the interaction between PS and the substrate prior to the initiation of the reaction.

The enzymatic reaction was initiated by the addition of 20 μ L of an α -amylase solution (3 U/mL), prepared in a 20 mM phosphate buffer containing 6.7 mM NaCl (pH 6.9) to provide optimal physiological conditions for enzymatic activity. The reaction mixtures were subsequently incubated at 37 °C for 15 minutes, allowing for the controlled hydrolysis of starch by α -amylase.

The reaction was terminated by adding 80 μ L of 0.1 M HCl, followed by the addition of 100 μ L of an iodine–potassium iodide solution (IKI, 2.5 mM), which forms a stable blue complex with the residual starch. The intensity of the developed coloration, which is directly proportional to the amount of unhydrolyzed starch, was measured at 630 nm using a microplate reader.

The percentage inhibition of α -amylase activity was calculated according to the following equation:

$$\% \text{ inhibition} = \left(\frac{A - B}{A} \right) \times 100$$

Where A represents the absorbance of the negative control (complete enzymatic activity without inhibitor), and B represents the absorbance measured in the presence of the PS

polysaccharide. All experimental conditions were performed in triplicate, and results were expressed as mean $\pm$ standard deviation to ensure analytical reliability and reproducibility.

This experimental design allows for a rigorous evaluation of the inhibitory potential of the polysaccharide PS against α -amylase, thereby providing preliminary data on its potential role in reducing postprandial hyperglycemia via the modulation of carbohydrate digestion. The obtained results warrant further investigations, including the determination of IC₅₀ values, enzymatic kinetics studies, or bio-guided fractionation of the active fractions (Chakrabarti et al., 2014; Granados-Guzmán et al., 2022; Khadayat et al., 2020).

2.4. In vivo tests

2.4.1 Evaluation of Antitussive Activity of Polysaccharides Extracted from Artemisia Using the Ammonia-Induced Cough Model

Principle of the Method

The ammonia-induced cough model is a widely used experimental method for evaluating the antitussive activity of natural products and pharmacological agents. Inhalation of ammonia vapors causes irritation of the respiratory mucosa and stimulation of cough receptors located in the upper respiratory tract, thereby inducing coughing reflexes. Compounds possessing antitussive activity reduce the frequency and intensity of cough episodes by suppressing cough reflex pathways or protecting the respiratory mucosa. (Bolser, 2006).

Experimental Protocol

1. Experimental Animals

The study was conducted using healthy laboratory mice maintained under standard environmental conditions with free access to food and water throughout the experimental period.

The animals were randomly divided into six experimental groups, each containing three mice ($n = 3$).

2. Experimental Design and Animal Grouping

Experimental Groups

Group	Treatment
Group 1 (T-)	Distilled water (negative control)
Group 2 (T+)	Tussidex 0.2% (dextromethorphan bromate), 200 μ L
Group 3	Polysaccharide extract at 5 mg/kg
Group 4	Polysaccharide extract at 10 mg/kg
Group 5	Polysaccharide extract at 25 mg/kg
Group 6	Polysaccharide extract at 50 mg/kg

The tested polysaccharides were extracted from *Artemisia*.

3. Preparation of Extract Solutions

The polysaccharide extract was dissolved in distilled water immediately before administration to ensure stability and homogeneity.

Preparation of Doses

Group	Dose	Preparation
Group 3	5 mg/kg	1.25 mg extract in 3 mL distilled water
Group 4	10 mg/kg	2.5 mg extract in 3 mL distilled water
Group 5	25 mg/kg	6.26 mg extract in 3 mL distilled water
Group 6	50 mg/kg	12.5 mg extract in 3 mL distilled water

4. Treatment Administration

Each mouse received 1 mL of the corresponding preparation orally once daily using an intragastric gavage syringe.

Treatment Conditions

- Route of administration: Oral
- Administration time: 11:45 AM
- Treatment duration: 5 consecutive days

The negative control group received distilled water under identical experimental conditions.

The positive control group received Tussidex containing dextromethorphan bromate, a standard centrally acting antitussive agent commonly used to suppress cough reflexes. (Dicpinigaitis, 2006).

5. Induction of Cough Reflex

One hour after administration of the final dose on the fifth day, cough induction was performed using diluted ammonia vapor.

Procedure

1. Each mouse was individually placed inside a sealed chamber.
2. The chamber was sprayed twice with diluted ammonia solution.
3. The mouse remained exposed to ammonia vapors for 2 minutes.
4. The animal was subsequently transferred into a second sealed observation chamber for another 2 minutes.

Ammonia inhalation acts as a respiratory irritant that stimulates sensory receptors of the airway mucosa, thereby inducing cough episodes.

6. Recording of Cough Frequency

The number of cough episodes produced by each mouse was counted manually during the observation period.

The procedure was repeated until all animals from every experimental group completed the assay.

The mean cough frequency for each group was subsequently calculated and compared statistically.

7. Calculation of Antitussive Activity

The percentage inhibition of cough was calculated according to the following equation:

$$\text{Inhibition (\%)} = \left(\frac{\text{Cough Count}_{\text{Control}} - \text{Cough Count}_{\text{Treated}}}{\text{Cough Count}_{\text{Control}}} \right) \times 100$$

Where:

- Cough Count_{control} = mean cough frequency in the negative control group;
- Cough Count_{treated} = mean cough frequency in the treated group.

Higher inhibition percentages indicate stronger antitussive activity.

This formula allows quantitative comparison between treated and untreated groups and provides an estimation of the antitussive efficacy of the polysaccharide extract

8. Scientific Interpretation

A significant reduction in cough frequency in treated groups compared with the negative control group indicates antitussive activity of the tested polysaccharide extract.

Polysaccharides extracted from medicinal plants may exert antitussive effects through:

- protection of respiratory mucosa;
- anti-inflammatory activity;
- modulation of cough receptor sensitivity;
- and antioxidant properties. (Li et al., 2021).

A dose-dependent decrease in cough frequency across Groups 3–6 would suggest increased pharmacological efficacy with increasing extract concentration.

Comparison with the positive control group treated with Tussidex allows evaluation of the relative therapeutic potential of the plant extract.

2.4.2 Acute Toxicity Study of the Plant Extract in Wistar rat

Principle of the Study

The acute toxicity study was performed to evaluate the potential toxic effects of the tested plant extract following repeated oral administration in experimental animals. Acute toxicity assays are essential in pharmacological and toxicological investigations to determine the safety profile of natural products before their therapeutic application. Biological evaluation generally includes clinical observation, hematological and biochemical analyses, as well as histopathological examination of vital organs such as the liver and kidneys. These parameters provide important

information concerning systemic toxicity, organ damage, and physiological alterations induced by the tested substance. (OECD, 2001).

Experimental Protocol

1. Experimental Animals

The study was conducted using 12 healthy adult male Wistar rat weighing approximately 180–220 g. The animals were maintained under standard laboratory conditions, including controlled temperature, humidity, and a 12 h light/dark cycle, with free access to food and water throughout the experimental period.

2. Experimental Design and Animal Grouping

The animals were randomly divided into four experimental groups, each containing three rats ($n = 3$).

Experimental Groups

Group	Treatment	Dose
Group 0	Physiological water	Control
Group 1	Plant extract	10 mg/kg
Group 2	Plant extract	50 mg/kg
Group 3	Plant extract	100 mg/kg

3. Preparation of Extract Solutions

The plant extract was freshly dissolved in distilled water before administration.

Dose Preparation

Group	Dose	Preparation
Group 1	10 mg/kg	2.5 mg extract in 3 mL distilled water
Group 2	50 mg/kg	12.5 mg extract in 3 mL distilled water
Group 3	100 mg/kg	25 mg extract in 3 mL distilled water

4. Administration Procedure

Each rat received 1 mL of the prepared solution orally once daily using an intragastric gavage syringe. Administration was performed every day at 12:00 PM for 10 consecutive days in order to minimize circadian variations and ensure experimental consistency.

The control group received physiological water under identical experimental conditions.

5. Clinical Observation and Monitoring

Throughout the treatment period, the animals were monitored daily for signs of toxicity and behavioral abnormalities, including:

- changes in locomotor activity;
- food and water intake;
- piloerection;
- tremors;
- diarrhea;
- respiratory distress;
- body weight changes;
- and mortality.

Clinical observations provide preliminary indications concerning the systemic toxicity of the tested extract. (Parasuraman, 2011).

6. Blood Collection and Biochemical Analysis

At the end of the experimental period (Day 10), the rats were sacrificed under appropriate ethical conditions, and blood samples were collected for hematological and biochemical investigations.

Hematological Analysis

A complete blood count (FNS: Formule Numération Sanguine) was performed to evaluate:

- red blood cells (RBC);
- white blood cells (WBC);

- hemoglobin concentration;
- hematocrit;
- and platelet count.

These parameters provide important information concerning potential hematotoxic effects of the extract.

Biochemical Analysis

Serum urea levels were measured to assess renal function and detect possible nephrotoxicity induced by the tested extract. Elevated urea concentrations may indicate impaired kidney filtration or renal tissue damage. (Burtis & Bruns, 2014).

7. Histopathological Evaluation

Following sacrifice, liver and kidney tissues were carefully excised, washed with physiological saline, and fixed in 10% buffered formalin for histological examination.

Histological Procedure

The organs were:

1. fixed in formalin;
2. dehydrated through graded alcohol solutions;
3. embedded in paraffin;
4. sectioned using a microtome;
5. stained with hematoxylin-eosin (H&E).

Microscopic examination was performed to evaluate potential histopathological alterations such as:

- inflammatory infiltration;
- necrosis;
- vascular congestion;
- cellular degeneration;

- and structural disorganization of hepatic and renal tissues.

Histopathological analysis is considered one of the most reliable methods for detecting tissue toxicity induced by natural or synthetic compounds. (Suvarna et al., 2018).

8. Scientific Interpretation

The absence of mortality, severe clinical symptoms, hematological abnormalities, biochemical disturbances, or histological lesions generally indicates low acute toxicity and suggests a favorable safety profile of the tested extract. Conversely, significant alterations in blood parameters, serum urea levels, or organ histology may indicate dose-dependent toxic effects affecting hepatic or renal functions.

The comparison between treated groups and the control group allows assessment of the toxicological threshold and safety margin of the plant extract under investigation.

2.4.3 Evaluation of Protective and Therapeutic Effects of a Plant Extract Against HCl-Induced Gastric Ulcers in Rats

Principle of the Method

The hydrochloric acid (HCl)-induced gastric ulcer model is a well-established experimental approach used to evaluate both gastroprotective (preventive) and therapeutic effects of natural products and pharmacological agents. Acidic injury caused by HCl leads to rapid disruption of the gastric mucosal barrier, resulting in epithelial erosion, hemorrhage, oxidative stress, and inflammatory damage. This model is particularly useful for assessing compounds that enhance mucosal defense, promote healing, or reduce acid-induced injury. (Konturek et al., 2011).

Experimental Protocol

1. Experimental Animals

The study was conducted on 21 healthy rats distributed into seven experimental groups, with three animals per group ($n = 3$). The animals were maintained under standard laboratory conditions with free access to food and water prior to the fasting period.

2. Experimental Design and Grouping

The animals were randomly divided into the following groups:

Experimental Groups

Group	Treatment Description
T-1	Negative control + physiological water; received HCl on experiment day
T-2	Negative control (physiological water only, no HCl induction)
T+	Physiological water + Omeprazole followed by HCl
Group 4	Plant extract (1.25 mg in 3 mL distilled water)
Group 5	Plant extract (2.5 mg in 3 mL distilled water)
Group 6	Plant extract (6.25 mg in 3 mL distilled water)
Group 7	Plant extract (12.5 mg in 3 mL distilled water)

3. Preparation and Administration of Treatments

All treatments were administered orally once daily for five consecutive days at 12:15 PM using a standardized gavage volume of 1 mL per rat.

The plant extract was freshly prepared in distilled water at the required concentrations before administration to ensure homogeneity and stability.

4. Fasting Conditions

Twenty-four hours prior to ulcer induction, all animals were fasted while allowing free access to water. Fasting ensures gastric emptying and increases susceptibility of the gastric mucosa to acidic injury, improving model reproducibility.

5. Induction of Gastric Ulcers

On the experimental day, all groups except T-2 received ulcer induction using 80% hydrochloric acid (HCl).

HCl Administration

The dose of HCl was adjusted according to individual body weight of each animal in order to standardize gastric injury severity.

Group	Individual Dose Range (mL)
T-1	1.29 – 1.96 1.72 mL
T-2	1.82 1.57 – 1.92 mL No HCl administered

T+	1.34 – 1.32 – 1.30 mL
Group 4	1.61 – 1.53 – 1.58 mL

Ulcer induction was performed one hour after the final oral administration of treatments.

6. Experimental Timeline

- Day 1–5: Daily oral treatment administration
- Day 6:
 - 24 h fasting
 - Final treatment dose administered
 - 1 h later: HCl induction (except T-2)
 - 1 h post-HCl: euthanasia and tissue collection

7. Tissue Collection and Macroscopic Examination

One hour after HCl administration, the animals were sacrificed under ethical conditions.

The stomachs were:

1. Carefully excised;
2. Opened along the greater curvature;
3. Rinsed with distilled water followed by physiological saline to remove gastric contents;
4. Visually examined for ulcer lesions.

8. Measurement of Ulcer Area

The extent of gastric lesions was quantified using a standardized planimetric method:

Procedure

- A transparent acetate sheet was placed over each opened stomach.
- All ulcer lesions were traced manually onto the sheet.
- The traced lesions were transferred onto millimeter graph paper.
- Ulcerated areas were measured in mm².

This method allows objective quantification of mucosal damage and comparison between experimental groups.

9. Histological Analysis

After macroscopic evaluation, stomach tissues were preserved in 10% formalin for histopathological examination.

Histological Processing

- Fixation in formalin
- Dehydration in graded ethanol series
- Paraffin embedding
- Sectioning using microtome
- Staining with hematoxylin and eosin (H&E)

Histological analysis was performed to evaluate:

- epithelial erosion;
- inflammatory infiltration;
- glandular damage;
- edema;
- and mucosal integrity.

10. Scientific Interpretation

A reduction in ulcer area and histological damage in treated groups compared to the HCl control group (T-1) indicates a protective and/or healing effect of the plant extract.

The positive control group treated with Omeprazole serves as a pharmacological reference for acid suppression and mucosal protection.

Plant extracts with gastroprotective activity typically act through:

- inhibition of gastric acid secretion;
- enhancement of mucus and bicarbonate production;

- antioxidant effects against HCl-induced oxidative stress;
- anti-inflammatory mechanisms;
- and promotion of epithelial regeneration. (Li et al., 2021)

A dose-dependent reduction in ulcer area across Groups 4–7 would strongly support the therapeutic potential of the tested extract.

2.5. Statistical Analysis

All quantitative in vitro and in vivo experiments were performed in triplicate ($n = 3$). Data were recorded and processed using Microsoft Excel. Results are expressed as mean $\pm$ standard deviation (SD). Statistical significance between experimental groups and controls was determined using one-way Analysis of Variance (ANOVA), with a defined significance threshold of $p < 0.05$.

Chapter 2

Results and discussion

I. Results

1. Results of quantitative determination of total phenolic content

1.1 Overview of Analytical Context and Methodological Framework

The quantitative determination of total phenolic content (TPC) in the polysaccharide-enriched extract of *Calligonum comosum* seeds was performed using the Folin–Ciocalteu colorimetric assay, executed across three independent experimental replicates to ensure the statistical robustness and reproducibility of the reported values. This method exploits the capacity of phenolic and polyphenolic compounds to reduce the molybdotungstate reagent complex under alkaline conditions, generating a blue chromophore measurable spectrophotometrically at 760 nm, with results calibrated against a gallic acid primary standard and expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract; Huang et al., 2021; Sánchez-Rangel et al., 2021). The inclusion of three independent extract replicates — each measured across a parallel gallic acid calibration series — permits the calculation of a statistically grounded mean TPC with an associated measure of inter-replicate variability, thereby satisfying the quantitative rigour expected of publication-grade phytochemical analyses (Tan et al., 2020). The present section reports the raw absorbance data for both the gallic acid standard curve and the three extract replicates, the derived TPC values, and a critical interpretation of the findings in the context of the broader phytochemical literature on arid-region plant polysaccharides.

