People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research

University of Ahmed Zabana - RELIZANE –
Faculty of the Natural and Life Sciences
Department of Biology

DOCTORAL THESIS LMD System – Third Cycle

Field: Biological Sciences

Specialty: Applied Biochemistry

Title of the Thesis:

Study of the Nutritional Properties and Antioxidant Activities of an Edible Mushroom and a Variety of Vegetables : underlying mechanisms and implications for the prevention of chronic diseases.

Presented by :

Mr : SI-LARBI Ali

Defence date: 16/04/2026

JURY MEMBERS

President	**Ms Oucif Hanane**	**MCA**	University of Relizane
Thesis Supervisor	**Ms Benahmed Fatiha**	**MCA**	University of Relizane
Co- Supervisor	**Ms Terbeche Rym**	**MCA**	University of Mohamed Buodiaf Oran
Examiner	**Ms Ait Hamadouche Nadia**	**Pr**	University Ahmed Benbella Oran 1
Examiner	**Ms Rached Wahiba**	**MCA**	University of Mostaganem
Examiner	**Ms Belkheir Khadidja**	**MCA**	University of Relizane

Academic Year: 2025/2026

Acknowledgments

First and foremost, I praise and thank Almighty God, who has granted me health, well-being, strength, patience, and determination to successfully complete this scientific work.

I express my sincere gratitude and appreciation to my beloved family, my mother and all my brothers and sisters, for the sincere love, constant support, and firm backing they have shown me, thus forming the foundation upon which I have built my academic and professional career.

I wish to express my deepest gratitude to my research supervisor, ***Dr. Benahmed Fatiha***, Senior Lecturer "A" at Ahmed Zabana University, Relizane, for her invaluable supervision, rigorous scientific guidance, patience, and constant support, all of which have had a decisive impact on the successful completion of this work. I also wish to thank ***Dr. Terbeche Rym***, Senior Lecturer "A" at the University of Mohamed Boudiaf of Oran, for serving as co-supervisor of this work.

I would like to express my highest consideration and profound gratitude to:

Dr. Oucif Hanane (Ahmed Zabana University, Relizane), who honored me by graciously accepting to chair the jury;

Professor Ait Hamadouche Nadia (University of Oran 1 Ahmed Ben Bella), for kindly agreeing to evaluate this work;

Dr. Rached Wahiba (University of Mostaganem), for her willingness to examine this thesis;

Dr. Belkheir Khadidja (Ahmed Zabana University, Relizane), for her kind acceptance to serve as an examiner of this thesis.

I would also like to express my sincere gratitude to my dear friend, ***Dr. Boualem Benamar Aissa***, a specialist in Applied Biochemistry, for his invaluable scientific support and fraternal companionship, which have been a solid foundation in my research.

I also acknowledge ***Dr. Toumi Mohammed Esseddik***, Professor of Applied Biochemistry at the Frères Mentouri 1 University in Constantine, for his support and valuable scientific contributions. I appreciate the assistance of ***Dr. Goujil Sabrina***, a specialist in Biochemistry, which has had a tangible impact on this work.

I extend my thanks to ***Fouad Meradsi,*** Professor at the University of Batna 2, for his valuable contributions and insightful guidance, which have significantly enriched this research.

I also give special recognition to ***Dr. Ahmed Fellah***, former coordinator of the Research Laboratory at the University of Relizane and currently a Lecturer at the same institution, for the facilities he provided during this research.

I wish to express my gratitude to **the Research Laboratory at the University of Relizane**, which provided a suitable scientific environment, as well as to the Natural Bio-Resources Laboratory at the Hassiba Benbouali University in Chlef and the National Center for Research in Biotechnology (CRBt) in Constantine, for their contributions in facilitating certain aspects of this work.

Finally, I would like to renew my deep gratitude to the professors of Relizane University for their invaluable role in our training, and to all my colleagues, friends, and teachers for their guidance, advice, and continuous encouragement, especially my classmates, each by name, as well as my colleagues at Tahri Miloud High School in Sidi Khettab, Relizane.

To all of them, I extend my highest appreciation and sincere gratitude.

Dedication

I dedicate this work to the soul of my late father, may God have mercy on him and grant him eternal peace;

To my beloved mother, my constant source of strength and support, may God bless and protect her;

To all my dear brothers and sisters, who have been a source of encouragement and motivation at every step;

To all my esteemed professors, from whom I have gained knowledge and guidance, and to everyone who has taught me a letter or a skill, thereby shaping my academic and professional journey;

To my friends and colleagues, who have shared this path with me, offering sincere advice, support, and companionship.

To all of you, I dedicate this humble effort as a token of my deep gratitude and enduring appreciation.

Si-Larbi Ali

الملخص

يهدف هذا البحث إلى تقييم الخصائص الغذائية والأنشطة البيولوجية للفطر الصالح للأكل *Pleurotus eryngii* وأوراق *Foeniculum vulgare* من غرب الجزائر، وذلك من خلال توصيف تركيبهما الغذائي، وتحليل مركباتهما الثانوية (الفينولات والفلافونويدات)، وتقييم نشاطهما المضاد للأكسدة والمضاد للالتهاب والمضاد للميكروبات، إضافةً إلى تقييم النشاط المثبط للانزيم ألفا اميلاز.

أظهرت التحاليل الغذائية للأجسام الثمرية لفطر *P. eryngii* (على أساس المادة الجافة) محتوى مرتفعًا من البروتين (14.81 ± 0.54 غ/100غ)، والسكريات الكلية (49.61 ± 3.16 غ/100غ)، والكربوهيدرات (77.26 ± 0.52 غ/100غ)، والمعادن (5.27 ± 0.21 غ/100غ)، إلى جانب محتوى منخفض نسبيًا من الدهون (2.67 ± 0.23 غ/100غ) وقيمة طاقوية مهمة (391.62 ± 1.55 كيلو كالوري/100غ). أما محتوى الرطوبة فكان في حدود 81.4 ± 1.68 %. في المقابل، أظهرت أوراق *F. vulgare* تركيبة غذائية أقل في البروتين (4.62 ± 0.37 غ/100غ) والدهون (1.54 ± 0.06 غ/100غ)، لكنها أغنى بالكربوهيدرات (90.14 ± 0.23 غ/ 100غ).

أوضحت التحاليل الفيتوكيميائية أن المستخلص المائي للفطر *P. eryngii* احتوى على نسب أعلى من الفينولات والفلافونويدات (20.65 ± 0.27 ملغ مكافئ حمض الغاليك/غ من المستخلص، 5.69 ± 0.07 ملغ مكافئ كيرسيتين/غ من المستخلص على التوالي)، بينما أظهرت أوراق *F. vulgare* الاتجاه المعاكس، حيث كان المستخلص الإيثانولي الأغنى بهذه المركبات (23.80 ± 0.19 ملغ مكافئ حمض الغاليك/غ من المستخلص، 17.62 ± 0.99 ملغ مكافئ كيرسيتين/غ من المستخلص على التوالي). كما كشفت تحاليل LC-MS/MS عن وجود ثلاثة مركبات فعالة رئيسية في *P. eryngii*، وكان حمض الترانس-سيناميك هو الأبرز، في حين تم تحديد عشرة مركبات في أوراق *F. vulgare*، كان حمض الكلوروجينيك هو المركب السائد.

أظهر تقييم النشاط المضاد للأكسدة أن الفطر *P. eryngii* كان أكثر فعالية في مستخلصه المائي وفقًا لاختبارات DPPH و FRAP و TAC و β-carotene bleaching وH_2O_2 scavenging، بينما أظهرت أوراق *F. vulgare* تفوق المستخلص الإيثانولي في نفس الاختبارات.

فيما يتعلق بالنشاط المضاد للالتهاب، أظهر المستخلص المائي للفطر *P. eryngii* قدرة قوية على تثبيط تمسخ الألبومين؛ غير أن نتائج أوراق *F. vulgare* كانت أكثر وضوحًا في المستخلص الإيثانولي مقارنة بالمائي. كما أظهرت دراسات الالتحام الجزيئي نشاطًا قويًا لمركبات مثل الروتين والكيرسيتين ضد بروتينات رئيسية مرتبطة بالاستجابة الالتهابية.

أما بالنسبة للنشاط المثبط للأنزيمات، فقد كان تثبيط إنزيم-α أميلاز أكثر وضوحًا في المستخلص المائي للفطر *P. eryngii* مقارنة بالمستخلص الإيثانولي، بينما كان العكس صحيحًا بالنسبة لأوراق *F. vulgare*.

فيما يخص النشاط المضاد للميكروبات، تم تقييمه بطريقة الانتشار من الحفر ضد أربع سلالات بكتيرية وفطر، حيث أظهرت المستخلصات الإيثانولية فعالية أعلى في كلا النوعين المدروسين.

بشكل عام، تؤكد هذه النتائج الإمكانات الغذائية والدوائية لكل من الفطر *P. eryngii* وأوراق *F. vulgare*، وتبرز قيمتهما كمصادر طبيعية للمركبات النشطة بيولوجيًا ذات الخصائص المضادة للأكسدة والمضادة للالتهاب والمضادة للميكروبات.

الكلمات المفتاحية: *Pleurotes eryngii* ، أوراق *Foeniculum vulgare*، التركيب الغذائي؛ النشاط المضاد للأكسدة؛ النشاط المضاد للالتهابات؛ النشاط المضاد للانزيم؛ النشاط المضاد للميكروبات؛ LC-MS/MS.

Abstract

This study evaluated the nutritional and biological activities of the edible mushroom *Pleurotus eryngii* and *Foeniculum vulgare* leaves from western Algeria by analyzing their composition, secondary metabolites (phenolic compounds and flavonoids), and antioxidant, anti-inflammatory, antimicrobial, and α-amylase inhibitory activity.

Nutritional analysis of *P. eryngii* (on a dry weight basis) revealed high protein (14.81 ± 0.54 g/100 g), sugars (49.61 ± 3.16 g/100 g), carbohydrates (77.26 ± 0.52 g/100 g), minerals (5.27 ± 0.21 g/100 g), low fat (2.67 ± 0.23 g/100 g), energy (391.62 ± 1.55 kcal/100 g), and moisture content (81.4 ± 1.68 %). *F. vulgare* leaves showed lower protein (4.62 ± 037. g/100 g) and fat (1,54 ± 006. g/100 g), but higher carbohydrates (90.14 ± 0.23 g/100 g).

Phytochemical analysis indicated that the aqueous extract of *P. eryngii* had higher polyphenols and flavonoids (20.65 ± 0.27 mg gallic acid equivalent/g extract, 5.69 ± 0.07 mg quercetin equivalent/g extract, respectively), while *F. vulgare* leaves were richest in the ethanolic extract (23.80 ± 0.19 mg gallic acid equivalent/g extract, 17.62 ± 0.99 mg quercetin equivalent/g extract, respectively). LC-MS/MS identified three main bioactive compounds in *P. eryngii* (predominantly trans-cinnamic acid) and ten in *F. vulgare* leaves (mainly chlorogenic acid).

Antioxidant activity was higher in the aqueous extract of *P. eryngii* (DPPH, FRAP, TAC, β-carotene, H_2O_2 assays), whereas *F. vulgare* leaves showed superior activity in the ethanolic extract. Regarding anti-inflammatory activity, the aqueous extract of *P. eryngii* showed a strong ability to inhibit albumin denaturation, whereas for *F. vulgare* leaves, the effect was more pronounced in the ethanolic extract. Molecular docking confirmed the activity of compounds such as rutin and quercetin against key proteins of the inflammatory response.

Enzymatic activity showed greater α-amylase inhibition in *P. eryngii* aqueous extract, and in *F. vulgare* ethanolic extract. Antimicrobial activity was assessed by the agar well diffusion method against four bacterial and fungal strains, with higher activity observed in ethanolic extracts for both species.

Overall, these results confirm the nutritional and pharmacological potential of *P. eryngii* and *F. vulgare* leaves as natural sources of bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties.

Keywords: *Pleurotus eryngii*; *Foeniculum vulgare* leaves; Nutritional composition; Antioxidant activity; Anti-inflammatory activity; enzyme inhibitory activity; Antimicrobial activity; LC-MS/MS.

Résumé

Cette étude a évalué les propriétés nutritionnelles et les activités biologiques du champignon comestible *Pleurotus eryngii* et des feuilles de *Foeniculum vulgare* de l'ouest de l'Algérie , en caractérisant leur composition nutritionnelle, leurs métabolites secondaires (composés phénoliques et flavonoïdes) et en évaluant leurs activités antioxydantes, anti-inflammatoires, antimicrobiennes et l'activité inhibitrice de l'α-amylase.

L'analyse nutritionnelle des corps fructifères de *P. eryngii* (exprimée sur la base de la matière sèche) a révélé une teneur élevée en protéines (14,81 ± 0,54 g/100 g), sucres totaux (49,61 ± 3,16 g/100 g), glucides (77,26 ± 0,52 g/100 g) et minéraux (5,27 ± 0,21 g/100 g), avec une faible teneur en lipides (2,67 ± 0,23 g/100 g), une valeur énergétique significative (391,62 ± 1,55 kcal/100 g) et une humidité de 81,4 ± 1,68 %. Les feuilles de *F. vulgare* présentaient moins de protéines (4.62 ± 0.37 g/100 g) et lipides (1,54 ± 0.06 g/100 g), mais plus de glucides (90.14 ± 0.23 g/100 g).

L'analyse phytochimique a montré que l'extrait aqueux de *P. eryngii* contenait plus de polyphénols et flavonoïdes (20.65 ± 0.27 mg équivalent acide gallique/g d'extrait, 5.69 ± 0.07 mg équivalent quercétine/g d'extrait, respectivement), tandis que pour les feuilles de *F. vulgare*, l'extrait éthanolique était le plus riche (23.80 ± 0.19 mg équivalent acide gallique/g d'extrait, 17.62 ± 0.99 mg équivalent quercétine/g d'extrait, respectivement). L'analyse LC-MS/MS a identifié trois principaux métabolites secondaires bioactifs dans *P. eryngii* (acide trans-cinnamique prédominant) et dix dans les feuilles de *F. vulgare* (acide chlorogénique majoritaire).

L'évaluation de l'activité antioxydante a montré que l'extrait aqueux de *P. eryngii* était plus efficace selon les tests DPPH, FRAP, TAC, β-carotène et piégeage de H_2O_2, tandis que pour *F. vulgare*, l'extrait éthanolique était supérieur.

Concernant l'activité anti-inflammatoire, l'extrait aqueux de *P. eryngii* a montré une forte capacité à inhiber la dénaturation de l'albumine, alors que pour les feuilles de *F. vulgare*, l'effet était plus marqué dans l'extrait éthanolique. Le docking moléculaire a confirmé l'activité de composés tels que la rutine et la quercétine contre des protéines clés de la réponse inflammatoire.

Pour l'activité enzymatique, l'inhibition de l'α-amylase était plus prononcée dans l'extrait aqueux de *P. eryngii,* et inversement pour les feuilles de *F. vulgare*. L'activité antimicrobienne, évaluée par diffusion en puits sur gélose contre quatre souches bactériennes et une souche fongique, était plus forte dans les extraits éthanoliques pour les deux espèces.

Dans l’ensemble, ces résultats confirment le potentiel nutritionnel et pharmacologique de *P. eryngii* et des feuilles de *F. vulgare*, et soulignent leur valeur comme sources naturelles de composés bioactifs aux propriétés antioxydantes, anti-inflammatoires et antimicrobiennes.

Mots-clés : *Pleurotus eryngii* ; feuilles de *Foeniculum vulgare* ; composition nutritionnelle ; activité antioxydante ; activité anti-inflammatoire ; activité antienzymatique; activité antimicrobienne ; LC-MS/MS.

Abbreviations

A0.5	The concentration at 0.50 absorbance
ATCC	American Type Collection Culture
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
BSA	Bovine Serum Albumin
CBB	Coomassie Brilliant Blue
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC_{50}	Half Maximal Effective Concentration
F	*Foeniculum*
FCR	Folin–Ciocalteu Reagent
FRAP	Ferric Reducing Antioxidant Power
H_2O_2	Hydrogen peroxide
HPLC	High Performance Liquid Chromatography
HRESIMS	High-Resolution Electrospray Ionization Mass Spectrometry
IC_{50}	Half Maximal Inhibitory Concentration
IL	Interleukins
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
MHA	Mueller–Hinton Agar
MIC	Minimum Inhibitory Concentration
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OS	Oxidative Stress
P	*Pleurotus*
PBS	Phosphate Buffered Saline

RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SDA	Sabouraud Dextrose Agar
T2DM	Type 2 Diabetes Mellitus
TAC	Total Antioxidant Capacity
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

List of figures

List of tables

TABLE OF CONTENTS

INTRODUCTION

Introduction

Since ancient times, humanity has relied on natural resources not only as food but also to treat various diseases due to their broad health benefits (Al Qaisi et al., 2024). More than 70% of the global population still depends directly on medicinal plants for primary health care, and the World Health Organization emphasizes integrating these plants into formal health systems (Ekor 2014, Barrahil et al., 2020 ; Vitale et al., 2022).

Facing growing global health challenges, scientific research increasingly explores untapped biological resources for bioactive compounds. Medicinal and aromatic plants, along with edible mushrooms, are rich “chemical reservoirs” of secondary metabolites with multiple therapeutic effects, boosting interest in nutrition and disease prevention (Badgujar et al., 2014 ; Karakas et al., 2023).

Proximate analysis (nutritional characterization) is essential for understanding the value of any natural resource. Edible mushrooms, especially of the Pleurotus genus, are rich in protein (up to 30%) and dietary fiber, with low fat content, making them ideal functional foods. Studies on *Pleurotus eryngii* show it is low in calories but rich in protein, fiber, and essential minerals like potassium, phosphorus, and selenium. Its polysaccharides, particularly beta-glucan, have biological activities including cancer cell growth inhibition, antioxidant, and anti-inflammatory effects, supporting its functional food status (Teniou et al., 2022 ; Caponio et al., 2025). Variations in nutritional content depending on cultivation conditions and substrate highlight the need to study local strains under specific environmental settings.

Similarly, *Foeniculum vulgare* (fennel), known locally in western Algeria as “basbas,” is widely used for food and medicinal purposes. Its bulbs and leaves are consumed as vegetables in various dishes, contributing to its prominence in Mediterranean cuisine and other global food traditions (Anka et al., 2020). Seeds contain 6.3% water, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber, and 42.3% carbohydrates. Fruits, with 10–12% oil, are a diverse source of fatty acids, including 0.6% petroselic acid, 22% oleic acid, 14% linoleic acid, and 4% palmitic acid (Zeeshan et al., 2023).

Research shows fennel leaves, often discarded as waste in Algeria, are actually the richest part in essential minerals and vitamins, supplying calcium, potassium, sodium, iron, phosphorus, thiamin, riboflavin, niacin, and vitamin C (Barros et al., 2010 ; Hao et al., 2021 ; Zeeshan, Akram et al. 2023). This composition underpins multiple functional properties, and regular consumption has been associated with improved memory, stress reduction, and overall health support without adverse effects (Anka et al., 2020).

Oxidative stress arises when the balance between free radicals and the body's antioxidant defenses is disrupted. This exposes cells to damage to lipids, proteins, and DNA, and is associated to chronic inflammation, cancer cell proliferation, and apoptosis dysfunction, with nitric oxide and peroxynitrite as key contributors (Ahmadi et al., 2022 ; Nardini 2023).

Plants and mushrooms contribute protective secondary metabolites, including phenols and flavonoids, which neutralize free radicals and reduce toxic effects (Nardini 2023, Caponio et al., 2025).

P. eryngii is rich in phenolic compounds and ergothionine, conferring potent antioxidant activity (Han et al., 2021). Fennel leaves are rich in phenolic acids (chlorogenic and caffeic) and flavonoids (quercetin, kaempferol, isorhamnetin), responsible for their high radical-scavenging capacity (Pacifico et al., 2018). Due to multiple oxidation mechanisms, comprehensive antioxidant assessment requires multiple assays (DPPH, FRAP, TAC), essential for accurate evaluation of extract effectiveness (Nerdy and Manurung 2018 ; Christodoulou et al., 2022).

Beyond antioxidant activity, other biologically relevant activities are important. Inflammation activates immune cells and mediators such as NO and TNF-α to maintain homeostasis, but chronic persistence leads to diseases like rheumatoid arthritis, asthma, multiple sclerosis, and cancer, justifying natural anti-inflammatory agents such as flavonoids. (Aziz et al., 2018). Protein denaturation inhibition is widely used for preliminary *in vitro* anti-inflammatory evaluation. Molecular docking simulates interactions of active compounds with key inflammatory targets, a successful approach for plant and mushroom extracts, including fennel and *P. eryngii* (Zhang et al., 2023 ; Fouda and Mohamed 2025).

α-Amylase inhibition is key for postprandial glucose control and managing type 2 diabetes (Kashtoh and Baek 2023). Plant and mushroom extracts are rich in α-amylase inhibitors; *F. vulgare* extracts show effective inhibition (Hadji et al., 2025), and *P. eryngii* polysaccharides exhibit promising antidiabetic activity *in vitro* and *in vivo* (Abd El-Zaher et al., 2025), confirming its potential relative to other mushrooms (Liu et al., 2025).

Evaluating antimicrobial activity is also essential as an alternative strategy against antibiotic-resistant pathogens, such as *Staphylococcus aureus*, as demonstrated with fennel and *Pleurotus* mushrooms (Gashaw et al., 2020 ; Alam et al., 2022).

Although many studies exist on *P. eryngii* and only a few on *F. vulgare* leaves separately, comprehensive evaluation within a single framework is lacking, especially from the same geographic region (western Algeria), where origin influences chemical composition and biological activity. This thesis addresses this gap through a holistic scientific approach focused on the nutritional and functional assessment of these natural resources. Specifically, this study aims to evaluate the nutritional composition and antioxidant properties of *P. eryngii* and *F. vulgare* leaves, as well as their underlying mechanisms of action and potential applications in the prevention of chronic diseases.

The thesis comprises three main parts. Part I consists of two chapters: the first addresses the theoretical foundations, including macronutrients and micronutrients, oxidative stress and antioxidant mechanisms, inflammatory pathways and molecular targets, the role of α-amylase in carbohydrate metabolism, and antimicrobial relevance. The second chapter reviews the literature on *P. eryngii* and *F. vulgare*, including their classification, morphology, distribution, ethnomedicinal and traditional uses, chemical composition, and biological activities.

Part II describes the materials and methods related to *P. eryngii* and *F. vulgare* leaves, including extraction techniques using safe solvents (water and ethanol), nutritional analysis, and the profiling of phenolic and flavonoid compounds using LC-MS/MS. It also covers the *in vitro* and *in silico* evaluation of antioxidant, anti-inflammatory, α-amylase inhibitory, and antimicrobial activities.

Part III presents the results and discussion, followed by a concluding section that highlights the key findings and outlines future application prospects, thereby emphasizing the scientific and applied value of these natural resources.

PART ONE

THEORETICAL BACKGROUND AND LITERATURE REVIEW

Part ONE: Theoretical background and literature review

1. Theoretical background

1.1. Nutritional importance of macronutrients and micronutrients

Macronutrients and micronutrients play fundamental roles in sustaining human health and development. Proteins, composed of amino acids linked by peptide bonds, are indispensable for growth, tissue maintenance, and energy provision alongside carbohydrates and lipids. Beyond their structural function, proteins participate in enzymatic catalysis, nutrient transport, immune defense, and cellular signaling. Adequate intake of high-quality proteins prevents muscle wasting, immune dysfunction, and age-related sarcopenia, highlighting their nutritional and biomedical significance (Awuchi et al., 2019; Twinomuhwezi et al., 2020; Bertoline et al., 2023; Hayes 2020).

Carbohydrates serve as the primary energy source through glycolysis and respiration, while also acting as structural components of nucleic acids and cell walls. They are further involved in immune regulation and the synthesis of bioactive secondary metabolites. Their unique properties—biocompatibility, low toxicity, and biodegradability—have expanded their application in nanomedicine and drug delivery systems, where they facilitate targeted release and molecular protection (Pifferi et al., 2021; Cao et al., 2022; Di et al., 2022).

Lipids and fatty acids provide dense energy storage, ensure membrane integrity, and regulate numerous physiological processes, including inflammation, cardiovascular protection, and cell signaling. Essential polyunsaturated fatty acids such as omega-3 and omega-6 are especially critical for metabolic and immune homeostasis. Advances in lipidomics have revealed extensive structural diversity, encompassing classes like phospholipids, triacylglycerides, and wax esters, which contribute to both cellular dynamics and nutritional value (De Carvalho and Caramujo 2018; Sud et al., 2007; Akoh, 2017).

Vitamins and minerals, though required in trace amounts, are equally indispensable for enzymatic activity, bone formation, and physiological regulation. Minerals such as calcium, iron, magnesium, and zinc act synergistically with vitamins (e.g., B-complex and vitamin E), influencing bioavailability and metabolic efficiency. Ash content analysis provides a reliable measure of mineral composition, indirectly reflecting the nutritional and vitamin potential of plant-based foods (Awuchi et al., 2020; Diosady et al., 2019; Puri et al., 2024).

Collectively, these nutrients represent an integrated framework of energy supply, structural support, and regulatory functions, underscoring their central role in health promotion, disease prevention, and the development of functional foods and therapeutic strategies.

1.2. Oxidative stress, reactive species, and antioxidant defense mechanisms

Oxidative stress arises from an imbalance between the production of reactive oxygen and nitrogen species (ROS and RNS) and the efficiency of antioxidant defense systems. Free radicals, defined as molecules or atoms with unpaired electrons, are generated both endogenously, mainly in mitochondria during oxidative phosphorylation and the electron transport chain (complexes I and III), as well as in peroxisomes, the endoplasmic reticulum, and immune cells, and exogenously through exposure to UV radiation, smoking, heavy metals, and environmental pollutants (Phaniendra et al., 2015; Singhal et al., 2015; Ponnampalam et al., 2022; Tumilaar et al., 2024; Morant-Ferrando et al., 2023). The most relevant ROS include superoxide anion (O2−), hydroxyl radical (OH·), hydrogen peroxide (H2O2), and peroxyl radicals (ROO·), while RNS comprise nitric oxide (NO) and peroxynitrite (ONOO−), all of which act as highly reactive oxidizing and nitrating agents (Apak et al., 2022; Sies et al., 2022). At physiological levels, ROS and RNS play beneficial roles in redox homeostasis, signaling, and immune responses (Vona et al., 2021), however, their excessive accumulation leads to oxidative damage in biomolecules. Proteins undergo oxidation and nitration of amino acid residues, impairing enzymatic and structural functions (Shcherbik and Pestov, 2019), lipids, especially polyunsaturated fatty acids, suffer peroxidation that produces cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) (Christodoulou et al., 2022; Venmathi Maran et al., 2022), and nucleic acids experience strand breaks and base modifications, with mitochondrial DNA being particularly vulnerable (Srinivas et al., 2019). These processes are strongly implicated in chronic diseases including neurodegenerative, cardiovascular, metabolic, and cancer pathologies, largely through the activation of pro-inflammatory signaling pathways such as NF-κB (Christodoulou et al., 2022; Venmathi Maran et al., 2022). To counteract oxidative damage, organisms rely on both enzymatic defenses such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), and non-enzymatic systems including reduced glutathione (GSH), NADPH, vitamins (C, E, and A), and, importantly, bioactive phytochemicals such as phenolic compounds and flavonoids (Shcherbik and Pestov 2019; Venmathi Maran et al., 2022). Polyphenols, which are abundant in natural sources like plants (fruits, vegetables, cereals, legumes) and mushrooms, represent crucial secondary metabolites with strong antioxidant activity (Lang et al., 2024; Li et al., 2020). Phenolic acids, such as caffeic, ferulic, chlorogenic, and gallic, exert their protective effect mainly by donating hydrogen atoms and scavenging free radicals, while flavonoids including flavonols, flavones, anthocyanins, and isoflavones act by neutralizing ROS, chelating transition metals, and stimulating antioxidant enzymes (Abotaleb et al., 2020; Saleem et al., 2022; Rodríguez De Luna et al., 2020; Oh et al., 2021). Due to these mechanisms, polyphenols from both plants and mushrooms play a vital role in preventing oxidative damage and mitigating the risk of inflammation, cardiovascular disorders, diabetes, and cancer (Hussain et al., 2018).

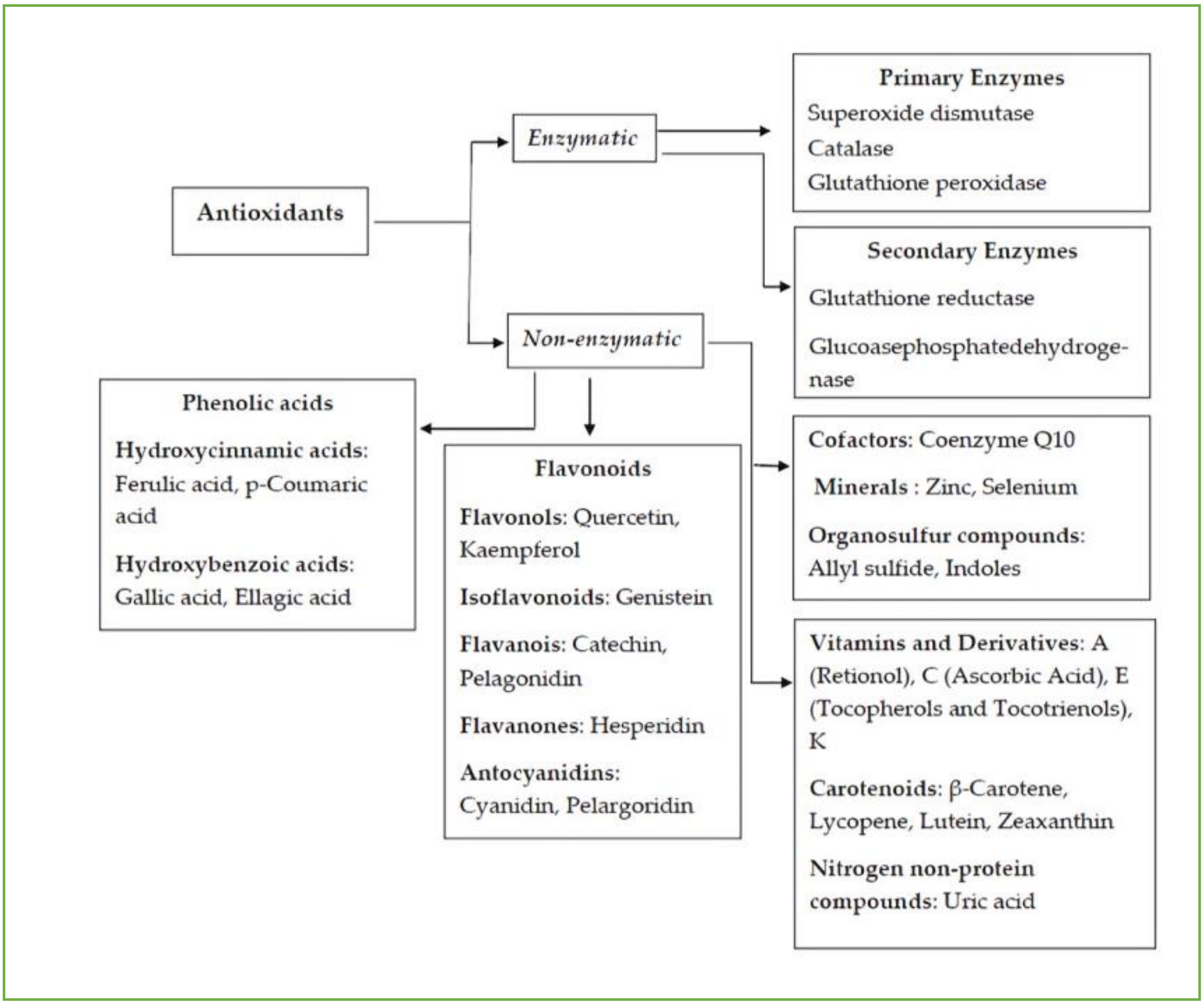


Figure 1. Classification of antioxidants (Munteanu and Apetrei 2021).

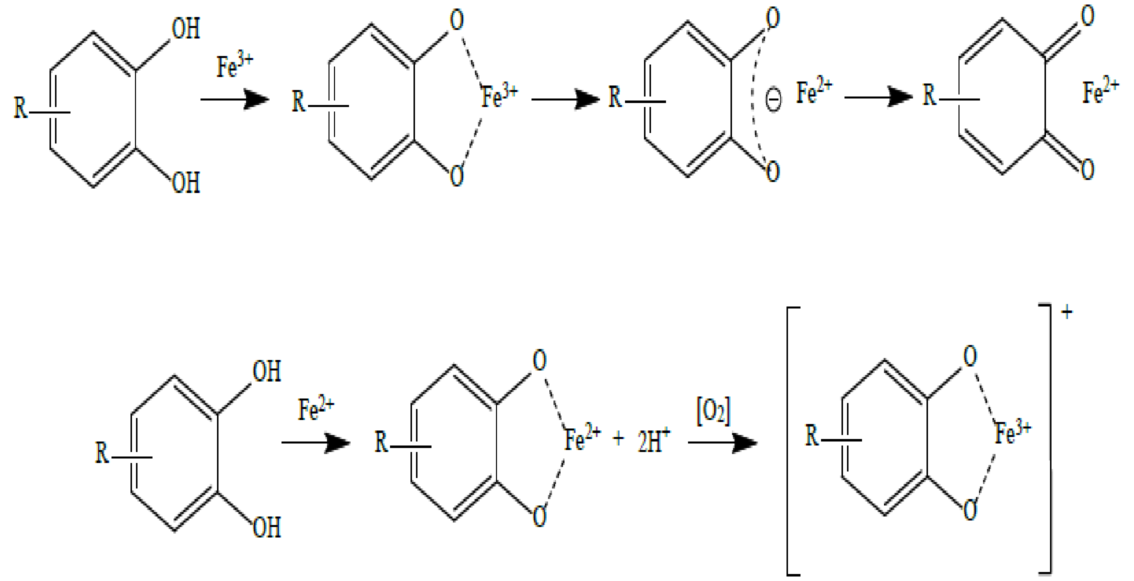


Figure 2. Phenolic acid derivative chelating Fe^{3+} and Fe^{2+} (Christodoulou et al., 2022).

1.3. Inflammation, molecular pathways, and the role of natural products

Inflammation is a vital defense mechanism triggered by infections, injuries, allergens, or cellular damage via pattern recognition receptors (PRRs) that detect PAMPs and DAMPs, activating innate immunity and releasing mediators such as nitric oxide, prostaglandins, C-reactive protein, and cytokines including IL-1β, IL-6, and TNF-α (Ginwala et al., 2019; Ma et al., 2018; Ysrafil et al., 2023). Acute inflammation is rapid and transient, supporting tissue repair, whereas chronic inflammation persists over months or years and is associated with autoimmune and metabolic disorders, neurodegeneration, atherosclerosis, diabetes, and cancer (Han et al., 2021; Obumnaeme et al., 2021). Its progression depends on a complex interplay of mediators, mainly cytokines and chemokines, which regulate immune cell activation, migration, and tissue responses (Harvanová et al., 2023; Griffiths et al., 2017). Central signaling pathways such as NF-κB, JAK/STAT, and Smad7 orchestrate inflammatory gene expression and represent major therapeutic targets (Duan et al., 2021). Natural products, particularly phenolic acids (shikimic, ferulic, p-coumaric) and flavonoids, effectively suppress pro-inflammatory cytokines, inhibit COX-derived mediators, modulate oxidative stress, and regulate immune signaling (Saleem et al., 2022; Khan et al., 2019). Nanotechnology-based delivery systems enhance their bioavailability, efficacy, and safety (Ysrafil et al., 2023). Plants (fruits, vegetables, cereals, legumes) and mushrooms provide abundant natural sources of these bioactive compounds, supporting the development of effective anti-inflammatory strategies.

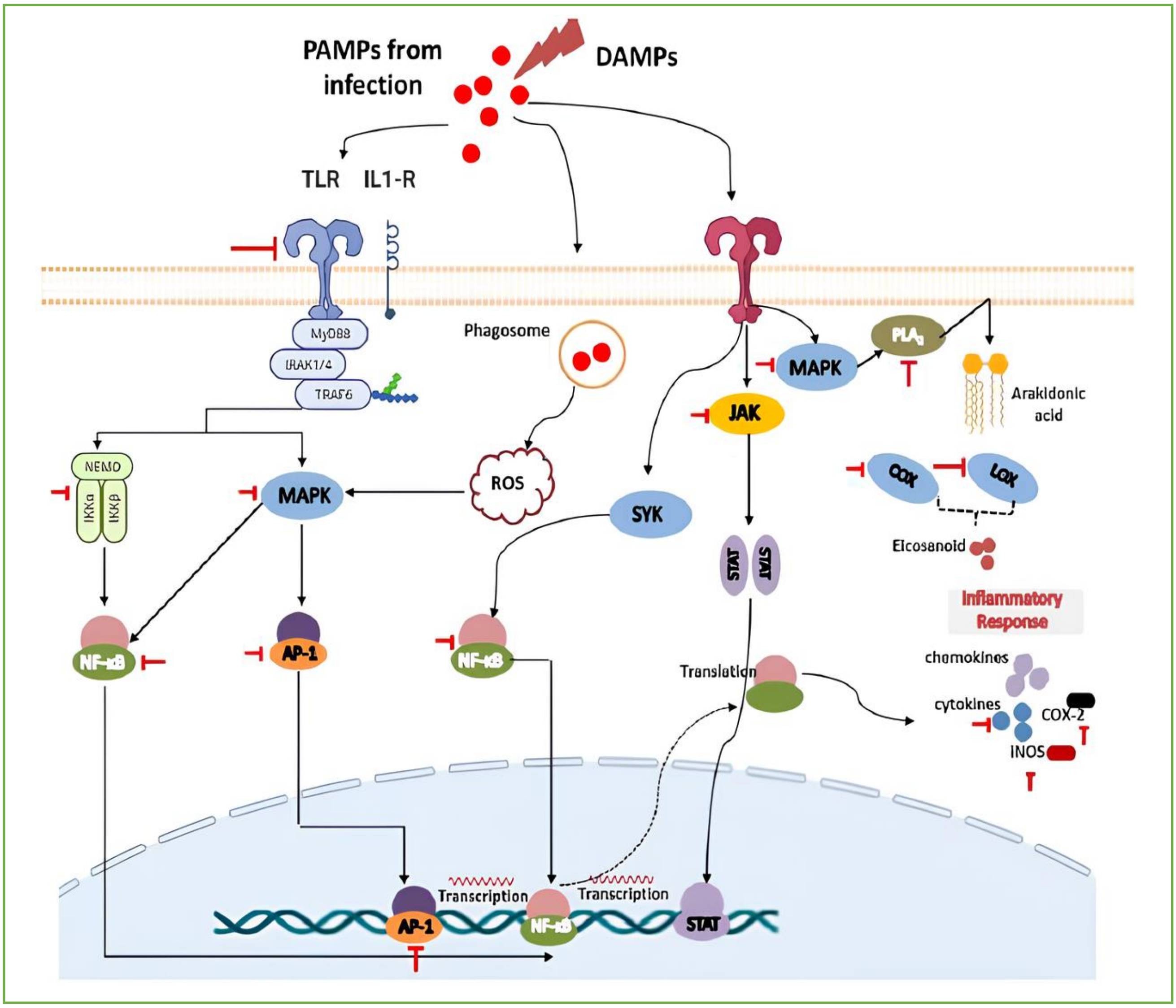


Figure 3. Schematic representation of the anti-inflammatory mechanisms of flavonoids (Ysrafil et al., 2023).

1.4. α-Amylase: physiological role, clinical relevance, and natural inhibitors

α-Amylase (EC 3.2.1.1) is a calcium-dependent enzyme that hydrolyzes α-1,4 glycosidic bonds in polysaccharides (amylose, amylopectin, glycogen, maltodextrins) into oligosaccharides, later converted to absorbable monosaccharides by α-glucosidase (Ćorković et al., 2022; Kashtoh and Baek, 2023). It facilitates carbohydrate digestion, contributes to energy metabolism, and acts synergistically with pancreatic lipase in lipid breakdown (Benrahou et al., 2022). In type 2 diabetes mellitus (T2DM), postprandial hyperglycemia drives complications such as neuropathy, retinopathy, nephropathy, and cardiovascular disease (Ćorković et al., 2022; Kashtoh and Baek, 2023). Inhibiting α-amylase and α-glucosidase is thus a key strategy to control glucose. Natural inhibitors from plants and mushrooms, rich in flavonoids (catechin, quercetin, rutin), phenolic acids (chlorogenic, gallic) and polysaccharides, bind to enzyme active sites or adjacent regions, altering structure or competing with substrates to reduce starch hydrolysis and glucose release (Cui and Li, 2025; Ćorković et al., 2022; Subramanian and Prasath, 2014; Sknepnek et al., 2025). These compounds offer safe dietary approaches to complement or substitute synthetic drugs like acarbose, highlighting the therapeutic potential of natural sources for T2DM management.

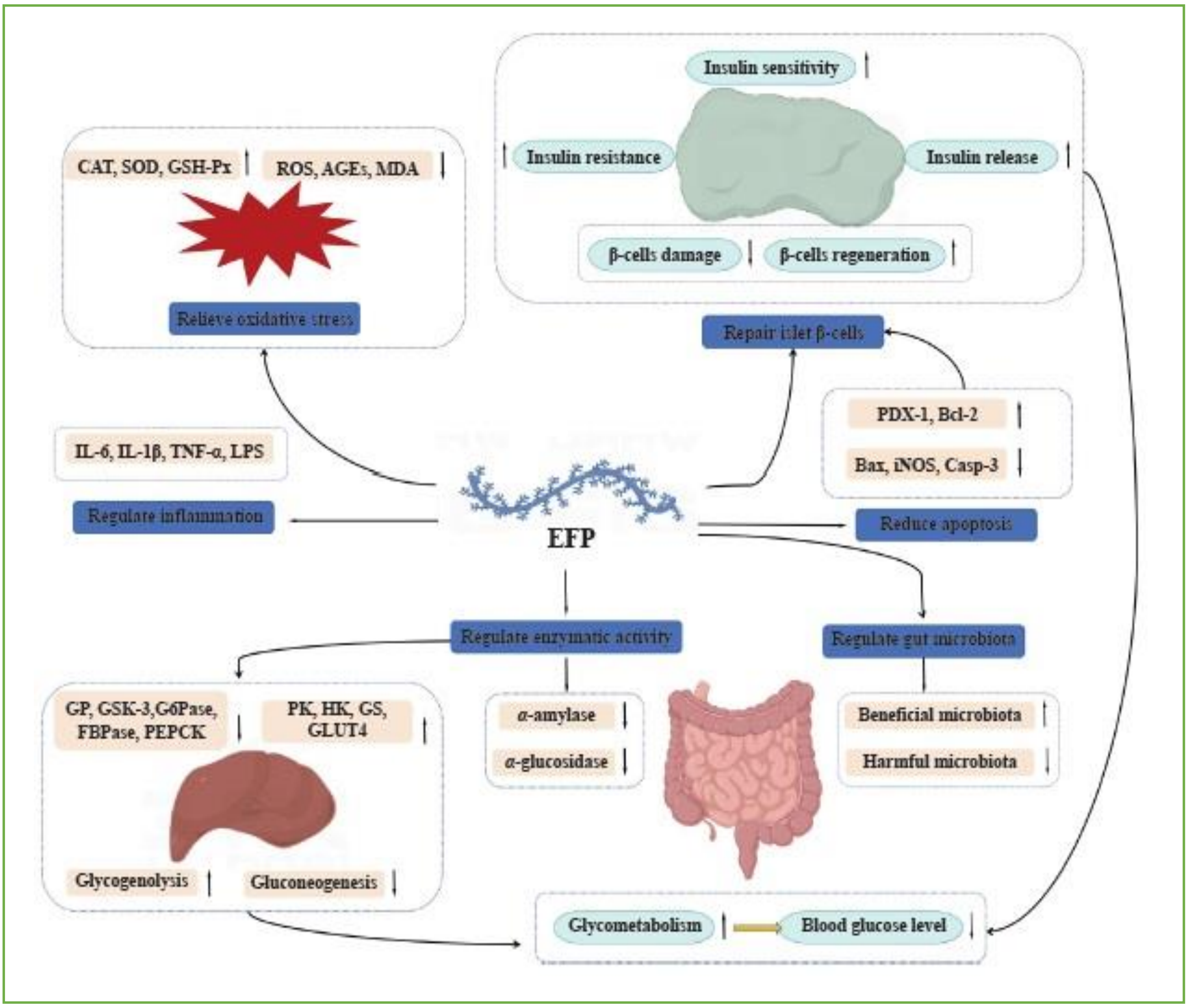


Figure 4. Hypoglycemic Mechanisms of Edible Fungus Polysaccharides (EFP) (Cui and Li 2025).

1.5. Antibacterial activity of natural compounds

Bioactive compounds from plants and mushrooms, including flavonoids, tannins, terpenoids, and alkaloids, exert antibacterial effects via multiple mechanisms such as disruption of cell walls and membranes, inhibition of protein and nucleic acid synthesis, enzyme deactivation, and interference with quorum sensing to prevent biofilm formation (Saquib et al., 2021). Acting on multiple bacterial targets, these compounds reduce the risk of resistance and may enhance the efficacy of conventional antibiotics or help mitigate antimicrobial resistance (AMR). (Yuan et al., 2021). Rich in phenols and flavonoids, plant- and mushroom-derived molecules show significant activity against Gram-positive and Gram-negative bacteria, including Staphylococcus aureus, and their safety, natural occurrence, and synergistic potential make them promising alternatives or complements to traditional antibiotics (Saquib et al., 2021; Yuan et al., 2021).

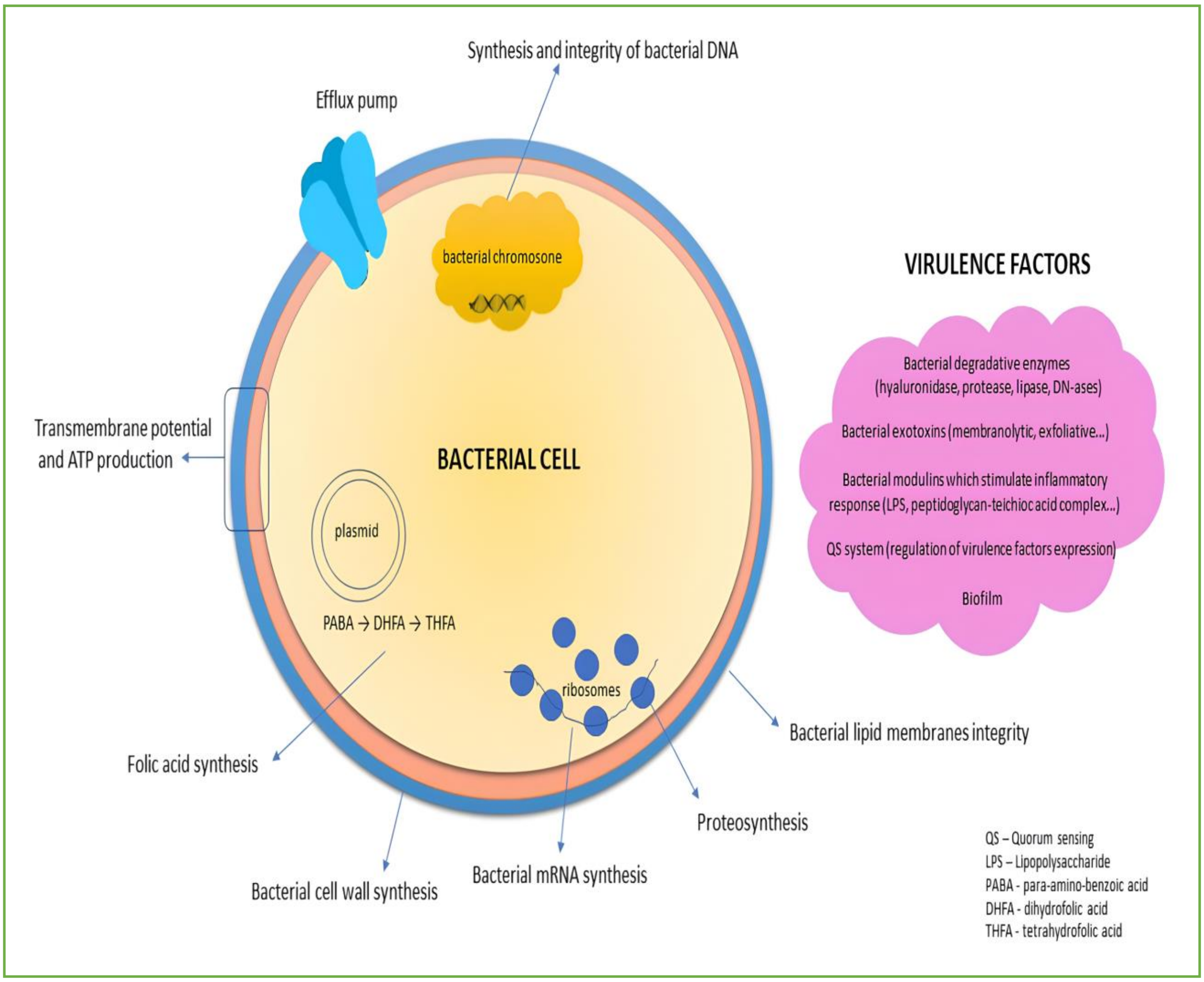


Figure 5. Molecular Targets of Bioactive Natural Antibacterial Compounds (Bittner Fialová et al., 2021).

2. Literature review

2.1. Mushroom : understanding their role in human health

Edible mushrooms are not only a dietary staple but also a rich source of proteins, fibers, vitamins, and minerals, along with bioactive compounds such as polysaccharides and polyphenols that provide antioxidant, anti-inflammatory, and immunomodulatory effects. Different species contain complete proteins, soluble and insoluble fibers, unsaturated fatty acids, ergosterol (a precursor of vitamin D), and tocopherols. The composition of these compounds varies depending on the species, harvest stage, and growth conditions, making edible mushrooms an excellent choice for health-promoting and functional nutrition. (Konsue et al., 2025).

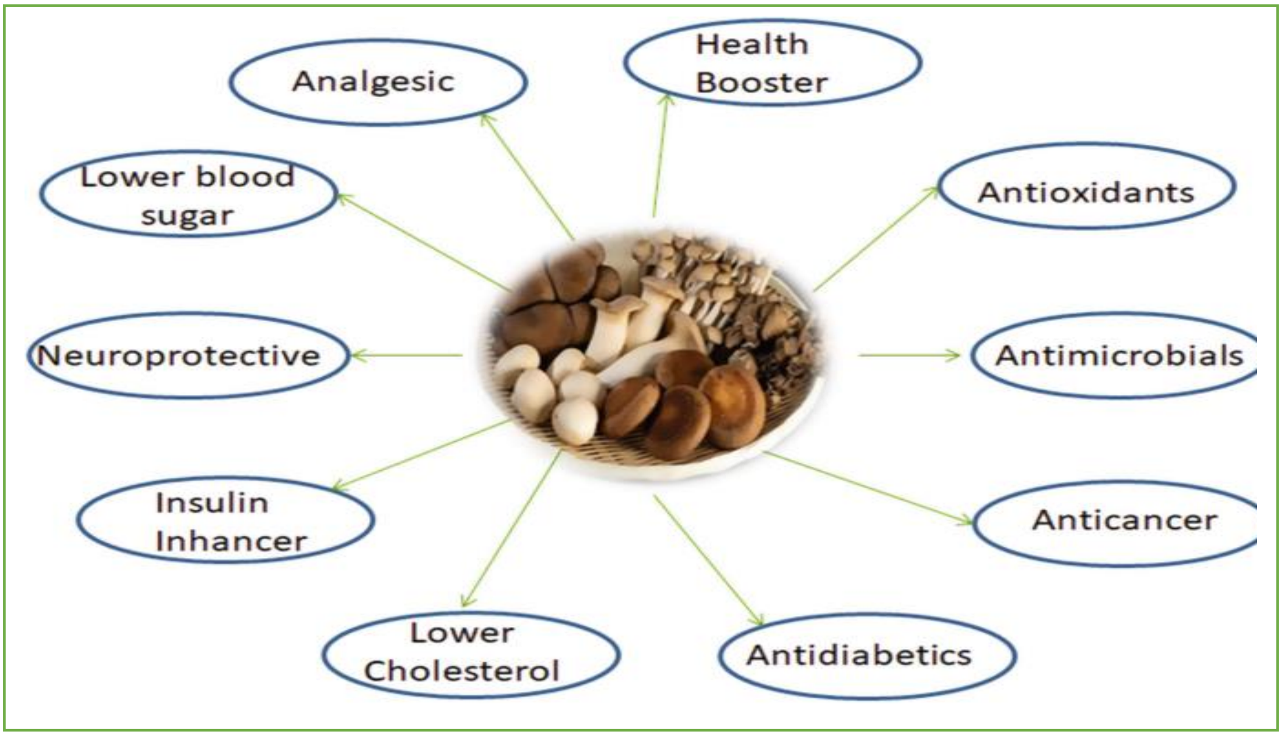


Figure 6. Nutritional values of edible mushrooms (Hait et al., 2025).

2.2. *Pleurotus eryngii*

2.2.1. Taxonomy

Pleurotus eryngii (DC.) Quél., 1872, belongs to the kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Agaricales, family Pleurotaceae, and genus *Pleurotus*. Recognized varieties include *P. eryngii* var. *eryngii*, var. *ferulae*, var. *tuoliensis*, and var. *elaeoselini*. Morphologically similar species such as *P. nebrodensis*, *P. fossulatus*, and *P. hadamardii* previously caused taxonomic confusion, but molecular analyses and detailed morphological studies now allow accurate differentiation (Zervakis et al., 2014).

Commonly known as the "royal oyster mushroom" (فطر المحار الملكي) or "Cardoncello," *P. eryngii* is valued for its edible and medicinal properties, being rich in proteins, vitamins, and minerals, while low in carbohydrates and cholesterol (Calabretti et al., 2021). It is widely cultivated and consumed, particularly in China (Xingbaogu), Europe, the Middle East, North America, and Asia (Yang et al., 2020; Zhang et al., 2020; Mariga et al., 2014).

Figure 7. Wild mushroom *Pleurotes eryngii.*

2.2.2. Morphological description and geographic distribution

Pleurotus eryngii typically develops on plant roots or at the base of stems, appearing singly or in small clusters from September to June. Its distribution spans from Morocco to China, covering southern and central Europe, parts of the Middle East, and western Asia, reflecting its adaptation to Apiaceae host plants (Zervakis et al., 2014).

Morphologically, it is characterized by a gray to grayish-brown cap (4–8 cm), a white stipe (3.5–15 cm), and relatively large basidiospores, often associated with *Eryngium* species (De Gioia et al., 2005; Parisa Maftoun et al., 2015). The culinary value of *P. eryngii* is linked to its dense plectenchyma tissue and low water content, enhancing flavor and suitability for cooking (Yu et al., 2018).

The species is also easily cultivated, yielding high productivity, and in Uzbekistan it grows on *Ferula* roots at altitudes of 1000–2500 m during spring (Zhang, Li, et al., 2020; Ilyor et al., 2025).

2.2.3. Ethnomedicinal and traditional uses

Beyond its nutritional value, *P. eryngii* has been used in traditional medicine as a general tonic and a source of fiber and nutrients, and its agricultural residues have been utilized as animal feed or for biodegradation of industrial compounds (Zervakis et al., 2014). In several cultures, it has been traditionally consumed to promote health and prevent disease (Yildirim et al., 2012).

In Uzbekistan, it is called “Shanbush” or the “white desert mushroom” and consumed in dishes such as Bichak, with believed antidiabetic properties (Ilyor et al., 2025). In traditional Oriental medicine, it was referred to as the “flower paradise mushroom” during the Song Dynasty (420–479 AD) and prepared as tea to enhance immunity and treat conditions such as weakness, fatigue, skin diseases, and musculoskeletal issues. Dried fruiting bodies were also employed in the treatment of cancer, sciatica, and vascular disorders, with similar uses recorded in folk medicine in Europe, South America, and Africa (Mariga et al., 2014).

Historically, the mushroom was used whole or as aqueous extracts, concentrates, alcoholic beverages, or powders. Pharmacological studies confirmed the cytotoxic activity of *P. eryngii* extracts against human cancers and immunomodulatory effects through stimulation of splenic and natural killer (NK) cells (Mariga et al., 2014).

2.2.4. Nutritional composition

Edible mushrooms of the genus *Pleurotus* are nutritionally rich, containing significant amounts of protein (15.4–28.6%), carbohydrates (61.3–84.1%), and dietary fiber (3–33.3%) (Raman et al., 2021). *P. eryngii* has higher protein content than vegetables and is rich in essential amino acids, particularly lysine, making it a promising alternative to animal and plant proteins (Wang et al., 2024). Its protein exhibits functional properties useful in food processing, including emulsification, foaming, and water and oil retention, with high stability (Chen et al., 2021; Lv et al., 2022).

This species also contains β-glucans, polysaccharides, unsaturated fatty acids, phenolic compounds, vitamins (C and B12), and minerals, with low fat content (Petraglia et al., 2023;

Yu et al., 2018). β-Glucans occur as linear or branched polysaccharides (β-(1,3-1,6) and β-(1,3) linkages) and occasionally as heteroglycans with sugars like glucuronic acid, xylose, galactose, mannose, arabinose, and ribose, with properties depending on linkage type, branching, molecular weight, and structure (Corrêa et al., 2016; Petraglia et al., 2023).

Commercially, *P. eryngii* is valued for its taste, capacity to degrade agricultural waste, and adaptability to various temperatures (Corrêa et al., 2016; Petraglia et al., 2023). It exhibits multiple functional and biological activities, including antitumor, antioxidant, immune-modulating, hepatoprotective, anti-inflammatory, antiviral, hypoglycemic, and lipid-lowering effects, confirming its role as a functional food (Corrêa et al., 2016).

2.2.5. Polyphenols and flavonoids and LC-MS/MS findings in previous studies

P. eryngii extracts are rich in polyphenols and flavonoids, with flavonoid content exceeding 5 mg/g (Yu et al., 2018; Ryu et al., 2018). HPLC analysis revealed a diverse phenolic profile, directly linked to strong antioxidant activity and cellular protection, supporting its use as a functional food (Yildirim et al., 2012).

Key phenolic compounds identified include chlorogenic, syringic, ferulic, p-coumaric, caffeic, t-cinnamic, and vanillic acids, along with naringenin (Gąsecka et al., 2016). Additional major compounds include gallic acid, protocatechuic acid, vanillin, naringin, hesperetin, formononetin, and biochanin A, reflecting the high diversity of phenolics and flavonoids and reinforcing *P. eryngii* as a natural antioxidant source for food and pharmaceutical applications (Alam et al., 2011).

2.2.6. Reported antioxidant activities

P. eryngii is a rich source of bioactive compounds with potent antioxidant properties, making it a valuable functional food. Chromatographic analysis identified phenolic compounds such as chlorogenic, syringic, ferulic, and p-coumaric acids, contributing to its antioxidant activity (Gąsecka et al., 2016). The mushroom also enhances cellular resistance to oxidative stress via activation of the transcription factor Nrf2 (Teniou et al., 2022).

Polysaccharides and β-glucans display antioxidant effects through free radical scavenging, iron (Fe^{2+}) chelation, inhibition of lipid peroxidation, and increased activity of antioxidant enzymes (SOD, CAT, GPx) (Corrêa et al., 2016; Petraglia et al., 2023). Their mechanism resembles that of phenolic compounds, involving hydrogen atom transfer in neutral polysaccharides or electron transfer in acidic polysaccharides, reducing ROS production and protecting against oxidative damage (Zhang et al., 2020; Petraglia et al., 2023).

In vitro and *in vivo* studies confirm that polysaccharides from *P. eryngii* fruiting bodies or cultures exert strong antioxidant activity through radical scavenging, iron chelation, inhibition of lipid peroxidation, and stimulation of antioxidant enzymes (Petraglia et al., 2023; Yan et al., 2019).

2.2.7. Anti-inflammatory activities

P. eryngii is recognized as a functional food of medical relevance due to its richness in bioactive compounds. Its consumption provides phenolic compounds and flavonoids, which exhibit strong antioxidant potential, modulate inflammatory pathways, and enhance immune defense (류혜숙 et al., 2018). These compounds act as free radical scavengers and modulators of signaling cascades involved in inflammation, supporting efficient immune responses.

The mushroom is also rich in polysaccharides (40–80 g/100 g) and proteins (10–35 g/100 g), both contributing to health-promoting effects. Polysaccharides demonstrate immunoregulatory, anti-inflammatory, and antioxidant activities, while proteins provide additional bioactivities, including antimicrobial and immune-modulating effects (Wu et al., 2023; Zhang et al., 2020).

P. eryngii exhibits a broad spectrum of health-promoting effects, including antibacterial, antioxidant, and immune-regulating activities, with anti-inflammatory activity being particularly well-documented (Wang et al., 2024). Its proteins have been shown to modulate cytokine production and interfere with pro-inflammatory pathways, potentially acting synergistically with polysaccharides and phenolic compounds to enhance anti-inflammatory effects (Wang et al., 2024).

Overall, *P. eryngii* represents a promising natural source of anti-inflammatory agents, combining secondary metabolites, polysaccharides, and proteins, supporting immune regulation and inflammation control, and highlighting its potential in functional foods and pharmaceutical applications.

2.2.8. α-Amylase inhibition

Medicinal mushrooms are a source of bioactive compounds capable of inhibiting carbohydrate-digesting enzymes such as α-amylase and α-glucosidase. Fatty acids (oleic, linoleic, palmitic), triterpenes, phenols, proteins, and polysaccharides can interact with enzymatic active sites, reducing starch hydrolysis and postprandial glucose absorption, explaining their antidiabetic effects (Zheng et al., 2020).

P. eryngii polysaccharides from fruiting bodies and mycelium are high molecular weight (≈ 4.06–4.18 $\times 10^6$ Da) with branched filamentous structures, composed of α-D-Glcp, β-D-Galp, and α-D-Manp with diverse linkages and side-chain variations. These polysaccharides exhibit significant competitive inhibition of α-glucosidase, supporting their potential as bioactive agents in food and pharmaceutical applications (Liu et al., 2010; Zheng et al., 2020).

2.2.9. Antimicrobial properties

P. eryngii is a natural source of bioactive compounds with potent antibacterial and antifungal properties (Akyuz and Kirbag, 2009). Extracts exhibit significant inhibition of bacterial species, including oral pathogens such as *Staphylococcus aureus*, *Streptococcus criceti*, *S. mutans*, *S. ratti*, *S. sobrinus*, and *Actinomyces viscosus* (Banerjee et al., 2018). Ethanolic and acetone extracts also show activity against multidrug-resistant strains, including *Enterobacter cloacae* and *Escherichia coli* (Yu et al., 2018), highlighting their potential as natural antimicrobial agents.

Polysaccharides from *P. eryngii* display neuroprotective activity, supporting prevention of neurological diseases (Zhang et al., 2020). The mushroom also offers additional benefits, such as improved liver function, enhanced immune response, reduced lipid and cholesterol levels, and roles in pluripotent cell reprogramming (Petraglia et al., 2023; Zheng et al., 2020), reinforcing its nutritional and functional value as a multi-application functional food.

2.3. Vegetables : understanding their role in human health

Vegetables play a crucial role in human health, not only as a source of essential macronutrients such as proteins and dietary fiber, but also through their by-products, which are frequently discarded during harvesting, processing, and consumption. These by-products, including peels, seeds, hulls, and pomace, are rich in proteins, dietary fiber, polyphenols, and other bioactive compounds that exhibit antioxidant, antimicrobial, anticancer, antidiabetic, and cholesterol-lowering activities. Efficient utilization of these vegetable and fruit by-products can help reduce food waste and provide functional ingredients for the development of nutritionally enhanced foods. Overall, vegetables and their by-products offer both direct nutritional benefits and potential applications in functional food development, thereby contributing to human health and food security (Lau et al., 2021).

2.4. *Foeniculum vulgare*

2.4.1. Taxonomy

According to botanical nomenclature, *Foeniculum vulgare* Mill. is the oldest valid name within the genus *Foeniculum*, as correctly published by Philip Miller in the eighth edition of the *Gardener's Dictionary* in 1768. Earlier attempts, such as by Hill, were not valid due to non-adherence to binomial nomenclature rules (Badgujar et al., 2014). In Arabic, it is commonly known as "الشمر", and in Algerian dialect as "البسباس".

F. vulgare Mill. is an aromatic and medicinal plant of the Apiaceae family, widely cultivated and historically recognized for its distinctive flavor, with over one hundred vernacular names documented. Taxonomically, it is classified as follows: Kingdom Plantae; Division Tracheophyta; Subdivision Spermatophytina; Class Magnoliopsida; Order Apiales; Family Apiaceae; Genus *Foeniculum*; Species *vulgare* (Vella et al., 2024).

2.4.2. Morphological description and geographical distribution

F. vulgare is a biennial or perennial aromatic herb native to the southern Mediterranean basin, now naturalized across Asia, North America, and Europe. It is cultivated in fields and grows spontaneously in the wild. In Algeria, particularly in western regions, it is also widely distributed and traditionally used. Historically, it was known to the ancient Egyptians, Romans, Indians, and Chinese, and later promoted by Emperor Charlemagne in Central Europe. Today, it remains important in French and Italian cuisines, with all aromatic parts used and fleshy shoots and leaves commonly consumed as vegetables, especially in southern Italy (Badgujar et al., 2014).

The plant has an erect, cylindrical, striated green stem with ascending branches. Leaves are finely divided (tripinnate to quadripinnate) with basal sheaths, ending in threadlike segments

(~0.5 mm wide, up to 4 cm long). Flowers are small, yellow, arranged in flat umbels with 5–30 rays, blooming between July and August. Fruits are ovate to elongated (3–5 mm × 1.5–2 mm) with prominent ribs; seeds are greenish-yellow and aromatic in cultivated forms (Badgujar et al., 2014).

In southern Italy, wild perennial forms occur up to 700 m altitude in rocky submontane and coastal areas, with striated stems and deeply divided leaves. Certain cultivars develop a "false bulb" (gromolo). Flowers are mainly hermaphroditic, arranged in yellow umbels, while narrow threadlike parts (~8 × 3 mm) exhibit strong aroma and sweet taste (Vella et al., 2024).

Figure 8. *F. vulgare* leaves.

2.4.3. Ethnomedicinal and traditional uses

F. vulgare is a traditional plant valued in both medicine and food. Ethnobotanical studies report the use of leaves, stems, fruits, and seeds in treating a wide range of disorders, including respiratory, renal, gastrointestinal, and metabolic conditions, as well as for galactagogue purposes. Seeds are employed against bad breath, and leaves and flowers provide natural pigments for cosmetics, textiles, wood, and food coloring (Badgujar et al., 2014).

In medical traditions such as Ayurveda, Unani, and Siddha, as well as in folk practices in India and Iran, fennel leaves are prescribed for bronchitis, chronic cough, hyperglycemia, kidney stones, diuretic relief, nausea, and eye diseases (Kooti et al., 2015). Historically, its fruits were chewed for dyspepsia and gastric disorders, and the plant has been noted for sedative effects on the nervous system since the 5th century (Vella et al., 2024).

Culinarily, all parts are used from fresh leaves in salads to soups, stews, and traditional dishes in Italian, Portuguese, and Spanish cuisines. This dual nutritional and medicinal role underscores the significance of *F. vulgare* as both an aromatic vegetable and a medicinal plant (Badgujar et al., 2014; Vella et al., 2024).

2.4.4. Nutritional composition

F. vulgare is rich in essential nutrients, including protein, fiber, carbohydrates, fats, vitamins, and minerals. It contains vitamin C, B vitamins (thiamin, riboflavin, niacin), and minerals such as calcium, potassium, phosphorus, and sodium. The plant is also abundant in essential fatty acids, contributing to nutritional balance and health (Anka et al., 2020).

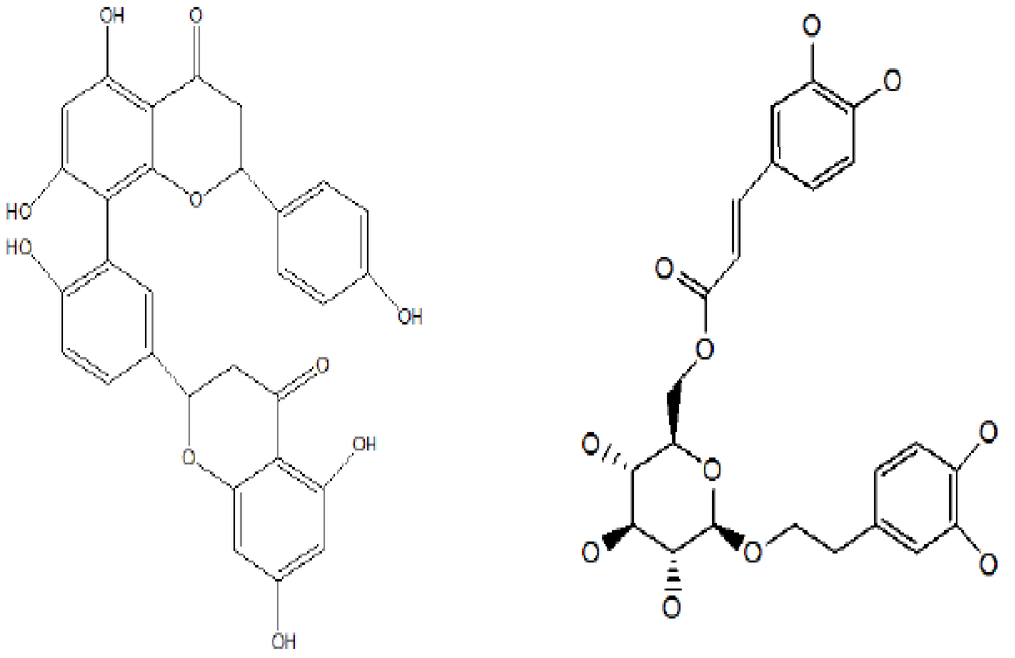
All plant parts, seeds, roots, leaves, and fruits, are utilized (Noreen et al., 2023). Fennel seeds contain 6.3% water, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber, and 42.3% carbohydrates. Leaves are rich in calcium, potassium, sodium, iron, phosphorus, and vitamins B1, B2, B3, and C (Farid et al., 2020; Noreen et al., 2023). Fruits contain 10–12% oil, with major fatty acids including petroselic (6%), oleic (22%), linoleic (14%), and palmitic (4%). Their essential oil contains 4–6% volatiles, mainly trans-anethole (50–80%) and limonene (5%), providing the characteristic aroma (Castaldo et al., 2021; Noreen et al., 2023). This nutritional profile underpins fennel’s health-promoting properties, supporting cardiovascular, metabolic, and overall well-being.

2.4.5. Polyphenols, Flavonoids and LC-MS/MS Findings in Previous Studies

F. vulgare is a rich source of phenolic and flavonoid compounds responsible for its biological activity. Methanolic and aqueous extracts of seeds, as well as essential oils, contain active compounds including caffeoylquinic acid derivatives, rosmarinic acid, eriodictyol-7-rutinoside, quercetin-3-O-galactoside, and kaempferol-3-O-glucoside, with trans-anethole as the main component of the essential oil, contributing to antioxidant and antimicrobial properties (Anka et al., 2020; Noreen et al., 2023; Vella et al., 2024).

LC-MS/MS and HRESIMS analyses identified additional phenolic acids (3-, 4-, 5-O-caffeoylquinic and di-caffeoylquinic derivatives) and flavonoids, including quercetin and kaempferol derivatives. These compounds neutralize free radicals by donating electrons or hydrogen atoms, preventing oxidative damage and supporting protective effects against cancer, inflammation, and cardiovascular disease (Noreen et al., 2023).

These chemical insights provide a foundation for exploring fennel’s applications in pharmacology, nutrition, and the development of natural therapeutic agents.



3, 8-binaringenin

3, 4-dihydroxy-phenethylalchohol-6-O-caffeoyl-β-D-glucopyranoside

Figure 9. Structure of phenolic compounds isolated from *F. vulgare* (Mehra et al., 2021).

2.4.6. Reported antioxidant activities

F. vulgare is a rich source of natural compounds that counteract oxidative stress by scavenging free radicals and interrupting harmful oxidative reactions. Its activity is attributed to phenolic compounds, flavonoids, and essential oils containing monoterpenes such as trans-anethole, D-limonene, and β-myrcene, which enhance antioxidant defenses and protect against chronic diseases including cardiovascular disorders, cancer, and inflammation (Vella et al., 2024).

Methanolic and aqueous extracts of fennel seeds effectively inhibit lipid peroxidation and neutralize free radicals, showing higher antioxidant capacity than some synthetic antioxidants (Anka et al., 2020). Active compounds include caffeoylquinic acids, rosmarinic acid, eriodictyol-7-rutinoside, quercetin-3-O-galactoside, and kaempferol-3-O-glucoside (Noreen et al., 2023). Studies using DPPH, H_2O_2, FRAP, and Folin-Ciocalteu assays confirmed that both aqueous and alcoholic extracts neutralize free radicals and surpass certain synthetic antioxidants such as BHA and BHT (Marinov and Valcheva-Kuzmanova, 2015; Noreen et al., 2023).

Mechanistically, phenolic and flavonoid compounds donate electrons and hydrogen atoms, chelate pro-oxidant metals like iron, and inhibit lipid peroxidation, reducing malondialdehyde (MDA) accumulation. For example, ethanolic extracts at 100 mg exhibited 99.1% efficacy, compared to 77.5% for aqueous extracts and 36.1% for α-tocopherol (Noreen et al., 2023).

2.4.7. Anti-inflammatory activities

Methanolic extracts of *F. vulgare* seeds and fruits exhibit significant anti-inflammatory effects in both acute and chronic models (Anka et al., 2020). Animal studies demonstrated that these extracts reduce carrageenan-induced plantar edema, arachidonic acid-induced swelling, and arthritis markers, likely via inhibition of cyclooxygenase (COX) and lipoxygenase pathways, similar to NSAIDs. They also decrease type IV hypersensitivity reactions, lower malondialdehyde (MDA) levels, and modulate antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), while increasing plasma high-density lipoprotein (HDL) concentration, indicating protection against oxidative stress and inflammation (Mostafa et al., 2021; Noreen et al., 2023).

The anti-inflammatory mechanism involves inhibition of proinflammatory cytokine production and reduction of oxidative stress through modulation of the balance between antioxidant enzymes and free radical generation (Noreen et al., 2023).

2.4.8. α-Amylase inhibition

F. vulgare and its essential oils may regulate blood glucose by inhibiting α-amylase, reducing starch hydrolysis and postprandial glucose absorption. In diabetic animal models, fennel oil lowered blood glucose and enhanced antioxidant enzyme activity, including glutathione peroxidase, suggesting its potential as a natural adjunct therapy for diabetes (Anka et al., 2020).

Aqueous extracts of fennel also demonstrated antidiabetic activity in streptozotocin-induced diabetic rats, significantly reducing glucose and cholesterol levels (Noreen et al., 2023). This activity is primarily attributed to phenolic compounds and flavonoids, which inhibit α-amylase and protect pancreatic tissue from oxidative stress (Noreen et al., 2023).