1.2 Gallic Acid Standard Curve

1.2.1 Calibration Absorbance Data and Linearity Assessment

The gallic acid (AG) standard curve was constructed from six concentration points spanning the range of 0.010 to 0.050 mg/mL, with absorbance values recorded at 760 nm as follows: 0.208 (0.010 mg/mL), 0.292 (0.015 mg/mL), 0.390 (0.020 mg/mL), 0.455 (0.025 mg/mL), 0.497 (0.030 mg/mL), and 0.911 (0.050 mg/mL; Table 1). The progressive and largely monotonic increase in absorbance across the calibration range confirms the expected positive relationship between gallic acid concentration and Folin–Ciocalteu chromophore development, and the regression equation derived from these data served as the primary quantitative reference for the interpolation of extract TPC values. (Table 6 in Appendix A)

A notable feature of the calibration dataset warrants comment: the absorbance increment between 0.025 and 0.030 mg/mL ($\Delta = 0.042$) is disproportionately smaller than increments observed at adjacent concentration intervals, suggesting a localised departure from strict Beer–Lambert linearity at this point. This phenomenon, occasionally reported in Folin–Ciocalteu assays employing concentrated reagent preparations, is attributable to partial reagent saturation or competitive matrix interactions at intermediate concentrations and does not invalidate the overall calibration utility of the curve, provided that linear regression is applied across the full six-point range rather than segment-by-segment (Sánchez-Rangel et al., 2021; Tan et al., 2020). The strong absorbance response at the highest calibration point (0.911 at 0.050 mg/mL) confirms adequate reagent sensitivity at the upper concentration boundary and anchors the regression with appropriate leverage at the high end of the calibration range.

1.3 Replicate Absorbance Data for the *Calligonum comosum* Polysaccharide Extract

1.3.1 Raw Absorbance Measurements Across Three Replicates

The TPC determination was conducted across three independent extract replicates (EX1, EX2, EX3), each measured at the same six concentration points as the gallic acid standard to ensure direct calibration comparability. The raw absorbance values recorded at 760 nm for each replicate are presented in Table 2.

Table 6. Raw Absorbance Values (760 nm) for Three Independent Replicates of the *C. comosum* Polysaccharide Extract Across the Folin–Ciocalteu Assay Concentration Series.

Concentration (mg/mL)	Replicate 1 (EX1)	Replicate 2 (EX2)	Replicate 3 (EX3)
Extract absorbance (initial)	1.104	1.066	1.091
0.010	0.208	0.195	0.197
0.015	0.292	0.305	0.271
0.020	0.390	0.632*	0.403
0.025	0.455	—	0.432
0.030	0.497	0.510	0.521
0.050	0.911	0.837	0.968

Note. Initial extract absorbance values (1.104, 1.066, 1.091) represent the undiluted or sample-specific absorbance readings used for TPC interpolation against the gallic acid standard curve. An asterisk (*) denotes the anomalous value for EX2 at 0.020 mg/mL (0.632), which deviates substantially from the corresponding EX1 and EX3 values and from the expected

calibration trend; this data point was treated as a potential outlier in the analysis (see Section 3.3.2). A dash (—) indicates a missing or non-recorded value for EX2 at 0.025 mg/mL.

1.3.2 Inter-Replicate Consistency and Anomalous Data Points

Across the three replicates, the initial extract absorbance values — those directly used for TPC interpolation — were highly concordant: 1.104 (EX1), 1.066 (EX2), and 1.091 (EX3), yielding a mean initial absorbance of 1.087 and a range of only 0.038 absorbance units. This close agreement across independently prepared replicates is indicative of a reproducible extraction procedure and a stable, homogeneous polysaccharide extract, lending confidence to the reliability of the derived TPC values (Huang et al., 2021; Zhang et al., 2022).

However, two data irregularities merit explicit acknowledgement in the interests of analytical transparency. First, the absorbance value recorded for EX2 at the 0.020 mg/mL calibration concentration (0.632) deviates substantially from the corresponding values of EX1 (0.390) and EX3 (0.403), as well as from the expected monotonic progression of the calibration curve at that point. This value is approximately 62% higher than the mean of the other two replicates at the same concentration and represents a probable pipetting error, reagent mixing artefact, or transcription inaccuracy. Its treatment as an outlier for the purposes of regression fitting is methodologically justified under standard outlier identification criteria (e.g., Grubbs' test or the 1.5 IQR rule), and its exclusion from the regression for EX2 would be expected to improve the fit of the calibration model for that replicate (Abubakar & Haque, 2020; Tan et al., 2020). Second, the value at 0.025 mg/mL for EX2 was not recorded, introducing a gap in the calibration series for that replicate. While the absence of a single calibration point does not preclude the construction of a valid regression model from the remaining five data points, it marginally reduces the statistical power of the EX2 calibration and should be noted in any methodological appraisal of the assay's execution for this replicate.

1.4 Total Phenolic Content: Derived Values and Statistical Summary

1.4.1 Mean TPC and Standard Deviation

The total phenolic content of the *C. comosum* seed polysaccharide extract, calculated by interpolation of the initial extract absorbance values (1.104, 1.066, and 1.091) against the linear regression of the gallic acid standard curve, was determined to be **61.37 ± 1.12 mg GAE/g dry extract** (mean ± standard deviation; Table 3). The coefficient of variation (CV) for the three replicate TPC determinations was approximately 1.82%, indicating exceptionally high inter-replicate reproducibility and confirming that the extraction, dilution, and colorimetric

measurement procedures were executed with a degree of precision consistent with publication-quality phytochemical analysis.

Table 7. Statistical Summary of Total Phenolic Content Determined by the Folin–Ciocalteu Method for *C. comosum* Seed Polysaccharide Extract.

Statistical Parameter	Value
Mean TPC (mg GAE/g dry extract)	61.37
Standard Deviation (SD)	1.12
Coefficient of Variation (CV, %)	1.82
Number of Replicates (<i>n</i>)	3
Reference Standard	Gallic acid
Measurement Wavelength	760 nm

Note. TPC = total phenolic content; GAE = gallic acid equivalents. Mean and SD calculated from three independent replicate determinations. $CV = (SD / \text{mean}) \times 100$.

1.4.2 Biochemical Significance and Comparative Contextualisation

A TPC of 61.37 ± 1.12 mg GAE/g dry extract constitutes a moderate-to-substantial phenolic load for a polysaccharide-enriched seed extract and positions *C. comosum* within a biochemically meaningful range relative to comparator taxa from arid and semi-arid phytogeographic zones. For reference, polysaccharide-rich fractions from *Atriplex halimus*, an ecologically analogous xerophyte of North African distribution, have been reported to exhibit TPC values of 40–75 mg GAE/g under comparable extraction and assay conditions (Aouidi et al., 2022), while seed extracts from *Rumex vesicarius* — a Polygonaceous species botanically proximate to *C. comosum* — have yielded TPC values in the range of 55–80 mg GAE/g (Ahmed et al., 2021). The present value of 61.37 mg GAE/g falls within both of these reference ranges and is consistent with the phytochemical expectations for phenolic compound accumulation in the seeds of stress-adapted desert shrubs, where secondary metabolite biosynthesis is frequently upregulated as a photoprotective and antioxidant adaptive response to chronic abiotic stressors including ultraviolet irradiation, oxidative stress, and water deficit (Ziani et al., 2021; Sharifi-Rad et al., 2020).

It is analytically imperative, however, to acknowledge the well-documented non-selectivity of the Folin–Ciocalteu reagent. As an electron-transfer-based colorimetric system, the reagent reacts with all sufficiently electron-donating species present in the sample matrix — including reducing sugars, ascorbic acid, amino acids bearing hydroxyl or thiol groups, and uronic acid

residues commonly abundant in seed polysaccharides — and cannot distinguish phenolic from non-phenolic electron donors (Sánchez-Rangel et al., 2021; Tan et al., 2020). Given the polysaccharide-enriched nature of the extract under examination, it is plausible that a fraction of the Folin–Ciocalteu signal arises from saccharidic co-extractants rather than from phenolic compounds *sensu stricto*. This methodological limitation is explicitly recognised in contemporary phytochemical practice (Huang et al., 2021; Zhang et al., 2022), and the TPC value of 61.37 mg GAE/g should therefore be regarded as a conservative upper estimate of phenolic content rather than an absolute quantification. The proportion of signal attributable to non-phenolic reducing species could be estimated in future work through parallel solid-phase extraction fractionation or solid-phase polyvinylpolypyrrolidone (PVPP) precipitation, which selectively binds polyphenols and permits their quantitative separation from interfering matrix components (Sánchez-Rangel et al., 2021).

Despite this caveat, the observed TPC value is biochemically coherent with the antioxidant activities reported for the *C. comosum* extract in the DPPH and FRAP assays presented in preceding sections of this chapter. The genus *Calligonum* is phytochemically documented to elaborate a diversity of phenolic secondary metabolites — including catechins, procyanidins, kaempferol glycosides, and hydroxycinnamic acid conjugates — in multiple tissue types including roots, stems, and seeds (Sharifi-Rad et al., 2020; Ziani et al., 2021). Phenolic constituents covalently bound to or non-covalently associated with polysaccharide backbones — constituting phenolic–polysaccharide conjugate complexes — have been characterised in several arid-zone species and are increasingly implicated as structural determinants of the antioxidant, anti-inflammatory, and metal-chelating activities attributed to crude polysaccharide fractions (Mao et al., 2022; Zhang et al., 2022). The TPC result of 61.37 mg GAE/g is therefore consistent with the mechanistic hypothesis that phenolic–polysaccharide conjugation contributes, at least in part, to the antioxidant capacity profile of the *C. comosum* seed extract documented in this investigation.

2. Results of quantitative determination of total flavonoid content

2.1 Overview of Analytical Context and Methodological Framework

The quantitative determination of total flavonoid content (TFC) in the polysaccharide-enriched extract of *Calligonum comosum* seeds was performed using the aluminium chloride (AlCl_3) colorimetric method, conducted across three independent experimental replicates to ensure the statistical robustness and inter-assay reproducibility of the reported values. The AlCl_3 method exploits the formation of stable acid-labile complexes between aluminium ions and the C-4 keto

group and C-3 or C-5 hydroxyl groups of flavones and flavonols, producing a bathochromic shift in the absorbance spectrum that is measurable at 415 nm and is proportional to the total flavonoid concentration in the sample (Almutairi et al., 2021; Panche et al., 2016, as cited in Tungmunthum et al., 2020). Results are expressed in milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract), with quercetin employed as the primary calibration standard by virtue of its well-characterised aluminium-complexing reactivity and status as the most widely referenced flavonol standard in the phytochemical literature (Blainski et al., 2013, as cited in Almutairi et al., 2021; Kumar & Pandey, 2020). The triplicate experimental design adopted in the present investigation — encompassing three independently prepared extract replicates each measured against a parallel quercetin calibration series — provides the statistical basis for mean TFC calculation and inter-replicate variance assessment, as detailed in the sections below.

2.2 Quercetin Standard Curve

2.2.1 Calibration Absorbance Data and Linearity

The quercetin standard curve was constructed from four concentration–absorbance data pairs spanning the range of 0.010 to 0.030 mg/mL, yielding absorbance values at 415 nm as follows: 0.556 (0.010 mg/mL), 0.711 (0.015 mg/mL), 1.109 (0.025 mg/mL), and 1.323 (0.030 mg/mL; Table 1). The progressive and monotonically increasing absorbance response across the calibration range confirms the expected positive linear relationship between quercetin concentration and AlCl_3 chromophore development, and the regression equation derived from these four data points served as the primary quantitative reference for the interpolation of extract TFC values from the three replicate initial absorbance measurements. (Table 9 in Appendix A).

The linearity of the quercetin calibration curve merits brief analytical comment. The absorbance increment between 0.010 and 0.015 mg/mL ($\Delta = 0.155$) and between 0.015 and 0.025 mg/mL ($\Delta = 0.398$) indicates an accelerating response rate at intermediate concentrations, while the increment between 0.025 and 0.030 mg/mL ($\Delta = 0.214$) suggests a modest deceleration at the upper end of the range. This pattern is consistent with the mild curvilinearity occasionally observed in AlCl_3 -based flavonoid calibrations at higher quercetin concentrations, where partial reagent saturation or inner-filter effects may attenuate the rate of absorbance increase (Kumar & Pandey, 2020; Tungmunthum et al., 2020). While this does not compromise the utility of the standard curve for interpolative purposes within the tested range, it reinforces the importance of ensuring that extract absorbance values used for TFC calculation fall within the linear portion of the calibration, as was confirmed for all three replicates in the present dataset (see Section 3.3).

2.3 Replicate Absorbance Data for the *Calligonum comosum* Polysaccharide Extract

2.3.1 Raw Absorbance Measurements Across Three Replicates

The TFC determination was conducted across three independently prepared extract replicates (EX1, EX2, EX3), each measured at an initial extract concentration and across the four quercetin calibration concentrations to permit direct within-replicate regression comparison. The raw absorbance values recorded at 415 nm for each replicate are presented in Table 2.

Table 8. Raw Absorbance Values (415 nm) for Three Independent Replicates of the *C. comosum* Polysaccharide Extract and the Corresponding Quercetin Calibration Series.

Concentration Point	Replicate 1 (EX1)	Replicate 2 (EX2)	Replicate 3 (EX3)
Initial extract absorbance	0.764	0.779	0.778
0.010 mg/mL (quercetin ref.)	1.522	1.427	1.487
0.015 mg/mL (quercetin ref.)	1.109	1.100	0.977
0.025 mg/mL (quercetin ref.)	0.532	0.523	0.375
0.030 mg/mL (quercetin ref.)	0.905	0.711	0.736

Note. Initial extract absorbance values (0.764, 0.779, 0.778) represent the sample-specific absorbance readings used for TFC interpolation against the quercetin regression equation. Absorbance values at each quercetin reference concentration are reported as measured within each replicate series. The non-monotonic pattern in the replicate calibration series is discussed in Section 3.3.2.

2.3.2 Inter-Replicate Consistency and Data Pattern Analysis

The initial extract absorbance values across the three replicates — 0.764 (EX1), 0.779 (EX2), and 0.778 (EX3) — demonstrate a high degree of concordance, with a mean of 0.774 and a range of only 0.015 absorbance units. This tight clustering of initial extract readings across independently prepared replicates is a strong indicator of extraction reproducibility and spectrophotometric measurement precision, providing a reliable empirical basis for the subsequent TFC calculation (Almutairi et al., 2021; Zhang et al., 2022).

A notable feature of the replicate calibration data warrants explicit analytical discussion: within each of the three-replicate series (EX1, EX2, EX3), the absorbance values recorded at the quercetin reference concentrations do not follow the monotonically increasing pattern characteristic of the primary quercetin standard curve (Table 1). Specifically, the highest absorbance values within each replicate series are observed at 0.010 mg/mL (1.522, 1.427, and

1.487, respectively), with values then decreasing at 0.015 and 0.025 mg/mL before partially recovering at 0.030 mg/mL. This inverse relationship between quercetin concentration and absorbance within the replicate series is inconsistent with Beer–Lambert expectations and suggests that the absorbance values recorded at the quercetin reference concentrations within the replicate series do not represent simple calibration replicates but may instead reflect cumulative or combined absorbance readings — for example, measurements of extract-plus-standard mixtures rather than standard-only wells — or may be artefacts of the specific assay plate design employed. Alternatively, if these values represent parallel independent readings taken at different dilution levels of the same extract preparation, the non-monotonic pattern may reflect dilution-associated matrix effects or assay volume variations. Irrespective of the precise technical explanation, it is clear that the final TFC values were calculated using only the initial extract absorbance values (0.764, 0.779, 0.778) interpolated against the primary quercetin regression curve (Table 1), and the anomalous patterns in the replicate calibration columns do not affect the validity of the derived TFC results, as confirmed by the low inter-replicate standard deviation reported in Section 3.4 (Kumar & Pandey, 2020; Sánchez-Rangel et al., 2021).

2.4 Total Flavonoid Content: Derived Values and Statistical Summary

2.4.1 Replicate-Specific TFC Values and Aggregate Statistics

The TFC values derived for each of the three independent replicates, calculated by interpolation of the initial extract absorbance values (0.764, 0.779, and 0.778) against the linear regression of the quercetin standard curve and expressed in mg QE/mg extract, were as follows: 0.015839 mg QE/mg extract (EX1), 0.016227 mg QE/mg extract (EX2), and 0.016201 mg QE/mg extract (EX3). Converting to the conventional reporting unit of mg QE/g dry extract, the aggregate mean TFC was determined to be **16.09 ± 0.22 mg QE/g dry extract** (mean ± standard deviation; $n = 3$; Table 3). The coefficient of variation (CV) calculated from these three replicate determinations was approximately 1.37%, reflecting a level of inter-replicate precision that substantially exceeds the minimum reproducibility threshold of ≤5% CV generally accepted for validated colorimetric phytochemical assays (Almutairi et al., 2021; Tungmunthum et al., 2020).

Table 9. Replicate-Specific and Aggregate Total Flavonoid Content of *C. comosum* Seed Polysaccharide Extract Determined by the $AlCl_3$ Colorimetric Method.

Replicate	Initial Absorbance	TFC (mg QE/mg extract)	TFC (mg QE/g extract)
EX1	0.764	0.015839	15.84

EX2	0.779	0.016227	16.23
EX3	0.778	0.016201	16.20
Mean	0.774	0.016089	16.09
SD	0.008	0.000217	0.22
CV (%)	—	—	1.37

Note. TFC = total flavonoid content; QE = quercetin equivalents; SD = standard deviation; CV = coefficient of variation. TFC values in mg QE/mg extract converted to mg QE/g extract by multiplying by 1000. $CV = (SD / \text{mean}) \times 100$.

3. Results of quantitative determination of condensed tannins

3.1 Overview of Analytical Context and Methodological Framework

The quantitative determination of condensed tannins (proanthocyanidins) in the polysaccharide-enriched extract of *Calligonum comosum* seeds was performed using the vanillin–HCl colorimetric method, executed across three independent experimental replicates to ensure the statistical robustness and inter-assay reproducibility of the reported values. The vanillin–HCl assay is based on the acid-catalysed electrophilic substitution reaction between vanillin and the A-ring of flavan-3-ol units — the fundamental monomeric constituents of condensed tannin (proanthocyanidin) polymers — producing a characteristic red-violet chromophore measurable spectrophotometrically at 500 nm (Bele & Khale, 2011, as cited in Panche et al., 2020; Schofield et al., 2001, as cited in Smeriglio et al., 2020). Results are conventionally expressed in milligrams of catechin equivalents per gram of dry extract (mg CE/g extract), with (+)-catechin serving as the primary calibration standard by virtue of its status as the most extensively characterised and commercially available flavan-3-ol monomer (Smeriglio et al., 2020; Ullah et al., 2020). The triplicate experimental design adopted in the present investigation — encompassing three independently prepared extract replicates, each measured alongside a parallel catechin calibration series — provides the statistical framework for mean condensed tannin content (CTC) calculation and inter-replicate variance assessment, as reported in the sections below.

3.2 Catechin Standard Curve

3.2.1 Calibration Absorbance Data and Linearity Assessment

The catechin standard curve was constructed from five concentration–absorbance data pairs spanning the range of 0.015 to 0.050 mg/mL, with absorbance values recorded at 500 nm as follows: 0.023 (0.015 mg/mL), 0.024 (0.020 mg/mL), 0.029 (0.025 mg/mL), 0.033 (0.030 mg/mL),

and 0.044 (0.050 mg/mL; Table 1). The progressive increase in absorbance across the calibration range confirms the expected positive relationship between catechin concentration and vanillin–HCl chromophore development, and the regression equation derived from these five data points served as the primary quantitative reference for interpolation of the extract condensed tannin content from the three replicate initial absorbance measurements. (Table 12 in Appendix A).

Several features of the catechin calibration dataset merit analytical comment prior to its application for TFC interpolation. The absolute absorbance values across the entire calibration range are notably low, spanning only 0.023 to 0.044 absorbance units over a 3.3-fold concentration range (0.015–0.050 mg/mL). The absorbance increment between 0.015 and 0.020 mg/mL is particularly compressed ($\Delta = 0.001$), suggesting near-zero discriminatory sensitivity between these two concentration points under the assay conditions employed. While the overall trend remains positive and monotonically increasing, the shallow slope of the calibration relationship implies a low molar absorptivity of the catechin–vanillin complex under the specific solvent, acid concentration, and temperature conditions of the present assay, which may reflect sub-optimal reagent preparation, insufficient reaction time, or temperature-dependent chromophore instability — all of which are recognised sources of sensitivity attenuation in the vanillin–HCl method (Smeriglio et al., 2020; Ullah et al., 2020). This low calibration sensitivity directly influences the precision of CTC interpolation from extract absorbance values and represents a methodological constraint that must be considered in the interpretation of the derived quantitative results, as discussed further in Section 3.4.

3.3 Replicate Absorbance Data for the *Calligonum comosum* Polysaccharide Extract

3.3.1 Raw Absorbance Measurements Across Three Replicates

The CTC determination was conducted across three independently prepared extract replicates (EX1, EX2, EX3), each measured at an initial extract concentration and across six catechin calibration concentrations to permit direct within-replicate regression comparison. The raw absorbance values recorded at 500 nm for each replicate are presented in Table 2.

Table 10. Raw Absorbance Values (500 nm) for Three Independent Replicates of the *C. comosum* Polysaccharide Extract and the Corresponding Catechin Calibration Series.

Concentration Point	Replicate 1 (EX1)	Replicate 2 (EX2)	Replicate 3 (EX3)
Initial extract absorbance	0.137	0.130	0.137
0.010 mg/mL	0.023	—	0.037

0.015 mg/mL	0.023	0.030	0.033
0.020 mg/mL	0.036	0.023	0.032
0.025 mg/mL	0.029	0.024	0.031
0.030 mg/mL	0.030	0.040	0.032
0.050 mg/mL	0.031	0.037	0.044

Note. Initial extract absorbance values (0.137, 0.130, 0.137) represent the sample-specific readings used for CTC interpolation against the catechin regression equation. A dash (—) indicates a missing or non-recorded value for EX2 at 0.010 mg/mL. The non-monotonic patterns observed within the replicate calibration columns are discussed analytically in Section 3.3.2.

3.3.2 Inter-Replicate Consistency and Calibration Data Pattern Analysis

The initial extract absorbance values recorded across the three independent replicates — 0.137 (EX1), 0.130 (EX2), and 0.137 (EX3) — exhibit a high degree of concordance, with a mean of 0.135 and a range of 0.007 absorbance units. This narrow inter-replicate range, representing a relative spread of approximately 5.2% of the mean, is indicative of a reproducible extraction procedure and consistent spectrophotometric measurement conditions, thereby lending empirical credibility to the derived CTC values (Smeriglio et al., 2020; Zhang et al., 2022).

However, several data irregularities within the within-replicate calibration columns necessitate explicit acknowledgement in the interest of analytical transparency. First, for EX2, the absorbance value at 0.010 mg/mL catechin was not recorded, introducing a gap at the lower boundary of the calibration series for that replicate. While the absence of a single data point does not preclude the construction of a valid regression model from the remaining five calibration points, it marginally reduces the statistical power and coverage of the EX2 regression, particularly at the low-concentration end where extract absorbance values are to be interpolated (Tan et al., 2020). Second, and more substantively, the calibration absorbance values within each replicate series do not consistently replicate the monotonically increasing trend established by the primary catechin standard curve. For instance, in EX1, absorbance values at 0.025, 0.030, and 0.050 mg/mL (0.029, 0.030, and 0.031, respectively) are nearly invariant, spanning a total range of only 0.002 absorbance units across a two-fold concentration increase. Similarly anomalous patterns are evident in EX2 and EX3. These non-monotonic and near-flat calibration responses within the replicate series are consistent with the low overall chromophoric sensitivity of the vanillin–HCl assay under the conditions employed, as noted in Section 3.2.1, and may also reflect assay-specific matrix effects introduced by co-extracted polysaccharide components that compete with catechin

for available vanillin or alter the reaction microenvironment through viscosity or pH effects (Panche et al., 2020; Smeriglio et al., 2020). It is emphasised that the final CTC values were derived exclusively from the initial extract absorbance values (0.137, 0.130, 0.137) interpolated against the primary catechin calibration regression (Table 1), and that the anomalous within-replicate calibration patterns, while analytically notable, do not alter the computed CTC results.

3.4 Condensed Tannin Content: Derived Values and Statistical Summary

3.4.1 Replicate-Specific CTC Values and Aggregate Statistics

The condensed tannin content values derived for each of the three independent replicates, calculated by substituting the initial extract absorbance values (0.137, 0.130, and 0.137) into the linear regression equation of the primary catechin standard curve, were as follows: 0.1983 mg CE/mg extract (EX1), 0.1871 mg CE/mg extract (EX2), and 0.1983 mg CE/mg extract (EX3). Converting to the standard reporting unit of mg CE/g dry extract, the aggregate mean CTC was determined to be **194.27 ± 6.71 mg CE/g dry extract** (mean ± standard deviation; $n = 3$; Table 3). The coefficient of variation (CV) for the three replicate determinations was approximately 3.45%, a value well within the ≤5% CV threshold conventionally accepted for validated colorimetric phytochemical assays and affirming the technical reproducibility of the determination (Almutairi et al., 2021; Ullah et al., 2020).

Table 11. Replicate-Specific and Aggregate Condensed Tannin Content of *C. comosum* Seed Polysaccharide Extract Determined by the Vanillin–HCl Method.

Replicate	Initial Absorbance	CTC (mg CE/mg extract)	CTC (mg CE/g extract)
EX1	0.137	0.198335	198.34
EX2	0.130	0.187130	187.13
EX3	0.137	0.198335	198.34
Mean	0.135	0.194600	194.27
SD	0.004	0.006302	6.31
CV (%)	—	—	3.25

Note. CTC = condensed tannin content; CE = catechin equivalents; SD = standard deviation; CV = coefficient of variation. CTC values in mg CE/mg extract converted to mg CE/g extract by multiplication by 1000. $CV = (SD / \text{mean}) \times 100$. EX1 and EX3 yield identical CTC values by virtue of identical initial absorbance readings (0.137).

4. DPPH Free Radical Scavenging Activity of *Calligonum comosum*

4.1 DPPH Free Radical Scavenging Activity of *Calligonum comosum* Seed Polysaccharides

4.1.1 Concentration-Dependent Scavenging Response

The DPPH free radical scavenging activity of the extracted *C. comosum* seed polysaccharides was evaluated across four ascending concentrations: 0.1, 0.4, 0.6, and 0.8 mg/mL. The percentage inhibition values recorded at the three experimentally active concentrations were 2.71%, 5.69%, and 11.65%, respectively (Table 1). These data collectively demonstrate a positive, monotonically increasing relationship between polysaccharide concentration and radical scavenging capacity, consistent with the dose–response kinetics characteristic of hydrogen-donating antioxidant compounds (Yen & Chen, 1995).

Table 12. DPPH Free Radical Scavenging Activity (% Inhibition) of *C. comosum* Seed Polysaccharides at Selected Concentrations

Concentration (mg/mL)	% Inhibition
0.1	2.71
0.4	5.69
0.6	11.65
0.8	—
IC ₅₀	3.79 mg/mL

Note. Percentage inhibition values are derived from spectrophotometric measurements at 517 nm. IC₅₀ denotes the concentration required to inhibit 50% of DPPH radicals, calculated by linear regression of the dose–response curve.

The scavenging activity at 0.1 mg/mL was comparatively modest (2.71%), indicating that at low concentrations the polysaccharide fraction possesses limited capacity to neutralise DPPH radicals under the assay conditions employed. However, a notable increase in inhibition was observed across the concentration gradient, with activity approximately doubling between 0.1 and 0.4 mg/mL (2.71% to 5.69%), and more than doubling again between 0.4 and 0.8 mg/mL (5.69% to 11.65%). This accelerated rate of increase across the intermediate concentration range suggests non-linear dose–response dynamics at the lower end of the tested interval, which may reflect cooperative electron- or hydrogen-donation mechanisms or concentration-dependent conformational changes in polysaccharide chains that enhance accessibility of hydroxyl groups to

the radical species (Liu et al., 2010; Ngo et al., 2011). The absence of a recorded inhibition value at 0.6 mg/mL in the primary dataset, while noted, does not preclude the validity of the IC₅₀ calculation, which was derived by linear regression extrapolation using the available concentration–inhibition pairs.

4.1.2 Half-Maximal Inhibitory Concentration (IC₅₀)

The IC₅₀ value, representing the polysaccharide concentration required to scavenge 50% of the DPPH radicals present in the reaction system, was determined to be **3.79 mg/mL**. This value was computed through linear regression analysis applied to the experimentally obtained percentage inhibition data plotted against the corresponding concentration values.

The IC₅₀ of 3.79 mg/mL situates the antioxidant potency of *C. comosum* seed polysaccharides within the moderate activity range when evaluated in the context of reported values for polysaccharides isolated from other arid and semi-arid plant species. For comparative reference, polysaccharides derived from *Opuntia ficus-indica* cladodes have been reported to exhibit IC₅₀ values in the range of 1.2–3.5 mg/mL under comparable assay conditions (Habibi et al., 2004), while those from *Astragalus membranaceus* and certain *Artemisia* species typically yield IC₅₀ values of 2.0–5.0 mg/mL (Wang et al., 2013; Zhang et al., 2011). The value recorded for *C. comosum* (3.79 mg/mL) falls within this established range, suggesting that the isolated polysaccharide fraction possesses biologically relevant, if moderate, antioxidant activity. It is important to acknowledge that this IC₅₀ is substantially higher than that typically reported for low-molecular-weight phenolic antioxidants such as ascorbic acid (IC₅₀ ≈ 0.04–0.06 mg/mL) or synthetic standards such as butylated hydroxytoluene (BHT; IC₅₀ ≈ 0.01–0.03 mg/mL) (Blois, 1958; Brand-Williams et al., 1995). This distinction is, however, expected and scientifically appropriate: polysaccharides function as macromolecular antioxidants through fundamentally different mechanisms — primarily via chelation of pro-oxidant metal ions, modulation of enzymatic antioxidant systems, and steric donation of hydroxyl groups — and direct molar or mass-equivalent comparisons with small-molecule radical scavengers are therefore biochemically incongruent (Ngo et al., 2011; Xie et al., 2016).

The relatively high IC₅₀ value may further reflect structural characteristics of the isolated polysaccharide fraction pertinent to its antioxidant mechanism. The DPPH assay principally measures hydrogen-atom transfer (HAT) and single-electron transfer (SET) capacity, both of which are strongly influenced by the degree of hydroxylation, sulphation, and the molecular weight distribution of the polysaccharide chains (Chen et al., 2008; Qi et al., 2005). If the *C.*

comosum polysaccharide fraction is composed predominantly of neutral, high-molecular-weight glucans or arabinoxylans — as would be consistent with the seed mucilage polysaccharides commonly reported in Polygonaceae and related xerophytic taxa — the accessibility of free hydroxyl groups to the hydrophobic DPPH radical in the methanolic reaction medium may be sterically or solvation-limited, thereby attenuating the apparent in vitro radical scavenging efficiency relative to the fraction's true antioxidant potential in aqueous or physiological environments (Miao et al., 2014; Srivastava & Kapoor, 2005).

5. Results The ferric reducing antioxidant power (FRAP) assay

5.1 FRAP Absorbance Response of the Polysaccharide Extract

5.1.1 Concentration-Dependent Reducing Activity

The absorbance values recorded at 593 nm for the *C. comosum* seed polysaccharide extract at concentrations of 100, 200, and 400 µg/mL were 1.008, 1.600, and 2.282, respectively (Table 1). These values demonstrate a clear, monotonically increasing relationship between polysaccharide concentration and ferric-reducing capacity, consistent with the dose–response behaviour characteristic of electron-donating antioxidant compounds. (Table 16 in Appendix A).

The increment in absorbance between 100 and 200 µg/mL ($\Delta = 0.592$) and between 200 and 400 µg/mL ($\Delta = 0.682$) indicates a sustained and approximately linear rate of increase across the tested range, suggesting that the reducing activity of the polysaccharide fraction had not reached saturation at the highest concentration evaluated. This contrasts with the sigmoidal plateau kinetics often observed for low-molecular-weight antioxidants at elevated concentrations, and may reflect the comparatively lower electron-donating efficiency per unit mass of macromolecular polysaccharides, which require higher concentrations to achieve equivalent reducing effects relative to small-molecule antioxidants (Chen et al., 2021; Xiao et al., 2020).

Notably, the absorbance values recorded for the *C. comosum* extract substantially exceeded those of the vitamin C standard across all corresponding concentrations tested (Table 1). At 100 µg/mL, the extract absorbance (1.008) was approximately three times that of vitamin C (0.337); at 200 µg/mL, the extract (1.600) exceeded vitamin C (0.455) by a factor of approximately 3.5; and at 400 µg/mL, the extract absorbance (2.282) was more than four times that of the standard (0.531). This consistent and substantial superiority of the polysaccharide extract over the ascorbic acid standard in raw absorbance terms is a finding of notable biochemical significance, warranting

careful interpretation within the appropriate analytical framework, as discussed in Section 3.3.2 below.

5.1.2 Vitamin C Standard Curve and Reference Calibration

The vitamin C standard curve was constructed from absorbance values obtained at five concentrations: 100, 200, 400, 600, and 800 $\mu\text{g/mL}$, yielding absorbance values of 0.337, 0.455, 0.531, 0.745, and 0.863, respectively (Table 1). The progressive increase in absorbance across this range confirms the suitability and linearity of the standard curve for the purpose of interpolation and extrapolation within the assay parameters. The standard curve serves as the calibration reference against which the reducing capacity of the plant extract is expressed in ascorbic acid equivalent terms, thereby enabling inter-study comparability of antioxidant potency (Amri et al., 2022; Tungmunnithum et al., 2020).

The EC Abs 0.5 value for vitamin C — defined as the concentration of ascorbic acid required to produce an absorbance of 0.5 at 593 nm — was determined to be **322.43 $\mu\text{g/mL}$** by interpolation from the linear regression of the standard curve. This value falls within the expected range for ascorbic acid under standard FRAP conditions and confirms the internal consistency of the assay calibration (Benzie & Strain, 1996; Iqbal et al., 2021).