2.4.9. Antimicrobial properties

F. vulgare extracts and essential oils exhibit significant antimicrobial activity against pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* spp. Active compounds such as dillapional, scopoletin, linoleic acid, 1,3-benzenediol, oleic acid, 2,4-undecaprenyl, 5-hydroxyfuranocoumarin, and anethole contribute to this efficacy, which involves disruption of bacterial cell walls and membranes as well as stimulation of nitric oxide (NO) and reactive oxygen species (ROS) in immune cells (Anka et al., 2020; Kwiatkowski et al., 2015; Noreen et al., 2023).

Aqueous and ethanolic extracts inhibit a wide range of bacteria, including *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus faecalis*, and *Acinetobacter baumannii*, with MIC values ranging from 20–80 mg/ml for aqueous extracts and 5–15 mg/ml for ethanolic extracts (Ahmed et al., 2019; Moradi et al., 2020).

Beyond its antimicrobial effects, fennel demonstrates anxiolytic, sedative, gastroprotective, estrogenic, lipid-lowering, hepatoprotective, and anticancer activities. Its anxiolytic and sedative effects are attributed to phytoestrogens acting on GABA-A receptors, while anticancer activity is linked to anethole-mediated suppression of NF-κB signaling and induction of apoptosis. Fennel also improves coronary circulation, reduces cholesterol and triglycerides, attenuates liver inflammation, inhibits lipid peroxidation, and prevents fibrosis, supporting its broad therapeutic potential (Anka et al., 2020; Kaveh et al., 2023; Noreen et al., 2023).

PART TWO

MATERIAL AND METHODS

PART TWO : MATERIAL AND METHODS

1. Plant and mushroom meterials

The fruiting bodies of *P. eryngii* were collected in March 2024 in Tiferet Mountain forest, located in the province of Saida, western of Algeria (34°56'12.7"N, 0°22'49.4"E), at a high altitude of more than 800 meters above sea level. All *P. eryngii* samples were mature and growing on Doum palm (*Hyphaene thebaica*) residues under the same environmental conditions. This mountainous region is characterized by a semi-arid continental climate, with hot, dry summers and cold winter.

The mushroom was identified and classified based on its macroscopic and microscopic characteristics by Dr. Lounis Youcef Khodja, (An Algerian Researcher specialized in Genetics, Evolutionary Biology, and Systematics of Mushrooms at the Department of Biology and Plant Ecology, Ferhat Abbas University of Setif).

The cultivated *F. vulgare* leaves were harvested in March 2024 in western Algeria, specifically in the Sira fields of Mostaganem province, at the coordinates: 35°46'01.1"N 0°11'18.3"E. This region is characterized by a Mediterranean climate, with rainfall between 350 mm and 400 mm, and moderate temperatures.

Immediately after collection, the fruiting bodies and the leaves were cleaned, sliced, and air-dried at room temperature (25 °C) for several days until complete dehydration. The dried materials were then ground into a fine powder. A powder was obtained and stored in airtight containers at room temperature until further analysis.

2. Nutritional composition, energy value, and physicochemical parameters

2.1. Determination of total proteins

- **Principle :** The Bradford method yielded higher protein content values in plant samples, suggesting its superior efficiency for such matrices (Zambari et al., 2021). The method is based on the binding of Coomassie Blue dye to proteins, with the efficiency of binding depending on the amino acid composition (Rekowski et al., 2021) (Figure 12).

- **Procedure:** Protein determination was performed according to the method of Bradford (1976) using Coomassie Brilliant Blue (CBB) as a reagent. The Bradford reagent was prepared by dissolving 100 mg of CBB in 50 mL of absolute ethanol, followed by the addition of 100 mL of orthophosphoric acid, and the final volume was made up to 1000 mL with distilled water.

Bovine serum albumin (BSA, Sigma) was used as the standard protein. A calibration curve was established using a stock solution of BSA (1 mg/mL).

To quantify the protein content of the sample, 100 µL of the sample was added to 5 mL of the Bradford reagent. After incubation at room temperature for 10 minutes, the absorbance was measured at 595 nm against a reagent blank. The protein concentration was calculated by comparison with the standard calibration curve. The protein content was expressed on a dry weight basis.

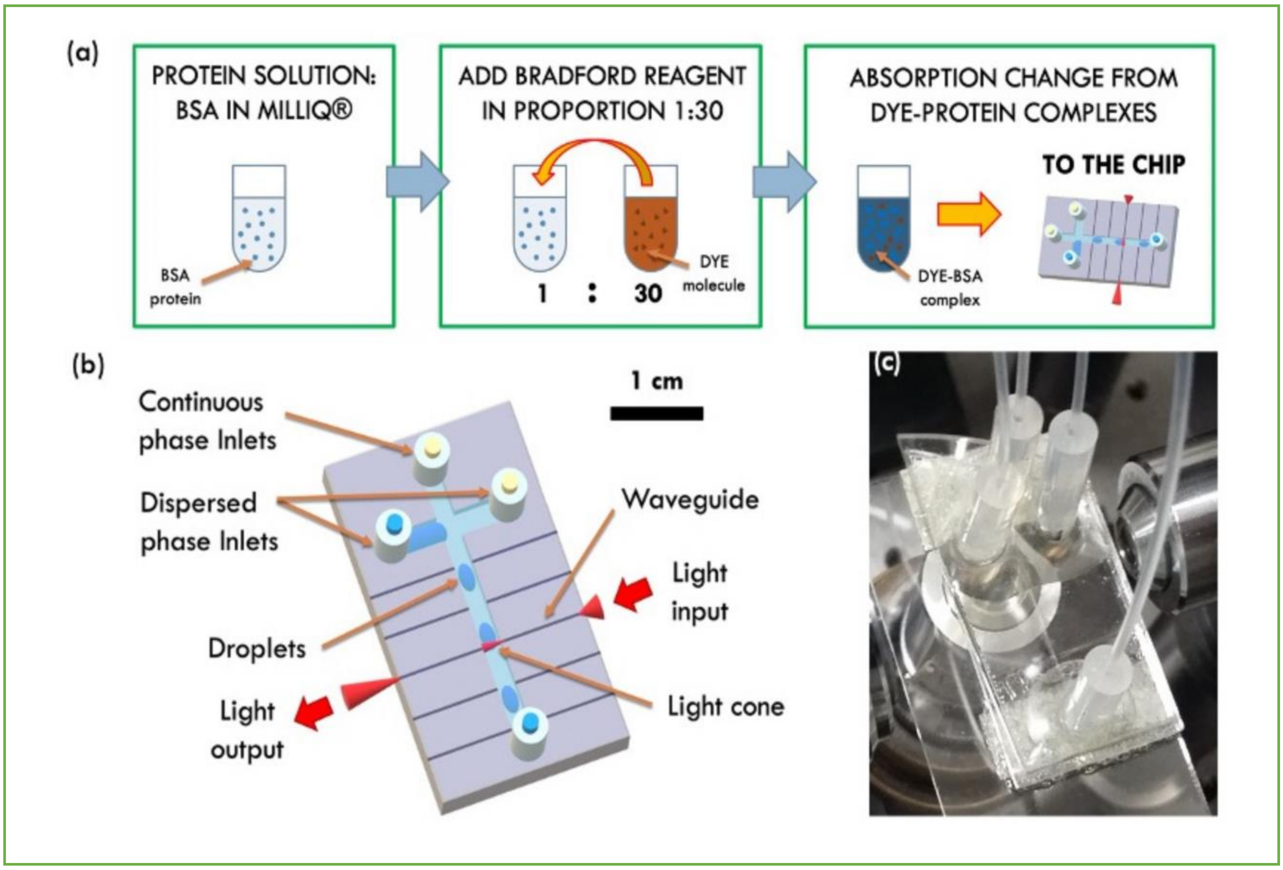


Figure 10. Schematic overview of the Bradford protein assay (Zanini et al., 2022).

2.2. Determination of the fat

- Principle : The principle of this method, as described by Goldsworthy et al. (1972), is based on the formation of pink-colored complexes between lipids and concentrated sulfuric acid in the presence of vanillin and orthophosphoric acid when heated. These complexes exhibit maximum absorbance at 530 nm, allowing the quantification of total lipids.

- **Procedure :** The total amount of lipids was extracted and quantified following the protocol described by Enneb et al. (2020) (based on Shibko et al. (1967) and Goldsworthy et al. (1972)), with minor modifications. First, 100 mg of finely powdered dry plant material were homogenized with 1 mL of pre-cooled trichloroacetic acid (TCA, 20%), followed by repeated and extended vortexing in an ice bath for 15 minutes (in place of sonication). The homogenate was then centrifuged at 5000 rpm for 10 minutes at 4 °C. The resulting pellet was extracted with 1 mL of a chloroform/ether mixture (1:1, v/v), vortexed vigorously, and centrifuged again under the same conditions. The lipid-containing supernatant was collected and evaporated completely to dryness.

The residue was resuspended in 1 mL of concentrated sulfuric acid (96%), and hydrolysis was carried out by heating at 100 °C for 10 minutes in a boiling water bath. After cooling to room temperature, 200 µL of the hydrolyzed sample were transferred to a new tube, and 2.5 mL of phospho-vanillin reagent were added for color development. The samples were incubated for 30 minutes in the dark, and absorbance was measured at 530 nm against a reagent blank. The phospho-vanillin reagent was prepared by dissolving 0.38 g of vanillin in 55 mL of hot distilled water and diluting to 250 mL with 85% orthophosphoric acid. Oleic acid was used as standard and diluted in concentrated sulfuric acid to obtain a final concentration of 1 mg/mL. The Fat content was expressed on a dry weight basis.

2.3. Determination of ash content

- **Principle :** The determination of total ash content is based on the method of incinerating organic matter at high temperatures until complete combustion is achieved. This leaves only the inorganic mineral residue (ash). The ash content is expressed as a percentage of the initial dry weight of the sample (Li et al., 2009).

- **Procedure:** Approximately 1 g of the previously dried sample was weighed into a pre-weighed porcelain crucible. The crucible was placed in a muffle furnace and subjected to incineration at 550 °C ± 2 °C for 8 hours, until white ash was obtained and a constant weight was reached. The mass loss during combustion reflects the amount of organic matter, while the residue corresponds to the total ash content. The ash percentage was calculated relative to the dry weight of the sample (Rao and Xiang 2009).

4.4. Determination of total sugars

- **Principle** : The total sugar content was determined following the phenol–sulfuric acid method as described by DuBois et al. (1956). This colorimetric technique is based on the reaction of carbohydrates with concentrated sulfuric acid and phenol, leading to the formation of orange-yellow chromophores. The intensity of the color developed is proportional to the sugar concentration and is measured spectrophotometrically at 485 nm (Figure 13).

- **Procedure** : According to the method adapted by Tajini et al. (2020), 1 mL of the sample was transferred into a clean test tube, followed by the addition of 50 µL of phenol (75% in water). Immediately afterward, 2.5 mL of concentrated sulfuric acid was rapidly added without letting it run along the tube walls. The reaction mixture was quickly vortexed under a fume hood due to the highly exothermic nature of the reaction. To stabilize the temperature and avoid degradation, the tubes were immediately cooled on ice, then incubated at 30 °C for 10 minutes. After incubation, the absorbance was measured at 485 nm using a spectrophotometer against a reagent blank. The total sugar content was expressed on a dry weight basis.

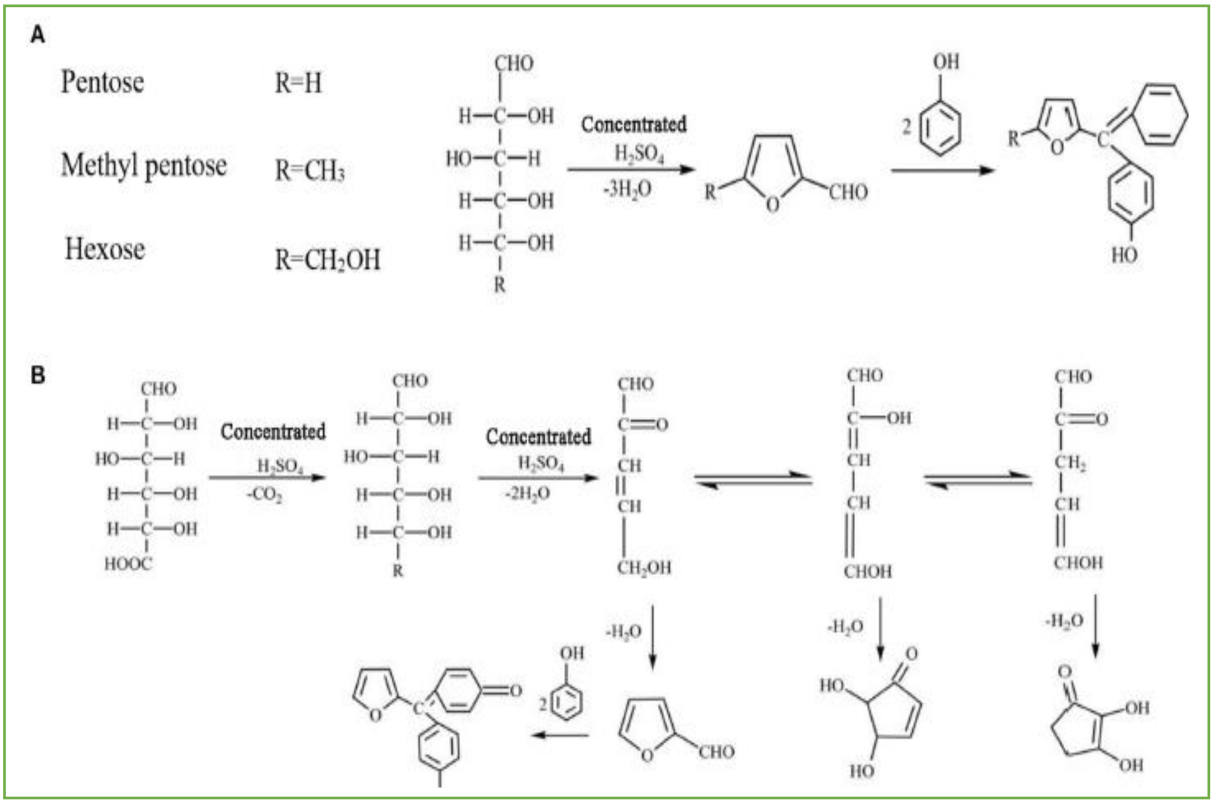


Figure 11. Principle of the phenol–sulfuric acid method Yue et al. (2022).

2.5. Determination of total carbohydrates

The total carbohydrate content was estimated by difference of the mean values, according to the method described by Md Noh et al. (2020), using the following formula:

Total carbohydrates (%) = 100 − [Ash (%) + Protein (%) + Lipid (%)]

where total carbohydrates include both soluble sugars and dietary fiber.

2.6. Determination of total energy

The total energy content was calculated according to the standard equation described by Irshad et al., (2023) :

Energy (kcal) = [4 × (grams of protein + grams of carbohydrates)] + [9 × grams of lipids]

2.7. Determination of moisture content

Moisture content was determined by drying 3.0 grams of fresh sample in an oven at 105 °C until a constant weight was achieved. The dried samples were then cooled to room temperature in a desiccator before being reweighed. The moisture content was calculated based on the weight loss during drying. It can also be expressed as: (Hamza et al., 2016).

Moisture content (%) = 100% − dry matter (%).

3. Study of secondary metabolites

3.1. Extraction of bioactive compound

3.1.1. Maceration extraction

The extraction process was conducted according to the method described by Hikmawanti et al. (2021). First, 10 grams of the studied sample powder were dissolved in 100 mL of 80% ethanol, solvent, then the mixture was stirred with an agitator for 5 hours at room temperature. After that, the filtrate was recovered using Whatman paper n° 3. The extraction process was repeated twice consecutively on the remaining residue using the same method. The collected filtrates were then evaporated using a rotary evaporator at 45 °C.

3.1.2. Decoction extraction

The extraction was performed according to the method of Chaoua et al. (2019) with some slight modifications. Briefly, 10 grams of the studied sample powder were dissolved in 100 mL of distilled water and heated at 65 °C for 30 minutes. After that, a rotary evaporator was used to completely evaporate the distilled water, under reduced pressure and at a temperature below 45 °C. Finally, the obtained extract was stored at 4 °C.

3.1.3. Yield calculation

The yield refers to the mass of the extract obtained and is expressed as a percentage relative to the initial mass of the biological material subjected to extraction. The extraction yield was determined using the following equation:

$$\textbf{Yield}\% = \left[\frac{M_1}{M_0}\right] \times 100$$

Where M_1 represents the mass of the dried extract obtained, and M_0 is the initial mass of the powdered sample used in the extraction process.

3.2. Quantitative analysis of bioactive compounds

3.2.1. Determination of total polyphenols (TPC)

- Principle : The determination of total polyphenol content using the Folin-Ciocalteu method is based on the reaction between the phenolic compounds present in the sample and the Folin-Ciocalteu reagent in an alkaline medium. The reducing capacity of the polyphenols reduces the phosphomolybdic-phosphotungstic acid complexes of the reagent, resulting in the formation of a blue-colored complex. The intensity of this color, measured spectrophotometrically at a wavelength of 765 nm, is proportional to the total concentration of phenolic compounds in the sample (Wabaidur et al., 2020) (Figure 14).

- Procedure: The content of polyphenols in the studied extract samples was estimated according to the method of Othman et al., (2007) using the Folin-Ciocalteu reagent (FCR), with slight modifications. Briefly, a volume of 0.2 mL of each extract was mixed with 2 mL of distilled water or diluted ethanol 80%, and incubated for 5 min, and then 1.5 mL of FCR (diluted 1/10) was added to the mixture. After incubation, 1.5 ml of fresh sodium carbonate (Na2CO3) solution was added and mixed with the samples. The resulting mixture was incubated at room temperature, in the dark, for 60 min.

The absorbance of the extracts against the blank sample was measured using a spectrophotometer at 765 nm, using the same protocol as the standard preparation. The TPC was expressed in milligram of **gallic acid** equivalents per gram of extract (mg EGA/g of Ext). The measurements were performed in triplicates.

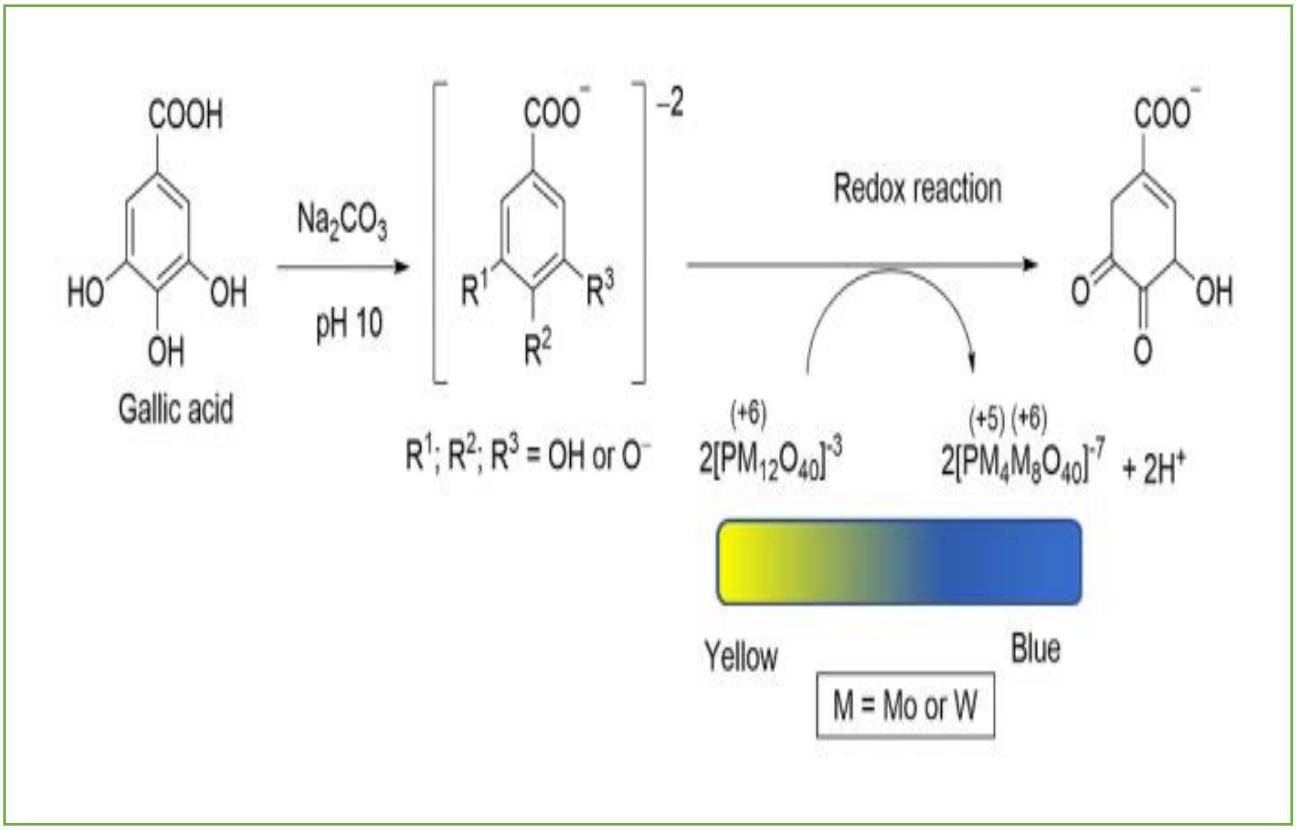


Figure 12. Schematic illustration of the redox mechanism underlying the Folin–Ciocalteu assay for total polyphenol quantification (Dominguez-López et al., 2024).

3.2.2. Determination of total flavonoids (TFC)

- Principle : The aluminum chloride colorimetric method for determining total flavonoid content relies on the ability of flavonoids to form stable yellow complexes with aluminum ions under acidic conditions. The resulting absorbance, measured at 415 nm, is directly proportional to the flavonoid concentration and is typically expressed in quercetin equivalents (Nurcholis et al. ,2021).

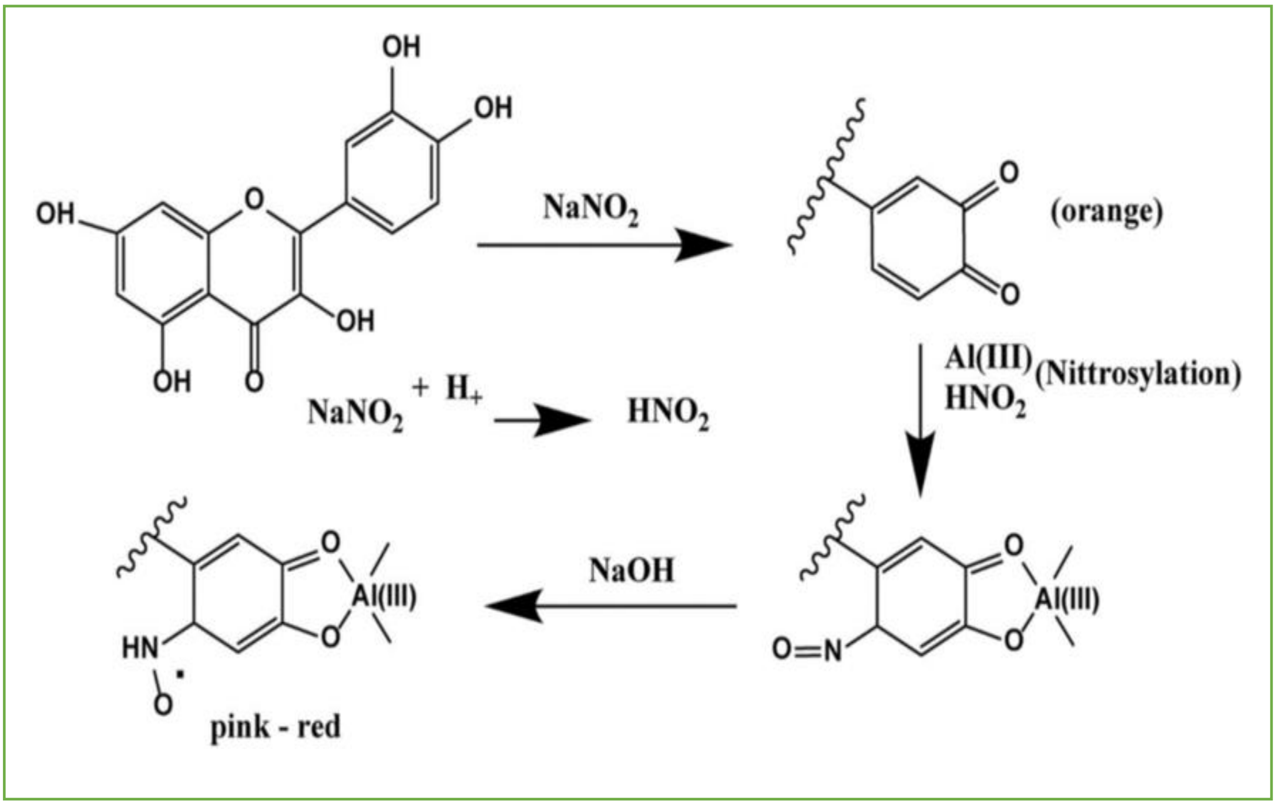


Figure 13. Formation of flavonoid complex with AlCl3 (Shraim et al., 2021).

- Procedure: TFC was estimated using a spectrophotometer according to the method described by Cherbal et al. (2023) with slight modifications. Briefly, 1.5% of aluminum chloride was added to 1.5 mL of the extract and to a standard solution (quercetin). After 45 min, the absorbance was measured at 430 nm. A blank solution, prepared under the same conditions as the samples, served as control.

Based on the calibration curve of quercetin, the TFC was calculated in mg equivalent to quercetin per gram of extract (mg EQ/g of Ext). The measurements were performed in triplicates.

3.3. LC-MS/MS analysis

- Principle : This technique is based on chromatographic separation of complex plant compounds using a C18 column under gradient mobile phase conditions, followed by detection via LC-MS/MS with a triple quadrupole system. Ionization is performed by electrospray (ESI), and detection is carried out in Multiple Reaction Monitoring (MRM) mode for high sensitivity and selectivity. Samples undergo liquid–liquid extraction with organic solvents (methanol, acetonitrile, water, hexane), followed by filtration before injection. This method is widely used for qualitative and quantitative analysis of bioactive compounds in plant extracts (de Oliveira Mozzaquatro et al., 2022).

- **Procedure**: LC-MS/MS analysis was performed at the Application and Research Center of Laboratory (ALUM), Igdir University, Igdir, Türkiye, according to Erenler et al. (2023). Due to the availability of only the ethanolic extract at the time of analysis, it was the only extract analyzed by LC-MS/MS.

Briefly, the samples were prepared at 50mg/mL of concentration in solvent solution containing acetonitrile, methanol and water in equal proportions (1:1:1). Vortex mixing was performed to ensure complete dissolution of the samples. Sonication was applied for samples that remained insoluble. After that, the extraction was performed by adding 0.8 ml of hexane to the extract, followed by a centrifugation at 7000 rpm for 5 min to separate the phases. The supernatant was then collected and diluted at the ratio of 1:4. Finally, an LC-MS/MS analysis was carried out after filtration using a 0.25 µm filter.

A volume of 4.00 µL was injected, with a flow rate of 0.400 ml/min and a total analysis time of 40.00 min. The chromatographic conditions were as follows: Phase A: Water containing 0.1% formic acid and 5 mM ammonium formate. Phase B: Acetonitrile with 0.1% formic acid.

The gradient program was set at 75.0%- 25.0% for 5 min, 25.0%-75.0% for 15 min, 0%- 100% for 16 and 20 min, and 85.0%- 15.0% for 22 and 40 min, respectively.

The experiment was conducted at 30°C, using an Agilent 6460 Triple Quad LCMS system equipped with a Poroshell 120 EC-C18 column (50 mm x 4.6 mm I.D., 2.7 µm).

4. Evaluation of biological activities

4.1. *In vitro* antioxidant activity

4.1.1. DPPH• radical scavenging activity

- **Principle :** The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is based on the reduction of the stable purple-colored DPPH radical by antioxidant molecules capable of donating hydrogen atoms or electrons. Upon reduction, the DPPH radical loses its characteristic absorbance at 517 nm and undergoes a visible color change from deep violet to pale yellow.

The extent of this discoloration reflects the radical scavenging capacity of the sample, thus providing an estimate of its antioxidant potential (Phuyal et al., 2020) (Figure 16).

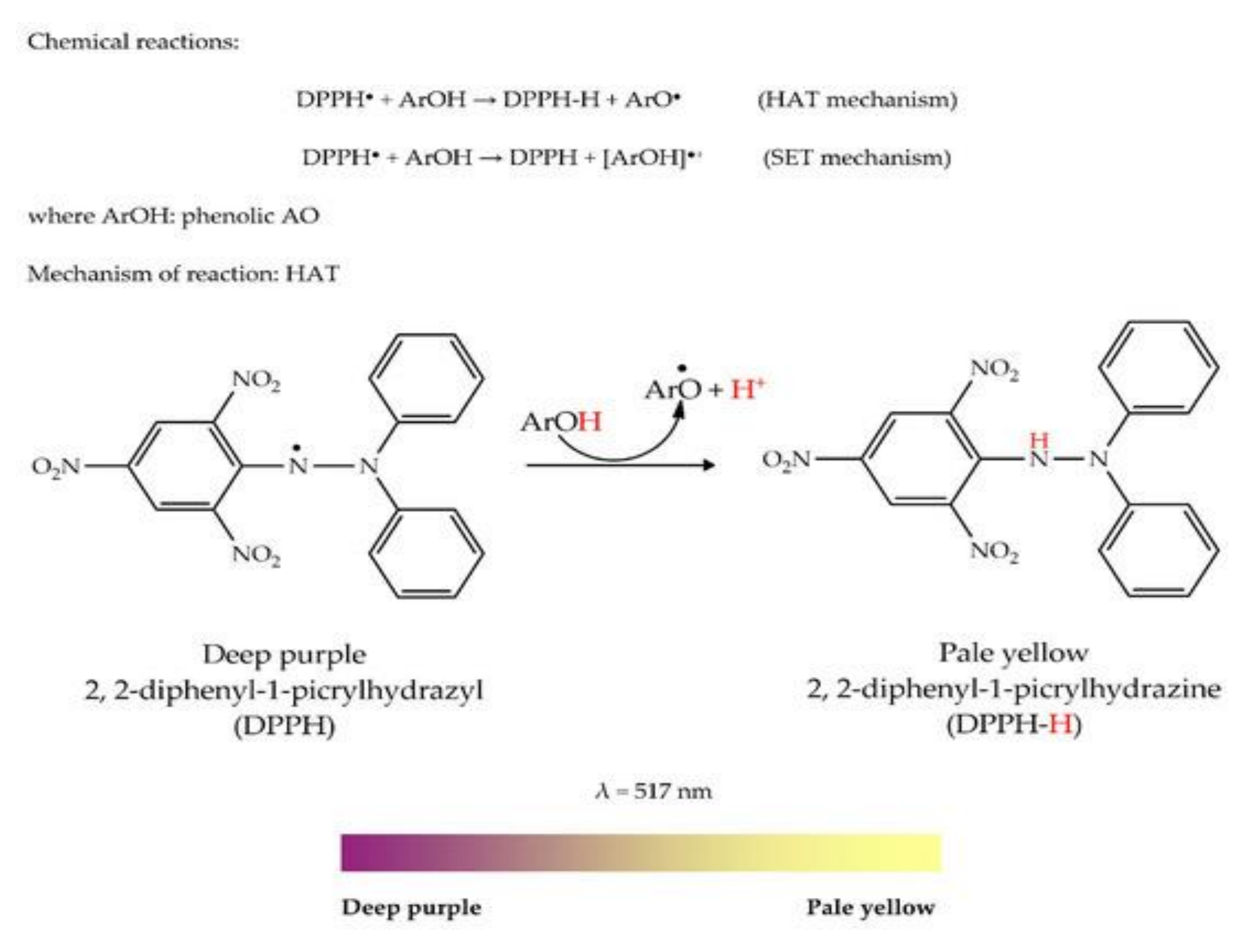


Figure 14. DPPH scavenging mechanisms by an antioxidant (AH) (Phuyal et al., 2020).

- **Procedure:** Based on the method described at Baliyan et al. (2022), the DPPH radical scavenging activity was evaluated by preparing a group of different concentrations of each extract, as well as a 0.004% DPPH solution in methanol. A volume of 50 µL of each concentration of the extract was transferred to test tubes, followed by the addition of 5 mL of the DPPH solution (0.004%). The test tubes were incubated in the dark and at room temperature for 30 minutes, then the absorbance was measured at 517 nm, and compared with the absorbance of the blank sample. To calculate the percentage of free radical inhibition, the following mathematical formula was used:

$$\boldsymbol{I\%_{DPPH}} = \left[\frac{Abs_0 - Abs_1}{Abs_0}\right] \times 100$$

Where **Abs_0** represents the absorbance of the negative control and **Abs_1** represents the absorbance of the solution containing the sample.

A dose-response curve of the percentage of inhibition against the concentration of the extract was used to determine the IC_{50} concentration which corresponds to 50% of inhibition of the free radical DPPH. Ascorbic acid was used as a positive control, and assays were conducted in triplicates.

4.1.2. Reducing power by the frap method

- **Principle :** The reducing power assay is a simple, rapid, and cost-effective method that does not require specialized equipment. Initially developed to evaluate the reducing capacity of plasma, its application has been extended to assess the antioxidant activity of various biological fluids, foods, and plant extracts (Zhong and Shahidi, 2015). This method, based on the potassium ferricyanide system, relies on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by antioxidant compounds present in the sample. In addition to evaluating antioxidant capacity, this assay has also been applied in the determination of reducing sugars in plant materials and in the analysis of dopamine hydrochloride in serum and pharmaceutical samples. During the reaction, different colored complexes may be formed depending on the nature of the sample; however, the assay is mainly characterized by the formation of a Prussian blue (blue–green) complex resulting from the reduction process (Figure 17).

- **Procedure**: The iron reducing power was evaluated by adding 1250 µL of phosphate solution (pH 6.6) to 500 µL of the extract with a concentration of 10 µg/mL, then 1250 µL of potassium ferricyanide 1% (K3Fe(CN)6)) was added to the mixture. After heating at 50°C, the mixture was kept at room temperature. Then, combined with 2.5 mL of 10% trichloroacetic acid and centrifuged at 3000 rpm for 5 min. Finally, 1.5mL of distilled water was mixed with 1.5 ml of the supernatant resulting from centrifugation with 0.2 ml of Ferric chloride solution 0.1% (FeCl3). Using a spectrophotometer, the absorbance of the samples and the absorbance of the blank sample were measured (Giriwono et al., 2020).

The results were expressed as $A_{0.5}$ and the EC_{50} values.

Where :

$A_{0.5}$: The concentration at 0.50 absorbance **EC_{50}** : Half Maximal Effective Concentration

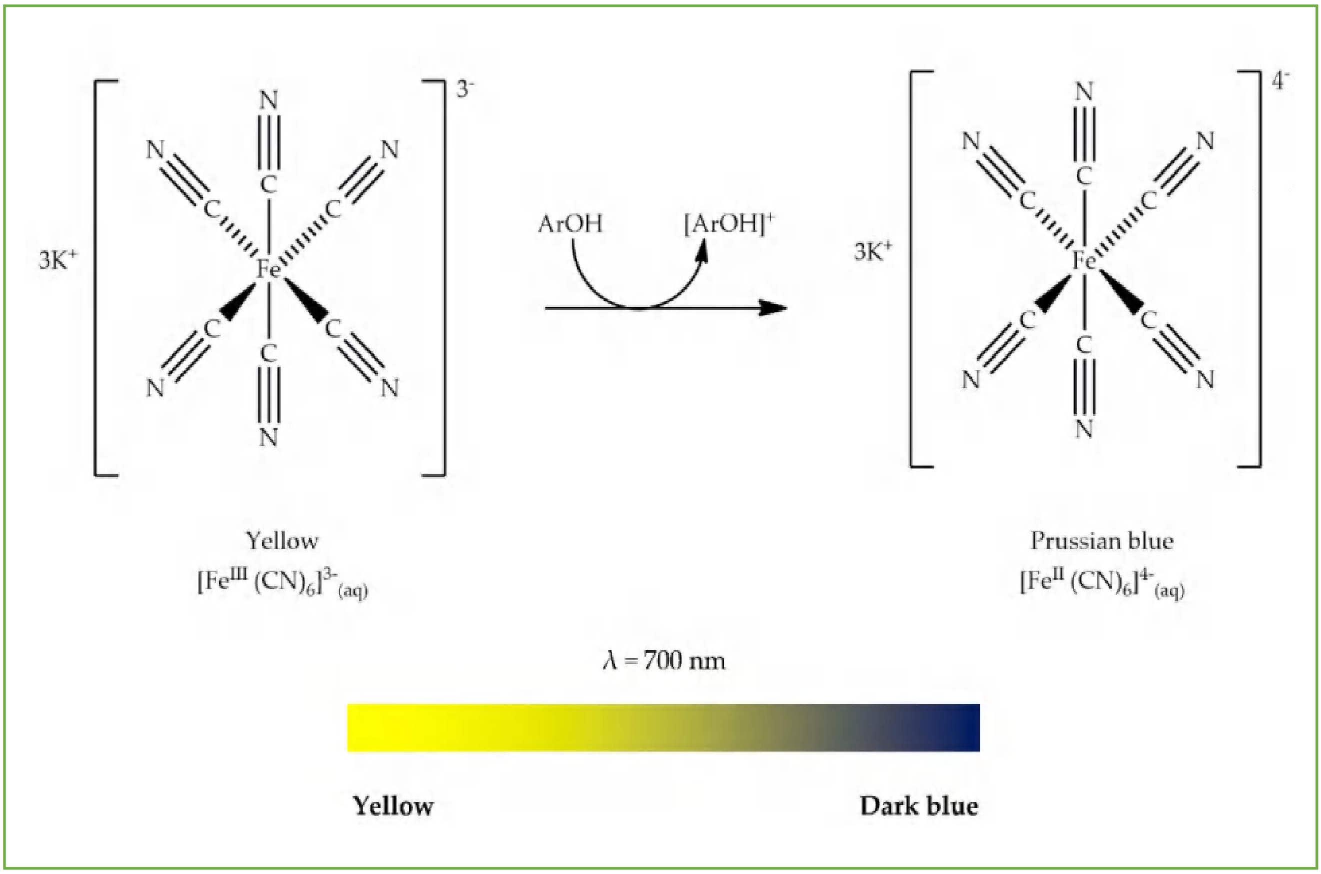


Figure 15. Reductive mechanism of the FRAP assay by antioxidant compounds. (Christodoulou et al., 2022)

4.1.3. Total antioxidant capacity (TAC) by the phosphomolybdenum method

- Principle : The principle of the phosphomolybdenum method lies in the reduction of molybdenum (VI) to molybdenum (V) by antioxidant compounds, resulting in the formation of a green complex. The intensity of this complex reflects the total antioxidant capacity of the tested sample (Asomadu et al., 2024).

- Procedure: According the method of Jan, et al. (2021) the TAC was evaluated using the phosphomolybide technique, where a series of different concentrations of extracts or Ascorbic acid were prepared. In each test tube, 0.3 ml of each concentration of the extract was mixed with 1 ml of the reagent containing three components in equal proportions (4mM ammonium molybdate, 28 mM sodium phosphate, 60mM sulfuric acid, 1 : 1 : 1). The test tubes were covered with aluminum foil, heated in a water bath at 90°C for 2.5 hours and then allowed to cool at room temperature. After that, the absorbance of the samples was measured against a blank sample. The assay was performed in triplicates to ensure results accuracy. The activity was expressed as mg of ascorbic acid equivalents per gram of extract (mg EAA/g of Ext).

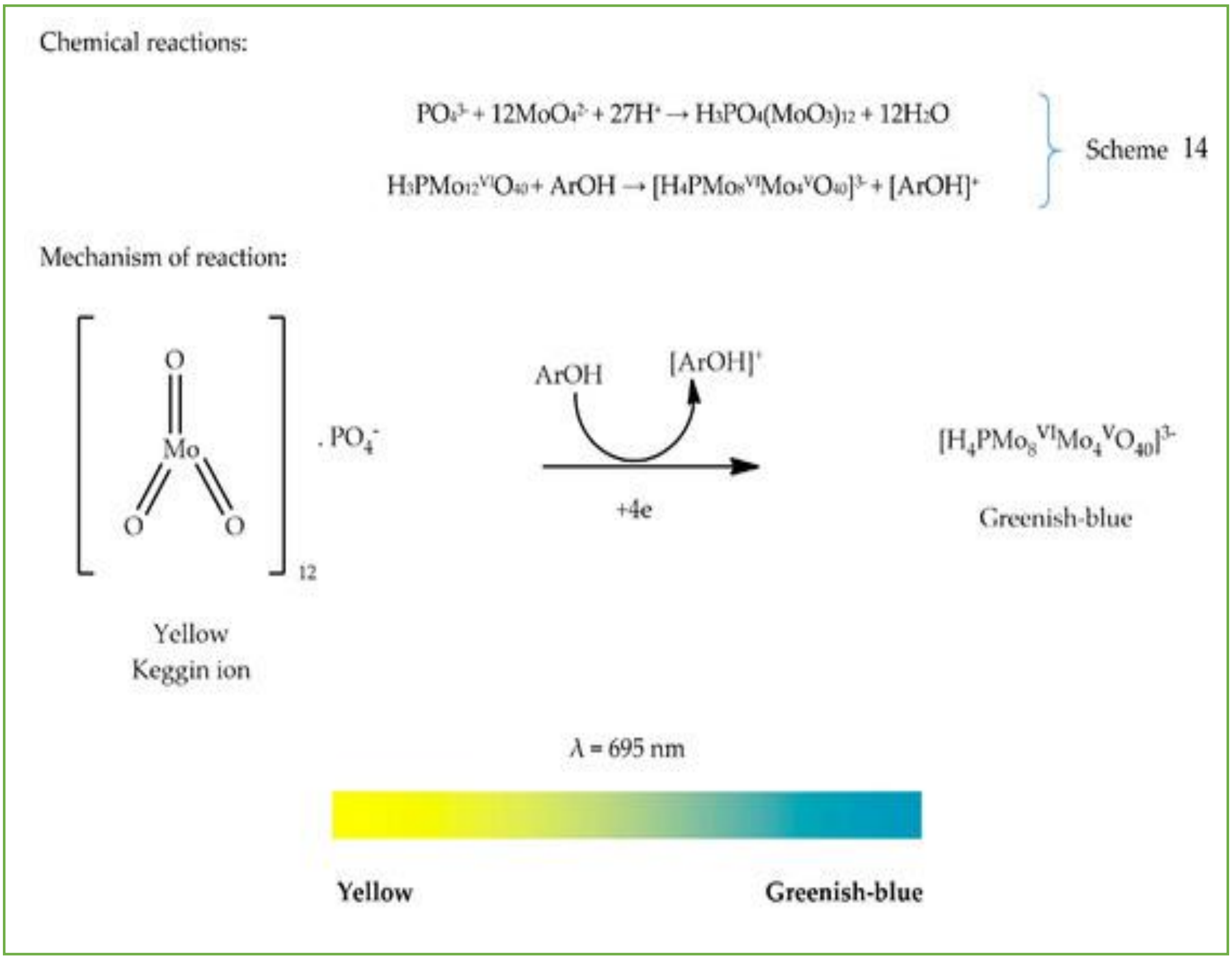


Figure 16. Phosphomolybdenum reaction mechanism (Phuyal, et al., 2020)

4.1.4. β-Carotene–linoleic acid bleaching method

- Principle : The antioxidant activity in the β-carotene bleaching assay is based on the ability of antioxidants to prevent the oxidative degradation of β-carotene caused by free radicals generated from linoleic acid. These radicals typically attack the conjugated double bonds of β-carotene, leading to a loss of its characteristic orange color. When antioxidant compounds are present, they neutralize these radicals, thereby reducing the extent of color loss (Figure 20).

This inhibition of β-carotene discoloration is used as an indicator of antioxidant potential (Loucif et al., 2020).

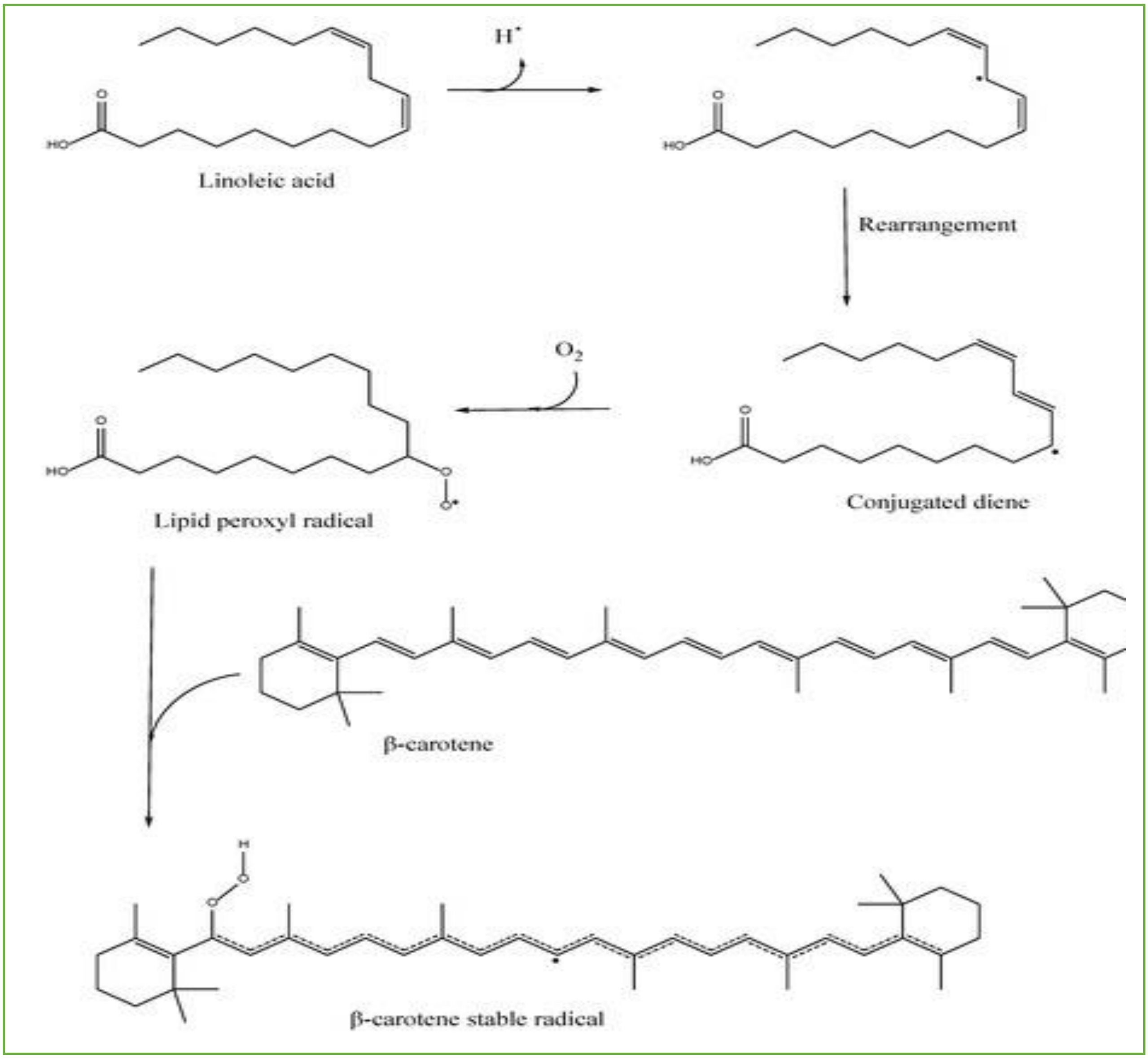


Figure 17. Mechanism of β-carotene stabilization (Christodoulou et al., 2022).

More specifically, this method involves an emulsion system where linoleic acid undergoes oxidation under thermal conditions, producing reactive species such as conjugated dienes and hydroperoxides. These reactive products then attack β-carotene, causing it to lose its color. Antioxidants help to stabilize the system by slowing down or inhibiting the formation of these oxidation products. As a result, the intensity of β-carotene color remains more stable in the presence of antioxidants. The degree of this protective effect, which reflects the antioxidant capacity of the tested compounds, is typically measured spectrophotometrically by monitoring absorbance changes over time (Modaressi et al., 2013).

- Procedure : β-carotene solution (0.5 mg/mL) was prepared in chloroform. Subsequently, 1 mL of this solution was mixed with 20 mL of linoleic acid and 200 mL of Tween 20. The chloroform was evaporated under vacuum at 45°C for 3 minutes. Then, 75 mL of distilled water was added to form an emulsion, which served as the working reagent. For each assay, 160 mL of 160 mL of freshly prepared β-carotene/linoleic acid emulsion was added to 40 mL of each extract. The emulsion was incubated in a water bath at 50°C for 2 hours. The absorbance was measured at 470 nm (Lekouaghet et al., 2020).

A control was prepared under the same conditions, replacing the extract with solvent. Antioxidant activity was expressed as the percentage of inhibition of β-carotene bleaching, calculated using the following formula:

$$\boldsymbol{I\%_{\beta-carotene}} = \left[\frac{\mathrm{As120} - \mathrm{Ac120}}{\mathrm{Ac0} - \mathrm{Ac120}}\right] \times 100$$

Where:

Ac_0 is the absorbance values of the control measured at the beginning of the experiment.

As_{120} and Ac_{120} are the absorbance values measured, after incubation for 120 min, for each sample and control, respectively.

4.1.5. Hydrogen peroxide (H_2O_2) scavenging activity

- **Principle** : One of the most common methods for evaluating the hydrogen peroxide scavenging capacity is based on the absorption of this molecule in the UV region (Magalhães et al., 2008).

More specifically, this method evaluates the ability of antioxidants present in the sample to scavenge hydrogen peroxide (H_2O_2), leading to a reduction in its absorbance at 230 nm. The extent of absorbance decrease indicates the scavenging potential of the extract (Hussen and Endalew 2023).

- Procedure: Antioxidant activity was evaluated by measuring the extract's ability to scavenge hydrogen peroxide (H_2O_2), based on the method reported in (Surana et al., 2022), with some necessary modifications. A 10 mM H_2O_2 solution was prepared in a phosphate buffer (pH 7.4). The reaction mixture consisted of 10 mM of the prepared H_2O_2 solution and varying concentrations of the extract. Ascorbic acid was used as a reference standard. Absorbance was measured at 240 nm. The percentage of H_2O_2 scavenging was calculated using the following equation:

$$I\%_{\mathbf{H2O2}} = \left[\frac{Abs_0 - Abs_1}{Abs_0}\right] \times 100$$

Abs_0 = the absorbance of the blank **Abs_1** = the absorbance of the extract

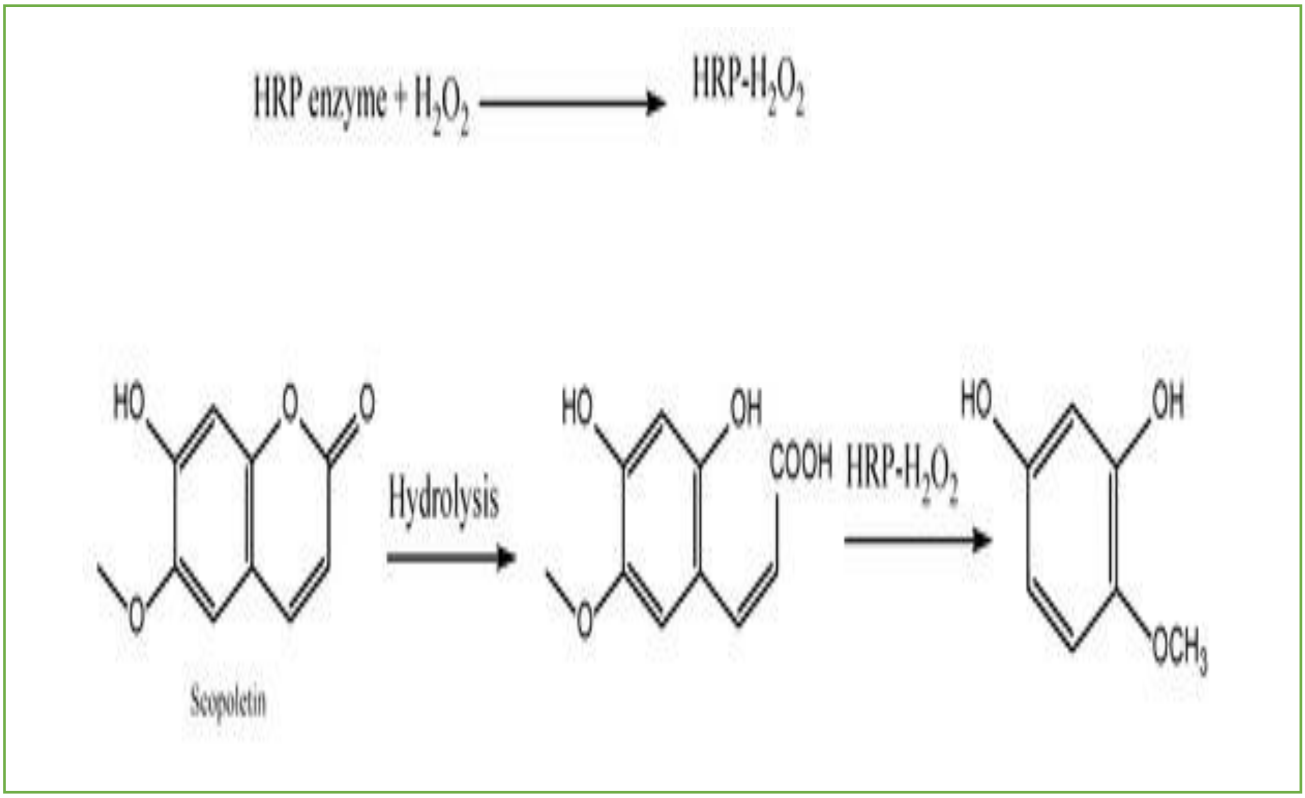


Figure 18. Overall mechanism of hydrogen peroxide scavenging by antioxidants (Christodoulou et al., 2022).

4.2. Anti-Inflammatory Activity

4.2.1. Anti-Inflammatory activity by *in vitro* albumin denaturation assay

Priciple : The denaturation of proteins is recognized as a significant trigger in the inflammatory process, as it disrupts protein structure and function, potentially leading to the generation of autoantigens , an event implicated in various forms of arthritis**.** While conventional anti-inflammatory treatments, including steroidal and non-steroidal drugs, remain widely used, their prolonged application often results in undesirable side effects such as gastric mucosal damage and local tissue irritation. This has led to a growing scientific focus on naturally derived compounds, particularly bioactive metabolites and phytochemicals from fungi, as safer alternatives with promising anti-inflammatory properties (Bains and Chawla 2020)

Procedure: The assay was conducted following to Bailey-Shaw et al. (2017) in which the inhibition of denaturation of the protein bovine serum albumin (BSA) by heat was studied. Serial dilutions of the extract were prepared, where 1 mL of 1% BSA solution in phosphate buffer saline (PBS) was added to 1 mL of each diltuion. Then PBS was added to maintain pH stability, the test tubes were incubated firstly at 37°C for 20 min, and secondly at 70°C for 5 min. The diclofenac was used as a reference standard for comparison, incubated first at 37°C for 20 min, then at 70°C for 5 min. PBS buffer was used as a blank. After allowing the tested samples to cool at room temperature, the absorbance was measured using a spectrophotometer at 660 nm, and the effect of the extract was evaluated by calculating of the percentage inhibition of BSA denaturation using the following equation:

$$\mathbf{I\%} = \left[\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right] \times 100$$

Where $Abs_{control}$ represents the absorbance of the negative control and A_{sample} represents the absorbance of the solution containing the sample.

4.2.2. *In silico* study

Principle : Computational approaches provide a robust theoretical framework for assessing anti-inflammatory activity through molecular docking studies. By simulating the interactions between candidate compounds and key inflammatory target proteins, such as the COX-2 enzyme, these methods enable the estimation and prediction of binding affinities at the molecular level. As illustrated in previous studies employing hybrid designs like IBU–TAX, this strategy underscores the role of computational modeling in guiding the identification of promising anti-inflammatory agents prior to experimental validation (Mousa et al., 2021).

Procedure : The 3D structures of the 10 flavonoid compounds identified from the plant extract through LC-MS/MS analysis, together with the reference drug diclofenac, were obtained in structure data file (sdf) format from PubChem (Kim et al., 2019)**.** The molecules were imported into the Schrödinger workspace and prepared using LigPrep with the OPLS3 force field at pH 7 (±2), employing Epik for ionization states (Balogun, Ipinloju et al. 2021)**.**

The crystallographic structures of the target proteins COX-2, IL1β, NFKβ, PLA2, and TNFα (PDB IDs: 1CX2, ILOB, 8TQD, 1CJY, and 5MU8, respectively) from Homo sapiens were retrieved from the Protein Data Bank (Berman et al., 2000). The monomeric chain (chain A) of each protein was prepared using the Protein Preparation Wizard of the Schrödinger Suite (2017 v2). Receptor grids were generated at the binding domains identified by Sitemap in Maestro, with the following grid coordinates: 1CX2 (x = 24.42, y = 21.81, z = 16.26), ILOB (x = 7.54, y = 6.03, z = 7.31), 8TQD (x = 19.98, y = 10.26, z = –7.91), 1CJY (x = 36.46, y = –1.31, z = 82.70), and 5MU8 (x = 31.19, y = 11.18, z = 17.90) (He et al., 2005; Rondeau et al., 2015).

The prepared ligands were then virtually screened against the defined binding sites of the target proteins. Binding affinity was estimated using the extra precision (XP) docking protocol to perform site-directed docking of the compounds. Finally, the 2D protein–ligand interactions of the docked complexes were visualized with Discovery Studio 2020 (Omoboyowa et al., 2023).

4.3. α-Amylase inhibitory activity

Principle : This method is designed to evaluate the inhibitory effect of crude extracts and their respective fractions, derived from the two studied plants, on pancreatic α-amylase activity. The assay relies on a colorimetric reaction involving 3,5-dinitrosalicylic acid (DNSA), which serves as an indicator of the amount of reducing sugars released during the enzymatic hydrolysis of starch.

Under alkaline and high-temperature conditions, the free aldehyde groups of the reducing sugars generated by enzymatic activity act as electron donors, reducing DNSA, a yellow-orange compound, into 3-amino-5-nitrosalicylic acid, which exhibits a reddish-orange coloration. This reduced form shows a strong absorbance peak at 540 nm when measured spectrophotometrically.

The intensity of the developed color is directly proportional to the concentration of reducing sugars in the reaction mixture and therefore reflects the residual α-amylase activity. A lower absorbance value indicates stronger enzyme inhibition by the tested extract or fraction. This method enables both qualitative and quantitative evaluation of α-amylase inhibition, offering valuable insight into the antidiabetic potential of the natural products under investigation (Thalapaneni et al., 2008).

Procedure: The α-amylase inhibitory activity of the aqueous and organic extracts was evaluated according to the protocol described in Mechchate et al. (2021), with slight modifications.

Briefly, 200 µL of each extract was mixed with 200 µL of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride and α-amylase solution. The mixture was incubated at 37 °C for 10 minutes. Subsequently, 200 µL of a 1% soluble starch solution was added, followed by a second incubation at 37 °C for 15 minutes. After incubation, 400 µL of DNSA (3,5-dinitrosalicylic acid) reagent was added. The reaction mixture was then heated in a boiling water bath (100 °C) for 5 minutes to allow color development. Absorbance was measured at 540 nm using a UV–Visible spectrophotometer to determine the residual enzymatic activity. A standard solution of acarbose was prepared in 0.02 M phosphate buffer (pH 6.9) at various concentrations and used as a positive control to assess the inhibitory potential of the tested extracts.

The percentage of inhibition (I%) was calculated using the following formula:

$$\boldsymbol{I}\% = \left[\frac{\mathrm{A}_{\mathrm{control}} - A_{sample}}{\mathrm{A}_{\mathrm{control}}}\right] \times 100$$

Where :

A$_{\textbf{control}}$ is the absorbance of the control

A$_{\textbf{sample}}$ is the absorbance of the test sample

The α-amylase inhibitory activity was also expressed as IC_{50} values (µg/mL), representing the concentration required to inhibit 50% of enzyme activity.

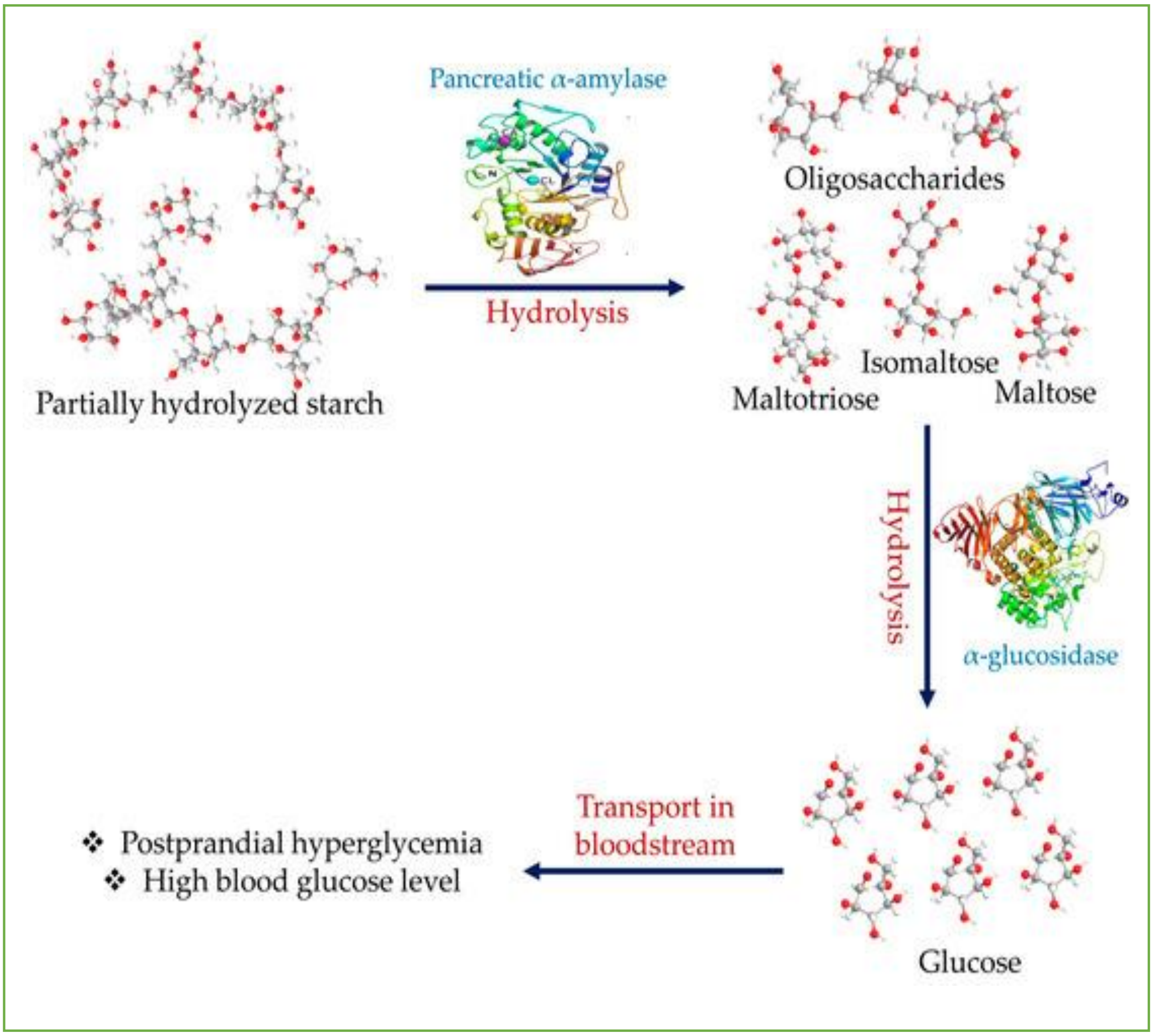


Figure 19. Schematic illustration of α-amylase involvement in starch hydrolysis leading to postprandial hyperglycemia (Kashtoh and Baek 2023).

4.4. Antimicrobial activities assays.

Principle : The agar well diffusion method relies on the radial diffusion of the test compound from a well created in a solid agar medium that has been uniformly inoculated with the target microorganism. When a defined volume of the sample is introduced into the well, the active molecules diffuse gradually through the medium, establishing a concentration gradient from the center outward. If the sample exhibits antimicrobial activity, it inhibits microbial growth in the surrounding area, producing a distinct inhibition zone devoid of growth. The diameter of this zone provides a qualitative and semi-quantitative measure of the antimicrobial potency of the sample, as larger inhibition areas are generally associated with higher efficacy or concentration. This principle underpins the use of the method as a preliminary, straightforward, and reliable approach for assessing antibacterial and antifungal activities, as well as for detecting antibiotic residues in food and environmental samples (Alaqeel et al., 2021).

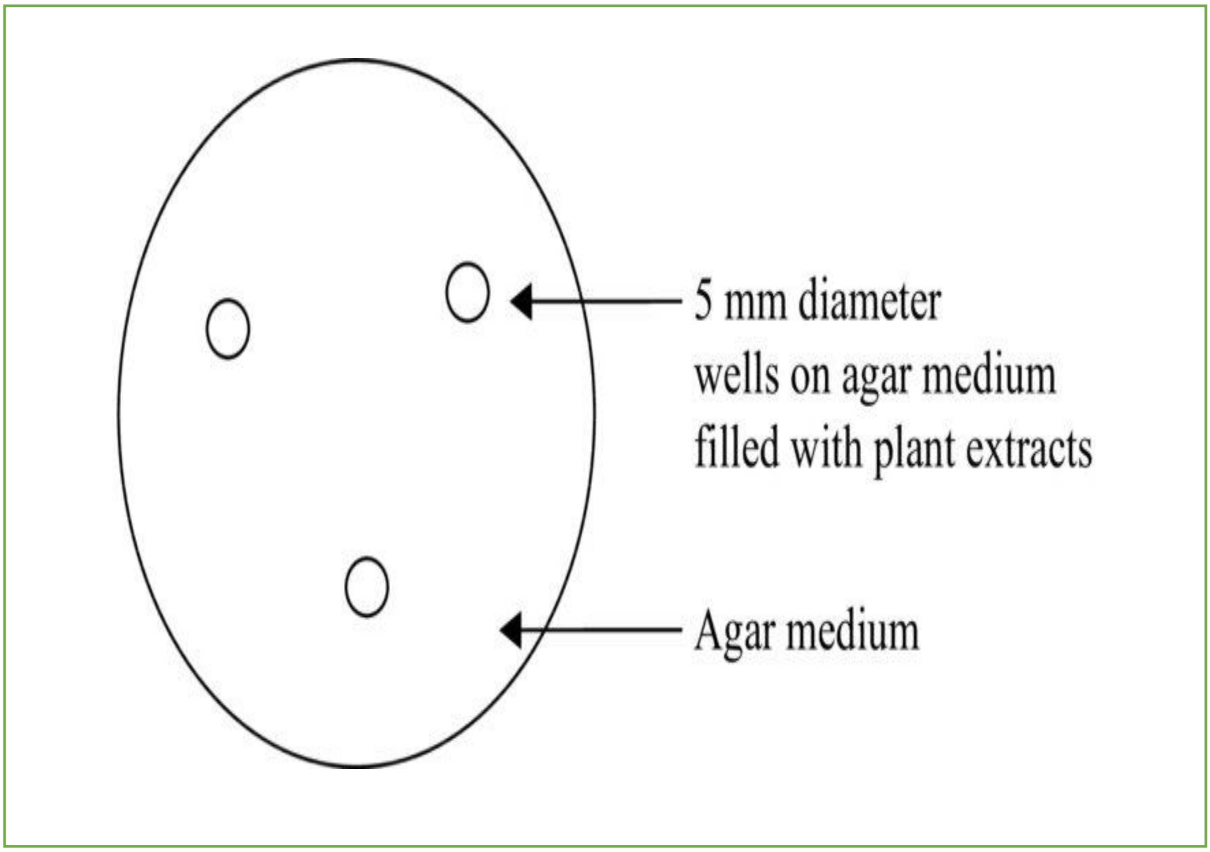


Figure 20. Diagram of the final step in the agar well diffusion method. (Navoda and Anupama 2022)

Procedure: The antimicrobial activity of the studied extracts was evaluated using the agar well diffusion method. The study involved four standard bacterial strains: Bacillus subtilis ATCC 25973, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 25923, as well as the pathogenic yeast Candida albicans ATCC 10231.

Mueller-Hinton Agar (MHA) was employed for antibacterial testing, whereas the anti-Candida activity was assessed on Sabouraud Dextrose Agar (SDA). Bacterial suspensions were prepared at an approximate density of 10^6 CFU/mL, and Candida albicans suspensions at 10^5 CFU/mL, all derived from fresh 24-hour cultures to ensure maximal biological activity.

Plates were uniformly inoculated using sterile cotton swabs, and wells of 6 mm diameter were aseptically created. Fifty microliters (50 µL) of each extract, at concentrations of 8000, 4000, 2000, and 1000 µg/mL, dissolved in sterile phosphate-buffered saline and filtered through a 0.22 µm filter to ensure sterility, were added to the wells. Sterile saline solution served as the negative control to exclude any solvent effects.

The plates were incubated at 37°C for 24 hours for bacterial strains and for 48 hours for Candida albicans. Antimicrobial activity was determined by measuring the diameter of the inhibition zones (mm) around the wells, with zones larger than 6 mm considered indicative of inhibitory activity (Kiehlbauch et al., 2000; Bonev et al., 2008).

5. Statistical analysis

Data normality was first assessed using the Shapiro-Wilk test, and homogeneity of variance was assessed using the Levene test. The means for each activity were compared using a one-way ANOVA, and post-hoc LSD and Dunnett tests were applied when significant differences ($P \leq 0.05$) were observed.

In addition, the general linear model (GLM) and MANOVA were used to compare chemical content (TPC and TFC) with various biological activities. Pearson's correlation coefficient (r) was also calculated to assess the relationship between chemical content and biological activities ($P \leq 0.05$).

All statistical analyses were performed using SPSS statistical software version 26 (IBM SPSS, 2019), and the results are presented as mean ± standard deviation (mean ± SD, n = 3).

PART THREE

RESULTS AND DISCUSSION

PART THREE : RESULTS AND DISCUSSION

1. Nutritional analysis

1.1. Nutritional analysis of algerian *P. eryngii*

The table 1 summarizes the physicochemical composition of the fruiting bodies of *P. eryngii* collected from the Tifrit region in Saïda (Western Algeria). The analyses revealed that this mushroom contains significant amounts of nutritional compounds, with total sugars showing the highest value at 49.61 ± 3.16 g/100 g, followed by proteins at 14.81 ± 0.54 g/100 g, and ash at 5.27 ± 0.21 g/100 g, while lipids recorded the lowest content at 2.67 ± 0.23 g/100 g. Furthermore, the total carbohydrate content was estimated at 77.26 ± 0.52%, with an energetic value of 391.62 ± 1.55 kcal/100 g. Moisture was determined at 81.4 ± 1.68%.

Table 1. Nutritional composition, energetic value, and physicochemical properties of *P. eryngii* and *F. vulgare* leaves.

Component / Parameter	*P. eryngii*	*F. vulgare* leaves
	Nutritional composition (g/100 g DM)	
Protein	14,81 ± 0.54	4,62 ± 0.37
Total sugar	49,61 ± 3.16	2,56 ± 0.17
Lipids	2,67 ±0.23	1,54 ± 0.06
Ash	5,27 ± 0.21	3,37 ± 0.45
Total carbohydrates	77,26 ± 0.52	90,14 ± 0.23
	Energy value (Kcal/100 g)	
Energy (Kcal/100 g)	391,62 ± 1.55	394,22 ± 1.63
	Physicochemical parameter (%)	
Moisture (%)	81,4 ± 1.68	79,56 ± 0.84
Dry matter (%)	18,6 ± 1.68	20,44 ± 0.84

Values are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3).

A comparison with the findings of Petraglia et al. (2023) on the same mushroom (*P. eryngii*) cultivated in Italy, a close similarity in nutritional composition is observed: proteins were reported at 12.45 ± 0.18 g/100 g (dry weight), ash at 6.50 ± 0.46 g/100 g, and lipids at 1.55 ± 0.09 g/100 g, along with higher carbohydrate content (79.49 ± 1.92 g/100 g) and an energetic value of 381.78 ± 12.27 kcal/100 g.

In contrast, Krüzselyi et al. (2016) reported somewhat different results for Hungarian *P. eryngii* fruiting bodies, with higher levels of proteins (8.91%), lipids (3.84%), and ash (6.25%), which resulted in a lower energetic value of 1512 kJ/100 g dry weight.

Similarly, a Korean study by 안명수 et al. (2006) found that *P. eryngii* powder contained a notably high protein content of around 20%, while lipid and ash values (2% and 5.2%, respectively) were consistent with our results.

In the same context, Reis et al. (2012) reported low levels of nutritional compounds in *P. eryngii* from Northeast Portugal, where protein, lipid, and ash contents did not exceed 1.21 g/100 g, 0.16 g/100 g, and 0.68 g/100 g, respectively.

Moreover, Khan and Tania (2012) indicated that most previous studies have placed protein concentrations within the range of 11–12 g/100 g, which is lower than in our sample, whereas lipid contents were reported between 7–8 g/100 g, considerably higher than those measured in the Algerian sample.

Additionally, Raman et al. (2021) reported protein content in *P. eryngii* samples at 11.95 g/100 g and ash at 4.89 g/100 g, both lower than in our results, while lipids were higher at 7.5 g/100 g.

A study conducted on *P. geesteranus* revealed that total sugars amounted to only 2.89 g/100 g, which is very low compared to the values recorded for Algerian *P. eryngii* (Kongkla and Poeaim 2016).

On another note, the nutritional composition of four Pakistani *Pleurotus* species (*P. ostreatus, P. sajor-caju, P. sapidus, P. columbinus*) was estimated, showing relatively high protein levels ranging from 16–25%, ash contents between 2.1–9.14%, and very low lipid levels not exceeding 1.88% (Irshad et al., 2023).

In a similar direction, Alam et al. (2008) reported elevated nutritional content in four Indian mushroom species, with proteins, ash, and lipids exceeding 23 g/100 g, 9 g/100 g, and 4 g/100 g, respectively, values considerably higher than those obtained in our sample.

In contrast, Hoa et al. (2015) reported that the protein and lipid contents in *P. cystidiosus* from Taiwan were close to our findings (19.52% and 2.05%, respectively), with a lower fat content not exceeding 1.32%.

The nutritional value of the Algerian mushroom is further highlighted when compared with 17 species of Patagonian Andean fungi studied by Rugolo et al. (2022), where its protein content was higher than in 13 of those species, and its lipid content exceeded the values reported in 10 species.

At the local level, the results of this study surpassed those obtained by Kebaïli (2022) on *Ganoderma lucidum*, where protein, lipids, and ash were found at 11.26 g/100 g, 1.58 g/100 g, and 1.9 g/100 g, respectively, all lower than the values recorded in *P. eryngii*.