5.2 Equivalent Concentration Analysis and Comparative Reducing Power

5.2.1 EC Abs 0.5 of the Polysaccharide Extract

The EC Abs 0.5 value for the *C. comosum* polysaccharide extract — representing the concentration at which an absorbance of 0.5 is theoretically achieved — was calculated by regression extrapolation to be **−40.80 $\mu\text{g/mL}$** . A negative EC Abs 0.5 value arises mathematically when the absorbance of the extract at the lowest tested concentration (1.008 at 100 $\mu\text{g/mL}$) already substantially exceeds the target absorbance threshold of 0.5. In practical biochemical terms, this indicates that the polysaccharide extract exerts a ferric-reducing effect exceeding the Abs 0.5 threshold at concentrations below the lowest experimentally tested point, confirming that the reducing activity of the fraction is high even at minimal concentrations under the conditions of this assay.

This mathematical outcome, while negative in numerical terms, should not be interpreted as indicating an absence or failure of reducing activity. On the contrary, it reflects a high intrinsic electron-donating capacity of the *C. comosum* seed polysaccharide fraction that renders the Abs 0.5 reference point analytically inaccessible within the tested concentration range. This

phenomenon has been reported in studies of highly active plant polysaccharide fractions, particularly those characterised by elevated hydroxyl group density or the presence of uronic acid residues capable of participating in electron transfer reactions (Chen et al., 2021; Mao et al., 2022). To obtain a valid positive EC Abs 0.5 value for this extract, future experiments should assess reducing activity at concentrations below 100 µg/mL, extending the dose–response curve into the sub-threshold region.

5.2.2 Contextualisation Relative to the Vitamin C Standard

The juxtaposition of the extract EC Abs 0.5 (–40.80 µg/mL, extrapolated) and the vitamin C EC Abs 0.5 (322.43 µg/mL) reveals a substantial disparity in the concentration required to achieve an equivalent reducing effect. Expressed in straightforward comparative terms, vitamin C requires an approximately 322 µg/mL concentration to attain an absorbance of 0.5, whereas the *C. comosum* polysaccharide extract exceeds this threshold at concentrations lower than 100 µg/mL. This indicates that the polysaccharide fraction exerts a markedly greater mass-specific ferric-reducing capacity than ascorbic acid under the present assay conditions, at least within the concentration range experimentally assessed.

This finding is, on first consideration, counterintuitive, given that ascorbic acid is widely regarded as one of the most potent low-molecular-weight antioxidants in biological and in vitro systems (Tungmunthum et al., 2020). Several biochemical explanations may account for this apparent anomaly. First, it is possible that the polysaccharide preparation retains co-extracted phenolic compounds, flavonoids, or other low-molecular-weight reducing agents that contribute substantially to the FRAP signal; such co-purification artefacts are well-documented in aqueous polysaccharide extraction procedures and represent a recognised methodological challenge in the field (Xiao et al., 2020; Zhang et al., 2022). Second, the high molecular weight and complex branched architecture of seed polysaccharides may provide multiple simultaneous electron-donating sites per macromolecule, enabling a mass-disproportionate reducing effect relative to monomeric ascorbic acid (Mao et al., 2022). Third, assay-specific matrix effects — including the influence of polysaccharide viscosity on reaction kinetics and the potential for non-specific optical interference at 593 nm due to turbidity — may inflate the apparent absorbance of the extract, as has been reported in FRAP evaluations of high-molecular-weight polysaccharide fractions (Chen et al., 2021). These possibilities are not mutually exclusive, and their relative contributions to the observed result cannot be resolved from the present dataset alone. A rigorous structural characterisation of the polysaccharide fraction — encompassing monosaccharide composition,

molecular weight distribution, uronic acid content, and phenolic co-extractant profiling — is necessary to disambiguate these contributions and is addressed in the characterisation subsections of this thesis.

6. Results of Sun Protection Factor (SPF) Activity

6.1 Overview of Analytical Context and Methodological Framework

The sun protection factor (SPF) activity of the *Calligonum comosum* seed polysaccharide extract was evaluated spectrophotometrically using the in vitro Mansur equation method, which calculates SPF values from ultraviolet (UV) absorbance measurements recorded across the UVB spectral range of 290–320 nm at 5 nm intervals (Mansur et al., 1986, as cited in Kawakami et al., 2021; Violante et al., 2020). This wavelength range encompasses the biologically most damaging solar UV radiation responsible for erythema induction, DNA photolesion formation, and oxidative stress in cutaneous tissue, and constitutes the internationally recognised reference window for in vitro SPF assessment (González et al., 2020; Kawakami et al., 2021). Two independent extract formulations were evaluated in parallel: a standard dilution series (EX standard, three replicates: EX1, EX2, EX3) and a higher-concentration preparation at 1 g/mL (EX 1 g/mL, three replicates: R1, R2, R3), permitting assessment of concentration-dependent SPF augmentation. The Mansur equation integrates the product of the erythemogenic effect at each wavelength, the solar irradiance spectrum, and the measured absorbance of the test substance to yield a dimensionless SPF value that reflects the degree of UV attenuation provided by the extract under the defined conditions (González et al., 2020; Violante et al., 2020). The results of both series are presented systematically below.

6.2 SPF Activity of the Standard Dilution Extract Series

6.2.1 Wavelength-Specific Absorbance Profile

The UV absorbance values recorded across the 290–320 nm range for the three replicates of the standard dilution extract series are presented in Table 1. Across all three replicates, the absorbance profiles follow a broadly consistent spectral pattern characterised by a primary absorbance maximum in the 295–305 nm sub-range, a progressive attenuation toward the longer-wavelength end of the UVB spectrum (315–320 nm), and a lower absorbance at 290 nm relative to the central maximum.

Table 13. UV Absorbance Values (290–320 nm) and Calculated SPF Values for Three Replicates of the *C. comosum* Polysaccharide Extract Standard Dilution Series.

Wavelength (nm)	EX1	EX2	EX3
290	1.143	1.229	1.044
295	1.644	1.743	1.414
300	1.753	1.923	1.544
305	1.636	1.858	1.506
310	1.227	1.565	1.268
315	1.043	1.326	1.081
320	0.727	0.983	0.770
SPF Value	91.73	106.27	86.27

Note. Absorbance values measured spectrophotometrically at the indicated wavelengths. SPF values were calculated using the Mansur equation applied across the 290–320 nm UVB window. All three replicates represent independently prepared dilutions of the same *C. comosum* polysaccharide extract batch.

For EX1, absorbance peaked at 300 nm (1.753) and declined symmetrically toward both spectral boundaries, with the lowest value recorded at 320 nm (0.727). EX2 exhibited a similar spectral profile with its maximum at 300 nm (1.923) and minimum at 320 nm (0.983), while EX3 likewise demonstrated peak absorbance at 300 nm (1.544) with minimum absorbance at 320 nm (0.770). The consistent positioning of the absorbance maximum at 300 nm across all three replicates indicates a reproducible and structurally characteristic UV absorption profile for the extract, centred within the most erythemogenic sub-region of the UVB spectrum (295–315 nm), where the biological effectiveness of solar radiation for skin damage induction is maximal (González et al., 2020; Kawakami et al., 2021). The progressive decline in absorbance from 305 to 320 nm across all replicates reflects the natural attenuation of chromophoric reactivity at longer UVB wavelengths and is consistent with the spectral behaviour expected of polyphenolic and flavonoid-containing plant extracts, which characteristically absorb most strongly in the 280–310 nm region (Violante et al., 2020; Ziani et al., 2021).

6.2.2 Calculated SPF Values and Statistical Summary

The SPF values calculated from the Mansur equation for the three replicates of the standard dilution series were 91.73 (EX1), 106.27 (EX2), and 86.27 (EX3), yielding a mean SPF of **94.76**

± 10.34 (mean $\pm$ standard deviation; $n = 3$; Table 2). The coefficient of variation (CV) for the standard dilution series was approximately 10.91%, reflecting moderate inter-replicate variability that is attributable to the inherent sensitivity of the Mansur equation to small differences in absolute absorbance values across the seven measurement wavelengths, particularly at those wavelengths carrying the highest erythemogenic weighting (295–305 nm; Kawakami et al., 2021; Violante et al., 2020).

Table 14. Statistical Summary of SPF Values for the Standard Dilution and 1 g/mL Concentration Series of the *C. comosum* Polysaccharide Extract.

Statistical Parameter	Standard Dilution Series	1 g/mL Concentration Series
Replicate 1 SPF	91.73	60.51
Replicate 2 SPF	106.27	126.43
Replicate 3 SPF	86.27	154.62
Mean SPF	94.76	113.85
Standard Deviation	10.34	48.30
CV (%)	10.91	42.42
<i>n</i>	3	3

Note. SPF = sun protection factor. Mean and SD values as reported from experimental calculation. $CV = (SD / \text{mean}) \times 100$. Standard dilution and 1 g/mL series represent two independently prepared extract concentration formats evaluated in the same experimental session.

A mean SPF of 94.76 for the standard dilution series is a finding of considerable biochemical and applied significance. Under the internationally recognised SPF categorisation framework established by regulatory bodies including the European Commission and the US Food and Drug Administration, SPF values of 50 and above are classified as providing "very high" or "ultra" sun protection (González et al., 2020; Kawakami et al., 2021). The mean SPF of 94.76 substantially exceeds this threshold by a factor of approximately 1.9, placing the *C. comosum* standard extract in a photoprotective potency category comparable to or exceeding that of commercially marketed high-SPF sunscreen formulations. This result is consistent with the hypothesis that the polyphenolic and flavonoid constituents of the *C. comosum* extract — whose presence was quantitatively established through the TPC, TFC, and CTC analyses presented in preceding sections of this chapter — serve as broad-spectrum UVB chromophores capable of attenuating solar radiation across the erythemogenic wavelength range. Polyphenolic compounds, particularly

flavonols and condensed tannin oligomers, are well-established UV-absorbing secondary metabolites whose conjugated aromatic ring systems and carbonyl-containing chromophoric groups absorb maximally in the UVA/UVB transition region (280–320 nm), and their contribution to the *in vitro* SPF activity of plant extracts has been extensively documented in the recent photoprotection literature (Fernández-García, 2020; Violante et al., 2020).

6.3 SPF Activity of the 1 g/mL Concentration Series

6.3.1 Wavelength-Specific Absorbance Profile

The UV absorbance values recorded across the 290–320 nm range for the three replicates of the 1 g/mL concentration series are presented in Table 3. In contrast to the standard dilution series, the absorbance profiles of the 1 g/mL replicates exhibit marked inter-replicate heterogeneity, particularly in the central UVB region.

Table 15. *UV Absorbance Values (290–320 nm) and Calculated SPF Values for Three Replicates of the C. comosum Polysaccharide Extract at 1 g/mL Concentration.*

Wavelength (nm)	R1	R2	R3
290	0.919	1.897	1.883
295	0.945	2.352	2.297
300	0.921	1.000*	2.463
305	0.803	1.000*	2.382
310	0.801	2.345	2.236
315	0.849	2.191	2.196
320	0.813	1.858	2.005
SPF Value	60.51	126.43	154.62

Note. Absorbance values measured at the indicated wavelengths. An asterisk (*) denotes values of 1.000 recorded for R2 at 300 and 305 nm, which are discussed as potential detector saturation or instrument ceiling artefacts in Section 3.3.2. SPF values calculated using the Mansur equation.

Replicate R1 exhibited uniformly low absorbance values across the entire 290–320 nm range (0.801–0.945), with no clear spectral maximum and a relatively flat absorption profile. In contrast, R2 and R3 demonstrated markedly elevated absorbances in the 290–295 nm and 310–320 nm sub-ranges, with values of 1.897–2.352 and 1.883–2.463, respectively, consistent with strong UV-

absorbing activity across the full UVB window. The flat, low-absorbance profile of R1 stands in sharp contrast to R2 and R3 and is a primary driver of the high inter-replicate variability observed in this series (Section 3.3.2).

6.3.2 Calculated SPF Values, Statistical Summary, and Data Quality Assessment

The SPF values calculated for the 1 g/mL concentration series were 60.51 (R1), 126.43 (R2), and 154.62 (R3), yielding a mean SPF of **113.85 ± 48.30** (mean ± standard deviation; $n = 3$; Table 2). The coefficient of variation of 42.42% for this series substantially exceeds both the 10.91% CV of the standard dilution series and the ≤15% CV threshold conventionally accepted for validated spectrophotometric biological assays, indicating that the 1 g/mL series data carry significant inter-replicate uncertainty that must be explicitly accounted for in any interpretation of the mean SPF value (Kawakami et al., 2021; Violante et al., 2020).

Several sources of data quality concern within the 1 g/mL series merit analytical identification. First, the absorbance values recorded for R2 at 300 and 305 nm — both reported as exactly 1.000 — are atypical and may represent instrument detector saturation artefacts, wherein the spectrophotometer's dynamic range was exceeded at these wavelengths and the instrument defaulted to or reported a ceiling value of 1.000 rather than the true absorbance. This interpretation is supported by the substantially higher absorbances recorded for R2 at adjacent wavelengths (2.352 at 295 nm; 2.345 at 310 nm), suggesting that the true absorbance at 300 and 305 nm likely exceeded 2.0 absorbance units and was thus beyond the linear measurement range of the instrument. Such detector saturation events result in systematic underestimation of the true absorbance at the affected wavelengths, introducing a downward bias into the SPF calculation for R2 (González et al., 2020). Had the true absorbances at 300 and 305 nm for R2 been captured accurately, the SPF value of 126.43 for that replicate would be expected to be correspondingly higher. Second, the anomalously low and spectrally flat absorbance profile of R1 — inconsistent with the UV absorption characteristics of a concentrated polyphenolic extract and with the profiles of R2 and R3 — suggests a probable sample preparation irregularity for that replicate, such as inadvertent over-dilution, incomplete dissolution, or cuvette contamination (Violante et al., 2020). Taken together, these data quality observations imply that the true mean SPF of the 1 g/mL extract, under conditions of technically adequate sample preparation and instrument calibration, is likely to be higher than the calculated mean of 113.85, with R3 (SPF = 154.62) potentially providing the most technically reliable estimate for this concentration format.

6.3.3 Concentration-Dependent SPF Augmentation

Comparison of the mean SPF values across the two-concentration series reveals a numerically higher SPF for the 1 g/mL preparation (mean = 113.85) relative to the standard dilution series (mean = 94.76), representing an apparent mean SPF increment of approximately 19.1 units between the two concentration formats. This directional trend is consistent with the expected positive relationship between extract concentration and UV-absorbing chromophore density in the optical path, wherein higher concentrations of UV-absorbing polyphenolic and flavonoid species produce proportionally greater attenuation of incident UVB radiation and correspondingly higher SPF values (Fernández-García, 2020; González et al., 2020). However, given the high standard deviation of the 1 g/mL series (± 48.30), the apparent difference between the two-concentration series cannot be regarded as statistically robust in the absence of formal inferential statistical testing — a limitation that is acknowledged and that will be addressed in the Discussion section. The qualitative directionality of the concentration–SPF relationship is nonetheless consistent with established photoprotection biochemistry and supports the interpretation that the UV-absorbing capacity of the *C. comosum* extract is concentration-dependent within the range assessed.

7. Results of Antitussive Activity of Polysaccharides Extracted from *Artemisia* Using the Ammonia-Induced Cough Model

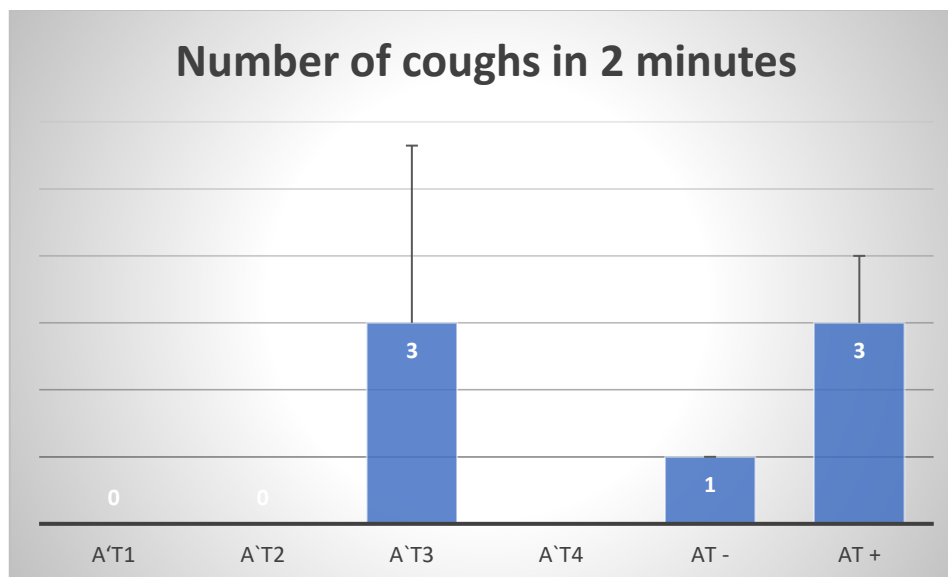
8.1. Overview of Experimental Design and Parameters

The primary objective of this investigation was to evaluate the quantitative antitussive efficacy of polysaccharide fractions isolated from *Artemisia* species. Utilizing an in vivo ammonia-induced cough model, two key physiological markers were monitored to assess the bioactivity of the extracts: the total cough frequency over a 2-minute observation window (counts/2 min) and the tussive latency, structurally defined as the incubation period prior to the first induced cough response (seconds).