Regarding carbohydrates, the percentage reported in this study (77%) is consistent with several previous reports, such as Reis et al. (2012), and higher than those described by Deepalakshmi and Sankaran (2014) for *P. ostreatus*, where the values ranged between 37–48 g/100 g.

The recorded moisture content in this study (81.4%) is relatively low compared to some previous reports, such as Petraglia et al. (2023) and Krüzselyi et al. (2016).

This variation in the estimation of nutritional compounds (particularly proteins and carbohydrates) between the present study and related previous works can be attributed to several factors, including mushroom strain, drying and extraction methods, which modify the concentration of bioactive compounds (Torres-Martínez et al., 2022 ; Irshad et al., 2023). Geographical environment, climate, and ecological factors also influence metabolic processes, and thus protein and carbohydrate contents (Rugolo et al., 2022). Harvest stage and moisture play an important role as well Hoa et al. (2015), as a distinction can be observed between fresh and dried mushrooms; drying generally increases nutrient concentration (Alam et al., 2008). Moreover, compositional differences exist between mushroom parts: caps and gills are richer in proteins and lipids, while stipes contain more fibers and carbohydrates (Alam et al., 2008). Protein content also varies depending on species, maturity stage, cultivation site, and nitrogen availability, which is inversely related to the carbon/nitrogen ratio (C/N) (Hoa et al., 2015).

Other influencing factors include morphological characteristics, growth stage, and sample nature (Sharif et al., 2016 ; Singh et al., 2020). Moisture content, in particular, is affected by mushroom age, species, post-harvest conditions, and growth temperature and humidity (Hoa et al., 2015).

The nutritional assessment of Algerian *P. eryngii* is consistent with previously reported data on the general composition of edible mushrooms. These organisms are widely recognized for their high nutritional value, particularly their elevated protein content on a dry weight basis, making them a valuable dietary source for mitigating protein deficiency, especially in vegetarian diets (Singh et al., 2020; Rugolo et al., 2022).

In addition, mushrooms are rich in dietary fiber and essential minerals while being low in fat, which contributes to their potential role in addressing malnutrition, particularly in developing countries (Alam et al., 2008; Rugolo et al., 2022). Carbohydrates represent the major fraction, including compounds such as trehalose and mannitol, which are involved in metabolic processes and cellular structure. Furthermore, mushrooms contain various organic acids, including malic, succinic, and fumaric acids, which participate in energy metabolism and the regulation of physiological functions (Rugolo et al., 2022).

They also provide bioactive compounds such as γ-aminobutyric acid (GABA), known for its role in supporting neurological functions, and ornithine, which contributes to muscle recovery following physiological stress or medical interventions (Raman et al., 2021).

Overall, the nutritional profile of Algerian *P. eryngii* falls within the range reported for *Pleurotus* species, confirming its consistency with the general nutritional patterns of this genus.

Species of *Pleurotus* spp. are characterized by a substantial carbohydrate content, which reached 77% in the present study. These carbohydrates consist primarily of simple sugars, including mono-, di-, and oligosaccharides, which are closely associated with the biosynthesis of structurally and functionally important polysaccharides. The latter encompass dietary fiber components such as chitin, β-glucans, cellulose, and various hemicelluloses, including mannans, galactans, and xylans (Raman et al., 2021).

In addition, *Pleurotus* spp. contain glucans with diverse glycosidic linkages, notably branched (1→3) and (1→6)-β-glucans, as well as linear (1→3)-α-glucans. A distinctive polysaccharide, pleuran, has also been identified and is recognized for its antitumor potential. These glucans contribute to both soluble and insoluble dietary fiber fractions (Khan and Tania, 2012), thereby enhancing digestive health and contributing to overall nutritional balance (Krüzselyi et al., 2016).

The elevated fiber content further supports the classification of these mushrooms as low-calorie foods, with significant implications for dietary management in conditions such as diabetes and obesity (Raman et al., 2021). This low-energy characteristic is primarily attributed to their high fiber concentration (Kebaïli, 2022).

Moreover, *Pleurotus* spp. represent a valuable source of high-quality proteins, providing a complete amino acid profile that includes all nine essential amino acids. This nutritional attribute places them at a level comparable to animal-derived proteins, supporting their use as an alternative in vegetarian diets and meat-substitution strategies (Torres-Martínez et al., 2022). Specifically, *P. eryngii* exhibits a superior protein profile due to the diversity of its protein fractions and a relatively high proportion of albumins, which are known for their high digestibility and bioavailability (Krüzselyi et al., 2016).

In terms of lipid composition, this genus is distinguished by a low fat content, with total lipids generally not exceeding 4% and only trace amounts of cholesterol, thereby reinforcing its classification as a low-calorie food. Linoleic acid (C18:2) has been identified as the predominant unsaturated fatty acid and plays a key role in the development of characteristic mushroom flavor compounds. Other fatty acids, including oleic acid (C18:1) and palmitic acid (C16:0), have also been reported (Torres-Martínez et al., 2022). This lipid profile contributes to the reduction of cholesterol accumulation, as diets incorporating *Pleurotus* spp. have been associated with decreased levels of LDL and VLDL, as well as reductions in total cholesterol and blood glucose (Raman et al., 2021). Furthermore, the presence of beneficial unsaturated fatty acids, particularly linoleic acid (PUFA) and oleic acid (MUFA), supports cardiovascular health (Rugolo et al., 2022).

Pleurotus spp. also constitute an important source of micronutrients, notably B-complex vitamins such as niacin, riboflavin, and folates, with levels comparable to those found in commonly consumed vegetables. Notably, folate bioavailability in these mushrooms exceeds that of certain vegetables, including peas and spinach, thereby enhancing their nutritional relevance. In particular, their richness in folic acid (vitamin B9) plays a crucial role in preventing neural tube defects during pregnancy (Raman et al., 2021).

Another notable characteristic of *Pleurotus* spp. is their balanced mineral composition, with minerals accounting for more than 56% of total ash content (Rugolo et al., 2022). These mushrooms are especially rich in potassium and phosphorus while maintaining low sodium levels, a combination that is beneficial for blood pressure regulation and proper nervous system function (Raman et al., 2021).

From a safety perspective, *P. eryngii* demonstrates a favorable profile, with reported absence of toxic heavy metals such as lead (Pb), mercury (Hg), and arsenic (As), supporting its suitability for human consumption. Consistently, studies on edible and medicinal mushrooms have shown that *P. eryngii* contains significant levels of both macro- and microelements, including magnesium, potassium, zinc, iron, copper, and sodium (Irshad et al., 2023). It is also recognized as one of the richest mushroom species in selenium (Raman et al., 2021). Furthermore, its relatively high phosphorus content supports bone health and energy metabolism, while comparatively moderate potassium levels may make it suitable for specific populations, such as individuals with kidney disorders (Krüzselyi et al., 2016; Raman et al., 2021).

Overall, these findings confirm that *P. eryngii* is a nutritionally rich species, characterized by substantial levels of carbohydrates, proteins, and essential minerals, thereby reinforcing its dietary and health-promoting value. These attributes support its classification as a “culinary-medicinal mushroom,” reflecting its dual nutritional and therapeutic potential (Irshad et al., 2023). Consequently, its inclusion in the diet may represent an effective strategy for alleviating deficiencies in essential micronutrients such as iron and zinc.

1.2. Nutritional analysis of algerian *Foeniculum vulgare* leaves

The nutritional profile of *Foeniculum vulgare* leaves harvested from the Sirat region in the Wilaya of Mostaganem (Western Algeria) revealed a remarkable nutritional value. Proteins were identified as the most abundant component, with an average content of 4.62 ± 0.37 g/100 g, followed by ash (3.37 ± 0.45 g/100 g), carbohydrates (2.56 ± 0.17 g/100 g), and fats (1.54 ± 0.06 g/100 g). Total carbohydrates were estimated at a high level of 90.14 ± 0.23 %, while the energy value reached 394.22 ± 1.63 kcal/100 g. Moisture content was 79.56 ± 0.84 %, reflecting a substantial water composition in the leaves of this plant (Table 1).

RESULTS AND DISCUSSION

In comparison with the study on Portuguese fennel leaves (Barros et al., 2010), the values recorded in our samples were significantly higher; protein did not exceed 1.16 g/100 g, fats were 0.61 g/100 g, and carbohydrates only 18.44 g/100 g, with a low energy value (83.90 kcal/100 g) and a moisture content of 76.36 g/100 g. Furthermore, the lipid content in the Portuguese study was inconsistent, with another reported value of 3.43 g/100 g, which is closer to our findings.

Our results are also consistent with the Tunisian fennel leaf study (Aider et al., 2020), where protein ranged from 5.47–6.63 %, ash from 3.53–4.4 %, and carbohydrates from 2.86–4.72 %. Moisture was comparable to Algerian values (79.42–80.31 %), while lipid levels were lower (1.04–1.17 %) compared to the Algerian counterpart.

At the seed level, the Indian fennel study reported protein contents ranging from 7.68–12.33 %, slightly higher than what we observed in Algerian fennel leaves. Conversely, seed carbohydrate and lipid contents were much higher (17.30–28.53 % and 9.29–19.10 %, respectively), confirming the marked compositional differences between leaves and seeds (Mehra et al., 2022).

Another study reported that green fennel stems contained lower levels of protein (~4 %) and fats (~2 %) compared to the leaves, while ash content reached 9 %. The energy value was comparable to our results (≈406 kcal/100 g) (Hao et al., 2021).

A comprehensive analysis of fennel structure reported total protein content of 9.38 %, lipids ranging from 9.76–10.71 %, and notably high ash levels (12.87–12.97 %) (Mehra et al., 2021). These findings highlight the nutritional importance of Algerian *F. vulgare* leaves, as our analysis was restricted to the foliar portion without accounting for other plant parts.

The discrepancies observed between the present findings and those reported in the literature can be attributed to a combination of genetic and environmental factors. These include plant species, the specific plant organ analyzed, genetic background, soil composition, and prevailing climatic conditions. Such variables are well known to induce significant variability, even within the same botanical family (Apiaceae) or across different geographical regions (Hao et al., 2021; Santoro et al., 2024).

Moisture content, in particular, is highly sensitive to environmental conditions, especially climate, as demonstrated in comparative studies conducted in Portugal and Morocco (Aider et al., 2024; Barros et al., 2010). In contrast, ash content—commonly used as an indicator of total mineral composition—tends to increase as moisture decreases, reflecting a well-established inverse relationship (Aider et al., 2024; Telci et al., 2009).

Carbohydrate levels are influenced by the stage of harvest, with delayed harvesting generally leading to reduced carbohydrate concentrations due to their metabolic utilization during plant senescence (Aider et al., 2024; Barroso et al., 2010). Similarly, protein content is modulated by multiple factors, including plant age, developmental stage, plant organ, environmental conditions, and soil nitrogen availability (Aider et al., 2024). Lipid composition also exhibits variability depending on cultivar type, genetic structure, and extraction methodology (Rebey et al., 2019).

Among plant organs, leaves are typically the richest in dietary fiber, reflecting their structural and physiological roles within the vegetative system (Santoro et al., 2024). In agreement with this, the present results indicate that Algerian *F. vulgare* leaves represent a valuable nutritional source, characterized by appreciable levels of proteins, lipids, carbohydrates, and dietary fiber (Saber and Eshra, 2019; Santoro et al., 2024).

Carbohydrates are the major macronutrient fraction, mainly composed of polysaccharides (starch and cellulose) and simple sugars (glucose, fructose, and sucrose) (Barros et al., 2010; Saber and Eshra, 2019; Santoro et al., 2024). The lower sugar content in leaves may be attributed to their developmental stage, where sugars are actively consumed in metabolic processes such as photosynthesis (Barros et al., 2010).

The lipid profile is characterized by a predominance of unsaturated fatty acids (66–80%), particularly polyunsaturated fatty acids (PUFAs). Linoleic acid (ω-6) and α-linolenic acid (ω-3) represent the major components, along with palmitic acid (Barros et al., 2010; Saber and Eshra, 2019; Santoro et al., 2024). Notably, *F. vulgare* leaves exhibit a favorable ω-6/ω-3 ratio (0.53), a low value that has been associated with a reduced risk of chronic diseases, including atherosclerosis, thrombosis, asthma, and certain cancers. Furthermore, data from the United States Department of Agriculture indicate that fennel is a rich source of essential vitamins and minerals, including potassium, calcium, and magnesium. It also contains significant levels of macronutrients (N, P, K) and trace elements (Cu, Zn, Fe), thereby enhancing its nutritional value and supporting its potential use as livestock feed. In this context, ash content serves as a direct indicator of mineral abundance (Hao et al., 2021).

In addition, *F. vulgare* leaves are characterized by high concentrations of β-carotene and chlorophyll, both of which are biologically active compounds with well-documented antioxidant, anti-mutagenic, and vision-supporting properties (Aider et al., 2024; Abdesslem et al., 2022). Overall, the nutritional profile of Algerian *F. vulgare* leaves highlights their relevance as a functional food with significant nutritional, therapeutic, and pharmaceutical potential.

2. Phytochemical composition and LC–MS analysis

2.1. Extraction yield

Extraction is defined as the process of transferring bioactive compounds from a solid or liquid matrix into a solvent phase in which these compounds exhibit greater solubility (Genthon, 2015).

In the present study, extraction yield was evaluated for two biological matrices, namely the fruiting bodies of *P. eryngii* and the leaves of *F. vulgare*, using distilled water and ethanol as extraction solvents. The choice of these solvents was not arbitrary but rather based on their well-documented efficiency in phytochemical extraction, as they are widely employed for the recovery of structurally diverse bioactive compounds (Dogan et al., 2020). In addition to their ability to achieve relatively high extraction yields (de Melo Macoris et al., 2017), distilled water and ethanol are recognized as environmentally friendly, non-toxic, and sustainable solvents. These characteristics further support their suitability for applications in pharmaceutical and nutraceutical analyses (Dogan et al., 2020).

Marked differences were observed depending on the plant material and solvent used (Table 2).

Table 2. Extraction yield % of *P. eryngii* and *F. vulgare* leaves extracts.

Sample	Extract	Mean ± SD (n=3)
P. eryngii	Ethanolic	7.47 ± 0.46^{b}
	Aqueous	10.76 ± 0.69^{a}
***F. vulgare* leaves**	Ethanolic	8.15 ± 1.55^{a}
	Aqueous	2.86 ± 0.96^{b}

The results are expressed as mean ± standard deviation (Mean ± SD). Values with different superscript letters (a, b, or c) in the same column are significantly different ($p < 0.05$).

For *P. eryngii*, the aqueous extract yielded 10.76 ± 0.69%, whereas the ethanolic extract yielded 7.47 ± 0.46%, indicating that water was significantly more effective for extracting polar, water-soluble compounds. In contrast, *F. vulgare* leaves showed a considerably higher yield with ethanol (8.15 ± 1.55%) compared to distilled water (2.86 ± 0.96%), which agrees with the findings of Starchenko et al. (2020) and Beyazen et al. (2017) that alcoholic solvents generally produce higher extraction yields due to their efficiency in solubilizing phenolic compounds.

Nevertheless, the yield of *F. vulgare* leaves in the present study was substantially lower than the 15.88% (158.8 ± 8.28 mg/g) reported by Beyazen et al. (2017), likely due to differences in extraction parameters such as solvent-to-sample ratio, particle size, or drying method. Conversely, the yield obtained for *P. eryngii* in the present work was higher than the 3.14% reported by Yu et al. (2018), suggesting that both the solvent choice and the physicochemical characteristics of the mushroom matrix strongly influence extraction efficiency.

As noted by Toumi et al. (2025), extraction yield is significantly affected by the polarity of the compounds and the solvent's nature. This aligns with our observations, where *P. eryngii*, rich in highly polar, water-soluble metabolites , favoured aqueous extraction, while *F. vulgare*, rich in phenolics, favoured ethanolic extraction. Dong et al. (2019) further highlighted that the physicochemical properties, bioactive components, and antioxidant activity of mushrooms are significantly influenced by the drying technique employed, a factor that may also explain inter-study variability.

Such discrepancies underscore the critical influence of the extraction technique and solvent system, as emphasized by Nawaz et al. (2020) and Lezoul et al. (2020)**,** who demonstrated that the recovery of bioactive compounds is highly dependent on solvent type and extraction conditions. Nonetheless, direct comparisons between studies remain challenging due to multiple influencing factors, including sampling site, drying method, processing temperature, compound polarity, and solvent-to-sample ratio (Ayouni et al., 2016).

2.2. Phytochemical profiling and quantification of bioactive compounds

Phenolic compounds are among the most extensively investigated classes of bioactive constituents, owing to their strong antioxidant capacity and wide range of biological activities, including anti-inflammatory, antimicrobial, antihypertensive, and anticancer effects (Shi et al., 2022). These compounds, which are abundant in plant-derived extracts, are increasingly recognized as natural therapeutic agents for the prevention and management of oxidative stress–related disorders, including cancer and diabetes.

Flavonoids, representing a major subclass of phenolic compounds predominantly found in edible plant parts, play a crucial role in these health-promoting effects through their antioxidant, antibacterial, and anti-inflammatory properties (Roy et al., 2022).

The growing interest in native plant resources as alternatives to synthetic compounds further underscores the importance of optimizing extraction strategies in order to maximize the recovery and bioavailability of these bioactive molecules.

2.2.1 TPC content

In this study, the total phenolic compound (TPC) content was evaluated in aqueous and ethanolic extracts of *P. eryngii* fruiting bodies and *Foeniculum vulgare* leaves.

The aqueous extract of *P. eryngii* showed higher phenolic content (20.65 ± 0.27 mg GAE/g extract) compared to its ethanolic extract (13.14 ± 0.54 mg GAE/g extract). Interestingly, this suggests that water was a more efficient solvent than ethanol for extracting polyphenols from *P. eryngii* (Table 3).

Table 3. TPC and TFC contents of *P. eryngii* and *F. vulgare leaves* extracts.

Parameter	**Extract**	***P. eryngii***	***F. vulgare leaves***
TPC (mg GAE/g)	Ethanolic	13.14 ± 0.54^{b}	23.80 ± 0.19^{a}
	Aqueous	20.65 ± 0.27^{a}	11.51 ± 0.61^{b}
TFC (mg QE/g)	Ethanolic	2.87 ± 0.05^{b}	17.62 ± 0.99^{a}
	Aqueous	5.69 ± 0.07^{a}	10.23 ± 0.24^{b}

The results are expressed as mean ± standard deviation (Mean ± SD). Values with different superscript letters (a, b, or c) in the same column are significantly different ($p < 0.05$).

The phenolic content values obtained in this study were significantly lower than those reported by Ait Hamadouche et al. (2024) in Algeria, who found a TPC of approximately 140 mg/g of extract using ethyl acetate in a Soxhlet apparatus, a markedly higher figure than ours. However, our results exceeded the TPC reported by Yu et al. (2018) in Korea, where various organic extracts of *P. eryngii* ranged between 1.31 ± 0.02 and 1.67 ± 0.05 mg GAE/g extract. Similarly, Choi et al. (2021) reported lower TPC values for aqueous and ethanolic extracts of the same mushroom in Korea, ranging between 1.03 ± 0.05 and 8.64 ± 0.09 mg GAE/g extract. Calabretti et al. (2021) in Italy also observed lower phenolic contents in various commercial and wild isolates of *P. eryngii*, varying from 0.24 ± 0.01 to 5.34 ± 0.05 mg/g, which are low compared to the Algerian *P. eryngii* studied here. Our findings align with those of Ryu et al. (2018) in Korea, who reported a TPC of 19.60 ± 0.10 mg/g in aqueous extracts. Additionally, Kongkla and Poeaim (2016) in Thailand found maximum phenolic contents of 17.63 ± 1.30 mg GAE/g in methanolic extracts of oyster mushrooms belonging to the same family.

Conversely, in *F. vulgare* leaves, the ethanolic extract exhibited higher polyphenol content (23.80 ± 0.19 mg GAE/g extract) than the aqueous extract (11.51 ± 0.61 mg GAE/g extract), indicating that solvent efficiency depends on the specific plant matrix and compounds (Table 3).

According to Pacifico et al. (2018), alcoholic extracts of freeze dried winter leaves of Italian *F. vulgare* showed a total phenolic content of 100.0 mg GAE/g of extrat, which is higher compared to the values obtained in the present study. The total phenolic content measured in the ethanolic extract in this study was also lower than the values reported by Singh et al., (2013) for ethanolic and hexane extracts of the same plant material, which were 44.11 ± 0.50 and 37.11 ± 1.73 mg GAE/g dry weight, respectively. However, the TPC observed herein was comparable to that reported for the aqueous extract (25.75 ± 1.73 mg GAE/g), and exceeded the concentration obtained using methanol (17.81 ± 0.49 mg GAE/g).

Furthermore, the TPC value in the current ethanolic extract of *Foeniculum vulgare* leaves was lower than those reported for *F. vulgare* seeds in the studies conducted by Benabdallah et al. (2022) and Cherbal et al. (2023), which recorded concentrations of 551.45 ± 0.010, 38.2 ± 2.3, mg GAE/g extract, respectively.

Nonetheless, the result obtained in the present investigation surpassed the value reported by Rebey et al. (2019) for Tunisian *F. vulgare* seeds, which was 15.69 ± 3.39 mg GAE/g DW.

2.2.2 TFC content

For *P. eryngii*, the aqueous extract contained significantly higher flavonoids (5.69 ± 0.07 mg QE/g extract) than the ethanolic extract (2.87 ± 0.05 mg QE/g extract), with this extract also showing the highest total phenolic content (Table 3).

Several previous studies have investigated flavonoid levels in *P. eryngii*. A Korean research team reported concentrations of 5.34 ± 0.12, 1.63 ± 0.03, and 1.31 ± 0.02 mg CE/g in acetone, ethyl acetate, and pure ethanol extracts, respectively (Yu et al., 2018), which align with the present findings. Another study from Korea reported flavonoid concentrations ranging from 24.71 ± 0.36 to 64.13 ± 2.10 µg/ml for extracts obtained at different temperatures (30, 55, and 80 °C) after hot-air or freeze-drying of *P. ostreatus* (Kim et al., 2020). Similarly, a Polish research group found values between 1.26 ± 0.17 and 1.89 ± 0.09 mg/g in ordinary king oyster mushrooms and zinc/selenium-enriched samples using methanolic extracts (Gąsecka et al., 2016). In another study, Choi et al. (2021) recorded flavonoid concentrations ranging from 5.57 ± 1.39 to 13.00 ± 2.04 µg QE/g, which are relatively low compared to the present results for Algerian *P. eryngii.* Conversely, Ryu et al. (2018) reported 15.21 ± 1.31 mg/g flavonoids in water extracts of *P. ostreatus,* a value higher than that observed in this study.

In contrast, flavonoid levels in *F. vulgare* leaves were greater in the ethanolic extract (17.62 ± 0.99 mg QE/g extract) compared to the aqueous extract (10.23 ± 0.24 mg QE/g extract) (Table 3), highlighting ethanol's enhanced ability to solubilize flavonoids and other bioactive compounds from plant tissues.

The flavonoid content in the leaf extracts investgated in yhis study was slightly lower than the 19.71 mg QE/g reported by Moumen et al. (2025) for a decoction of the seeds. However, it exceeded the flavonoid levels recorded in aqueous (14.78 mg/g) and ethanolic extracts (5.08 mg/g), as well as those reported in Tunisian seeds by Khammassi et al. (2022) and Kalleli et al. (2019), which were 16.42 ± 0.20 mg QE/g and 12.65 ± 0.88 mg QE/g, respectively. These findings suggest that *F. vulgare* leaves may represent a superior source of flavonoids compared to the seeds, underscoring their potential as a valuable natural reservoir of bioactive compounds.

Although solvent polarity is the main determining factor in the extraction process (Farooq et al., 2020), given that different phenolic compounds possess varying degrees of polarity (Veljović et al., 2017), the observed differences in total phenolic compound (TPC) and total flavonoid content (TFC) between the studied extracts of *P. eryngii* and *F. vulgare* leaves, especially considering the inversion in the results, cannot be solely attributed to solvent quality or extraction methods due to their similarity. Instead, these contradictory findings suggest a specific interaction between the solvent and the type of plant matrix analyzed.

Variations observed in comparison with previous studies can be attributed to differences in extraction conditions, including the extraction technique, solvent properties, extraction time and temperature, solvent-to-solid ratio, and the intrinsic composition of the plant matrix (Senapati and Behera, 2023; Putra et al., 2024). In addition, environmental and agronomic factors such as climatic conditions, genetic background, plant maturity, and harvest time are known to significantly influence the yield and composition of bioactive compounds (Goudjil et al., 2024; Bentahar et al., 2025).

Mushrooms produce a range of ligninolytic enzymes, including manganese peroxidase and laccase, which play key roles in the degradation of lignin, cellulose, and hemicellulose in the growth substrate, whether under natural or controlled conditions. This degradation process facilitates the utilization of these macromolecules as precursors for the biosynthesis of simple phenolic compounds. In parallel, phenylalanine ammonia-lyase (PAL) catalyzes the conversion of phenylalanine and tyrosine into phenolic acids, thereby promoting polyphenol biosynthesis. Consequently, variations in polyphenol content in fruiting bodies can be explained by differences in the activity of these enzymes, which are involved in both substrate degradation and phenolic metabolism (Kebaïli, 2022). According to Moumen et al. (2025), extracts of *F. vulgare* are particularly rich in bioactive constituents, notably polyphenols and phenolic acids, which confer strong antioxidant and antimicrobial properties. The ethanolic extract has been reported to be especially efficient in recovering flavonoids and polyphenols, as well as concentrating key bioactive compounds such as quercetin-3-glucuronide, quercetin-3-di-xyloside, apigenin, and rutin.

Overall, the occurrence of phenolic and flavonoid compounds in mushrooms and plant matrices underscores their importance as significant sources of antioxidant molecules with protective roles against various human diseases. Therefore, optimizing extraction parameters remains essential to maximize the recovery of these compounds and to enhance their potential applications in pharmaceutical and food industries.

2.2.3. Phenolic compounds determination

To identify the bioactive compounds present in the ethanolic extracts, LC-MS/MS analysis was performed on both *P. eryngii* fruiting bodies and *F. vulgare* leaves.

The analysis of the ethanolic extract of *P. eryngii* revealed the presence of three bioactive compounds. The primary constituent was trans-cinnamic acid, with a concentration of 45.88 µg/g extract of extract. The second identified acid was gentisic acid, present at 8.39 µg/g extract of extract, while p-coumaric acid was found at the lowest concentration of 1.72 µg/g extract (Table 4).

Table 4. LC-MS/MS analysis data of detected metabolites from the ethanolic extract of *P. eryngii* mushroom.

Nº	Compound name	Retention time	Ethanolic extract (µg /g)	Prec Ion	Prod Ion	Dwell	Frag (V)	CE (V)	Polarity
1	**Gentisic acid**	3,37	8.39	152.8	108.9	9	100	13	Negative
2	**p-Coumaric acid**	4,45	1.72	163.1	119.2	9	85	12	Negative
3	**trans-cinnamic acid**	10.81	45.88	149.1	131.1	18	90	6	Positive

These compounds exhibit a range of significant biological activities. Trans-cinnamic acid, identified as the major constituent, is a phenolic compound known for its broad spectrum of bioactivities, particularly its strong antioxidant capacity (Sova, 2012). It also demonstrates anti-inflammatory properties and retains its biological activity following digestion (Pagliari et al., 2023). Moreover, trans-cinnamic acid has been reported to promote wound healing by stimulating laminin and type I collagen synthesis, as well as enhancing extracellular matrix regulation and fibroblast migration (De Aquino et al., 2021).

The second identified compound, gentisic acid, exhibits a higher antioxidant capacity than its precursor, attributable to its molecular structure, which enables selective transformation into bioactive derivatives that modulate inflammatory responses (Abedi et al., 2020). Its therapeutic potential has been demonstrated in studies showing its ability to mitigate diabetes-induced renal damage through the reduction of creatinine and urea levels and the regulation of the renin–angiotensin system in kidney tissues (Altinoz and Ozpinar, 2019). Furthermore, Razliqi et al. (2023) reported that gentisic acid exerts nephroprotective effects by increasing urinary nitrogen and albumin levels while reducing oxidative stress markers, including malondialdehyde. It also enhances antioxidant defense systems by activating key enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, while downregulating pro-inflammatory mediators, including NF-κB, TNF-α, and IL-1β, and upregulating anti-inflammatory IL-10 levels (Razliqi et al., 2023).

The least abundant compound, p-coumaric acid, also displays relevant biological activities, including antimicrobial, antidiabetic, antigout, and antioxidant effects (Kaur and Kaur, 2022). Its therapeutic potential in inflammatory conditions has been highlighted, particularly in the context of diseases such as rheumatoid arthritis (Pragasam et al., 2013).

Chromatographic and spectrometric investigations of phenolic compounds in *P. eryngii* remain relatively limited. Gąsecka et al. (2016) reported the presence of p-coumaric and trans-cinnamic acids among several detected compounds, but did not identify gentisic acid, which was detected in the present study and may represent a distinguishing feature of Algerian samples. In contrast, Li and Shah (2013) identified eight compounds, including gentisic and cinnamic acids, whereas Alam et al. (2011) reported ten phenolic constituents, namely gallic acid, protocatechuic acid, chlorogenic acid, vanillin, ferulic acid, naringin, naringenin, hesperetin, formononetin, and biochanin A. More recently, Ait Hamadouche et al. (2024) detected a single phenolic compound, myricetin, in *P. ostreatus*, and two compounds—quercetin and 2,3,4,5,7-pentahydroxyflavone (2,3,4,5,7-PHF)—in *P. dryinus* using HPLC analysis.

The absence or variation of certain compounds in the present study compared to previous reports may be attributed to differences in extraction methodologies, which are known to significantly affect the recovery and detectability of phenolic compounds. Additionally, environmental and climatic conditions, along with variations in fungal metabolism and developmental stage at the time of sampling, may further contribute to these discrepancies.

Similarly, the ethanolic extract of *F. vulgare* leaves was analyzed, revealing the presence of 10 bioactive phenolic compounds. Chlorogenic acid was the most abundant, measured at 95.73 µg/g extract, followed by trans-ferulic acid (18.32 µg/g extract), salicylic acid (15.97 µg/g extract), rutin (15.53 µg/g extract), trans-cinnamic acid (12.34 µg/g extract), gentisic acid (12.09 µg/g extract), vanillin (3.31 µg/g extract), quercetin (2.54 µg/g extract), morin (2.01 µg/g extract), and caffeic acid (1.01 µg/g extract) (Table 5).

Table 5. LC-MS/MS analysis data of detected metabolites from the ethanolic extract of *F. vulgare* Leaves.

Nº	Compound name	Retention time	EE (µg/g)	Prec Ion	Prod Ion	Dwell	Frag (V)	CE (V)	Polarity
1	**Chlorogenic acid**	2.54	95.73	353	191	9	110	12	Negative
2	**Trans-Ferulic acid**	5.85	18.32	195.1	176.9	9	75	10	Positive
3	**Salicylic acid**	7.25	15.97	137	93.1	38	85	18	Negative
4	**Rutin**	2.95	15.53	609.1	300.2	9	185	40	Negative
5	**Trans-cinnamic acid**	10.81	12.34	149.1	131.1	18	90	6	Positive
6	**Gentisic acid**	3.37	12.09	152.8	108.9	9	100	13	Negative
7	**Vanillin**	4.97	3.31	152.9	124.9	9	65	13	Positive
8	**Quercetin**	11.63	2.54	300.8	179	18	130	20	Negative
9	**Morin**	11.64	2.01	302.9	136.8	18	145	32	Positive
10	**Caffeic acid**	3.22	1.01	178.9	135	9	80	16	Negative

Morin exerts neuroprotective and anti-inflammatory effects primarily through the inhibition of acetylcholinesterase activity, leading to increased acetylcholine levels and improved cognitive function, particularly in cerebral ischemia models. In addition, it has demonstrated anti-angiogenic and analgesic activities *in vivo* (Jung et al., 2010).

Caffeic acid is also recognized for its multifaceted pharmacological properties. It promotes vasodilation via modulation of vascular smooth muscle activity, adrenergic receptors, nitric oxide release, and ion channel regulation. Furthermore, its antioxidant, anti-inflammatory, and anti-angiogenic effects contribute to protection against atherosclerosis and ischemia/reperfusion injury. It has also been shown to downregulate pro-inflammatory cytokines and malondialdehyde levels, while upregulating IL-10 and total antioxidant capacity, thereby supporting its therapeutic potential in inflammatory bowel diseases (Silva and Lopes, 2020; Wan et al., 2021). Chlorogenic acid was identified as the predominant compound in the present study, in agreement with previous reports on Moroccan *F. vulgare* seed extracts (Moumen et al., 2025) and Tunisian *Pimpinella anisum* L. extracts (Rebey et al., 2019), both belonging to the Apiaceae family. Similarly, comparable phytochemical profiles have been reported by Kalleli et al. (2019) and Khammassi et al. (2022) in Tunisian *F. vulgare* seeds, where chlorogenic acid, caffeic acid, trans-ferulic acid, rutin, and quercitrin were identified, in close agreement with the present findings.

Although most previous studies have focused on *F. vulgare* seeds, to the best of our knowledge, the present work represents the first LC–MS/MS-based comprehensive phytochemical profiling of *F. vulgare* leaves. This provides novel insights into their chemical composition and highlights their potential biological and functional relevance.

3. *In vitro* Evaluation of biological activities

3.1. Antioxidant activity

To maintain cellular homeostasis, a dynamic equilibrium between pro-oxidant and antioxidant systems is essential, ensuring that reactive oxygen species (ROS) remain at physiological levels. Oxidative stress (OS) arises when this balance is disrupted in favor of oxidants, leading to the excessive accumulation of ROS and subsequent oxidative damage to nucleic acids, proteins, lipids, and carbohydrates, ultimately contributing to cell dysfunction and death (Pereira et al., 2021; Venmathi Maran et al., 2022; Tumilaar et al., 2024). This condition has been implicated in the pathogenesis of numerous chronic diseases, including cancer, neurodegenerative disorders such as Alzheimer’s disease, cardiovascular pathologies, gastrointestinal disorders, and the aging process (Skoryk and Horila, 2023).

Antioxidants mitigate oxidative damage by scavenging ROS through electron or hydrogen donation, thereby stabilizing free radicals and interrupting chain oxidation reactions. This rapid interaction, particularly characteristic of low-molecular-weight antioxidants, limits oxidative propagation and protects cellular components from damage (Tumilaar et al., 2024).

Antioxidants are broadly classified according to their solubility and mode of action into water-soluble compounds (e.g., vitamin C, polyphenols), lipid-soluble compounds (e.g., vitamin E), and enzymatic and non-enzymatic systems comprising molecules such as flavonoids, phospholipids, and ceruloplasmin (Skoryk and Horila, 2023; Tumilaar et al., 2024).

Due to the potential toxicity associated with synthetic antioxidants such as BHA and BHT, increasing attention has been directed toward natural antioxidants derived from plant sources. Phenolic-rich plant matrices represent a major reservoir of bioactive compounds capable of neutralizing ROS and reducing oxidative stress, supporting their applications in pharmaceutical, nutritional, and cosmetic fields, particularly flavonoid-rich extracts (Tumilaar et al., 2024). Similarly, bioactive compounds from functional food systems with diverse antioxidant profiles contribute to improved oxidative stability and biological activity (Laila et al., 2025).

The investigated species in this study are characterized by a high content of phenolic compounds, structurally defined by hydroxyl groups attached to aromatic rings. These structural features enable hydrogen atom or electron donation, leading to the stabilization of reactive radicals and termination of oxidative chain reactions (Çayan et al., 2020).

Given the complexity of oxidative mechanisms and the diversity of antioxidant compounds with different polarities, no single assay can comprehensively evaluate antioxidant capacity. Therefore, multiple complementary assays are generally required to obtain a reliable and holistic assessment of antioxidant activity (Diallo, 2019).

In this study, the antioxidant activity of aqueous and ethanolic extracts of *P. eryngii* and *F. vulgare* leaves was evaluated using five complementary assays: DPPH, FRAP, total antioxidant capacity (TAC), β-carotene bleaching, and hydrogen peroxide scavenging assays. This integrated approach allows the assessment of different antioxidant mechanisms, including radical scavenging, reducing power, and inhibition of lipid peroxidation.

The DPPH assay, widely used due to its simplicity and reproducibility, evaluates the hydrogen or electron donating ability of antioxidants toward the stable DPPH radical, making it particularly suitable for phenolic-rich extracts (Munteanu and Apetrei, 2021; Gulcin and Alwasel, 2023).

The FRAP assay measures the reducing potential of antioxidants by their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, reflecting their electron-donating capacity (Jayakumar et al., 2011).

The total antioxidant capacity (TAC) assay, based on the phosphomolybdenum method, quantifies the overall antioxidant potential through the reduction of molybdenum (VI) to molybdenum (V), representing a total electron transfer-based measurement (Aadesariya et al., 2017; Hussen and Endalew, 2023).

The β-carotene bleaching assay evaluates the ability of antioxidants to inhibit lipid peroxidation by preventing oxidative degradation of β-carotene in an emulsion system, thereby reflecting both hydrophilic and lipophilic antioxidant activity (Sadowska-Bartosz and Bartosz, 2022).

Finally, the hydrogen peroxide scavenging assay assesses the ability of extracts to neutralize H_2O_2, a reactive oxygen species capable of generating highly reactive hydroxyl radicals in biological systems. This assay is often used as a complementary confirmation of radical scavenging potential (Kim et al., 2023).

Table 6 presents the results of antioxidant activity, as determined by the five assays (DPPH, FRAP, TAC, β-carotene bleaching, and hydrogen peroxide scavenging), conducted for both *P. eryngii* and *F. vulgare* leaves.

Table 6. Antioxidant activity of *P. eryngii* and *F. vulgare* leaves and antioxidant standards.

Sample		**DPPH**	**β-Carotene Bleaching**	**H_2O_2 Scavenging**	**TAC**	**FRAP**	
			IC_{50} (µg/mL)		**mg AAE/ g extract**	**EC_{50} (µg/mL)**	**$A_{0.5}$ (µg/mL)**
P. eryngii	**Ethanolic extract**	1222.46 ± 130.34^{c}	1170.92 ± 61.68^{c}	701.59 ± 58.12^{c}	120.48 ± 3.02^{b}	734.62 ± 3.91^{c}	107.75 ± 3.91^{c}
	Aqueous extract	714.57 ± 59.51^{b}	717.65 ± 12.52^{b}	393.89 ± 73.61^{b}	129.63 ± 1.14^{a}	687.89 ± 1.96^{b}	35.51 ± 1.87^{b}
***F. vulgare* leaves**	**Ethanolic extract**	15.39 ± 0.96^{b}	19.36 ± 0.16^{b}	128.33 ± 1.00^{b}	1769.6 ± 3.03^{c}	207.8 ± 4.04^{b}	25.92 ± 2.91^{b}
	Aqueous extract	386.91 ± 4.14^{c}	260.32 ± 17.67^{c}	504.21 ± 12.85^{c}	1589.12 ± 17.48^{b}	316.5 ± 2.45^{c}	51.32 ± 1.51^{c}
Antioxidant standards	**Ascorbic acid**	5.61 ± 2.83^{a}	14.99 ± 1.44^{a}	7.75 ± 0.65^{a}	-	150.91 ± 4.42^{a}	7.66 ± 1.33^{a}
	BHA	5.98 ± 5.38^{a}	-	13.80 ± 4.46^{a}	-	79.25 ± 2.19^{a}	6.15 ± 1.00^{a}
	BHT	-	-	-	7970.63 ± 171.75^{c}	-	-

IC_{50}: the concentration required to inhibit 50% of activity ; EC_{50}: the effective concentration required to produce 50% of the maximal effect ; $A_{0.50}$: the concentration corresponding to an absorbance value of 0.50. Values are expressed as mean ± SD.

3.1.1. DPPH radical scavenging activity

Both the aqueous and 80% ethanolic extracts of Algerian *P. eryngii* mushrooms exhibited notable efficacy in scavenging DPPH free radicals, with IC_{50} values of 714.57 ± 59.51 µg/mL and 1222.46 ± 130.34 µg/mL, respectively (Table 6). In both extracts, the percentage of inhibition increased progressively across the tested concentrations (0.03125, 0.0625, 0.125, 0.25, 0.5, and 1 mg/mL), reaching maximal activity at 1 mg/mL. The aqueous extract consistently demonstrated a higher radical scavenging capacity than the 80% ethanolic extract. At all tested concentrations, both extracts significantly inhibited the DPPH radical in a dose-dependent manner ($P < 0.05$ vs. control), with Pearson's correlation coefficients ranging from 0.87 to 0.98, indicating strong positive associations.

The DPPH radical scavenging capacity of *P. eryngii* has been reported in several prior studies, though results vary widely. Kongkla and Poeaim (2016) reported an IC_{50} of 8.48 ± 0.87 mg/mL for aqueous extracts, whereas Gąsecka et al. (2016) observed a lower IC_{50} of 3.35 ± 0.11 mg/mL in methanolic extracts from zinc- and selenium-enriched *P. eryngii*. In a study on Korean *P. eryngii*, Choi et al. (2021) recorded IC_{50} values of 2479.07 ± 7.87 µg/mL for aqueous, 2610.77 ± 9.62 µg/mL for 70% ethanolic, and 5123.60 ± 34.45 µg/mL for 95% ethanolic extracts. These values are markedly higher (indicating lower activity) than those obtained in the present work, supporting our observation that aqueous extracts possess superior radical scavenging capacity compared to ethanolic extracts, consistent with the findings of Choi et al. (2021). Similar trends were reported by Lin et al. (2014), who evaluated *P. eryngii* harvested at different fruiting stages and found ethanol extract IC_{50} values ranging from 1.08 ± 0.06 to 1.30 ± 0.10 mg/mL. In contrast, our results diverge from those of Li and Shah (2013), who reported substantially higher activity (IC_{50} = 32.61 ± 0.63 µg/mL) in freeze-dried samples.

Comparative analyses with related *Pleurotus* species also reveal variability in antioxidant potential. Lee et al. (2007) demonstrated that ethanol, hot water, and cold water extracts of *P. citrinopileatus* effectively scavenged free radicals, with the highest activity in ethanol extracts (1.33 ± 0.01 mg/mL), in line with the performance of our ethanolic extract. In the same study, hot and cold water extracts exhibited weaker activity (15.24 ± 0.06–16.47 ± 0.06 mg/mL) than the Algerian *P. eryngii* extracts reported here. Stastny et al. (2022) evaluated the DPPH scavenging activity of several *Pleurotus* species (*P. flabellatus* 5013, *P. pulmonarius* KZ50, *P. opuntiae* 5012, *P. ostreatus* Sylvan Ivory, and *P. ostreatus* 5175 Florida) using diluted methanol extractions. Their results indicated that most species displayed stronger activity than *P. eryngii* in our study, except *P. ostreatus* 5175 Florida (IC_{50} = 1134.0 ± 65.8 µg/mL), which was comparable to our ethanolic extract. Moreover, Randhawa and Shri (2018) reported significantly greater activity in *P. florida* (IC_{50} = 239.02 ± 0.22 µg/mL).

Evaluation of antioxidant activity using the DPPH assay revealed a progressive increase in free radical neutralization with rising concentrations of both ethanolic and aqueous extracts of *F. vulgare* leaves, demonstrating a clear dose-dependent response and exhibiting higher activity than the *P. eryngii* extracts reported in the present work. Percent inhibition was also assessed using ascorbic acid and BHA as reference standards. At a concentration of 200 µg/mL, the ethanolic extract achieved an inhibition rate of 70 ± 0.81%, with an IC_{50} value of 15.39 ± 0.96 µg/mL. In contrast, the aqueous extract reached a lower inhibition rate of 65.71 ± 0.34% at a concentration of 1 mg/mL, corresponding to an IC_{50} of 386.91 ± 4.14 µg/mL (Table 6). These findings indicate that both extracts possess notable antioxidant capacity, particularly at higher concentrations.

Conversely, ascorbic acid and BHA demonstrated markedly stronger antioxidant activity, effectively inhibiting DPPH free radicals even at low concentrations. Their activity levels were substantially higher than those of the aqueous extract and only slightly higher than those of the ethanolic extract of *F. vulgare* leaves, underscoring the superior efficacy of the reference antioxidants.

When compared to prior research, the alcoholic extracts of freeze-dried winter leaves of Italian *F. vulgare* demonstrated lower antioxidant activity, with an IC_{50} of 40.12 µg/mL (Pacifico et al., 2018)**.** In the same context, the essential oil from Algerian *F. vulgare* leaves achieved a DPPH scavenging activity of 82.04 ± 0.47 mg/mL, which appears weaker than the activity observed in the present study (Hamada Saoud et al., 2024). Likewise, the ethanolic extract investigated herein exhibited stronger activity than the hydromethanolic leaf extract of *F. vulgare* reported by Beyazen et al., (2017), which showed an IC_{50} of 69.68 ± 2.28 µg/mL, and further surpassed the aqueous leaf extract evaluated in the current work.

In addition, studies Kalleli et al. (2019) and Khammassi et al. (2022) on Tunisian *F. vulgare* seed extracts prepared using organic solvents such as ethanol and methanol reported IC_{50} values of 27.17 ± 2.82 mg/mL and 23.66 mg/mL, respectively, while an aqueous seed extract evaluated by Benabdallah et al. (2022) showed weaker activity with an IC_{50} of 30.91 ± 0.49 mg/mL. All these values were much lower than those recorded in the present study.

Finally, within the same botanical family (Apiaceae), *Pimpinella anisum* L. examined in Rebey et al. (2019) displayed markedly higher activity, with an IC_{50} of 12.87 µg/mL, thus exceeding the values recorded for *F. vulgare* in the present work, particularly for the Algerian variety.

3.1.2. Ferric reducing antioxidant power (FRAP)

The results of the FRAP assay were expressed as $A_{0.5}$ (the concentration yielding an absorbance of 0.5) and EC_{50} (half maximal effective concentration) values for the studied extracts, in comparison with standard compounds (ascorbic acid, BHA, and BHT).

Regarding *P. eryngii*, the aqueous extract exhibited $A_{0.5}$ and EC_{50} values of 35.51 ± 1.86 µg/mL and 687.89 ± 1.96 µg/mL, respectively, whereas the ethanolic extract recorded values of 107.74

± 3.91 µg/mL and 734.62 ± 3.91 µg/mL, respectively. In contrast, the reference antioxidants displayed clearly superior activity, with $A_{0.5}$ values of 7.66 ± 1.33 µg/mL for ascorbic acid and 6.15 ± 1.00 µg/mL for BHA, and EC_{50} values of 150.91 ± 4.42 µg/mL and 79.25 ± 2.19 µg/mL, respectively (Tables 8).

According to Dunnett's test, both aqueous and ethanolic extracts showed statistically significant differences ($p < 0.001$) when compared with standard antioxidants. These findings indicate a moderate reducing ability for both extracts, in contrast to the strong reducing power of ascorbic acid and BHA. Nevertheless, the aqueous extract demonstrated a higher reducing capacity than its ethanolic counterpart from Algerian *P. eryngii.*

These observations are consistent with trends observed in the DPPH radical scavenging assay, supporting the hypothesis of a strong relationship between phenolic compound content and oxidative stress inhibition capacity.

For comparison, Choi et al. (2021) reported EC_{50} values of 2205.67 ± 2.89, 4653.67 ± 15.28, and 5178.72 ± 10.64 µg/mL for water, 70% ethanol, and 95% ethanol extracts, respectively, which are markedly higher than the values obtained in the present study, indicating lower reducing activity. Similarly, Lin et al. (2014) found EC_{50} values ranging from 2.58 ± 0.12 to 2.97 ± 0.16 mg/mL in ethanolic extracts of *P. eryngii* fruiting bodies at different developmental stages, while Lee et al. (2007) reported EC_{50} values ranging from 1.11 ± 0.01 to 2.28 ± 0.01 mg/g for ethanolic and aqueous extracts of *P. citrinopileatus.*

The relatively low EC_{50} values obtained in the current study reflect a strong reducing capacity, underscoring the potential of Algerian *P. eryngii* as a significant source of antioxidants. Furthermore, the superior performance of aqueous extracts in reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) compared to ethanolic extracts is in agreement with the findings of Choi et al.(2021) and Lee et al. (2007), and was also confirmed by Yalcin et al. (2020) in their investigation of *Ganoderma carnosum* and *Ganoderma pfeifferi*, reinforcing the notion that polar solvents are more effective in extracting water-soluble compounds responsible for high reducing activity.

The results presented in Table 6 show that the ethanolic extract of *F. vulgare* leaves exhibited a lower $A_{0.5}$ value (25.92 ± 2.91 µg/mL) than the aqueous extract (51.32 ± 1.51 µg/mL), with a highly significant difference between them ($p \leq 0.001$). In contrast, the reference standards (ascorbic acid and BHA) displayed markedly lower $A_{0.5}$ values ($p \leq 0.001$), confirming their superior reducing power compared to both extracts.

Similarly, for EC_{50} values, the ethanolic extract recorded a lower value (207.8 ± 4.04 µg/mL) than the aqueous extract (316.50 ± 2.45 µg/mL), with a highly significant difference between them ($p \leq 0.001$), while the reference antioxidants again demonstrated clearly stronger activity, with substantially lower EC_{50} values than those of the plant extracts ($p \leq 0.001$). These findings highlight the moderate reducing ability of the extracts relative to the potent activity of the standard antioxidants.

When compared with data from previous studies, the reducing capacity of the ethanolic extract in this work falls within the upper range of values reported for the methanolic extract of fennel seeds from various Tunisian regions (41.85–253.32 µg/mL) (Kalleli et al., 2019). These results are also close to the activity recorded for the hydroethanolic extract of Moroccan fennel seeds obtained by Soxhlet extraction (153.60 µg/mL) according to Moumen et al. (2025). Notably, the same study found that the aqueous extract prepared by decoction was more effective than that obtained by Soxhlet, highlighting the role of extraction method in enhancing reducing capacity through improved recovery of active phenolic compounds.

Regarding essential oils, Tunisian *F. vulgare* leaf essential oil showed EC_{50} values ranging from 0.78 ± 0.01 to 0.29 ± 0.01 mg/mL (Aider et al., 2024), while the Egyptian fennel essential oil exhibited an activity of 3.23 mg/mL according to Shahat et al. (2011).

Finally, for another Apiaceae species, Rebey et al. (2019) reported FRAP-based reducing capacities in the mature stage of *Pimpinella anisum* of 122.76 ± 4.8 µg/mL (Turkish sample), 565.11 ± 9.0 µg/mL (Egyptian sample), and 652.47 ± 8.0 µg/mL (Tunisian sample), most of which were less effective than the ethanolic leaf extract of fennel in this study.

3.1.3. Total antioxidant capacity (TAC)

As shown in Table 6, the total antioxidant capacity (TAC) of Algerian *P. eryngii* was 129.63 ± 1.14 µg AAE/g for the aqueous extract and 120.48 ± 3.02 µg AAE/g for the 80% ethanolic extract, with no statistically significant difference observed between them. However, the aqueous extract exhibited a slightly higher reducing ability in mitigating oxidative stress compared to the ethanolic extract ($p \leq 0.01$), a trend consistent with the results obtained from the FRAP and DPPH assays. Both extracts, nonetheless, showed considerably lower TAC values than the synthetic antioxidant BHT (7970.63 ± 171.75 µg AAE/g) ($p \leq 0.001$).

This observation aligns with the findings of Untea et al. (2018), who also reported a strong correlation and agreement between the DPPH and TAC assays when assessing the antioxidant potential of certain medicinal plants. Numerous studies have similarly highlighted the considerable total antioxidant capacity of both wild and cultivated mushrooms, particularly within the Pleurotus genus. For instance, Jegathchandran et al. (2024) reported TAC values of approximately 4.8 mg AAE/g for aqueous extracts of *P. ostreatus* and 2.5 mg AAE/g for *P. eryngii*. Likewise, Sirimanne and Liyanage (2024) found that *P. ostreatus* cultivated on rice straw and coconut straw exhibited a molybdenum-reducing ability of 4.50 mg AAE/g, values considered significant and notably higher than those obtained in the present study. In the same context, Krishna et al. (2023) reported total antioxidant capacities of 5.11 and 9.16 mg AAE/mg extract for acetonic and methanolic extracts of *P. eryngii*, respectively, further illustrating the variability in TAC values for this species depending on the extraction solvent and conditions.

In contrast, when comparing our results with those of Devi et al. (2014), who reported a TAC of 276 ± 24 µg AAE/mg for ethanolic extracts of *Lentinula edodes* (shiitake), the Algerian *P. eryngii* still demonstrated substantial antioxidant potential. This reductive activity becomes even more evident when set against the data from Chu et al. (2023), who observed markedly lower TAC values in ethanolic extracts of various mushrooms, including shiitake (24.52 ± 1.2 µg AAE/g), portobello flat (8.70 ± 0.78), Swiss brown (7.84 ± 0.1), and brown portobello (6.78 ± 0.17 µg AAE/g).

The present findings, together with those reported by Dandapat and Sinha (2015), as well as the significant TAC values documented by Jegathchandran et al. (2024) and Krishna et al. (2023), underscore the impact of using water (a highly polar solvent) in enhancing the extraction of antioxidant compounds.

When considered alongside earlier studies, the results of the current work reveal the remarkable reductive potential of Algerian *P. eryngii*, highlighting its promise as a valuable natural source of dietary antioxidants.

The results of the present study also showed that the ethanolic extract of *F. vulgare* leaves exhibited a total antioxidant capacity (TAC) of 1769.60 ± 3.03 µg AAE/g, which was statistically higher ($p < 0.01$) than that of the aqueous extract (1589.12 ± 17.48 µg AAE/g). In addition, the reference synthetic antioxidant BHT recorded an even stronger TAC, with highly significant differences ($p < 0.001$) compared to both extracts (Table 6).

When compared with values reported in the literature, Beyazen et al. (2017) documented the highest TAC in the chloroform leaf extract, with a value of 1.94 ± 0.76 mg AAE/g, whereas the study on fennel seeds using a methanolic extract reported a wide range between 51.76 and 94.06 mg GAE/g DE for samples from different regions (Khammassi et al., 2022). In the study by Majdoub et al. (2017), aqueous extracts from zinc-treated and untreated samples ranged from 27.121 ± 0.465 to 42.700 ± 0.462 mg AAE/mL. The work of Moumen et al. (2025) showed that the hydroethanolic extract obtained by Soxhlet reached 53.87 mg AAE/g, and also indicated that the aqueous extract prepared by decoction exhibited higher activity than the aqueous extract obtained by Soxhlet. In Nigeria, Beyazen et al. (2017) reported that fennel seeds displayed the highest activity in the methanolic extract and the lowest in the chloroform extract, while a recent Indonesian study found high activity for the ethanolic extract, reaching 103.513 ± 0.047 mg AAE/g at a concentration of 1 mg (Fajar et al., 2024).

These comparisons highlight that variations among studies are largely attributable to differences in the plant part used, the type of solvent, and the extraction method, making the results of each study highly dependent on its specific experimental conditions and the phytochemical composition of the material under investigation.

3.1.4. β-Carotene bleaching assay

In the β-carotene bleaching assay, the *P. eryngii* extracts in our study demonstrated significant differences in antioxidant activity depending on the solvent used. The aqueous extract showed an IC_{50} value of 717.65 ± 12.52 µg/mL, with an inhibition percentage of 55.23 ± 2.48% at 1 mg/mL ($p \leq 0.01$). In contrast, the ethanolic extract recorded a higher IC_{50} of 1170.92 ± 61.68 µg/mL and a lower inhibition percentage of 39.49 ± 5.58% ($p \leq 0.01$). Ascorbic acid exhibited near-complete inhibition at the same concentration, with an IC_{50} of 14.99 ± 1.44 µg/mL ($p \leq 0.001$) (table 6). Although these values are lower than those of the reference, they reflect the appreciable capacity of the aqueous extract.

This is consistent with previous studies. For example, Krishna et al. (2023) reported that acetone and methanol extracts of *P. eryngii* achieved inhibition percentages of 72.58 ± 0.28% and 47.12 ± 0.17%, respectively, at 1 mg/mL, demonstrating solvent- and sample-dependent efficacy. Likewise, Lin et al. (2014) indicated that the antioxidant capacity of ethanolic extracts of *P. eryngii* fruiting bodies collected on different days after fruiting induction was highest on day 10 (EC_{50} = 2.17 mg/mL; inhibition = 46.42%), followed by day 12 (EC_{50} = 4.44 mg/mL; inhibition = 38.30%) and day 15 (EC_{50} = 9.77 mg/mL; inhibition = 34.75%). These values are comparable to the activity of the aqueous extract in our study, despite differences in harvest date and cultivation conditions.

Regarding hot aqueous extracts, Abdullah et al. (2012) reported an IC_{50} of 24.71 ± 0.54 mg/mLThese findings highlight the influence of thermal extraction conditions and extract concentration on antioxidant activity.

On the other hand, the acetone extract of *Calocybe indica* reached 63.83% inhibition at 0.5 mg/mL, surpassing our extracts at the same concentration, possibly due to interspecific differences or solvent effects (Alam et al., 2019).

Furthermore, the methanolic extract of *P. flaccida* from Morocco showed high efficacy (IC_{50} = 0.22 ± 0.01 mg/mL) (Erbiai et al., 2023), whereas Moroccan *Lactarius sanguifluus* showed an IC_{50} of 0.56 mg/mL (Erbiai et al., 2021). *P. squarrosulus* and *Austroboletus hygrometricus* had IC_{50} values of 3.794 and 0.377 mg/mL, respectively (Khatua et al., 2013), and *Polyporus badius* from Turkey showed an IC_{50} of 1.78 mg/mL (Orhan and Üstün 2011). In contrast with our findings**,** Gharib et al. (2022) observed that in *Ganoderma lucidum*, the ethanolic extract was the most active, followed by the methanolic extract, while the aqueous extract showed the least efficacy, a trend differing from our observations, as in our study the aqueous extract exhibited greater efficacy than the organic solvents.

Regarding *F. vulgare*, the ethanolic leaves extract in our study recorded an IC_{50} of 128.33 ± 1.0 µg/mL with 81.66 ± 0.65% inhibition, while the aqueous extract showed an IC_{50} of 504.21 ± 12.85 µg/mL with 67.81 ± 1.31% inhibition ($p \leq 0.001$). In both cases, ascorbic acid was significantly stronger than the extracts ($p \leq 0.001$) (Table 6).

These values are close to those reported by Kalleli et al. (2019) for methanolic extracts of fennel seeds (114.43–211.45 µg/mL), although with differences in the plant part used.

A study on fennel seeds from Pakistan ranked the distilled water extract highest (66.63 ± 0.05% inhibition at 1 mg/mL), followed by 80% ethanol and then 80% acetone, reflecting variations due to solvent and geographical origin (Noreen et al., 2024). Additionally, Abdellaoui et al. (2020) reported IC_{50} values for the essential oil of fennel seeds in Morocco of 0.634 ± 0.027 mg/mL for the wild and 0.872 ± 0.031 mg/mL for the cultivated, with differences attributable to sample nature. Finally, the salinity study on acetone extracts of fennel seeds in Tunisia and Egypt reported values of 125.86 ± 0.23 and 137.05 µg/mL, respectively, illustrating the combined influence of geographical origin and solvent used (Daaloul-Jedidi et al., 2017).

3.1.5. H2o2 hydrogen peroxide inhibition

In the hydrogen peroxide inhibition assay, the aqueous extract of *P. eryngii* recorded an IC_{50} value of 393.89 ± 73.61 µg/mL, with an inhibition percentage of 59.38 ± 2.31% at 1 mg/mL. By comparison, the ethanolic extract exhibited a higher IC_{50} value of 701.59 ± 58.12 µg/mL and a slightly lower inhibition percentage of 53.54 ± 1.2% ($p \leq 0.01$). Meanwhile, ascorbic acid demonstrated exceptional scavenging activity against hydrogen peroxide free radicals, achieving an inhibition percentage of 90.64 ± 0.22% and an IC_{50} of 7.75 ± 1 µg/mL, whereas BHA showed an inhibition percentage of 88.15% with an IC_{50} of 13.80 ± 4.46 µg/mL. Both reference antioxidants were significantly more active than the extracts ($p \leq 0.001$) (Table 6).

In comparison, Krishna et al. (2023) using the same species, *P. eryngii*, showed inhibition percentages of 47.46 ± 0.16% and 66.48 ± 0.13% for acetone and methanol extracts, respectively. For the methanolic extract of Indian *P. ostreatus*, Macwan and Patel (2023) reported an IC_{50} of 96.54 ± 2.32 µg/mL, while Thomas et al. (2022) indicated a value of 84.07 µg/mL for the same extract. Additionally, Bains and Tripathi (2017*)* recorded inhibition percentages of 62.21 ± 0.16% and 56.7 ± 0.21% for *P. floridanus* and *P. ostreatus*, respectively, at 1 mg/mL. In Jayakumars et al. (2011), the ethanolic extract of *P. ostreatus* grown by layer spawning showed 60.02% inhibition at 10 mg/mL, with an IC_{50} of 8 mg/mL. Conversely, Kim et al. (2012) reported activities of 68.4% for the hot aqueous extract and 78.7% for the ethanolic extract of Korean *P. ferulae* at 12 mg/mL. In Ozen et al. (2011), the Turkish mushroom *B. edulis* exhibited 67.17% inhibition at 100 µg/mL, while the Chinese Shiitake mushroom presented an IC_{50} of 153.6 ± 3.0 µg/mL (Huang et al. 2011). Likewise, Babu and Rao (2013) showed 76.1% activity at 2 mg/mL against *Agaricus bisporus*. Udeh et al. (2021) confirmed the superior activity of *P. ostreatus* against H_2O_2 compared to species such as *P. ostreatus, Agaricus bisporus* var. *albidus*, and *A. bisporus* var. *bisporus.* Overall, our results place *P. eryngii* favorably within the Pleurotaceae family, demonstrating similar or higher efficacy relative to related species despite variations in experimental conditions.

Regarding fennel leaves, the ethanolic extract exhibited stronger free radical scavenging activity than the aqueous extract, with inhibition percentages of 80.96 ± 1.01% and 71.85 ± 1.01%, respectively, at 1 mg/mL. The IC_{50} values were 19.36 ± 0.16 µg/mL for the ethanolic extract and 260.32 ± 17.67 µg/mL for the aqueous extract. Both extracts demonstrated significantly lower antioxidant activity compared to the reference standard ($p \leq 0.001$) (Table 6).

Romojaro et al. (2013) reported an inhibition percentage of 63.5 ± 4.46%, which aligns closely with our findings. Moreover, Di Napoli et al. (2022) documented an IC_{50} of 100 µg/mL for *F. vulgare* leaf oil, which indicates a lower antioxidant activity compared to ours. In cintrast, Chatterjeei et al. (2012) observed an inhibition percentage of 71.61% at 240 µg/mL for the methanolic seed extract, which wasg higher than ours. Although our study shows lower antioxidant activity compared to standards like ascorbic acid and BHA, the activity remains notable and consistent with previous research on fennel.

The antioxidant evaluation revealed distinct and systematic differences between the studied matrices depending on the extraction solvent. Overall, aqueous extracts of *P. eryngii* consistently exhibited superior antioxidant performance across all assays (DPPH IC_{50}, FRAP EC_{50}, FRAP $A_{0.5}$, β-carotene bleaching IC_{50}, and H_2O_2 scavenging IC_{50}), whereas *F. vulgare* leaves demonstrated higher antioxidant efficiency in ethanolic extracts. These contrasting patterns suggest a solvent-dependent differential extraction of bioactive compounds with varying polarity and redox properties.

Prior to statistical inference, data normality was confirmed using the Shapiro–Wilk test, while homogeneity of variances was validated via Levene's test, ensuring the suitability of parametric analyses. Multivariate analysis of variance (MANOVA), followed by ANOVA, demonstrated that extract type exerted a statistically significant effect on total phenolic content, flavonoid content, and all antioxidant activity indices, confirming the strong dependence of antioxidant potential on both biological source and extraction medium.

Correlation analysis using Pearson's coefficient revealed a near-perfect concordance among antioxidant assays within each extract group ($r \approx 1$), indicating a high level of methodological consistency and robustness of the applied assays. Furthermore, a strong positive association was observed between phenolic/flavonoid content and antioxidant performance, reinforcing the central role of these metabolites in governing redox activity.

The antioxidant capacity of the studied extracts is primarily attributed to their high phenolic and flavonoid contents, which are well-documented for their electron- and hydrogen-donating properties (Shalihah et al., 2021; Shi et al., 2022). LC-MS profiling further confirmed the presence of multiple bioactive phenolics, including chlorogenic acid, whose synergistic interactions likely contribute to the overall antioxidant efficacy (Bhattacharya, 2011; Belahcene et al., 2023). The collective presence of structurally diverse antioxidant metabolites suggests a synergistic rather than additive mode of action.

Variability in antioxidant responses among extracts and between studies can be attributed to multiple interdependent factors, including the anatomical part used, solvent polarity, extraction methodology, environmental and geographical conditions, developmental stage, and genetic variability. Importantly, total phenolic content alone does not fully explain antioxidant behavior, as abiotic and biotic stressors, as well as intrinsic metabolic regulation, significantly modulate phytochemical profiles (Bennour et al., 2020; Khammassi et al., 2022).

Previous reports consistently demonstrate a strong correlation between phenolic composition and antioxidant capacity across diverse plant systems, confirming that higher total phenol content generally corresponds to enhanced radical scavenging activity (Mendonça et al., 2022).

In mushrooms, phenolic compounds are widely distributed, with concentrations in *Pleurotus* species typically ranging from 2 to over 30 mg/g, strongly correlating with antioxidant potential. Flavonoid occurrence is species-dependent and may be absent in certain taxa such as *P. columbinus* and *P. ostreatus*, further highlighting interspecies biochemical variability (Elhusseiny et al., 2021). The antioxidant potency of mushrooms is therefore mainly associated with phenolic-driven redox mechanisms (Raharjo and Haryoto, 2019).

Hydrogen peroxide scavenging activity reflects the ability of extracts to neutralize H_2O_2, a precursor of highly reactive hydroxyl radicals involved in oxidative cytotoxicity. Mushroom-derived polyphenols play a crucial role in this defense system, while additional antioxidant constituents contribute to lipid peroxidation inhibition, as evidenced by β-carotene bleaching assays. These effects are consistent with their ability to reduce Fe^{3+} to Fe^{2+} and inhibit oxidative chain reactions, as previously reported (Krishna et al., 2023).

Similarly, TAC, DPPH, and ABTS assays collectively confirmed a significant positive correlation between antioxidant activity, extraction yield, and total phenolic content (Khammassi et al., 2022).

In *F. vulgare*, leaves are particularly rich in flavonoids (rutin, quercitrin, kaempferol) and phenolic acids (chlorogenic, ferulic, sinapic acids) (Palukuri and Subramaneyaan, 2022). The observed decrease in DPPH IC_{50} values with increasing phenolic content further confirms the direct involvement of phenolic concentration in radical scavenging efficiency. Comparable findings have been reported in cumin seeds, where antioxidant capacity strongly correlates with phenolic accumulation (Rebey et al., 2019). Interestingly, chelating and reducing power exhibited a negative correlation with total phenolic content ($r = -0.9$; $p < 0.05$), suggesting a complex, non-linear relationship between antioxidant mechanisms and phytochemical composition.

The antioxidant activity of fennel is largely mediated by phenolic compounds, including polyphenols, flavonoids, and tannins, which neutralize DPPH radicals through hydrogen atom donation (Khammassi et al., 2022). The reducing capacity, measured via the phosphomolybdenum (TAC) assay, reflects the electron-donating potential and reductone content of the extracts, which contributes to chain-breaking antioxidant activity.

At the molecular level, phenolic acids exert their antioxidant effects through hydroxyl and methoxy substitutions on aromatic rings, enabling hydrogen donation and radical stabilization. For instance, caffeic acid derivatives present in fennel have been identified as potent radical scavengers (Christodoulou et al., 2022).

Flavonoids, characterized by multiple hydroxylated aromatic structures, exhibit antioxidant mechanisms involving radical quenching, metal ion chelation, and modulation of endogenous antioxidant enzymes such as superoxide dismutase and catalase. Their activity is strongly influenced by structural features including hydroxylation pattern, glycosylation, and acylation (Christodoulou et al., 2022).

Beyond phenolic compounds, the extracts also contain a wide range of bioactive metabolites, including polysaccharides, glycosides, tannins, tocopherols, and ascorbic acid, all contributing to overall antioxidant capacity (Krishna et al., 2023). Notably, polysaccharides from *P. eryngii* have demonstrated significant radical scavenging activities, including 64.90% DPPH inhibition, 66.09–77.29% hydroxyl radical scavenging, 93.71% hydrogen peroxide scavenging, and 78.9% ferrous ion chelation, highlighting the contribution of primary metabolites to antioxidant defense systems (Barbosa et al., 2020).

Overall, the results demonstrate that the antioxidant potential of *P. eryngii* and *F. vulgare* leaves arises from a synergistic interaction between secondary metabolites (phenolics and flavonoids) and primary metabolites (notably polysaccharides). This integrated biochemical network enhances radical scavenging efficiency and oxidative stability, underscoring the relevance of these natural matrices as promising sources of multifunctional antioxidant compounds.

3.2. Anti-inflammatory activity

Inflammation is a highly coordinated and essential physiological process that preserves tissue integrity and organismal homeostasis in response to harmful stimuli, including pathogenic microorganisms, infectious agents, and chemical or physical insults (Lee et al., 2020). Although this response is fundamentally protective, its sustained activation or dysregulation may result in chronic inflammation, a pathological condition strongly associated with progressive tissue injury and the development of numerous chronic diseases.

Conventional anti-inflammatory therapies primarily include corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and diclofenac. These agents are widely used due to their potent inhibitory effects on inflammatory mediators; however, their long-term administration is often limited by adverse effects, including gastrointestinal toxicity, gastric ulceration, and local tissue irritation (Bains and Chawla, 2020).

In this context, growing attention has been directed toward natural bioactive compounds as safer therapeutic alternatives. Plant- and fungi-derived phytochemicals have emerged as promising candidates due to their multi-target anti-inflammatory potential and comparatively lower toxicity profiles, highlighting their relevance in the development of novel anti-inflammatory strategies.

3.2.1. Anti-inflammatory activity – *in vitro*

From a molecular perspective, protein denaturation is considered a key physicochemical event contributing to the initiation and amplification of inflammatory processes. It involves the disruption of non-covalent intramolecular interactions, including hydrogen bonds, ionic interactions, and hydrophobic forces, which collectively stabilize the native conformation of proteins. Exposure to external stressors such as elevated temperature or extreme pH conditions leads to structural destabilization, resulting in the loss of secondary and tertiary structures and a consequent decline in biological functionality (Cherbal et al., 2023).

Such conformational alterations may promote the exposure of cryptic epitopes and the formation of neoantigens, which have been implicated in the pathogenesis of autoimmune disorders, including rheumatoid arthritis (Al Bashera et al., 2021). Accordingly, the stabilization of protein structure and the prevention of denaturation have been proposed as relevant mechanisms through which anti-inflammatory agents exert protective effects.

In this regard, *in vitro* models remain essential for the preliminary evaluation of anti-inflammatory potential, particularly given the established interrelationship between oxidative stress, protein destabilization, and inflammatory signaling pathways. Among these models, the bovine serum albumin (BSA) denaturation assay is widely recognized for its simplicity, reproducibility, and biological relevance. This method assesses the ability of test compounds to inhibit heat- or chemically induced protein denaturation, with results typically benchmarked against standard non-steroidal anti-inflammatory drugs such as diclofenac (Bausa et al., 2024).

The percentage inhibition observed in this assay is generally interpreted as an indicator of the compound's capacity to preserve native protein conformation and thereby mitigate inflammatory progression.

In the present study, aqueous and ethanolic extracts of the investigated species were evaluated for their ability to inhibit BSA denaturation as a proxy for anti-inflammatory activity, using diclofenac as a reference standard for comparative analysis.

In this study, the anti-inflammatory potential of Algerian *P. eryngii* extracts was evaluated based on their capacity to prevent heat-induced denaturation of albumin protein, a well-established *in vitro* model for screening anti-inflammatory agents. The results were expressed as inhibition percentages and IC_{50} values (Table 7). At a concentration of 1 mg/mL, the aqueous extract achieved an inhibition rate of 59.59 ± 1.26%, with an IC_{50} of 1022.86 ± 25.99 µg/mL ($P \leq 0.01$). In contrast, the ethanolic extract exhibited a lower inhibitory effect, with 49.85 ± 0.89% inhibition and a higher IC_{50} of 1411.07 ± 44.86 µg/mL ($P \leq 0.01$).

Both extracts, while demonstrating moderate anti-inflammatory activity, were less potent than the reference drug diclofenac, which reached 91.15 ± 0.38% inhibition at 60 µg/mL with an IC_{50} of 30.56 ± 0.29 µg/mL ($P \leq 0.001$).

Table 7. Anti-inflammatory activity (IC_{50}) of *P. eryngii* and *F. vulgare* leaves extracts.

Sample		$IC_{50} \pm SD$
P. eryngii	**Ethanolic extract**	1411.07 ± 44.86^{c}
	Aqueous extract	1022.90 ± 25.99^{b}
***F. vulgare* leaves**	**Ethanolic extract**	111.53 ± 0.26^{b}
	Aqueous extract	737.36 ± 12.79^{c}
Reference drug	**Diclofenac**	30.56 ± 0.29^{a}

IC_{50} values represent mean ± SD (n=3).. Values with different superscripts (a, b, c, d, e, or f) in the same columns are significantly different ($p < 0.05$).

The comparative literature supports these observations. Panda et al. (2021), working on *Russula violeipes*, reported higher activity for aqueous extracts compared to ethanol and chloroform extracts in preventing protein denaturation, a trend consistent with the present findings. Bains and Tripathi (2017) documented even higher inhibition rates for aqueous extracts of *P. ostreatus* (72.2 ± 0.6%) and *P. floridanus* (80.15 ± 0.7%) at the same concentration of 1 mg/mL. Similarly, Ghosh et al. (2022), studying *Termitomyces heimii*, found IC_{50} values close to ours — 865 ± 1.02 µg/mL for decoction and 900 ± 0.89 µg/mL for diffusion-prepared aqueous extracts. The anti-inflammatory potency of *P. eryngii* in this study also approximates that reported for *Calocybe indica* with an IC_{50} of 1188.60 ± 3.26 µg/mL (Prabu and Kumuthakalavalli 2014). Conversely, the results here far exceed those obtained for *Hericium erinaceus* by Suleiman et al. (2022), who recorded only 6.55% inhibition at 300 µg/mL. These comparative data reinforce the positioning of *P. eryngii* as a mushroom with notable anti-inflammatory properties.

It is worth noting that Kebaïli (2022) reported markedly lower activity for the aqueous extract of the Algerian wild *Ganoderma lucidum* (reishi mushroom), with an IC_{50} of 1494.33 ± 15.73 µg/mL, suggesting that *P. eryngii* possesses a relatively stronger anti-inflammatory effect in the protein denaturation assay.