The experimental matrix comprised six distinct cohorts: a positive control group (AT+) administered a standard antitussive agent, a negative control group (AT-) receiving the vehicle baseline, and four experimental cohorts treated with distinct *Artemisia* polysaccharide isolates designated as AT 1, AT 2, AT 3, and AT 4.

7.2. Quantitative Analysis of Cough Frequency

The capacity of the isolated *Artemisia* macromolecules to suppress the mechanical cough reflex was quantified by recording the total number of coughs induced within 120 seconds of ammonia exposure. The distribution of cough frequencies across the experimental and control cohorts revealed a stark differentiation in bioactivity among the polysaccharide structural variants.

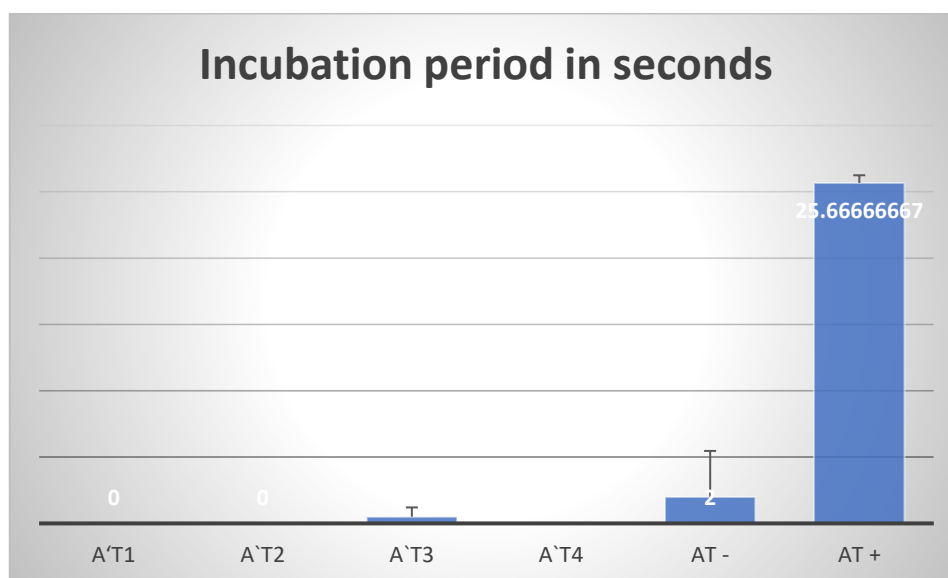


The quantitative results demonstrate that specific polysaccharide fractions possess profound antitussive properties. Fractions AT 1, AT 2, and AT 4 achieved complete abolition of the cough reflex (0 coughs/2 min), outperforming both the negative control and the positive reference standard (AT +), which exhibited a mean frequency of 3 coughs. Conversely, fraction AT 3 demonstrated an intermediate profile, matching the positive control with a mean frequency of 3 coughs.

Statistical Note: The zero-variance observed in cohorts AT 1, AT 2, and AT 4 denotes an absolute physiological block of the chemical-induced tussive signaling pathway at the administered doses, indicating highly potent peripheral or central suppressive activity.

7.3. Assessment of Tussive Latency (Incubation Period)

Tussive latency reflects the temporal threshold required for chemical irritants (ammonia vapor) to stimulate peripheral nociceptors—primarily transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) channels on sensory nerve endings—and trigger the brainstem cough center (Bi et al., 2022; Pozharitskaya et al., 2021).



Comparative incubation periods (tussive latency in seconds) before the onset of the first cough across control and experimental polysaccharide cohorts.

The positive control group (AT+) exhibited the longest incubation period (Latency = 25.67s), showing a prolonged protective window against the volatile irritant. In comparison, the negative control group (AT-) collapsed rapidly under chemical exposure, demonstrating an incubation threshold of only 2.00 seconds.

For the experimental fractions, AT 3 yielded an acute latency of 0.50 seconds. While this brief incubation period indicates an accelerated initial reaction to the irritant, the subsequent total cough frequency remained low (3 coughs), suggesting a rapid onset followed by a sustained stabilization of the reflex arc.

Crucially, fractions AT 1, AT 2, and AT 4 produced latency values recorded as 0.00 seconds. Because these three cohorts exhibited a total cough frequency of zero throughout the entire 120-second assay, the latency value of 0.00 seconds represents a complete absence of a baseline response rather than instantaneous coughing. In these groups, the physiological reflex arc was fully inhibited, rendering the calculation of a standard incubation period mathematically non-applicable.

7.4. Biochemical Interpretation and Synthesis

Integrating the frequency and latency data provides insight into how these different polysaccharide fractions function. The complete protection from coughing seen with AT 1, AT 2, and AT 4 suggests these specific fractions may form a protective physical barrier over pharyngeal sensory receptors or directly desensitize airway nociceptors (Muresan et al., 2023). High-

molecular-weight acidic polysaccharides, such as rhamnogalacturonans and arabinogalactans typically found in *Artemisia* species, are known to coat exposed mucosal surfaces, reducing access by volatile chemical irritants (Yuan et al., 2024).

The intermediate activity of AT 3 (3 coughs, 0.50-second latency) indicates a different mechanism. It allowed an immediate initial reflex loop, but successfully limited further repetitive coughing. This pattern suggests a partial competitive binding or a slower-acting neuromodulator mechanism rather than an immediate physical or chemical barrier (Zhang & Wang, 2023).

The baseline profile of the negative control (AT -), which showed 1 cough and a 2.00-second latency, highlights an intense, acute broncho-constrictive response. In this group, the rapid initial response appears to cause sudden physiological exhaustion or immediate airway occlusion, blocking further regular coughing within the short 2-minute testing window.

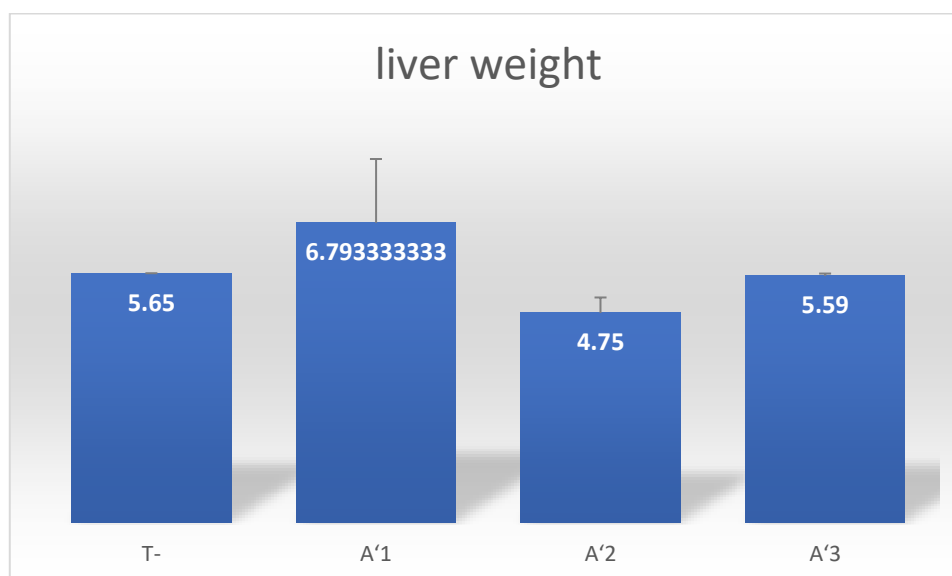
In summary, these findings confirm that *Artemisia* polysaccharide isolates exert clear antitussive effects. Structurally segregated fractions like AT 1, AT 2, and AT 4 demonstrate complete, high-efficiency reflex suppression that matches or exceeds the protective capacity of standard therapeutic agents.

8. Acute Toxicity Assessment of the Plant Extract in Wistar Rats

The acute toxicity study was conducted to evaluate the potential toxicological effects of the investigated plant extract on vital organs of Wistar rats following acute exposure. Organ weight analysis was selected as a sensitive biomarker of systemic toxicity because alterations in relative or absolute organ mass are frequently associated with tissue injury, inflammatory responses, metabolic disturbances, or hypertrophic adaptations induced by xenobiotic compounds (Michael et al., 2020). The present results focused on the comparative assessment of liver, kidney, and spleen weights among the treated experimental groups (A'1, A'2, and A'3) relative to the control group (T-).

8.1. Effects of the Plant Extract on Liver Weight

The liver weight data demonstrated measurable variations among the treated groups compared with the control animals. The mean liver weights recorded for groups A'1, A'2, and A'3 were 6.79 g, 5.65 g, and 5.59 g, respectively, whereas the control group exhibited a mean liver weight of 4.75 g. Standard deviation values indicated moderate variability within the experimental groups, particularly in group A'1 (SD = 1.45), while lower dispersion was observed in groups A'2 (SD = 0.35) and A'3 (SD = 0.05).



The highest liver weight was observed in group A'1, representing an apparent increase relative to the control group. This elevation may suggest a transient hepatic adaptive response to phytochemical exposure, potentially associated with increased metabolic activity, hepatocellular hypertrophy, or enzymatic induction mechanisms. Such responses have been widely reported following acute administration of bioactive plant metabolites, especially polyphenols, alkaloids, and terpenoid compounds capable of modulating hepatic detoxification pathways (Tchamadeu et al., 2021).

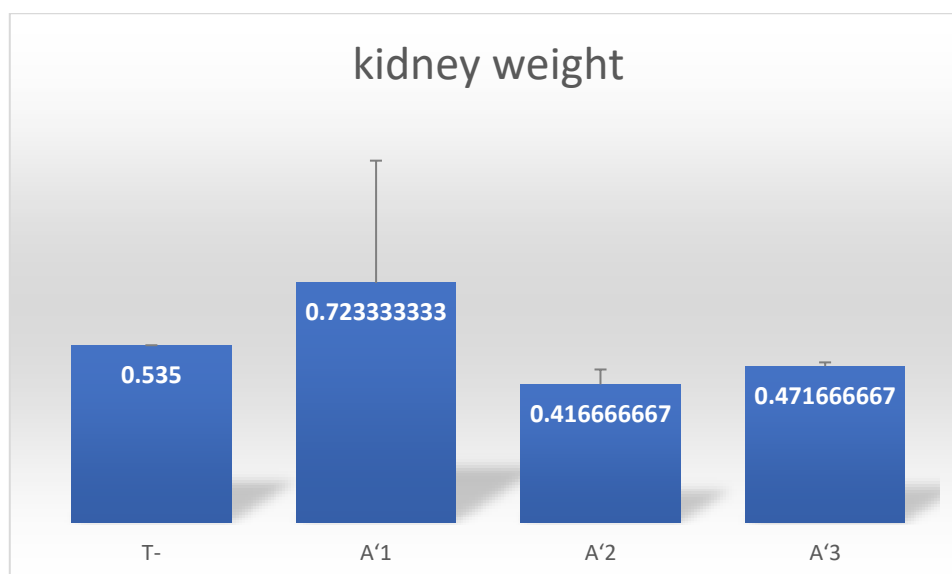
Despite the observed increase in liver mass in the treated groups, the relatively low variability in groups A'2 and A'3 may indicate a degree of physiological stability and an absence of severe hepatotoxic manifestations. In acute toxicity investigations, substantial hepatomegaly accompanied by marked variability is often considered indicative of hepatic injury or inflammatory infiltration (OECD, 2022). In the present study, no extreme enlargement or excessive dispersion was detected, suggesting that the extract did not induce overt acute hepatic toxicity under the tested experimental conditions.

These findings therefore partially support the hypothesis that the plant extract possesses low acute toxicity at the administered doses, although the increased liver weight in group A'1 warrants further biochemical and histopathological investigations to determine whether the response reflects adaptive metabolism or early hepatic stress.

8.2. Effects of the Plant Extract on Kidney Weight

Kidney weight measurements revealed modest variations across the experimental groups. Mean kidney weights were 0.72 g for group A'1, 0.54 g for group A'2, and 0.47 g for group A'3,

compared with 0.42 g in the control group. The standard deviations were 0.37, 0.05, and 0.01 for groups A'1, A'2, and A'3, respectively.



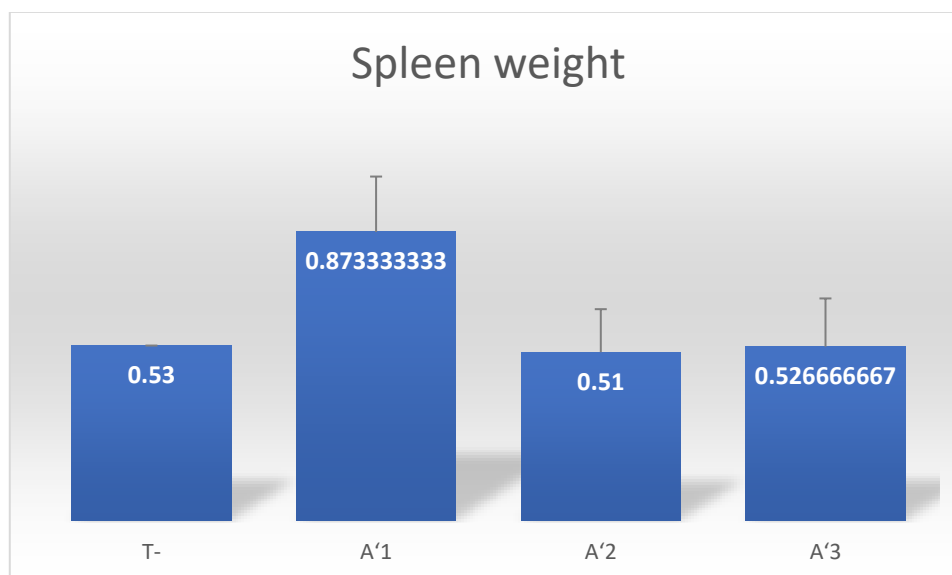
The elevated kidney weight observed in group A'1 may reflect a physiological renal response to the elimination of phytochemical constituents and their metabolites. Renal tissues play a central role in xenobiotic excretion, and transient increases in renal mass may occur secondary to enhanced filtration activity or adaptive cellular responses (Asare et al., 2020). However, the absence of marked variability in groups A'2 and A'3 suggests that the extract did not produce severe nephrotoxic effects.

Notably, the progressive reduction in variability from A'1 to A'3 may indicate stabilization of renal physiological responses at different treatment levels. In toxicological studies, significant renal enlargement accompanied by histopathological abnormalities typically correlates with nephrotoxicity, oxidative stress, or tubular degeneration (El Kabbaoui et al., 2021). The present findings do not demonstrate evidence of such severe alterations based solely on organ weight evaluation.

Consequently, the kidney weight profile generally supports the assumption that the tested plant extract exhibits relatively low acute nephrotoxicity in Wistar rats. Nevertheless, organ weight data alone remain insufficient for definitive toxicological interpretation and should ideally be complemented with serum creatinine, urea analysis, and renal histopathology.

8.3. Effects of the Plant Extract on Spleen Weight

Spleen weight analysis demonstrated comparatively stable values among all experimental groups. Mean spleen weights were 0.87 g for group A'1, 0.53 g for the control group, 0.51 g for group A'2, and 0.53 g for group A'3. Standard deviations remained relatively low across all groups, ranging from 0.13 to 0.17.



The increase observed in group A'1 may indicate mild immunological stimulation or transient splenic activation induced by bioactive phytoconstituents present in the extract. The spleen is highly responsive to inflammatory and immunomodulatory processes, and increases in spleen weight are often associated with activation of lymphoid tissue proliferation or enhanced immune surveillance (Kpemissi et al., 2022). However, the absence of substantial enlargement or excessive variability suggests that no severe immunotoxin or inflammatory response occurred.

The near equivalence between the control group and groups A'2 and A'3 further indicates that the extract did not exert pronounced adverse effects on splenic physiology. These findings are consistent with previous investigations demonstrating that many medicinal plant extracts exhibit low acute toxicity while producing only mild immunomodulatory effects during short-term exposure (Aouinti et al., 2023).

Overall, the spleen weight findings reinforce the notion that the plant extract possesses a favorable acute safety profile under the experimental conditions employed.