Regarding *F. vulgare* leaves, the results (Table 7) contrasted sharply with the pattern observed for *P. eryngii*. Here, the ethanolic extract outperformed the aqueous extract, demonstrating notably higher inhibition of serum albumin denaturation. The ethanolic extract achieved 71.53

± 1.22% inhibition at 0.5 mg/mL with a remarkably low IC_{50} of 111.53 ± 0.26 µg/mL (P ≤ 0.001), while the aqueous extract reached 66.00 ± 0.46% inhibition at 1 mg/mL and a much higher IC_{50} of 737.36 ± 12.79 µg/mL (P ≤ 0.001). This large disparity in IC_{50} values indicates that the ethanolic extract requires a substantially lower concentration to achieve 50% inhibition, reflecting greater potency. Compared with diclofenac, the ethanolic extract's activity profile aligned more closely with the reference drug, which reached 30.56 ± 0.29 µg/mL (P ≤ 0.001), suggesting that ethanol was more effective than water in extracting the bioactive compounds responsible for the anti-inflammatory effect in *F. vulgare* leaves.

These findings partially align with those of Saleem et al. (2020), who observed higher activity of aqueous extracts in certain contexts; however, in the present case, ethanol clearly yielded superior potency. Interestingly, both extracts of *F. vulgare* leaves here exhibited stronger activity than the seed extracts reported by Cherbal et al. (2023), where inhibition did not exceed 38% at 250 µg/mL. Nonetheless, their activity was still lower than that reported by Marrelli et al. (2020) for the essential oil of *F. vulgare* leaves, with an IC_{50} of 95.9 ± 2.4 µg/mL. Furthermore, Mehra et al. (2023) reported lower activity, with an IC_{50} of 676.44 ± 0.96 µg/mL, and noted that hexane seed extracts displayed higher anti-inflammatory potential than ethanolic ones within the same study.

Our results demonstrated a clear differential pattern of anti-inflammatory activity between the tested extracts, depending on both the plant source and extraction solvent. Specifically, the aqueous extract of *P. eryngii* exhibited the strongest inhibition of albumin denaturation, whereas *F. vulgare* leaves showed higher activity in the ethanolic extract. Notably, these variations closely paralleled the distribution of total phenolic and flavonoid contents, as well as the antioxidant profiles obtained across the different assays, suggesting a strong interdependence between redox status and anti-inflammatory potential.

The observed bioactivity can be largely attributed to the differential accumulation of flavonoids, as confirmed by quantitative phytochemical analysis. Flavonoids are well documented for their ability to modulate inflammatory responses through the downregulation of key mediators, including prostaglandins, nitric oxide, and pro-inflammatory cytokines (Shalihah et al., 2021). In parallel, phenolic compounds contribute to anti-inflammatory effects by targeting multiple enzymatic pathways, particularly cyclooxygenase (COX) and lipoxygenase (LOX), while simultaneously attenuating oxidative stress through free radical scavenging activity (Lekouaghet et al., 2020). This dual antioxidant–anti-inflammatory behavior highlights their central role in maintaining cellular homeostasis under inflammatory conditions.

The inhibitory effect on albumin denaturation may also be mechanistically linked to the presence of specific bioactive metabolites identified via LC-MS/MS analysis. Among the detected compounds, chlorogenic acid and trans-cinnamic acid have been previously reported to interact with protein amino acid residues through hydrogen bonding and hydrophobic interactions, thereby stabilizing native protein conformations and preventing structural unfolding (Belahcene et al., 2023). Such interactions provide a plausible molecular basis for the observed protection against thermal or chemical denaturation of albumin.

Beyond low-molecular-weight phenolics, higher-order biomolecules such as polysaccharides and proteins may also contribute significantly to the anti-inflammatory potential of the extracts. These macromolecules have been increasingly recognized for their immunomodulatory and anti-inflammatory properties, particularly through their capacity to regulate inflammatory signaling pathways and maintain physiological equilibrium (Zheng et al., 2020; Wu et al., 2023). Their involvement suggests that anti-inflammatory activity in natural extracts is not solely dependent on secondary metabolites but rather results from a synergistic interplay between multiple biochemical classes.

Furthermore, the inhibition of albumin denaturation is widely accepted as a predictive *in vitro* model for anti-inflammatory activity, reflecting mechanisms analogous to those of nonsteroidal anti-inflammatory drugs (NSAIDs). In addition to inhibiting prostaglandin biosynthesis, NSAIDs are known to stabilize protein structures and prevent denaturation processes that may lead to the formation of autoantigens implicated in chronic inflammation (Derbel et al., 2023). Accordingly, the ability of the tested extracts to prevent albumin denaturation suggests a dual protective mechanism involving both enzyme inhibition and protein stabilization.

3.2.2. Anti-inflammatory activity – *in silico*

Computer-assisted drug target identification methods are an effective tool in drug discovery, offering several advantages over traditional approaches, which are often costly and time-consuming. These methods enable the precise identification of disease-associated targets and their binding sites, thereby reducing the extent of experimental work and associated costs, while facilitating the evaluation of binding affinities between predicted active sites and drug candidates prior to clinical trials (Liao et al., 2022).

These approaches are complemented by *in silico* molecular modeling studies, which provide a powerful framework for assessing the binding affinity of bioactive molecules toward target proteins, thereby allowing the interpretation of compound bioactivity at the molecular level (Khirallah et al., 2022).

In the present study, an *in silico* analysis was conducted on five key inflammation-related proteins, given their central role in inflammatory pathways. In addition, LC-MS/MS analysis was used to identify the bioactive compounds present in the extracts. The objective was to explore their potential interactions with these proteins and to elucidate their mechanisms of action in inflammation inhibition, thereby supporting experimental findings related to protein denaturation prevention.

Based on available databases and computational tools, these approaches can be applied individually or in combination, thereby improving the efficiency of candidate compound identification, providing a framework for result interpretation and tool comparison, and offering directions for future optimization. Consequently, they represent a key strategic step in accelerating drug development while reducing the risks and costs associated with pharmaceutical research (Liao et al., 2022).

Building on the in vitro anti-inflammatory activity, the ethanolic extract of *F. vulgare* leaves, which exhibited the highest inhibitory effect and the richest profile of bioactive compounds, was selected for molecular docking analysis. The study evaluated interactions with key inflammatory targets: COX-2 (PDB: 1CX2), IL-1β (1LOB), NF-κB (8TQD), PLA2 (1CJY), and TNF-α (5MU8). These proteins play central roles in inflammatory pathways, where COX-2 is involved in prostaglandin biosynthesis, IL-1β and TNF-α mediate cytokine-driven responses, NF-κB regulates pro-inflammatory gene expression, and PLA2 initiates arachidonic acid release for eicosanoid production. Diclofenac was used as the reference drug. Docking was performed using Schrödinger (XP mode), and binding affinities were reported in kcal/mol, with more negative values indicating stronger interactions. The docking results are summarized in Table 8.

Table 8. Molecular docking scores of natural compounds and reference drug diclofenac obtained using Schrödinger (XP precision mode).

Compounds	**Pubchem ID**	**Docking Score (kcal/mol)**				
		COX-2 (1CX2)	**IL1β (1LOB)**	**NFKβ (8TQD)**	**PLA2 (1CJY)**	**TNFα (5MU8)**
Chlorogenic acid	1794427	-5.958	-5.921	-4.308	-6.033	-5.832
Ferulic acid	445858	-6.774	-3.390	-1.917	-3.917	-3.560
Salicylic acid	338	-5.468	-3.683	-2.954	-3.882	-3.313
Rutin	5280805	0.000	-7.244	-5.231	-10.351	-10.278
Trans-cinnamic acid	444539	-5.258	-1.545	-1.750	-2.607	-2.256
Gentisic acid	3469	-5.630	-3.826	-3.760	-4.420	-3.699
Vanillin	1183	-6.555	-4.865	-2.296	-3.768	-3.710
Quercetin	5280343	-8.793	-5.040	-3.479	-6.018	-5.094
Morin	5281670	-7.663	-3.324	-3.338	-7.267	-4.647
Caffeic acid	689043	-7.275	-4.138	-1.588	-4.814	-4.532
Diclofenac	3033	-7.977	-2.102	-2.808	-2.426	-3.242

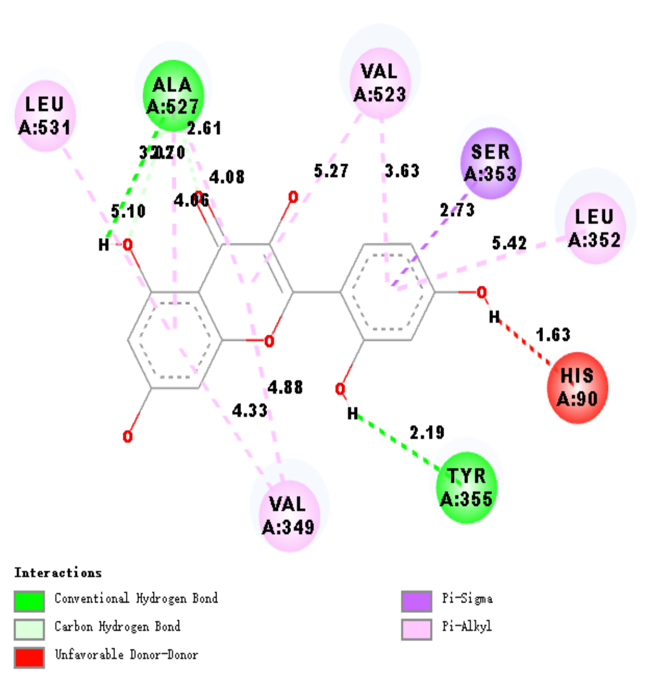
Out of the ten phenolic compounds analyzed, five (rutin, quercetin, chlorogenic acid, morin, and ferulic acid) showed significant binding affinities (≤ −5 kcal/mol) with at least one target, a value generally considered indicative of biologically relevant interactions. Rutin stood out, demonstrating favorable binding across all evaluated targets except COX-2, highlighting its broad potential as a multi-target anti-inflammatory agent.

Among all tested compounds, quercetin showed the strongest interaction with COX-2 (−8.793 kcal/mol), surpassing that of diclofenac (−7.977 kcal/mol), suggesting its strong potential to inhibit prostaglandin production. Additional compounds such as morin, caffeic acid, ferulic acid, chlorogenic acid, and salicylic acid also displayed meaningful interactions with COX-2, indicating possible additive or synergistic anti-inflammatory effects. For IL-1β, rutin emerged as the most potent binder (−7.244 kcal/mol), followed by chlorogenic acid and quercetin, indicating its capacity to modulate cytokine activity. Notably, rutin was the only compound to exhibit a significant binding score for NF-κB (−5.231 kcal/mol), suggesting it may disrupt key transcriptional regulatory mechanisms, even if direct inhibition of transcription factors is typically uncommon. In the case of PLA2, four compounds—rutin, morin, chlorogenic acid, and quercetin, showed strong affinities, with rutin again ranking highest (−10.351 kcal/mol), underscoring its potential to inhibit the release of phospholipid-derived inflammatory mediators. Rutin also displayed the strongest interaction with TNF-α (−10.278 kcal/mol), further emphasizing its potential role as a broad-spectrum modulator of inflammation.

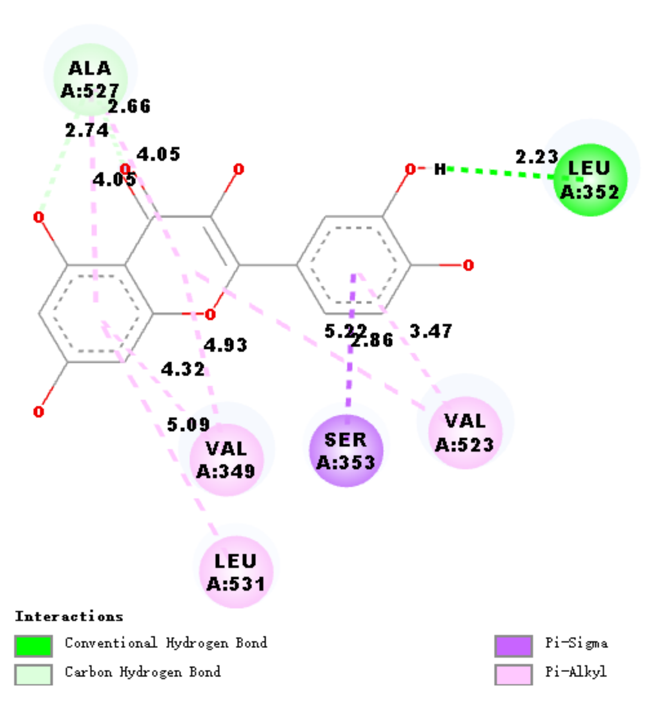
The interaction diagrams for the top-scoring compounds, along with diclofenac, are presented in Figure 21(a–e). These visualizations highlight the involvement of key functional groups such as hydroxyl, carboxyl, and aromatic rings in stabilizing the ligand–protein complexes. These moieties contribute to various non-covalent interactions, including hydrogen bonds, π–π stacking, and hydrophobic contacts, which collectively enhance the binding stability, specificity, and overall affinity of the compounds for their targets.

Molecular docking results shed light on how quercetin and rutin interact with several inflammation-related proteins at the atomic level. Quercetin displayed a notable binding preference for COX-2, forming a conventional hydrogen bond with Leu352 and carbon hydrogen bonds with Ala527. It also established π–π stacking interactions with hydrophobic residues such as Val349, Val523, Leu531, and Ser353, which likely contribute to a stable binding conformation (Figure 21a). Rutin, meanwhile, showed a rich interaction pattern across multiple inflammatory targets. In its complex with IL-1β, it engaged in several hydrogen bonds involving Glu64, Lys63, Glu37, Lys65, Ser21, and Val41, and formed an additional carbon hydrogen bond with Gln38 (Figure 21b). For NF-κB, key interactions included hydrogen bonds with Thr124, Arg163, Glu119, Asp120, and Glu159, alongside carbon hydrogen bonds with Glu119 and Asp120 (Figure 21c).

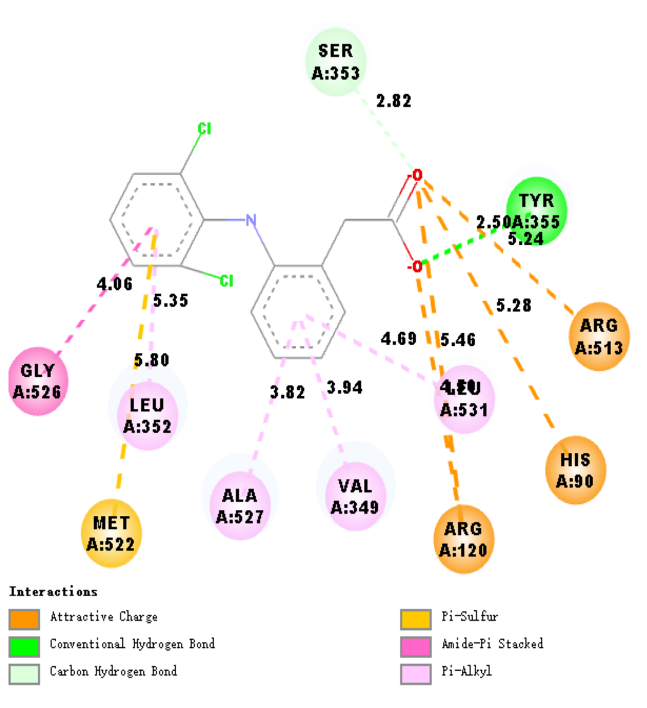
The docking pose with PLA2 (Figure 21d) highlighted an extensive hydrogen bonding network comprising Glu717, Asp633, Lys632, Lys626, Asp629, and Asn627, further supported by carbon hydrogen bonds with Glu714, Pro625, and Gln721. Finally, rutin’s binding to TNFα was stabilized through hydrogen bonds with Gln61, Ser60, and Leu120, as well as a carbon hydrogen bond with Tyr151 (Figure 21e). Altogether, these interaction profiles suggest that both flavonoids form complexes within the active sites of their targets, reinforcing their promise as multi-target modulators of inflammatory pathways.



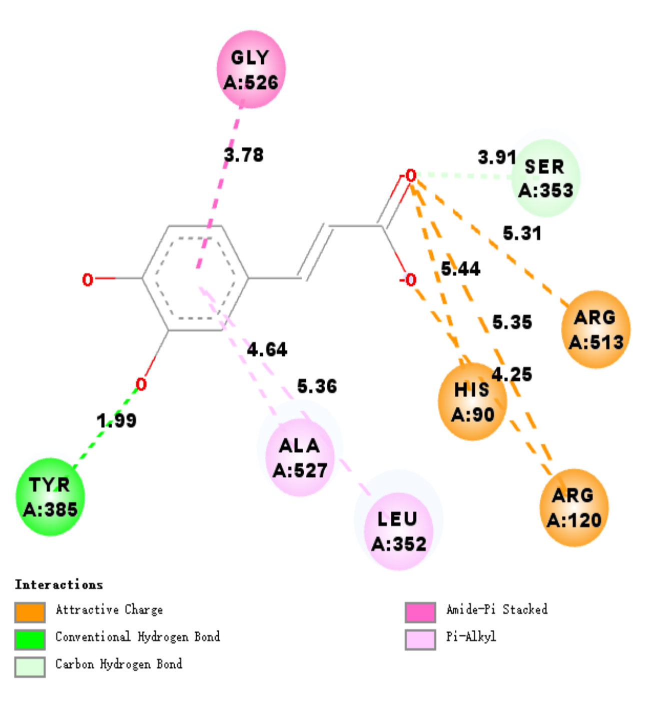
Morin -1CX2



Quercetin -1CX2

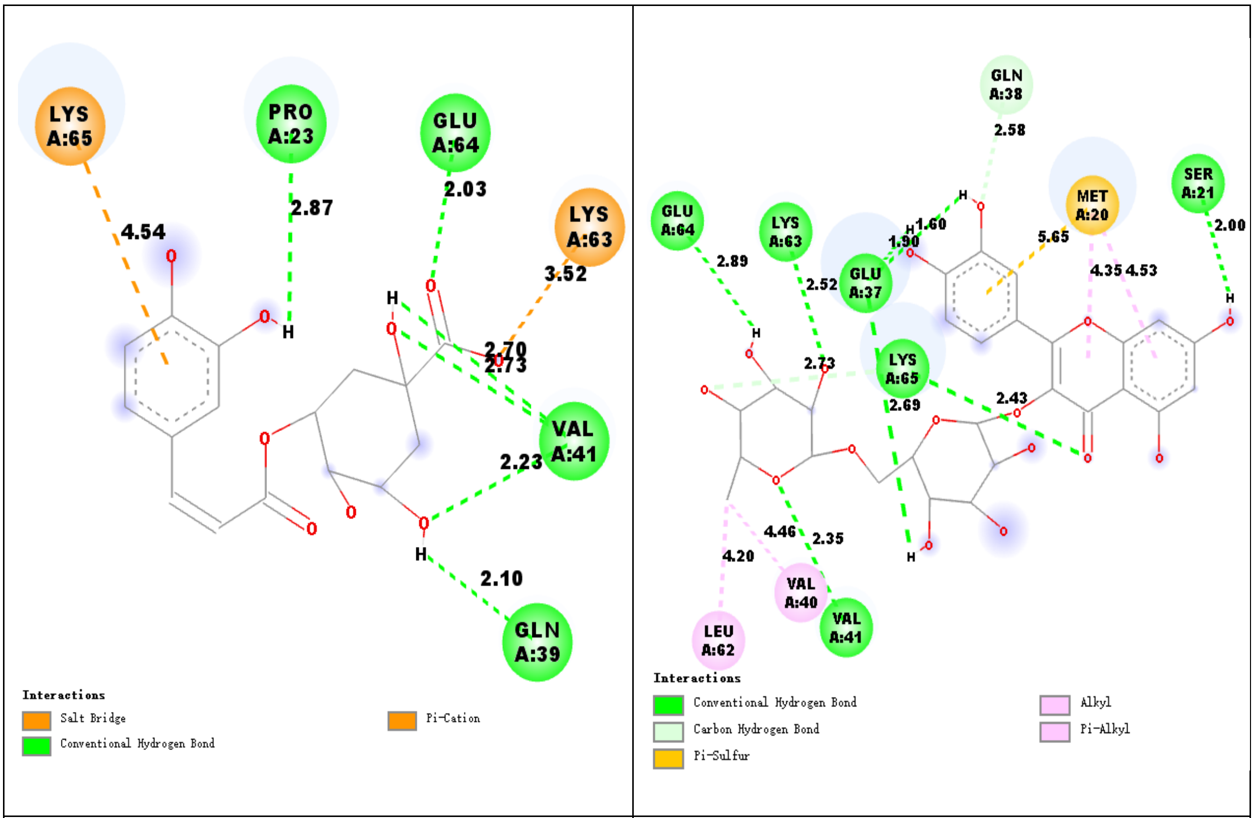


Diclofenac -1CX2



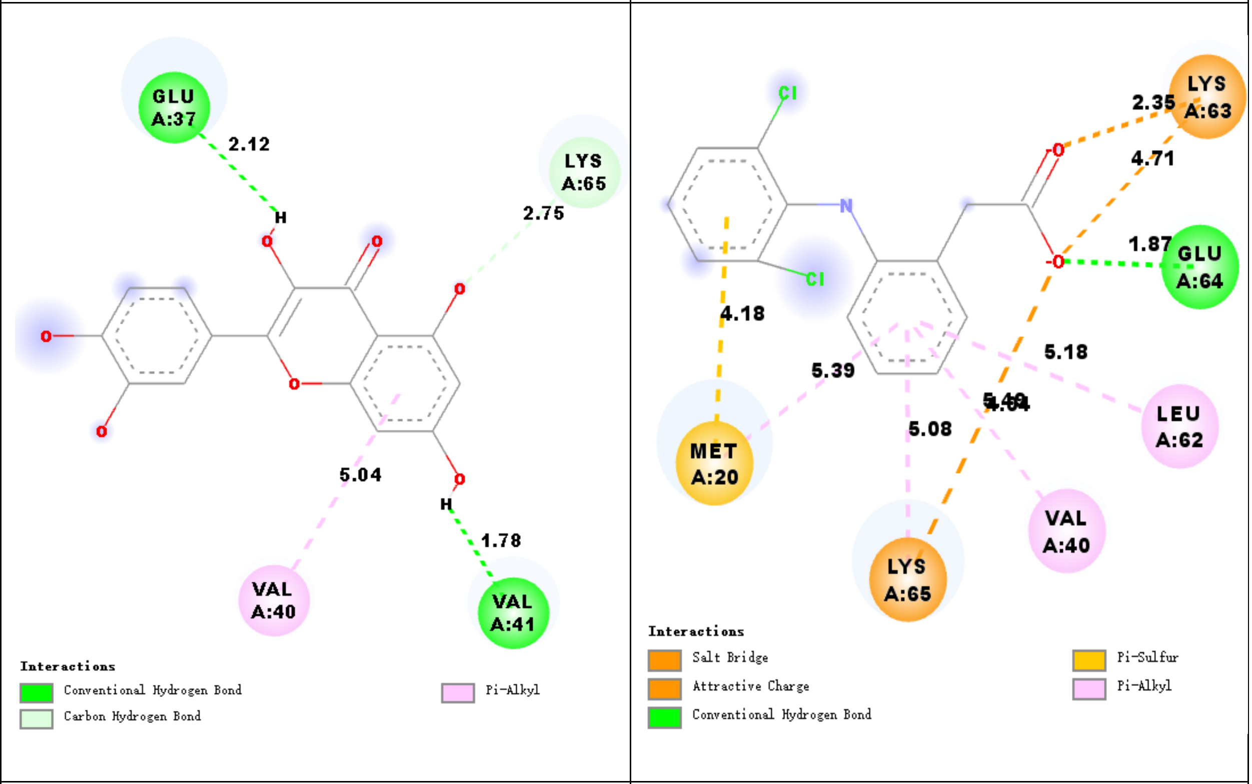
Caffeic acid -1CX2

Figure 21a. Predicted 2D binding interactions of selected phenolic compounds with the active site of COX-2.



Chlorogenic acid- IL1β

Rutin- IL1β



Quercetin- IL1β

Diclofenac- IL1β

Figure 21b. Predicted 2D binding interactions of selected phenolic compounds with the active site of IL1β (1LOB).

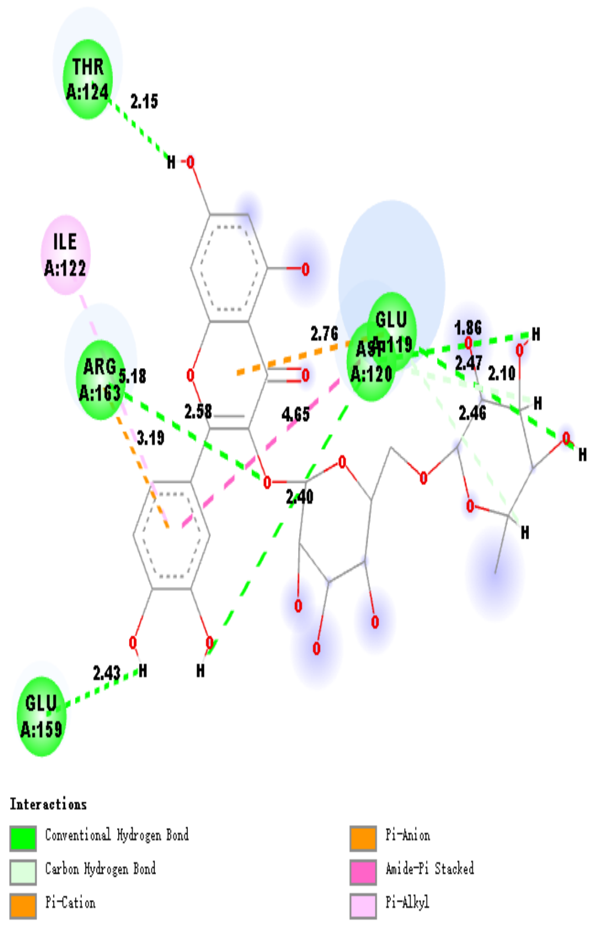
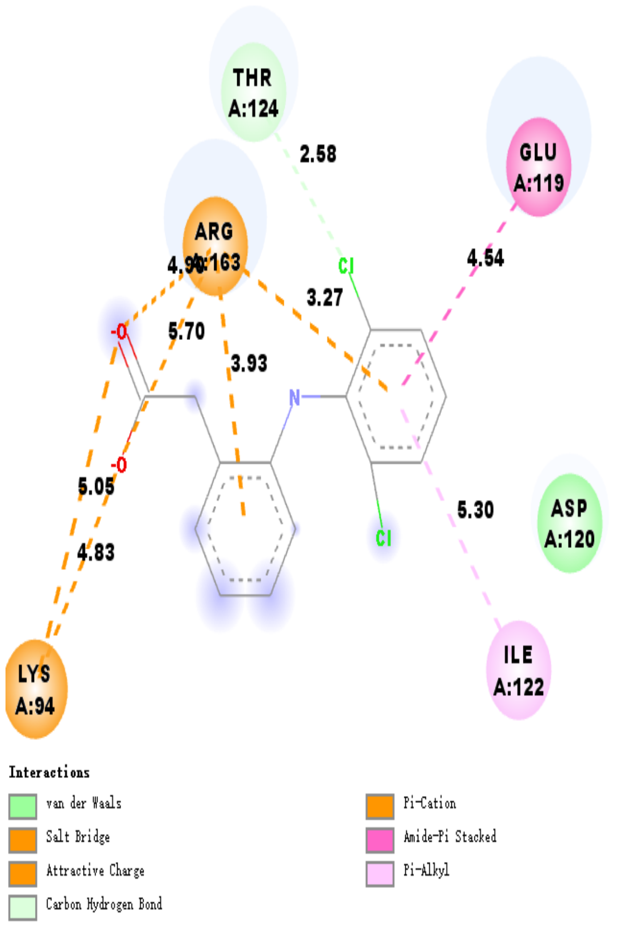
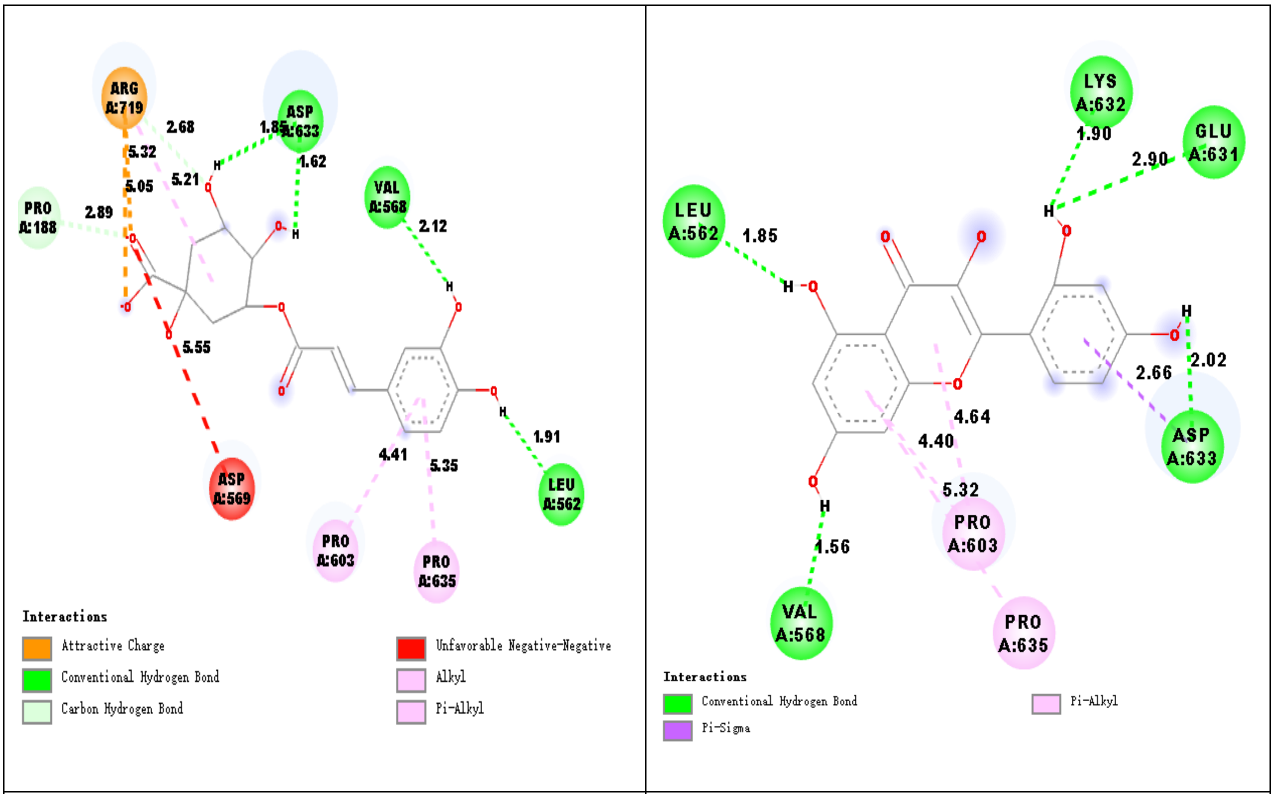
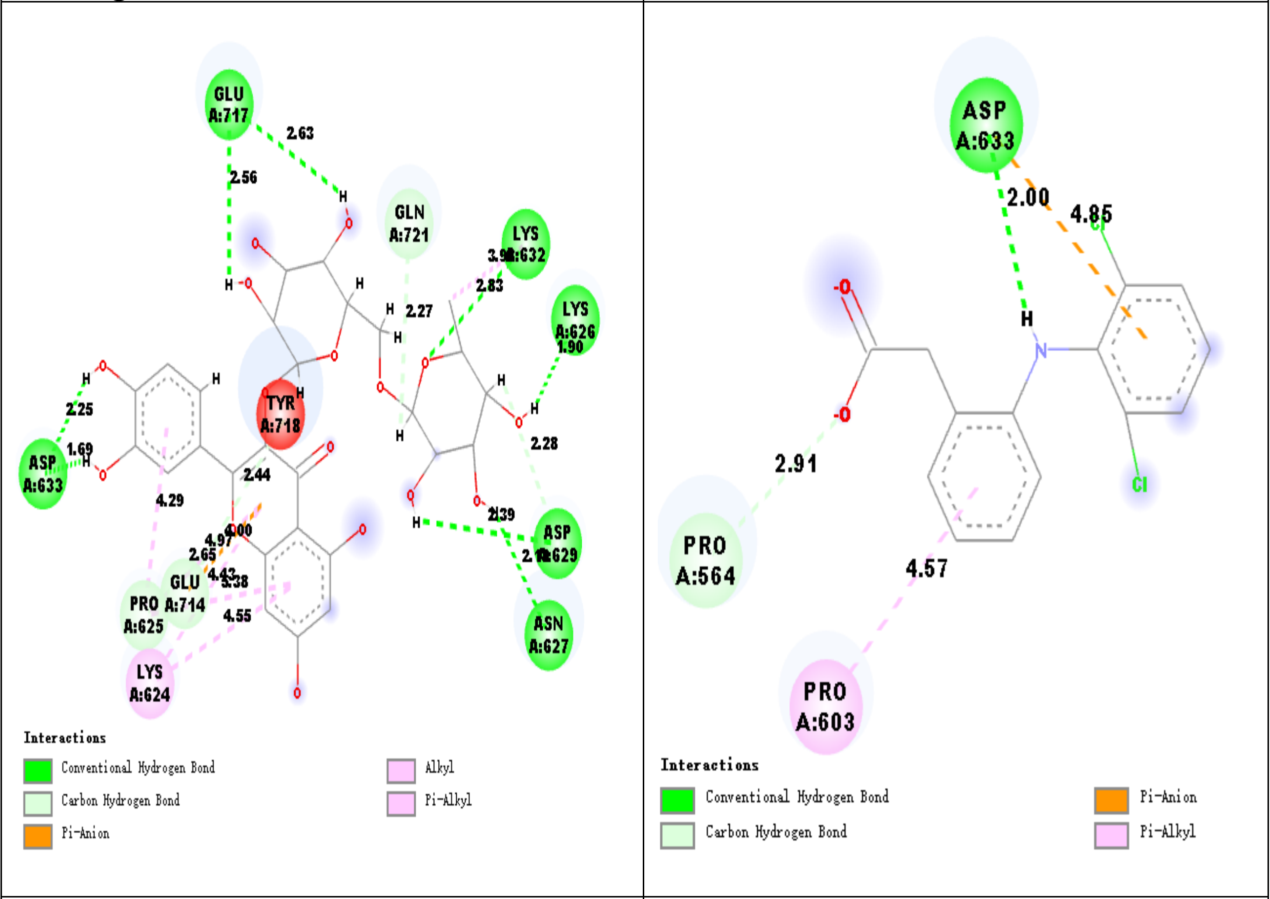
	
RUTIN- NFKβ	**Diclofenac- NFKβ**

Figure 21c. Predicted 2D binding interactions of selected phenolic compounds with the active site of NFKβ (8TQD).



Chlorogenic acid - PLA2

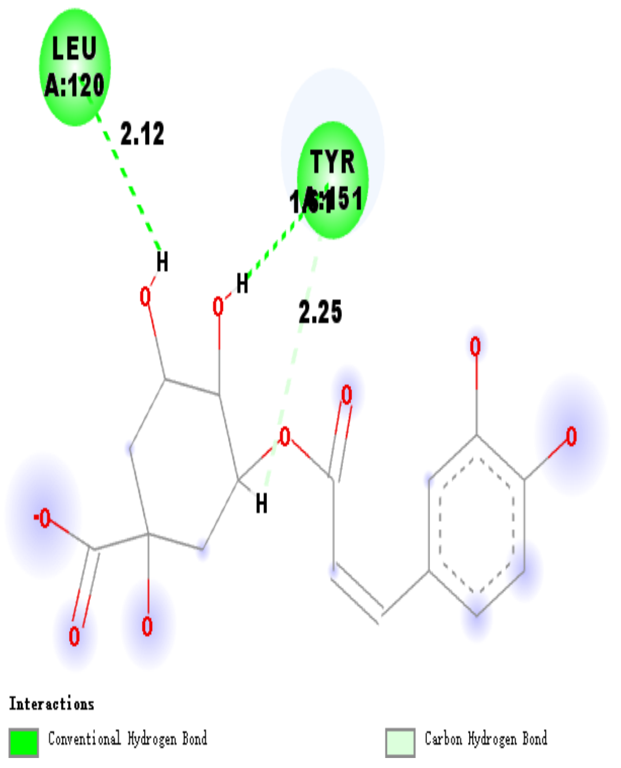
Morin - PLA2



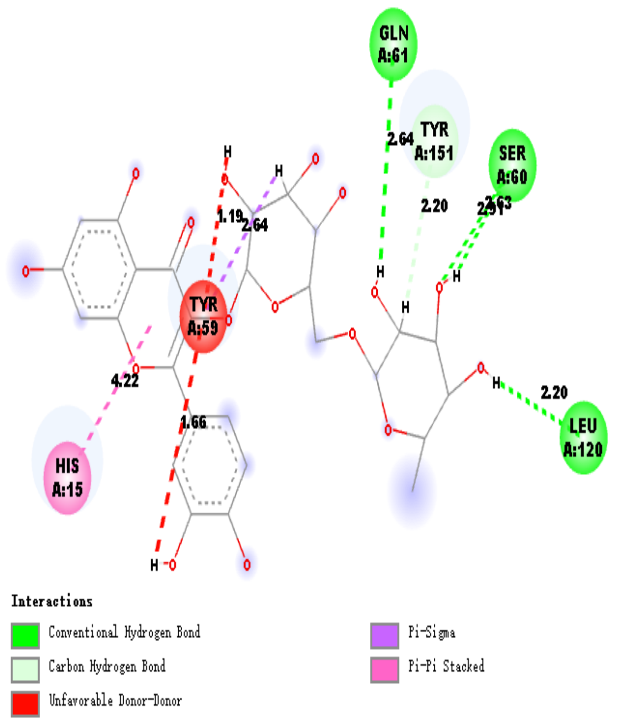
Rutin- PLA2

Diclofenac - PLA2

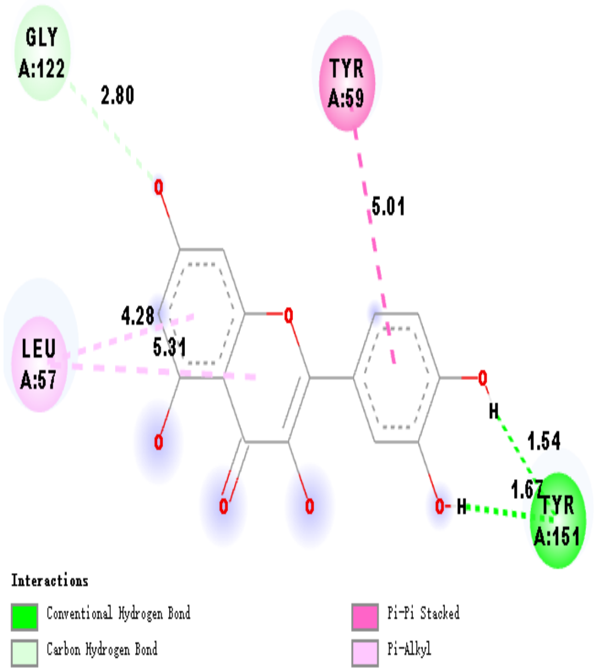
Figure 21d. Predicted 2D binding interactions of selected phenolic compounds with the active site of PLA2 (1CJY).