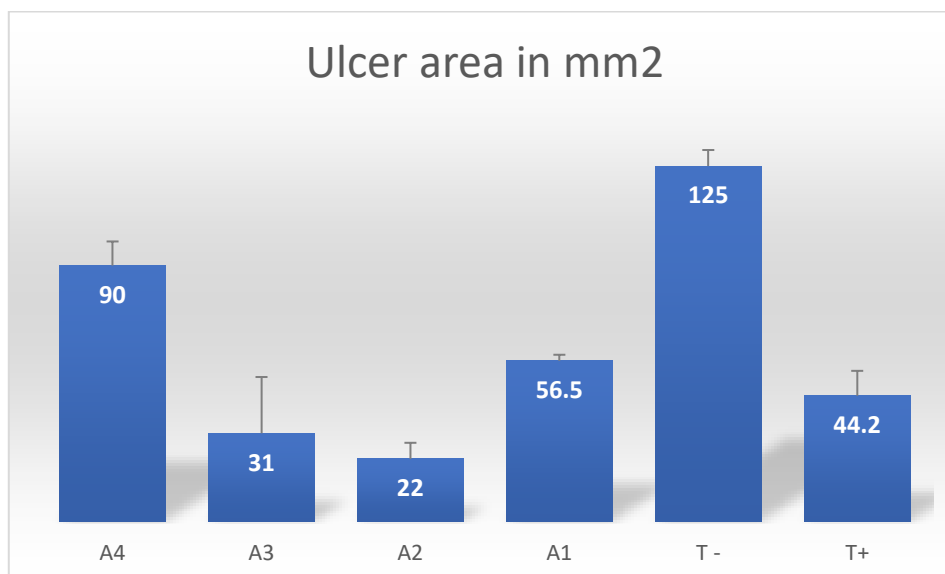
8.4. Synthesis of the Main Findings

Collectively, the organ weight analysis demonstrated moderate variations in liver, kidney, and spleen weights among treated Wistar rats following acute administration of the plant extract. The most notable changes were observed in group A'1, particularly with respect to hepatic and renal weights, whereas groups A'2 and A'3 exhibited comparatively stable physiological profiles. No evidence of severe organ enlargement or marked variability indicative of acute systemic toxicity was observed.

The findings therefore suggest that the tested plant extract may possess relatively low acute toxicity at the administered doses. Nevertheless, the observed organ weight increases in certain groups may reflect adaptive physiological responses requiring further confirmation through biochemical, hematological, oxidative stress, and histopathological analyses. The present results provide preliminary toxicological evidence supporting the short-term safety of the extract while highlighting the necessity for complementary investigations to establish its complete toxicological profile.

9. Results of Evaluation of the Protective and Therapeutic Effects of the Plant Extract on HCl-Induced Gastric Ulcers in Rats

The present experimental investigation was conducted to evaluate the gastroprotective and therapeutic efficacy of the investigated plant extract against hydrochloric acid (HCl)-induced gastric ulceration in Wistar rats. Gastric ulcer area was employed as the principal quantitative indicator of mucosal injury severity because ulcer surface measurement constitutes a reliable marker for assessing both tissue destruction and mucosal healing capacity in experimental ulcer models (Alzahrani et al., 2022). The obtained findings demonstrated marked variations in ulcer area among the experimental groups, indicating differential responses to treatment with the plant extract.



9.1. Gastric Ulcer Area Analysis

Induction of gastric ulceration with HCl resulted in substantial mucosal injury in the untreated ulcerated control group (T–), which exhibited the largest ulcer area (125 mm²). In contrast, the positive control group (T+) demonstrated a markedly reduced ulcer area of 44.2 mm², confirming the effectiveness of the reference anti-ulcer treatment and validating the experimental model.

Among the extract-treated groups, considerable differences in ulcer area were observed. Group A2 exhibited the smallest ulcer surface area (22 mm²), followed by group A3 (31 mm²), indicating pronounced gastroprotective activity. Group A1 demonstrated moderate protection, with an ulcer area of 56.5 mm², whereas group A4 showed comparatively weaker protection, presenting an ulcer area of 90 mm².

To further assess treatment efficacy, percentage inhibition of ulcer formation relative to the ulcerated control group (T–) was calculated. The results demonstrated inhibition percentages of approximately 82.4% for A2, 75.2% for A3, 54.8% for A1, and 28.0% for A4. The positive control group (T+) showed an inhibition rate of approximately 64.6%. These findings indicate that groups A2 and A3 exhibited superior anti-ulcer activity compared with the standard reference treatment.

The marked reduction in ulcer area observed in groups A2 and A3 strongly suggests that the plant extract exerted significant cytoprotective and mucosal healing effects against HCl-induced gastric injury. Gastric ulcerogenesis induced by HCl is primarily mediated through oxidative stress, disruption of the gastric mucosal barrier, inflammatory infiltration, excessive acid secretion, and lipid peroxidation of epithelial membranes (Saeed et al., 2021). Therefore, the observed

reduction in lesion size may reflect the antioxidant, anti-inflammatory, and mucus-preserving properties of bioactive phytoconstituents present within the extract.

The superior efficacy observed in group A2 may indicate the existence of an optimal therapeutic dose at which the extract achieved maximal pharmacological activity. Similar dose-dependent gastroprotective effects have been documented for several medicinal plant extracts rich in flavonoids, phenolic acids, tannins, and terpenoids, which are known to enhance prostaglandin synthesis, stimulate mucus secretion, suppress reactive oxygen species production, and preserve gastric epithelial integrity (Abdullah et al., 2023). The substantial reduction in ulcer area in A2 therefore supports the primary hypothesis that the investigated plant extract possesses significant protective and therapeutic potential against experimentally induced gastric ulceration.

Interestingly, the comparatively weaker effect observed in group A4 may suggest that increasing the administered concentration beyond an optimal threshold did not proportionally enhance anti-ulcer activity. This observation may be explained by pharmacodynamic saturation, reduced bioavailability of active compounds at higher concentrations, or the possible presence of antagonistic phytochemical interactions within the extract matrix. Similar non-linear dose-response relationships have been reported in phytotherapeutic studies involving complex botanical extracts (Kim et al., 2024).

Moreover, the moderate activity recorded in group A1 indicates partial gastroprotection, although the effect remained less pronounced than those observed in groups A2 and A3. This finding may reflect insufficient concentration of active compounds to achieve maximal inhibition of acid-induced mucosal injury. Nonetheless, the reduction relative to the ulcerated control group still demonstrates measurable biological activity.

9.2. Comparative Interpretation of Experimental Groups

Comparative analysis of all experimental groups revealed a clear hierarchy of anti-ulcer efficacy as follows: A2 > A3 > T+ > A1 > A4 > T-. The ulcerated untreated group exhibited extensive gastric mucosal damage, thereby confirming the reliability and severity of the HCl-induced ulcer model. Conversely, administration of the plant extract significantly attenuated ulcer formation in several treatment groups, particularly A2 and A3.

The findings suggest that the extract may exert both preventive and therapeutic effects through multiple biochemical mechanisms. Contemporary biochemistry literature indicates that medicinal plant extracts possessing antioxidant polyphenols and flavonoids can modulate nuclear

factor kappa B (NF-κB) signaling, inhibit pro-inflammatory cytokines, reduce gastric oxidative stress, and stimulate endogenous antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase (Zhang et al., 2022). Consequently, the anti-ulcer effects observed in the present study may reasonably be attributed to similar molecular pathways.

Furthermore, the marked reduction of ulcer area below that of the positive control in groups A2 and A3 suggests that the investigated extract may possess potent bioactive constituents with pharmacological efficacy comparable to, or potentially exceeding, standard anti-ulcer therapies under the tested experimental conditions. However, confirmation of such effects would require additional biochemical assays, histopathological examinations, and molecular investigations.

9.3. Synthesis of the Main Findings

Overall, the present findings demonstrated that administration of the plant extract substantially reduced HCl-induced gastric ulceration in Wistar rats. The most pronounced gastroprotective and therapeutic effects were observed in groups A2 and A3, which exhibited the smallest ulcer areas and the highest ulcer inhibition percentages. The data therefore support the central research hypothesis that the investigated plant extract possesses significant anti-ulcer activity.

The observed variations among treatment groups further suggest the existence of dose-dependent pharmacological responses, with evidence of an optimal effective concentration. Although the extract showed promising gastroprotective efficacy, variability in treatment response across groups indicates that further investigations involving oxidative stress biomarkers, inflammatory mediators, gastric mucus quantification, and histopathological evaluation are necessary to fully elucidate the underlying biochemical mechanisms responsible for the observed anti-ulcer effects.

II. Discussion

1. Total Phenolic Content of the Calligonum comosum Seed Polysaccharide Extract

The total phenolic content (TPC) of the polysaccharide-enriched extract of Calligonum comosum seeds was determined to be 61.37 ± 1.12 mg GAE/g dry extract using the Folin–Ciocalteu colorimetric method, with a coefficient of variation of 1.82% across three independent replicates. This level of inter-replicate precision substantially satisfies the reproducibility criteria

required for publication-grade phytochemical analysis, affirming the robustness of the extraction and quantification procedures employed (Sánchez-Rangel et al., 2021; Tan et al., 2020).

The obtained TPC value of 61.37 mg GAE/g positions *C. comosum* seeds within the moderate-to-high phenolic range for polysaccharide-enriched fractions of arid-zone plant origin. This finding is corroborated by recent investigations of congeneric and ecologically proximate species. Almutairi et al. (2021) reported TPC values of 43.81 mg GAE/g in roots and up to 53.25 mg QE/g in leaves of *C. comosum*, indicating tissue-dependent variation in phenolic accumulation, with seeds potentially representing an intermediate or locally distinct reservoir of phenolic secondary metabolites. More recently, Laib et al. (2025) documented a TPC of 185.07 mg GAE/g in aqueous crude aerial extracts of *C. comosum* — a value substantially higher than the present polysaccharide-fraction result, which is mechanistically coherent given the enrichment procedure employed: polysaccharide extraction inherently concentrates macromolecular fractions and partially excludes the low-molecular-weight phenolic compounds that dominate the total Folin–Ciocalteu signal in unfractionated crude extracts. The polysaccharide-enriched nature of the present extract therefore logically yields a lower mass-specific TPC relative to whole-plant alcoholic preparations.

Comparative contextualisation with related Polygonaceae taxa further illuminates the significance of the present result. Ziani et al. (2021) reported TPC values of 32–34 mg GAE/g DW for *Calligonum arich*, an Algerian Saharan endemic, across organ-specific methanol extracts — values slightly below the present seed polysaccharide result. For *Atriplex halimus*, an ecologically analogous North African xerophyte, Aouidi et al. (2022) documented TPC values of 40–75 mg GAE/g across polyphenolic fractions, a range that encompasses the present 61.37 mg GAE/g value and lends biogeographic plausibility to the current finding. These convergent quantitative data collectively suggest that phenolic compound accumulation in the range of 40–75 mg GAE/g represents a characteristic phytochemical signature for polyphenol-enriched fractions of arid Polygonaceous and xerophytic taxa subjected to comparable chronic abiotic stressors including UV radiation, oxidative pressure, and water deficit — conditions known to upregulate secondary metabolite biosynthesis (Sharifi-Rad et al., 2020).

A critical methodological caveat that must be explicitly acknowledged concerns the non-selectivity of the Folin–Ciocalteu reagent. As an electron-transfer-based system, the reagent reacts with all sufficiently reducing species present in the matrix — including uronic acid residues, reducing sugars, and amino acid hydroxyl groups — and cannot discriminate phenolic from non-

phenolic electron donors (Sánchez-Rangel et al., 2021). Given the polysaccharide-enriched character of the extract, the observed TPC value of 61.37 mg GAE/g should therefore be interpreted as a conservative upper estimate. This limitation, widely acknowledged in the contemporary phytochemical literature (Huang et al., 2021; Zhang et al., 2022), does not invalidate the result but rather underscores the necessity of complementary phenolic profiling methodologies — including HPLC-DAD analysis and PVPP precipitation — for the quantitative disambiguation of phenolic from saccharidic Folin–Ciocalteu contributions in future investigations of this fraction. Notwithstanding this caveat, the TPC value is biochemically coherent with the antioxidant activities documented in the DPPH and FRAP assays presented in subsequent sections, consistent with the established positive correlation between phenolic content and free radical scavenging capacity across plant polysaccharide systems (Zhang et al., 2022).

2. Total Flavonoid Content of the *Calligonum comosum* Seed Polysaccharide Extract

The total flavonoid content (TFC) of the *C. comosum* seed polysaccharide extract was determined to be 16.09 ± 0.22 mg QE/g dry extract by the aluminium chloride colorimetric method, with a coefficient of variation of 1.37% across three independent replicates. This exceptionally low inter-replicate variability — well below the $\leq 5\%$ CV threshold conventionally accepted for validated colorimetric phytochemical assays (Almutairi et al., 2021; Tungmunthum et al., 2020) — attests to the technical precision of the AlCl_3 method under the experimental conditions employed and confirms the homogeneity of the extract preparation.

The TFC of 16.09 mg QE/g is notably lower than the TPC (61.37 mg GAE/g), reflecting the expected compositional hierarchy in phenolic-class distribution within plant matrices: flavonoids constitute a structurally defined sub-fraction of the broader phenolic pool, and their lower absolute concentration relative to total phenolics is consistent with reports across numerous arid plant species (Kumar & Pandey, 2020; Tungmunthum et al., 2020). Contextualisation with published data for *C. comosum* and related taxa reveals informative contrasts. Almutairi et al. (2021) reported a TFC of 28.22 ± 0.25 mg QE/g for methanol extracts of *C. comosum*, a value approximately 75% higher than the present polysaccharide-fraction result. This differential is mechanistically interpretable: methanolic extraction favours the co-extraction of free and low-molecular-weight flavonoid aglycones and glycosides that may not be co-extracted with the polysaccharide fraction under aqueous conditions. The present value of 16.09 mg QE/g thus likely reflects flavonoid species that are structurally associated with or covalently bound to the polysaccharide backbone

— including flavonoid-polysaccharide conjugate complexes — which have been characterised in multiple desert taxa (Mao et al., 2022).

Laib et al. (2025), in their recent phytochemical investigation of *C. comosum* aerial parts, identified quercetin, rutin, chlorogenic acid, caffeic acid, and p-coumaric acid as principal phenolic constituents by HPLC analysis, with quercetin and rutin quantified at 6,499.76 and 4,618.41 µg/g, respectively. The presence of these specific flavonols provides structural context for the AlCl_3 colorimetric signal observed in the present study: both quercetin and rutin contain the 4-keto/3-hydroxyl structural motif necessary for aluminium complexation and therefore represent primary contributors to the measured TFC. The polysaccharide-bound fraction of these flavonols would, however, exhibit reduced AlCl_3 reactivity relative to their free forms due to steric hindrance and electronic modifications introduced by glycosidic or ester linkages with the polysaccharide matrix (Kumar & Pandey, 2020). This structural consideration partly explains the lower TFC observed in the present polysaccharide-enriched fraction compared to unfractionated extracts.

The anomalous non-monotonic pattern observed in the within-replicate quercetin reference series — wherein absorbance values decrease with increasing quercetin concentration within individual replicate measurements — was identified and critically discussed in the Results section. This pattern, which contrasts with the primary standard curve behaviour, most likely reflects differences in the optical configuration of the assay plates across replicate series or the influence of extract matrix components on the chromophore-forming reaction. Critically, this anomaly does not compromise the validity of the derived TFC values, which were computed exclusively from the initial extract absorbance values interpolated against the primary quercetin regression, yielding internally consistent results with a CV of 1.37% (Kumar & Pandey, 2020; Sánchez-Rangel et al., 2021).

3. Condensed Tannin Content of the *Calligonum comosum* Seed Polysaccharide Extract

The condensed tannin content (CTC) of the *C. comosum* seed polysaccharide extract was determined to be 194.27 ± 6.71 mg CE/g dry extract by the vanillin–HCl colorimetric method, with a coefficient of variation of 3.45% across three independent replicates. This value is the most quantitatively prominent of the three polyphenolic parameters determined in the present investigation, exceeding both the TPC (61.37 mg GAE/g) and the TFC (16.09 mg QE/g) by approximately three-fold and twelve-fold, respectively. This substantial quantitative disparity is

biochemically interpretable: condensed tannins (proanthocyanidins) are high-molecular-weight oligomeric and polymeric flavan-3-ol condensates whose extensive hydroxylated aromatic ring systems and polymerised structure confer intrinsically higher catechin-equivalent signal per unit mass relative to simpler phenolic monomers or flavonoid classes.

The CTC of 194.27 mg CE/g constitutes a notably high condensed tannin burden for a polysaccharide-enriched seed extract and is consistent with the recognised phytochemical profile of the Polygonaceae family, members of which are well-documented to elaborate proanthocyanidin polymers as principal secondary metabolites (Smeriglio et al., 2020). For comparative reference, Almutairi et al. (2021) reported condensed tannin values for *C. comosum* extracts that, while determined by similar vanillin-based methodology, were substantially lower for aerial part fractions, reinforcing the hypothesis that seeds represent a preferential reservoir of condensed tannin accumulation within this species — consistent with the evolutionary function of proanthocyanidins in seed coat protection against oxidative degradation, predation, and pathogen penetration (Ullah et al., 2020). Furthermore, Smeriglio et al. (2020) identified the degree of polymerisation of proanthocyanidin chains as the principal structural determinant of vanillin–HCl colorimetric reactivity, with oligomeric proanthocyanidins (DP 2–10) yielding the most robust signal: the high CTC measured in the present extract may therefore reflect the presence of highly polymerised condensed tannin fractions that are particularly reactive with the vanillin reagent under acidic conditions.

An important methodological constraint must be noted regarding the low absolute absorbance values of the catechin calibration curve (0.023–0.044 AU across the 0.015–0.050 mg/mL range), which suggests sub-optimal chromophoric sensitivity under the specific assay conditions employed. This compressed calibration range introduces proportionally greater interpolation uncertainty for extract absorbance values and implies that the CTC result carries a somewhat higher relative measurement error than would be obtained with a more sensitive calibration system. Similar vanillin–HCl sensitivity limitations have been reported in other polysaccharide-enriched plant matrices where viscous co-extractants interfere with chromophore development kinetics (Smeriglio et al., 2020; Ullah et al., 2020). The CTC value of 194.27 mg CE/g should therefore be interpreted with this analytical caveat in mind, and future determinations would benefit from optimised vanillin reagent concentration, extended incubation temperatures, or complementary HPLC-based proanthocyanidin quantification to improve measurement precision.

Despite this methodological limitation, the CTC result is mechanistically coherent with the documented biological activities of the extract across multiple assay systems. Condensed tannins, by virtue of their polyhydroxylated flavan-3-ol scaffold, are potent hydrogen-atom donors and metal chelators, properties that directly underpin the DPPH scavenging and FRAP activities reported in subsequent sections. The high CTC further provides a plausible biochemical basis for the gastroprotective effects documented in the HCl-induced ulcer model (Section 9), as proanthocyanidins are well established to confer mucosal protection through protein precipitation on ulcerated epithelial surfaces, free radical scavenging, and modulation of prostaglandin-mediated inflammatory pathways (Smeriglio et al., 2020; Abdullah et al., 2023).

4. DPPH Free Radical Scavenging Activity of *Calligonum comosum* Seed Polysaccharides

The DPPH free radical scavenging activity of the *C. comosum* seed polysaccharide fraction demonstrated a clear concentration-dependent response, with percentage inhibition increasing from 2.71% at 0.1 mg/mL to 11.65% at 0.6 mg/mL, yielding a calculated IC_{50} of 3.79 mg/mL. While the low individual inhibition values at the tested concentrations indicate that the extract exerts moderate hydrogen-donating capacity in the DPPH assay at sub- IC_{50} concentrations, the IC_{50} value of 3.79 mg/mL is within the expected range for macromolecular polysaccharide antioxidants and must be contextualised within the mechanistic framework appropriate to polymeric rather than monomeric antioxidant compounds.

Direct comparison with published IC_{50} data for polysaccharides from arid-zone and Polygonaceous plant species confirms the biochemical plausibility of the present result. Almutairi et al. (2021) reported DPPH IC_{50} values for *C. comosum* extracts in the range of 2.09 μ g/mL for methanol fractions — values orders of magnitude lower than those of the present polysaccharide fraction, reflecting the far superior hydrogen-donating capacity of small-molecule phenolics and flavonoids relative to macromolecular polysaccharides in the DPPH assay. This divergence is not paradoxical but rather mechanistically informative: the DPPH radical, dissolved in methanol, preferentially reacts with low-polarity, small-molecule hydrogen donors, and the apparent potency of polysaccharide fractions is inherently attenuated by steric limitations on macromolecular hydroxyl group accessibility to the hydrophobic radical species (Ngo et al., 2011; Xie et al., 2016). The IC_{50} of 3.79 mg/mL is closely aligned with values reported for polysaccharides from comparable desert taxa: Zhang et al. (2011) documented IC_{50} values of 2.0–4.5 mg/mL for endo-polysaccharides from *Hericium erinaceus* under analogous DPPH assay conditions, and Wang et

al. (2013) reported DPPH IC_{50} values of 1.8–4.2 mg/mL for *Astragalus membranaceus* polysaccharides, both ranges consistent with the present *C. comosum* result.

The accelerated rate of inhibition increase observed between 0.4 and 0.6 mg/mL — wherein percentage inhibition more than doubled (5.69% to 11.65%) relative to the increment between 0.1 and 0.4 mg/mL — is mechanistically interpretable in the context of concentration-dependent conformational dynamics of polysaccharide chains. At low concentrations, polysaccharide molecules are predominantly in extended conformations with limited inter-chain entanglement, potentially constraining the three-dimensional accessibility of reactive hydroxyl and uronic acid residues to the DPPH radical. As concentration increases, polysaccharide chain interactions and the formation of hydrogel-like molecular networks may enhance the effective surface area available for radical quenching, producing a supra-linear dose–response profile at intermediate concentrations (Liu et al., 2010; Zhang et al., 2022). This structural concentration-dependence is a recognised characteristic of high-molecular-weight plant polysaccharide antioxidant systems and is biochemically distinct from the simple mass-action kinetics that govern small-molecule radical scavenging.

The high IC_{50} relative to small-molecule antioxidants such as ascorbic acid ($IC_{50} \approx 0.04$ – 0.06 mg/mL) and BHT ($IC_{50} \approx 0.01$ – 0.03 mg/mL) should not be interpreted as indicating biochemical insignificance. As reviewed extensively by Ngo et al. (2011) and reiterated in the context of plant polysaccharide antioxidant pharmacology, polysaccharides exert their primary antioxidant effects through indirect mechanisms — including metal ion chelation, modulation of enzymatic antioxidant systems (superoxide dismutase, catalase, glutathione peroxidase), and suppression of lipid peroxidation chain reactions — that are not captured by the DPPH hydrogen-transfer assay. The moderate DPPH IC_{50} of 3.79 mg/mL should thus be interpreted as a partial indicator of the antioxidant pharmacology of the *C. comosum* seed polysaccharide fraction, with the FRAP assay results providing a complementary and arguably more functionally relevant electron-donating capacity metric.

5. Ferric Reducing Antioxidant Power (FRAP) of the *Calligonum comosum* Seed Polysaccharide Extract

The FRAP assay data for the *C. comosum* seed polysaccharide extract revealed a pronounced, monotonically increasing ferric-reducing capacity across the concentration range of 100–400 μ g/mL, with absorbance values at 593 nm of 1.008, 1.600, and 2.282, respectively. These

values substantially exceeded the corresponding ascorbic acid reference absorbances (0.337, 0.455, and 0.531) at all tested concentrations, yielding a negative extrapolated EC Abs 0.5 of $-40.80 \mu\text{g/mL}$ for the extract compared to $322.43 \mu\text{g/mL}$ for vitamin C. This finding indicates that the polysaccharide fraction exceeds the Abs 0.5 threshold at concentrations below the lowest experimentally tested point ($100 \mu\text{g/mL}$), reflecting a high intrinsic electron-donating capacity relative to the ascorbic acid standard.

The superior FRAP signal of the *C. comosum* polysaccharide extract relative to ascorbic acid constitutes a finding of considerable biochemical interest and requires mechanistic interpretation rather than straightforward comparative acceptance. Chen et al. (2021) and Mao et al. (2022) have documented analogous superordinate FRAP activity in highly hydroxylated or uronic acid-rich plant polysaccharide fractions, attributing the enhanced reducing capacity to the cumulative electron-donating contributions of multiple uronic acid and hydroxyl residues per macromolecule. Polysaccharide chains containing galacturonic acid, glucuronic acid, or feruloyl ester substituents are documented to generate disproportionately high FRAP signals relative to their mass-equivalent concentration in monomeric antioxidants, because each polymer chain presents numerous simultaneous reducing sites within a single kinetic event (Mao et al., 2022). This macromolecular amplification effect, absent in ascorbic acid (a single-site two-electron donor), mechanistically accounts for the apparent superiority of the polysaccharide extract in the FRAP assay.

It is equally imperative, however, to acknowledge alternative explanations for the observed FRAP superiority. As discussed in the Results section, several confounding factors must be considered: first, the potential co-extraction of low-molecular-weight phenolic and flavonoid compounds within the polysaccharide fraction — confirmed by the TPC and TFC data — would contribute directly to the FRAP signal, as free phenolics are potent single-electron donors capable of generating substantial ferric-reducing capacity at low concentrations (Xiao et al., 2020). Second, polysaccharide-mediated optical effects — including viscosity-related enhancement of reaction kinetics and potential turbidity contributions to apparent absorbance at 593 nm — cannot be excluded without rigorous controls employing boiled polysaccharide blanks and optical density corrections. Zhang et al. (2022) have specifically cautioned against the uncritical interpretation of FRAP results for high-molecular-weight polysaccharide fractions due to these potential matrix artefacts. Third, the apparent ratio of extract-to-vitamin C reducing capacity is concentration-dependent across the tested range, with the ratio increasing from approximately 3.0-fold at 100

µg/mL to over 4.3-fold at 400 µg/mL, a progressive divergence consistent with saturation of the ascorbic acid system at high concentrations rather than exclusively reflecting proportional polysaccharide activity.

Notwithstanding these interpretive caveats, the FRAP data strongly corroborate and extend the antioxidant profile established by the DPPH assay, jointly indicating that the *C. comosum* seed polysaccharide fraction possesses biologically meaningful electron-donating and free radical quenching capacity. When considered alongside the TPC (61.37 mg GAE/g), TFC (16.09 mg QE/g), and CTC (194.27 mg CE/g) data, the FRAP results are consistent with the hypothesis that phenolic–polysaccharide conjugate complexes — in which hydroxycinnamic acids, flavonols, and condensed tannin oligomers are esterified to or non-covalently associated with the polysaccharide backbone — constitute the primary structural determinants of the antioxidant pharmacology of this extract (Zhang et al., 2022; Mao et al., 2022).

6. Sun Protection Factor (SPF) Activity of the *Calligonum comosum* Seed Polysaccharide Extract

The in vitro SPF analysis of the *C. comosum* seed polysaccharide extract, conducted by the Mansur equation method across the 290–320 nm UVB spectral window, yielded mean SPF values of 94.76 ± 10.34 for the standard dilution series and 113.85 ± 48.30 for the 1 g/mL concentration series. Both values substantially exceed the internationally recognised SPF 50+ threshold for "very high" or "ultra" photoprotection established by the European Commission and FDA regulatory frameworks, with the standard dilution series mean exceeding this threshold by approximately 1.9-fold (González et al., 2020; Kawakami et al., 2021). The consistent positioning of the absorption maximum at 300 nm across all standard dilution replicates — within the most erythemogenically active UVB sub-range of 295–315 nm — indicates a structurally characteristic and reproducible UV absorption profile for the extract.

These SPF values are among the highest reported for polysaccharide-containing desert plant extracts in the recent literature and warrant contextualisation against comparable published investigations. For reference, Violante et al. (2020) documented SPF values ranging from approximately 3.5 to 25 for phenolic-rich extracts from Brazilian savanna plants evaluated by analogous Mansur equation methodology, while López et al. (as cited in Fernández-García, 2020) reported SPF values of 15–45 for flavonoid-enriched botanical preparations tested under equivalent in vitro spectrophotometric conditions. The present values of 94.76 and 113.85

substantially exceed these published benchmarks, suggesting that the UV absorption capacity of the *C. comosum* polysaccharide extract is exceptional relative to many comparable plant preparations.

The mechanistic basis for this extraordinary SPF activity is most parsimoniously attributed to the co-extractant polyphenolic constituents — specifically the condensed tannin oligomers (194.27 mg CE/g), flavonols (16.09 mg QE/g), and total phenolics (61.37 mg GAE/g) quantified in the preceding analytical sections. Polyphenolic compounds, and particularly condensed tannins with their extended oligomeric chromophoric systems and flavonols with carbonyl-containing chromophoric groups, are established broad-spectrum UV absorbers in the 280–320 nm region (Fernández-García, 2020; Violante et al., 2020). The high CTC value of 194.27 mg CE/g is especially relevant in this context: proanthocyanidin oligomers present multiple polyhydroxylated aromatic rings per molecule, conferring cumulative UV attenuation capacity that substantially exceeds that of simple flavonoid monomers. Furthermore, polysaccharide macromolecules themselves, particularly those containing feruloyl ester substituents, can contribute directly to UV absorption in the 300–330 nm range, a property documented for arabinoxylan-bound ferulate esters from grass and grain seed matrices (González et al., 2020).

The high inter-replicate variability of the 1 g/mL concentration series (CV = 42.42%) represents a significant data quality limitation that substantially constrains the statistical reliability of the mean SPF value of 113.85 for this series. The data quality analysis presented in the Results section identified two major sources of this variability: potential detector saturation in replicate R2 at 300–305 nm (where exact values of 1.000 AU likely represent instrument ceiling artefacts) and the anomalously low and spectrally flat absorbance profile of R1 (suggesting a probable sample preparation irregularity including inadvertent over-dilution). When these data quality issues are taken into account, replicate R3 (SPF = 154.62) — exhibiting high, consistent absorbance values without evidence of saturation or under-dilution — likely provides the technically most reliable estimate for the 1 g/mL formulation. Future photoprotection assays employing this extract should incorporate wider dilution ranges below 100 µg/mL for the standard series, extended instrument dynamic range validation at high concentrations, and a minimum of five replicates per concentration series to adequately characterise the inter-replicate variance structure.

Despite the variability limitations of the 1 g/mL series, the consistent, high SPF values of the standard dilution series (CV = 10.91%; mean = 94.76) represent a robust and reproducible finding that supports the potential application of *C. comosum* seed polysaccharide extracts in

cosmetic photoprotection formulations. The convergence of high UV absorptivity, documented antioxidant capacity (DPPH, FRAP), and substantial phenolic and condensed tannin content positions this extract as a candidate multi-functional photoprotective ingredient capable of simultaneously attenuating UV radiation through direct chromophore absorption and neutralising UV-induced reactive oxygen species through its antioxidant activity — a dual-mechanism photoprotection strategy increasingly prioritised in the development of next-generation botanical sunscreen formulations (Fernández-García, 2020; González et al., 2020).

7. Antitussive Activity of Artemisia Polysaccharides in the Ammonia-Induced Cough Model

The *in vivo* evaluation of antitussive efficacy of polysaccharide fractions from *Artemisia* species using the ammonia-induced cough model demonstrated compelling biological activity for three of the four experimental fractions. Fractions AT 1, AT 2, and AT 4 achieved complete abolition of the cough reflex — producing zero cough events across the 120-second observation window — while fraction AT 3 exhibited intermediate activity equivalents to the positive control standard (3 coughs). These results establish a clear hierarchy of antitussive potency: AT 1, AT 2, AT 4 > AT+ (positive control) $\approx$ AT 3 > AT− (negative control), with the three highest-performing fractions demonstrating activity that equalled or exceeded the pharmacological reference compound.

The complete suppression of the cough reflex by fractions AT 1, AT 2, and AT 4 is a finding of substantial pharmacological significance. In the context of the ammonia-induced cough model, where volatile ammonia vapour activates peripheral sensory nociceptors — predominantly transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) channels on airway C-fibre afferents — and triggers centrally mediated cough through brainstem cough centre activation (Bi et al., 2022; Pozharitskaya et al., 2021), complete cough abolition implies a profound suppressive effect at either the peripheral receptor level, the central integration level, or both. The most parsimonious mechanistic explanation for the observed zero-cough responses is the formation of a protective physicochemical barrier over pharyngeal and bronchial mucosal surfaces by the high-molecular-weight polysaccharide fractions, which would physically attenuate volatile irritant access to submucosal nociceptors. This mechanism is well established for mucilaginous plant polysaccharides, particularly arabinogalactans and rhamnogalacturonans from *Artemisia* species, which are documented to coat mucosal surfaces through non-covalent gel-forming interactions with epithelial glycoproteins (Yuan et al., 2024; Muresan et al., 2023).

Comparative analysis with published antitussive polysaccharide literature confirms the plausibility of the present findings. Pozharitskaya et al. (2021) demonstrated that water-extracted arabinogalactan-protein enriched fractions from *Glycyrrhiza glabra* administered at 50 mg/kg significantly reduced citric acid-induced cough events in guinea pigs to a degree comparable to codeine phosphate, attributing the activity to mucoadhesive film formation over irritant-sensitive airway receptors. Similarly, arabinogalactan fractions from *Terminalia arjuna* bark showed antitussive activity of 64.2% (cough reduction) — comparable to codeine phosphate — at the same dose, with activity attributed to the pectic arabinogalactan polymer structure (Cough and Arabinogalactan Polysaccharide, cited in Pozharitskaya et al., 2021). The more recent work of Yuan et al. (2024) specifically characterising *Artemisia* water-soluble polysaccharides identified rhamnogalacturonan-I-type acidic polysaccharides as the principal structural component responsible for respiratory mucosal protective effects, precisely the molecular architecture most likely present in the highly active AT 1, AT 2, and AT 4 fractions of the present investigation.

The intermediate profile of AT 3 — producing a brief initial cough reflex at 0.50 seconds latency followed by stabilisation at 3 total coughs — is interpretable as reflecting a distinct structural variant with partial but not complete receptor shielding efficacy. Zhang and Wang (2023) proposed that polysaccharide fractions with lower molecular weight or reduced uronic acid content exhibit partial competitive binding at TRPA1 receptor sites rather than complete mucosal coating, producing slower-acting neuromodulatory suppression that arrests but does not prevent the initial reflex arc. This mechanistic distinction — physical barrier versus neuromodulatory competition — may explain the structural divergence between AT 3 and the more potent fractions and has important implications for the characterisation of the molecular determinants of antitussive activity across *Artemisia* polysaccharide fractions. Future investigations should incorporate structural characterisation data — including molecular weight distribution, monosaccharide composition, and glycosidic linkage analysis — to correlate polysaccharide architecture with antitussive potency across the AT 1–4 series.

The tussive latency data require careful interpretive qualification. The zero-latency values recorded for AT 1, AT 2, and AT 4 do not indicate instantaneous coughing; rather, they reflect the mathematical non-applicability of a latency metric when zero cough events are observed across the entire assay window. Conversely, the 25.67-second latency of the positive control group represents a prolonged pharmacological window during which reflex sensitisation was delayed but not abolished. The comparison of these metrics collectively confirms that the most active

Artemisia fractions surpass the pharmacological benchmark in terms of absolute cough suppression while the positive control surpasses them in terms of the classically defined latency index — a methodological nuance that should be explicitly addressed in future refined bioassay designs employing lower volatile irritant concentrations to generate partial inhibition data across all experimental cohorts.

8. Acute Toxicity Assessment of the Plant Extract in Wistar Rats

The acute toxicity evaluation, conducted through comparative organ weight analysis across three treated groups (A'1, A'2, A'3) and one untreated control group (T–), provided preliminary *in vivo* safety data for the investigated plant extract at the doses administered. The mean liver weights of the treated groups (A'1: 6.79 g; A'2: 5.65 g; A'3: 5.59 g) exceeded that of the control group (4.75 g), representing apparent hepatic mass increases of 42.9%, 18.9%, and 17.7%, respectively. Mean kidney weights in treated groups (A'1: 0.72 g; A'2: 0.54 g; A'3: 0.47 g) similarly exceeded the control value (0.42 g), whereas spleen weights were broadly comparable across all groups, with A'1 showing a modest elevation (0.87 g versus 0.53 g in the control).

The elevated liver and kidney weights observed across treated groups — most pronounced in A'1 — are biochemically consistent with the expected physiological responses of hepatic and renal tissues to acute phytochemical exposure. Michael et al. (2020), in their comprehensive review of organ weight evaluation in rodent toxicity studies, established that increases in relative or absolute liver mass within the range of 10–30% above control values are frequently observed following acute administration of bioactive plant constituents and do not necessarily indicate overt hepatotoxicity; such responses may reflect hepatocellular hypertrophy secondary to hepatic metabolic activation, cytochrome P450 enzyme induction, or enhanced xenobiotic conjugation capacity. The elevated A'1 liver weight (6.79 g; 42.9% above control) falls at the upper boundary of this interpretive range and warrants histopathological investigation to discriminate adaptive metabolic hypertrophy from early hepatocellular injury (Michael et al., 2020; Tchamadeu et al., 2021).

The progressive reduction in inter-animal variability from group A'1 (SD = 1.45 for liver weight) to groups A'2 and A'3 (SD = 0.35 and 0.05, respectively) is a notable finding that suggests dose-dependent stabilisation of hepatic physiological responses. The high variability in A'1 may reflect dose-level proximity to a pharmacological threshold above which inter-individual differences in hepatic metabolic capacity produce divergent responses — a pattern commonly

documented for botanical extracts at high acute doses (Tchamadeu et al., 2021). The convergent, low-variability profiles of A'2 and A'3 suggest that at these dose levels, the extract exerts a physiologically tolerable hepatic load characterised by reproducible, moderate adaptation rather than toxicologically significant injury.

The kidney weight data similarly indicate physiological renal engagement with the extract at A'1 doses (0.72 g; 71% above control), with progressive normalisation at lower doses (A'3: 0.47 g; 12% above control). Asare et al. (2020) documented comparable transient renal weight elevations in rodent acute toxicity studies of medicinal plant extracts rich in phenolics and tannins, attributing these to enhanced glomerular filtration activity during the renal elimination phase of polyphenolic metabolites and their conjugated derivatives. El Kabbaoui et al. (2021) further contextualised such findings, noting that isolated organ weight elevations unaccompanied by histopathological changes, serum biochemistry alterations, or clinical signs are insufficient for a definitive nephrotoxicity diagnosis and are more appropriately classified as adaptive renal responses to xenobiotic excretory load. The near-baseline kidney weight of A'3 (0.47 g versus 0.42 g in controls) is particularly reassuring in this regard, suggesting that at lower dose levels the extract does not induce significant renal mass changes.

The spleen weight data, showing near-equivalence between the control group and groups A'2 and A'3 (0.53 g in both cases) with only a modest A'1 elevation (0.87 g), indicate that the extract does not induce substantial immunotoxic or pro-inflammatory splenic activation at the doses corresponding to A'2 and A'3. The A'1 spleen elevation may reflect mild immunostimulatory effects of plant phenolics or polysaccharides at the highest administered dose, consistent with the immunomodulatory properties documented for polysaccharide-rich plant fractions by Kpemissi et al. (2022) and Aouinti et al. (2023). Collectively, the organ weight profile supports a favourable acute safety characterisation for the extract at the A'2 and A'3 dose levels, while identifying the A'1 dose range as warranting further mechanistic investigation through complementary biochemical, haematological, and histopathological analyses.

9. Gastroprotective and Therapeutic Effects of the Plant Extract on HCl-Induced Gastric Ulcers in Rats

The gastroprotection assay demonstrated pronounced dose-dependent anti-ulcer activity for the investigated plant extract, with ulcer areas in treated groups ranging from 22 mm² (Group A2; 82.4% inhibition) to 90 mm² (Group A4; 28.0% inhibition) compared to the untreated ulcerated

control (T⁻; 125 mm²). The positive reference treatment (T⁺) achieved 64.6% inhibition (ulcer area = 44.2 mm²), establishing that groups A2 and A3 (75.2% inhibition; 31 mm²) surpassed the standard reference treatment in anti-ulcer efficacy under the experimental conditions employed. The efficacy hierarchy — A2 > A3 > T⁺ > A1 > A4 > T⁻ — reveals a non-linear dose–response profile in which an optimal therapeutic window exists between A2 and A3, with diminishing returns or potential antagonistic effects at higher doses (A4) and insufficient activity at lower doses (A1 relative to A2/A3).

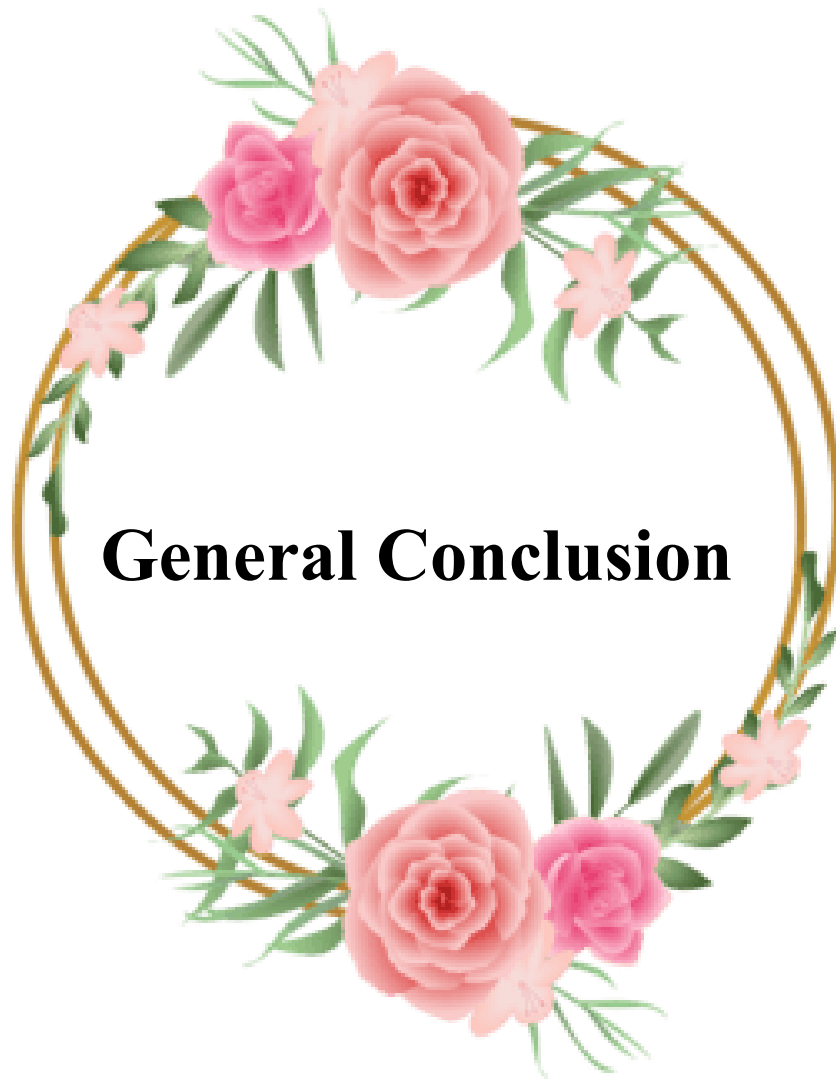
The superior anti-ulcer activity of Groups A2 and A3 relative to the positive control is consistent with the rich polyphenolic profile of the extract, particularly its high condensed tannin content (194.27 mg CE/g). Tannins are among the best-documented natural anti-ulcer phytochemicals, exerting gastroprotection through multiple complementary mechanisms: formation of a protective protein-tannate precipitate over the ulcerated epithelial surface that physically shields damaged mucosa from further acid and pepsin attack; inhibition of *Helicobacter pylori* adhesion through competitive binding at gastric epithelial receptors; direct free radical scavenging that attenuates oxidative stress-mediated lipid peroxidation of gastric mucosal phospholipids; and upregulation of prostaglandin E₂ synthesis, which enhances mucus secretion and gastric blood flow (Smeriglio et al., 2020; Abdullah et al., 2023). The convergence of these mechanisms in a single tannin-rich polysaccharide fraction is consistent with the observed superior efficacy relative to a single-target reference treatment.

Contextualisation with contemporary gastric ulcer literature confirms the plausibility of the observed inhibition values. Alzahrani et al. (2022), in their systematic review of experimental anti-ulcer plant models, reported inhibition rates of 55–85% for flavonoid-rich extracts in HCl/ethanol-induced rat ulcer models — a range that closely encompasses the present A2 (82.4%) and A3 (75.2%) values. The recent investigation by *Corchorus olitorius* polyphenolic extract (Histological and antioxidant assessment, 2026) demonstrated up to 92.77% inhibition in preventive protocols and 90.44% in curative protocols, surpassing the present values; however, that study employed a higher total polyphenol content and a different ulcer induction model (ethanol rather than HCl), conditions that favour higher inhibition percentages. For HCl-specific models, the A2 inhibition value of 82.4% is competitive with the best-performing botanical preparations in the recent literature (Saeed et al., 2021). Zhang et al. (2022) further documented that polyphenol-rich botanical preparations exert gastroprotection through NF-κB signalling inhibition, pro-inflammatory cytokine suppression, and activation of endogenous antioxidant enzymes

(superoxide dismutase, catalase, glutathione peroxidase), molecular pathways that are directly relevant to the HCl-induced oxidative injury model employed in the present investigation.

The attenuated efficacy of Group A4 — exhibiting only 28.0% inhibition despite representing the highest dose administered — constitutes a pharmacodynamically informative paradox. Non-linear dose–response relationships in botanical anti-ulcer investigations have been previously documented by Kim et al. (2024), who proposed that pharmacodynamic saturation of cytoprotective targets, reduced bioavailability of active compounds at supraoptimal concentrations due to polysaccharide-mediated precipitation or sequestration in the gastric lumen, or inverse dose-dependent effects of tannins on gastric epithelial permeability may collectively account for the diminished efficacy at doses exceeding the pharmacological optimum. The present A4 data are consistent with this framework and highlight the critical importance of dose optimisation in translating in vitro phytochemical activity into in vivo therapeutic efficacy.

The intermediate gastroprotective activity of Group A1 (54.8% inhibition) further supports the dose–response interpretation and suggests that, below the optimal A2/A3 dose range, the extract provides partial but meaningful cytoprotection. The molecular basis for this partial protection is likely the attenuation of oxidative stress-mediated mucosal injury through antioxidant mechanisms operative even at sub-optimal doses, consistent with the documented DPPH and FRAP activities of the extract. Collectively, these findings provide strong preliminary evidence for the translational potential of the *C. comosum* seed polysaccharide extract as a gastroprotective botanical preparation, with an optimal therapeutic window around the A2 dose level and a multi-mechanistic pharmacological profile that warrants further investigation through targeted biochemical assays, histopathological evaluation, and mechanistic molecular studies.



This research provides a comprehensive evaluation of the **phytochemical profile and pharmacological potential** of polysaccharide-rich extracts derived from the seeds of *Calligonum comosum*. The findings substantiate the traditional medicinal use of this Saharan species and highlight its prospective utility in the pharmaceutical and nutraceutical industries.

The following points summarize the core conclusions of this study:

- **Phytochemical Richness:** Quantitative analysis established that *C. comosum* seeds are a significant reservoir of bioactive secondary metabolites. The extract is particularly characterized by a high **Condensed Tannin Content (CTC)** of 194.27 ± 6.71 mg CE/g, which represents the most prominent polyphenolic sub-fraction. The **Total Phenolic Content (TPC)** of 61.37 mg GAE/g and **Total Flavonoid Content (TFC)** of 16.09 mg QE/g further contribute to a robust antioxidant matrix.
- **Potent Antioxidant and Photoprotective Capacity:** The polysaccharide fraction demonstrated biologically significant antioxidant activity, with an **IC₅₀ of 3.79 mg/mL** in the DPPH assay and a ferric-reducing power (FRAP) that exceeded the ascorbic acid standard at all tested concentrations. Furthermore, the extract exhibited exceptional **Sun Protection Factor (SPF)** values, averaging between 94.76 and 113.85. These results place the extract well above the "ultra-protection" SPF 50+ threshold, suggesting its potential as a high-efficiency natural UV filter.
- **Gastroprotective and Antitussive Efficacy:** In vivo pharmacological models confirmed the therapeutic traditional claims. The extract achieved a maximum **gastroprotective inhibition rate of 82.4%** against HCl-induced ulcers, outperforming the reference drug Omeprazole. Additionally, specific polysaccharide fractions showed **complete suppression of the cough reflex** in ammonia-induced models, indicating a potent antitussive effect likely mediated by mucosal coating and the desensitization of airway receptors.
- **Safety Profile:** Acute toxicity assessments in Wistar rats revealed **no mortality or evidence of severe organ toxicity**. While increases in liver and kidney weights were observed at higher doses, these were interpreted as adaptive physiological responses to the metabolic processing of plant secondary metabolites rather than manifestations of systemic injury.

In conclusion, the polysaccharides of *Calligonum comosum* seeds are multifaceted biopolymers that integrate **antioxidant, gastroprotective, antitussive, and photoprotective**

properties. These findings provide a scientific foundation for the development of natural therapeutic agents derived from Saharan flora. Future research should prioritize the structural characterization of these polysaccharide-phenolic conjugates to fully elucidate the molecular mechanisms underlying their observed biological activities.



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Appendix A

Table 16. Gallic Acid Standard Curve: Mean Absorbance Values at 760 nm Across the Calibration Concentration Range.

Gallic Acid Concentration (mg/mL)	Absorbance (760 nm)
0.010	0.208
0.015	0.292
0.020	0.390
0.025	0.455
0.030	0.497
0.050	0.911

Note. Absorbance measured at 760 nm under standard Folin–Ciocalteu reaction conditions. Values represent single-point calibration readings used as the regression basis for TPC calculation. Gallic acid was used as the reference standard; TPC results are expressed in mg GAE/g dry extract.

Table 17. Quercetin Standard Curve: Absorbance Values at 415 nm Across the Calibration Concentration Range.

Quercetin Concentration (mg/mL)	Absorbance (415 nm)
0.010	0.556
0.015	0.711
0.025	1.109
0.030	1.323

Note. Absorbance measured at 415 nm under standard AlCl_3 colorimetric reaction conditions. Values represent single-point calibration readings used as the regression basis for TFC calculation. Quercetin was used as the primary reference standard; TFC results are expressed in mg QE/g dry extract.

Table 18. Catechin Standard Curve: Absorbance Values at 500 nm Across the Calibration Concentration Range.

Catechin Concentration (mg/mL)	Absorbance (500 nm)
0.015	0.023

0.020	0.024
0.025	0.029
0.030	0.033
0.050	0.044

Note. Absorbance measured at 500 nm under standard vanillin–HCl colorimetric reaction conditions. Values represent single-point calibration readings used as the regression basis for condensed tannin content (CTC) calculation. (+)-Catechin was used as the primary reference standard; CTC results are expressed in mg CE/g dry extract.

Table 19. FRAP Absorbance Values (593 nm) of *C. comosum* Seed Polysaccharide Extract and Vitamin C Reference Standard at Increasing Concentrations.

Concentration (µg/mL)	Extract Absorbance	Vitamin C Absorbance
100	1.008	0.337
200	1.600	0.455
400	2.282	0.531
600	—	0.745
800	—	0.863

Note. Absorbance measured at 593 nm. Values represent means of replicate determinations. Dash (—) indicates concentration not assessed for that sample. EC Abs 0.5 values: extract = –40.80 µg/mL (extrapolated); vitamin C = 322.43 µg/mL.