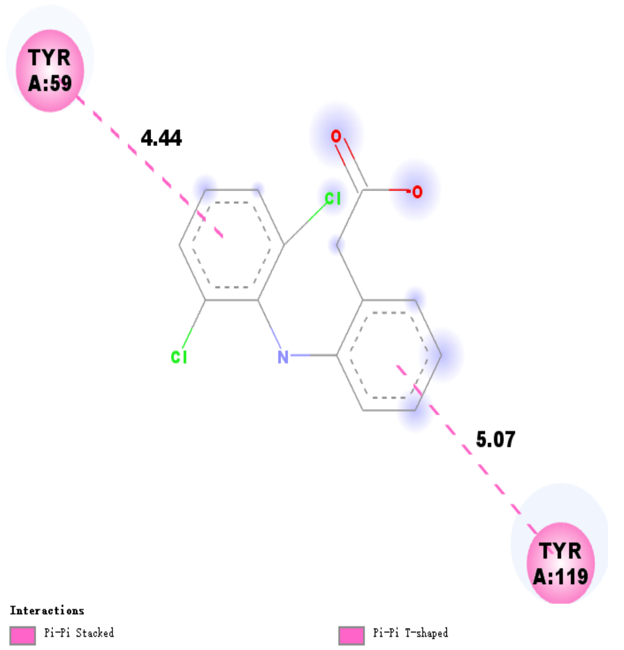
Chlorogenic acid- TNFα



Rutin- TNFα



Quercetin- TNFα



Diclofenac TNFα

Figure 21e. Predicted 2D binding interactions of selected phenolic compounds with the active site of TNFα (5MU8).

The binding affinities obtained in this study suggest that Rutin may act as a lead multi-target anti-inflammatory agent, likely due to its polyhydroxylated flavonoid structure and glycosylated moiety that enable extensive hydrogen bonding and stabilizing interactions within protein active sites (Sharma et al., 2021; Pentu et al., 2025). Its affinity toward NF-κB further suggests a potential indirect role in modulating pro-inflammatory gene expression, in agreement with reports on polyphenols interfering with nuclear translocation and DNA-binding processes (Forouzanfar et al., 2025). Similarly, Quercetin exhibited broad interactions with inflammation-related enzymes and signaling pathways, consistent with its reported ability to downregulate IL-1β, TNF-α, and IL-6 via NF-κB modulation (Bastin et al., 2023; Triwardhani et al., 2023). Chlorogenic acid and morin also showed consistent binding to COX-2 and PLA2, with morin suggesting potential involvement in phospholipid metabolism inhibition, a key inflammatory step (Hong et al., 2025; Touny et al., 2025).

Collectively, these *in silico* profiles provide mechanistic support for previously reported *in vitro* anti-inflammatory activity. The top-scoring compounds are well known for antioxidant and enzyme-inhibitory properties, consistent with experimental observations. Overall, the results suggest that the anti-inflammatory potential of *F. vulgare* is driven by a synergistic multi-target effect rather than a single dominant compound.

However, interpretation must be cautious, as binding energies alone represent an oversimplified predictor of inhibitory activity. Molecular docking assesses geometric and energetic complementarity under static and simplified conditions and does not necessarily reflect biological activity. Thus, more negative binding energies do not directly correlate with inhibition or IC_{50} values.

Moreover, discrepancies were observed between favorable binding energies and interaction patterns, particularly for rutin complexes with PLA2 and TNF-α, which involved a significant proportion of weak interactions (alkyl , π-alkyl) and unfavorable donor–donor repulsions. Such interactions may reduce complex stability and functional efficiency, suggesting that high binding scores may result from non-specific interaction accumulation rather than strong directional bonding.

Additionally, docking assumes a rigid receptor, whereas biological systems are dynamic and influenced by solvent effects, pH, and intracellular competition. The absence of molecular dynamics (MD) simulations and experimental validation therefore limits these findings to a predictive level. Binding at non-functional sites also remains possible, meaning strong affinity does not necessarily imply functional inhibition.

Accordingly, although rutin and quercetin show promising multi-target interaction profiles, they cannot be considered definitive inhibitors without further validation. These results should be regarded as preliminary screening evidence, requiring molecular dynamics simulations and *in vivo* studies to confirm pharmacological relevance and biological efficacy.

3.3. α-Amylase inhibitory activity

Diabetes mellitus is a widespread metabolic disorder marked by chronic hyperglycemia and severe systemic complications (Agarwal and Gupta, 2016). This is partly mediated by α-amylase, which hydrolyzes complex carbohydrates into absorbable sugars, driving postprandial glucose spikes (Kaur et al., 2021).

Although inhibitors such as acarbose, miglitol, and voglibose are effective, their use is limited by gastrointestinal side effects, prompting interest in natural alternatives. Plant-derived phenolics and flavonoids have shown α-amylase inhibitory activity and glycemic regulation potential (Santoso et al., 2022).

Accordingly, this study evaluates the α-amylase inhibitory activity of the investigated extracts.

The α-amylase inhibitory activity of *P. eryngii* shows that both extracts exhibited a relatively weak inhibitory capacity compared to the reference drug (Acarbose). The IC_{50} value for the ethanolic extract was 2396.49 ± 93.21 µg/mL, versus 1816.29 ± 188.26 µg/mL for the aqueous extract, while the IC_{50} of Acarbose was very low, 151.40 ± 15 µg/mL. Despite this difference, the aqueous extract showed relative superiority over the ethanolic extract, as indicated by its lower IC_{50} value. This relative superiority is further reinforced by the results of the inhibition percentage at a concentration of 2 mg/mL, where the aqueous extract achieved 47.19 ± 3.20% inhibition, compared to 42.43 ± 1.07% for the ethanolic extract, indicating consistency between the two evaluation methods (IC_{50} and inhibition percentage) (Table 9).

At the level of statistical correlations, Pearson's analysis revealed a strong and significant relationship between TPC and TFC with IC_{50} values, reflecting that higher content of phenolic compounds and flavonoids is associated with lower IC_{50} values and an increase in enzyme inhibitory activity. These results confirm that the α-amylase inhibitory activity is partially related to the chemical composition of the extracts, particularly in terms of phenols and flavonoids, although their efficacy remains considerably lower than that of the reference drug (Acarbose). This suggests that *P. eryngii* could represent a potential complementary source of compounds with antidiabetic activity, without being a direct substitute for pharmacological treatment.

Table 9. IC_{50} values (µg/mL) of anti-α-amylase activity of *P. eryngii* extracts and *F. vulgare* leaves extracts.

Sample		IC_{50} ± SD
P. eryngii	**Ethanolic extract**	2396.49 ± 93.21^{b}
	Aqueous extract	1816.29 ± 188.26^{c}
***F. vulgare* leaves**	**Ethanolic extract**	697.74 ± 24.64^{b}
	Aqueous extract	1972.19 ± 75.82^{c}
Reference drug	**Acarbose**	151.40 ± 15.51^{a}

IC_{50} values represent mean ± SD (n=3).. Values with different superscripts in the same columns are significantly different ($p < 0.05$).

Compared to previous studies, the current results align with reports on the ability of edible mushrooms to inhibit α-amylase, although the IC_{50} values recorded in this study are higher, indicating relatively lower efficacy. For example, Kim et al. (2020) reported that the ethanolic extract of *P. eryngii* had an IC_{50} of 1.68 mg/mL, a value close to the limits recorded in our study, reflecting a similar overall trend despite differences in the level of inhibitory activity. Similarly, Deveci et al. (2021) indicated that the methanolic extract of *P. ostreatus* exhibited significant activity (IC_{50} = 0.74 ± 0.17 mg/mL), while Tamboli et al. (2018) reported values for *P. ostreatus* mycelial extracts ranging from 224 to 383 µg/mL, values lower than those obtained in our study but confirming the presence of inhibitory activity within the Pleurotus genus.

Regarding other species, extracts of *Agaricus bisporus* showed high inhibition percentages (76.3–83.5% at 1 mg/mL) according to Kim et al. (2023), and Deveci et al. (2023) reported that hexane extracts from several edible mushroom species exhibited strong inhibitory activity (47.82–81.06% at 0.5 mg/mL). Overall, these findings confirm that edible mushrooms, including *P. eryngii*, possess a considerable capacity to inhibit α-amylase, even though the efficacy of the extracts in the present study is relatively lower than some previous reports.

The α-amylase inhibitory activity of fennel leaves exhibited a pattern opposite to that observed in *P. eryngii*. The ethanolic extract of fennel showed markedly higher efficacy than the aqueous extract, with an inhibition percentage at 2 mg/mL of 81.86 ± 1.20% versus 47.26 ± 1.86% for the aqueous extract. This difference was also reflected in the IC_{50} values, where the ethanolic extract recorded 697.74 ± 24.64 µg/mL compared to 1972.19 ± 75.82 µg/mL for the aqueous extract, clearly highlighting the superiority of ethanol in enhancing α-amylase inhibition, although both extracts were less potent than the reference drug Acarbose (151.40 ± 15.51 µg/mL (Table 9).

Statistical analyses revealed a strong positive correlation between phenols and flavonoids and a clear negative correlation between these compounds and IC_{50} values, indicating that the high content of these bioactive components in the ethanolic extract directly contributes to the observed inhibition. This confirms that the activity is closely linked to the chemical structure of ethanol-soluble compounds rather than being incidental.

These findings suggest that in fennel, the extraction of antidiabetic compounds is primarily associated with phenolic and flavonoid compounds highly soluble in organic solvents such as ethanol, unlike *P. eryngii*, where aqueous extracts showed relatively better activity. This contrast reflects inherent differences in the predominant chemical profiles of the two species, influencing solubility and enzyme interaction.

Compared to previous studies, the results are consistent with reports highlighting significant α-amylase inhibition by fennel extracts). Sayah et al. (2020) reported IC_{50} values of 1026.50 ± 6.5 µg/mLfor boiled aqueous leaf extract and 194.30 ± 4.8 µg/mL for roots, aligning with our observation of ethanolic extract superiority. Similarly, Mehra et al. (2023) indicated a hexane seed extract IC_{50} of 227.77 ± 0.64 µg/mL, while Sayahet al. (2020) recorded 117 ± 2.3 µg/mL for seeds, confirming the efficacy of organic solvent extraction.

Moreover, Palukuri and Subramaneyaan (2022) showed ethanolic fennel extract at 1 mg/mL achieved 87.14 ± 1.30% inhibition, close to 81.86 ± 1.20% at 2 mg/mL in our study, reinforcing the consistency of the findings. Karamat et al. (2024) also reported 67.8% inhibition for aqueous extract and 72.97% for methanolic extract, supporting the general trend that organic solvents provide higher α-amylase inhibition than aqueous extracts.

Overall, the comparative analysis demonstrates that *F. vulgare* extracts exhibit a higher α-amylase inhibitory potential, whereas *P. eryngii* shows moderate activity, with aqueous extracts occasionally displaying enhanced inhibitory effects. These differences underscore the critical influence of solvent polarity and phytochemical profile on enzyme inhibition efficiency across both species.

The observed α-amylase inhibitory activity can be attributed to the combined presence of multiple bioactive classes, including polysaccharides, phenolic compounds, flavonoids, and organic acids, which act through complementary molecular mechanisms. In *P. eryngii*, polysaccharides have been shown to exert concentration-dependent inhibition, reaching approximately 56–58% at 6 mg/mL, indicating a clear dose–response relationship, although their efficacy remains lower than that of the reference inhibitor acarbose (Zheng et al., 2020).

At the molecular level, phenolic compounds from mushrooms are reported to inhibit α-amylase by interfering with enzyme–substrate complex formation through hydrogen bonding interactions with amino acid residues at the catalytic site, thereby reducing starch hydrolysis into glucose units (Mbarga et al., 2020). In the present study, LC-MS/MS analysis confirmed the presence of key phenolics such as p-coumaric acid and cinnamic acid, which are structurally consistent with previously reported α-amylase inhibitory molecules, supporting their direct involvement in the observed bioactivity (Stojković et al., 2019).

Similarly, the inhibitory activity observed in *F. vulgare* leaves is strongly associated with its rich phytochemical composition, particularly phenolic acids and flavonoids such as quercetin, ferulic acid, p-coumaric acid, and cinnamic acid, all of which were identified in the ethanolic extract via LC-MS/MS analysis. These compounds are known to act synergistically by targeting the enzyme active site and surrounding binding regions, thereby reducing substrate accessibility and limiting the breakdown of starch into oligosaccharides and glucose. This coordinated inhibition ultimately contributes to the reduction of postprandial hyperglycemia (Karamat et al., 2024).

In addition, FTIR spectral analysis further supports these findings by revealing the presence of functional groups such as hydroxyl (-OH), carbonyl (-C=O), methyl ($-CH_3$), and nitro ($-NO_2$) moieties, which are capable of forming non-covalent interactions with amino acid residues within the enzyme active site. These interactions may further stabilize enzyme–ligand complexes and hinder catalytic activity, reinforcing the overall inhibitory effect (Sayah et al., 2020).

Collectively, α-amylase inhibition in both *P. eryngii* and *F. vulgare* is governed by a multi-target mechanism involving direct enzyme binding interference, synergistic interactions among phytochemical constituents, and structural modulation of enzyme–substrate recognition. The observed differences in inhibitory potency between the two species can be primarily attributed to variations in phytochemical composition, polarity-dependent extraction efficiency, and the relative abundance of key active metabolites.

3.4. Antibacterial and anti-candida activities

Rising antibiotic resistance has intensified the search for plant-derived antimicrobials, as medicinal plants are rich in bioactive compounds capable of disrupting microbial growth and enzyme activity (Hossain 2024). With over 20,000 documented medicinal plants and more than 1,340 showing antimicrobial activity, they represent a major reservoir of potential therapeutics. Their compounds may act alone or synergistically with antibiotics via multitarget and resistance-inhibiting mechanisms (Vaou et al., 2021), justifying the evaluation of the present extract's antimicrobial potential.

The antimicrobial activity results of *P. eryngii* extracts, both ethanolic and aqueous, showed a variable capacity to inhibit the growth of the bacteria and fungi studied. For the ethanolic extract, it inhibited *Escherichia coli* at the concentration of 8000 µg/mL with an inhibition zone of 20 mm, while no inhibition was observed at lower concentrations (4000, 2000, and 1000 µg/mL). In *Pseudomonas aeruginosa*, inhibition was limited and observed only at the highest concentration (8 mm). The extract also inhibited *Staphylococcus aureus* at 8000 µg/mL (11 mm), with a progressive decrease of the inhibition zone at 4000, 2000, and 1000 µg/mL (9, 8, and 8 mm, respectively). For *Bacillus subtilis*, activity was observed only at the highest concentration of 8000 µg/mL (15 mm). Regarding *Candida albicans*, the ethanolic extract showed inhibition at 8000 µg/mL (14 mm) and 2000 µg/mL (10 mm), while no inhibition was observed at the other concentrations.

For the aqueous extract, inhibition of *Escherichia coli* was observed only at 8000 µg/mL (9 mm), and *Pseudomonas aeruginosa* was inhibited at the same concentration (10 mm). Weak inhibition of Staphylococcus aureus was recorded at 8000 µg/mL (8 mm), while no activity was detected against *Bacillus subtilis* at any concentration. In *Candida albicans*, significant inhibition was observed at 8000 µg/mL (16 mm), with the inhibition zone decreasing progressively at 4000 and 2000 µg/mL (10 and 8 mm, respectively).

Table 10. Antimicrobial activity (inhibition zone, mm ± SD) of *P. eryngii* and *F. vulgare* leaves extracts and gentamicin against different microbial strains.

Samples		**Microbial strains**				
		E. coli	***P. aeruginosa***	***S. aureus***	***B. subtilis***	***C. albicans***
P. eryngii **(8 mg/ mL)**	**Aqueous extract**	9.00 ± 1.25	10.03 ± 0.95	8.00 ± 1.1	0.00 ± 0.00	16.03 ± 1.55
	Ethanolic extract	20.00 ± 0.36	08.00 ± 0.20	11.00 ± 0.30	15.00 ± 0.40	14.00 ± 0.26
F. vulgare **leaves (8 mg/ mL)**	**Aqueous extract**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.09 ± 0.26
	Ethanolic extract	15.00 ± 0.30	07.00 ± 0.20	10.00 ± 0.40	0.00 ± 0.00	21.93 ± 0.40
Reference drug (25 µg/mL)	**Gentamicin**	20.00 ± 0.45	17.00 ± 0.30	16.00 ± 0.76	16.00 ± 0.60	0.00 ± 0.00

Values are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3).

When comparing the current results with previous studies, it appears that the ethanolic extract of *P. eryngii* exhibited antibacterial and antifungal activity at varying degrees. In the present study, the ethanolic extract showed clear efficacy against *Escherichia coli* at the highest concentration of 8000 µg/mL (20 mm inhibition zone), with no activity at lower concentrations. This pattern is similar to acetone extracts that showed high activity against *E. coli*, while ethanolic and ethyl acetate extracts showed weak or absent activity (Yu et al., 2018). The ethanolic extract also displayed activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, reflecting broader efficacy compared to some previous studies.

In previous studies, the ethanolic extract only exhibited weak activity against *Staphylococcus aureus* among six bacterial species tested (류혜숙 et al., 2018), highlighting the improved effectiveness of the extract in the present study. The aqueous extract generally showed limited activity, especially against Gram-negative bacteria, consistent with previous observations on aqueous and ethanolic mushroom extracts, where aqueous extracts typically exhibited lower activity compared to organic extracts such as methanol or ethanol (Akyuz and Kirbag 2009). For instance, methanolic extracts of *P. eryngii* var. *ferulae* demonstrated variable inhibition against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*, with inhibition zones ranging from 7.7 to 10.3 mm (Akyuz and Kirbag 2009), lower than the values recorded here for the ethanolic extract, indicating the role of active compound solubility in organic solvents in determining antimicrobial activity.

Comparative studies on other Pleurotus species, such as *P. ostreatus* and *P. florida*, have shown that methanolic or ethanolic extracts cultivated on nutrient-rich substrates can achieve maximum antibacterial efficacy, with inhibition zones of 19.8 mm against *Escherichia coli* and 16.4 mm against *Pseudomonas aeruginosa* (Gashaw et al., 2020), supporting the importance of active compound concentration and culture medium in antimicrobial potency.

Overall, the ethanolic extract of *P. eryngii* in this study exhibited broader and higher antibacterial and antifungal activity against most of the bacterial and fungal species tested compared to the aqueous extract. The activity varied according to the bacterial species and extract concentration, reflecting the combined effect of the active chemical compounds and their solubility in different solvents, partially in agreement with previous studies.

The results related to the antimicrobial activity of both ethanolic and aqueous *F. vulgare* leaves extracts demonstrated variable inhibition of the growth of the bacteria and fungi studied.

The ethanolic extract inhibited the growth of *Escherichia coli* at concentrations of 8000, 4000, and 2000 µg/mL, with zones of inhibition measuring 15, 13, and 10 mm, respectively, while no inhibition was observed at 1000 µg/mL. It also inhibited the growth of *Pseudomonas aeruginosa* at 8000, 4000, and 2000 µg/mL (7, 9, and 8 mm, respectively). For *Staphylococcus aureus*, the extract demonstrated clear activity at all concentrations from 1000 to 8000 µg/mL, with zones of inhibition of 12, 11, 9, and 10 mm, respectively. No activity was observed against *Bacillus subtilis* at any concentration.

Regarding the fungus *Candida albicans*, the extract exhibited notable activity at 8000 µg/mL (22 mm), as well as at 4000 and 2000 µg/mL (8 and 10 mm, respectively), whereas no inhibition was observed at 1000 µg/mL.

The aqueous extract did not show antimicrobial activity against the bacteria (*E. coli, P. aeruginosa, S. aureus, B. subtilis*) at any concentration. Against *C. albicans*, it demonstrated clear inhibitory activity at the highest concentrations, with inhibition zones of 16 mm at 8000 µg/mL, 10 mm at 4000 µg/mL, and 8 mm at 2000 µg/ml, while no inhibition was observed at 1000 µg/mL.

In support of previous findings, studies have demonstrated that leaf and seed extracts of *F. vulgare* exhibit antibacterial and antifungal activity, which varies according to extract type and concentration. For instance, the aqueous-methanolic extract (20:80) was most effective against Gram-positive bacteria, showing the highest growth inhibition of *Bacillus megaterium* (8.74 ± 0.12 mm) and moderate inhibition of *Enterococcus gallinarium* (8.02 ± 0.43 mm), while chloroform and purely aqueous extracts showed no significant effect (Beyazen et al., 2017). In contrast, Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* were less sensitive, likely due to the LPS-rich outer membrane that restricts the entry of active compounds (Beyazen et al., 2017).

The essential oil from the leaves of *F. vulgare* subsp. *vulgare* var. *vulgare* displayed broad-spectrum antibacterial activity, with *E. coli* being the most sensitive, reaching 100% mortality at 200 µg/mL, and complete mortality against *Staphylococcus aureus* at the same concentration (Di Napoli et al., 2022). Similarly, the Algerian essential oil from fennel seeds showed strong antifungal activity against *Candida albicans* (MIC = 0.06%), attributed to its high estragole content (84.8%) (Ferhat and Yessad 2018 ; Belabdelli et al., 2020).

The methanolic extract of fennel seeds also exhibited significant activity against both Gram-positive and Gram-negative bacteria at full concentration, with inhibition zones ranging from 7.33 to 8.66 mm, sometimes surpassing reference antibiotics such as norfloxacin and ofloxacin (Wany et al., 2022). Additionally, some studies reported that *F. vulgare* seeds inhibit the growth of Gram-positive strains, whereas Gram-negative strains remain largely unaffected (Sulaiman et al., 2024).

Notably, the ethanolic extracts demonstrated strong inhibition against *S. aureus* and *E. coli* (zones of inhibition 16–17 mm), while showing no activity against *C. albicans* (Ahmed et al., 2025). These observations align with our results, where the ethanolic extract was more effective against bacteria and mushrooms than the aqueous extract, which showed limited activity only against the mushroom.

The antibacterial activity of the investigated extracts can be rationalized through multiple complementary biochemical mechanisms, as supported by previous literature. Kebaïli (2022) reported that the most potent antimicrobial compounds are generally hydrophobic in nature, and therefore preferentially extracted using low-polarity organic solvents. This provides a mechanistic explanation for the higher antibacterial efficacy observed in the ethanolic extract compared to the aqueous extract in the present study.

The antibacterial activity of mushrooms is linked to bioactive metabolites such as polysaccharides, triterpenoids, phenolics, alkaloids, and tannins, which often act synergistically. These compounds exert multi-target effects by increasing membrane permeability, disrupting electron transport, and inhibiting oxidative phosphorylation, leading to growth inhibition of Gram-positive and Gram-negative bacteria (Cör et al., 2018).

Among these metabolites, cationic polysaccharides interact electrostatically with negatively charged bacterial cell wall components, increasing membrane permeability, causing leakage of intracellular contents, and leading to bacterial death (Kandasamy et al., 2020).

The antibacterial activity of *F. vulgare* leaf extracts is also attributed to phenolics, flavonoids, and anethole, which disrupt membranes and inhibit enzymes (Ahmed et al., 2025). Fennel extracts further show strong anti-biofilm effects at low concentrations by interfering with bacterial adhesion and biofilm formation (Di Napoli et al., 2022). In addition, glycosidic compounds may contribute by targeting cell wall integrity and essential metabolic enzymes (Wany et al., 2022). This multifactorial action highlights *F. vulgare* as a potential source of bioactive antimicrobial agents for pharmaceutical and food applications.

The agar well diffusion method constitutes a preliminary screening approach that provides only a limited assessment of antimicrobial activity under simplified *in vitro* conditions; thus, the obtained results cannot be considered definitive evidence of biological efficacy.

Although inhibitory effects were observed against certain microbial strains, these findings do not capture the dynamic complexity of biological systems or the underlying molecular mechanisms *in vivo*, but rather indicate a promising potential requiring further validation. Accordingly, confirmation of these results necessitates more advanced methodologies, including quantitative *in vitro* assays such as MIC and MBC determination, microbial kill-kinetics, and biofilm inhibition studies, in addition to *in silico* analyses (molecular docking and molecular dynamics simulations). Furthermore, *in vivo* studies remain essential to establish pharmacological relevance and accurately evaluate the therapeutic efficacy of these natural extracts.

Conclusion

The objective of this study was to evaluate the nutritional properties and biological activities of the edible mushroom *Pleurotus eryngii* and fennel leaves (*Foeniculum vulgare*) in western Algeria, as no comprehensive study had been conducted on these species to date. The importance of this study lies in the fact that fennel leaves are often discarded as waste, in addition to the limited local use of the mushroom for both dietary and therapeutic purposes.

Nutritional analyses revealed that the mushroom contains high levels of proteins and carbohydrates (including sugars), with relatively low fat content, while the fennel leaves showed low protein and fat levels but high carbohydrate content.

Two environmentally friendly and low-toxicity extracts were prepared for each species, aqueous and ethanolic, to evaluate biological activities and phenolic compounds. The aqueous extract of the mushroom showed the highest content of polyphenols and flavonoids, while LC-MS/MS analysis of the ethanolic extract identified three major phenolic compounds, most notably trans-cinnamic acid. The aqueous extract also exhibited the strongest antioxidant activity, confirmed by five complementary techniques, as well as anti-inflammatory activity through albumin denaturation inhibition and α-amylase inhibitory activity. However, antimicrobial activity was stronger in the ethanolic extract compared to the aqueous extract.

Regarding fennel leaves, the ethanolic extract showed superiority in polyphenol and flavonoid contents, and the analysis revealed ten important phenolic compounds, including chlorogenic acid, quercetin, and rutin. Antioxidant, anti-inflammatory, α-amylase inhibitory, and antimicrobial activities were also more pronounced in the ethanolic extract. Furthermore, an *in silico* study indicated that several compounds present in fennel leaves are capable of inhibiting key proteins involved in inflammation, including COX-2 (PDB ID: 1CX2), IL-1β (1LOB), NF-κB (8TQD), PLA2 (1CJY), and TNF-α (5MU8), reinforcing the therapeutic potential of these plants.

The results suggest that *P. eryngii* and *F. vulgare* leaves constitute important natural sources of bioactive compounds with antioxidant, anti-inflammatory, antimicrobial, and enzyme-inhibitory properties, highlighting their potential use as dietary supplements or eco-friendly therapeutic ingredients.

Future perspectives

1. Conduct comprehensive LC–MS/MS analyses on all extracts, particularly aqueous ones, to achieve a complete characterization of phenolic compound diversity and to clarify the influence of solvent polarity on the chemical composition.
2. Perform in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) studies of the bioactive compounds to predict their pharmacokinetic behavior, bioavailability, and safety profiles prior to in vivo investigations.
3. Conduct additional in vivo studies using appropriate animal models to confirm the in vitro findings and to strengthen their biological relevance.
4. Determine MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values against a broader range of microbial strains to accurately differentiate between bacteriostatic and bactericidal effects.
5. Perform comprehensive nutritional profiling, including trace element analysis using ICP-MS and vitamin determination, to provide a more complete assessment of nutritional value.
6. Apply advanced extraction techniques, such as ultrasound-assisted extraction, to improve the yield and efficiency of bioactive compound recovery.
7. Focus future research on the valorization of *Pleurotus eryngii* and *Foeniculum vulgare* for food and pharmaceutical applications, particularly in the development of functional foods and natural dietary supplements.

REFERENCES

Refrences

Aadesariya, M. K., Ram, V. R., & Dave, P. N. (2017). Evaluation of antioxidant activities by use of various extracts from *Abutilon pannosum* and *Grewia tenax* in the Kachchh region. *MOJ Food Processing & Technology, 5*(1), 216–230.

Abd El-Zaher, E. H., Tousson, E. M., Mostafa, A. A., El-Gaar, E. M., Yahya, G., & Mahmoud, Y. A.-G. (2025). Revitalizing *Pleurotus eryngii* polysaccharides: Gamma irradiation boosts antidiabetic and antioxidant potential. *Bioresources and Bioprocessing, 12*(1), 44.

Abdellaoui, M., Derouich, M., & El-Rhaffari, L. (2020). Essential oil and chemical composition of wild and cultivated fennel (*Foeniculum vulgare* Mill.): A comparative study. *South African Journal of Botany, 135*, 93–100.

Abdullah, N., Ismail, S. M., Aminudin, N., Shuib, A. S., & Lau, B. F. (2012). Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. *Evidence-Based Complementary and Alternative Medicine*, 2012, 464238.

Abedi, F., Razavi, B. M., & Hosseinzadeh, H. (2020). A review on gentisic acid as a plant-derived phenolic acid and metabolite of aspirin. *Phytotherapy Research, 34*(4), 729–741.

Abedinzadeh, Z. (2001). Sulfur-centered reactive intermediates derived from the oxidation of sulfur compounds of biological interest. *Canadian Journal of Physiology and Pharmacology, 79*(2), 166–170.

Abotaleb, M., Liskova, A., Kubatka, P., & Büsselberg, D. (2020). Therapeutic potential of plant phenolic acids in cancer treatment. *Biomolecules, 10*(2), 221.

Agarwal, P., & Gupta, R. (2016). Alpha-amylase inhibition can treat diabetes mellitus. *Research & Reviews: Journal of Medical and Health Sciences, 5*(4), 1–8.

Ahmadi, A., Jamialahmadi, T., & Sahebkar, A. (2022). Polyphenols and atherosclerosis: Effects on LDL oxidation. *Pharmacological Research, 184*, 106414.

Ahmed, A. F., Shi, M., Liu, C., & Kang, W. (2019). Comparative analysis of antioxidant activities of fennel (*Foeniculum vulgare* Mill.) seed extracts. *Food Science and Human Wellness, 8*(1), 67–72.

Ahmed, N., Rasool, A., Manan, A., Shah, G., Khan, M., Ahmed, M., Kaleem, I., Ullah, K., & Manzoor, R. (2025). Antibacterial and antifungal activity of *Foeniculum vulgare* extracts. *Pakistan's Multidisciplinary Journal for Arts & Science, 6*(2), 11–23.

Aider, M., Elbaz, M., Ben Abdesslem, S., St-Gelais, A., Fortin, S., Boulares, M., Ben Moussa, O., Timoumi, M., & Hassouna, M. (2024). Chemical composition, antioxidant properties and antibacterial activity of Tunisian fennel (*Foeniculum vulgare* Mill.) leaves grown under conventional and organic cultivation conditions. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.4707094

Akoh, C. C. (2017). *Food lipids: chemistry, nutrition, and biotechnology*. CRC Press.

Akyuz, M., & Kirbag, S. (2009). Antimicrobial activity of *Pleurotus eryngii* var. *ferulae*. *Eurasian Journal of BioSciences, 3*(1), 58–63.

Al Bashera, M., Mosaddik, A., Batiha, G. E. S., et al. (2021). Anti-inflammatory and antioxidant activities of plant extracts. *Applied Sciences, 11*(19), 9073.

Al Qaisi, Y., Alfarrayeh, I., Alsarayreh, A., Khleifat, K., & Abu-Nawas, N. (2024). Antioxidant and anticancer activity of methanolic plant extracts. *Phytomedicine Plus, 4*(2), 100534.

Alam, A., Foudah, A. I., Salkini, M. A., Raish, M., & Sawale, J. (2022). Fennel essential oil nanogel: formulation and antibacterial activity. *Gels, 8*(11), 736.

Alam, N., Amin, R., Khan, A., Ara, I., Shim, M. J., Lee, M. W., & Lee, T. S. (2008). Nutritional analysis of cultivated mushrooms. *Mycobiology, 36*(4), 228–232.

Alam, N., Sikder, M. M., Karim, M. A., & Amin, S. M. R. (2019). Antioxidant and antityrosinase activities of mushrooms. *Bangladesh Journal of Botany, 48*(4), 1065–1073.

Alam, N., Yoon, K. N., Lee, K. R., Lee, J. S., & Lee, T. S. (2011). Phenolic compounds and antioxidant activities of *Pleurotus eryngii*. *Advances in Environmental Biology, 5*(6), 1104–1113.

Alaqeel, S. I., Almansour, A. I., Arumugam, N., Kumar, R. S., Ponmurugan, K., & Al-Dhabi, N. A. (2021). Antimicrobial activities of heterocycles. *Journal of Infection and Public Health, 14*(12), 1870–1874.

Altinoz, M. A., & Ozpinar, A. (2019). Acetylsalicylic acid and gentisic acid in psychiatric disorders. *Behavioural Pharmacology, 30*(8), 626–640.

Anka, Z., Gimba, S., Nanda, A., & Salisu, L. (2020). Phytochemistry and pharmacological activities of *Foeniculum vulgare*. *IOSR Journal of Pharmacy, 10,* 1–10.

Apak, R., Calokerinos, A., Gorinstein, S., et al. (2022). Methods to evaluate antioxidant activity. *Pure and Applied Chemistry, 94*(1), 87–144.

Asomadu, R. O., Ezeorba, T. P. C., Ezike, T. C., & Uzoechina, J. O. (2024). Antioxidant potential of endophytic fungi. *3 Biotech, 14*(5), 127.

Awuchi, C. G., Igwe, V. S., & Echeta, C. K. (2019). Functional properties of foods and flours. *International Journal of Advanced Academic Research, 5*(11), 139–160.

Ayouni, K., Berboucha-Rahmani, M., Kim, H. K., Atmani, D., Verpoorte, R., & Choi, Y. H. (2016). Metabolomic analysis of *Fraxinus angustifolia*. *Industrial Crops and Products, 88,* 65–77.

Aziz, N., Kim, M.-Y., & Cho, J. Y. (2018). Anti-inflammatory effects of luteolin: A review of in vitro, in vivo, and in silico studies. *Journal of Ethnopharmacology, 225,* 342–358.

Babu, D. R., & Rao, G. N. (2013). Antioxidant properties and electrochemical behavior of cultivated commercial Indian edible mushrooms. *Journal of Food Science and Technology, 50*(2), 301–308.

Badgujar, S. B., Patel, V. V., & Bandivdekar, A. H. (2014). *Foeniculum vulgare* Mill.: A review of its botany, phytochemistry, pharmacology, contemporary applications, and toxicology. *BioMed Research International,* 2014, 842674.

Bailey-Shaw, Y. A., Williams, L. A., Green, C. E., Rodney, S., & Smith, A. M. (2017). In vitro evaluation of the anti-inflammatory potential of selected Jamaican plant extracts using the bovine serum albumin protein denaturation assay. *International Journal of Pharmaceutical Sciences Review and Research, 47*(1), 145–153.

Bains, A., & Chawla, P. (2020). In vitro bioactivity, antimicrobial and anti-inflammatory efficacy of modified solvent evaporation-assisted *Trametes versicolor* extract. *3 Biotech, 10*(9), 404.

Bains, A., & Tripathi, A. (2017). Evaluation of antioxidant and anti-inflammatory properties of aqueous extract of wild mushrooms collected from Himachal Pradesh. *Asian Journal of Pharmaceutical and Clinical Research, 10*(3), 467–472. https://doi.org/10.22159/ajpcr.2017.v10i3.16547

Baliyan, S., et al. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules, 27*(4), 1326.

Balogun, T. A., et al. (2021). Computational evaluation of bioactive compounds from *Colocasia affinis* Schott as a novel EGFR inhibitor for cancer treatment. *Cancer Informatics, 20,* 11769351211049244.

Banerjee, R., Hilmi, I. N., Ran, Z., & Wu, K. (2018). Antibacterial effects of *Pleurotus eryngii* extract on oral bacteria. *Journal of Korean Society of Dental Hygiene, 18*(1), 9–18.

Barbosa, J. R., dos Santos Freitas, M. M., da Silva Martins, L. H., & de Carvalho Junior, R. N. (2020). Polysaccharides of mushroom *Pleurotus* spp.: New extraction techniques, biological activities and development of new technologies. *Carbohydrate Polymers, 229,* 115550.

Barros, L., Carvalho, A. M., & Ferreira, I. C. F. R. (2010). The nutritional composition of fennel (*Foeniculum vulgare*): Shoots, leaves, stems and inflorescences. *LWT – Food Science and Technology, 43*(5), 814–818.

Bastin, A., et al. (2023). In vitro and molecular docking analysis of quercetin as an anti-inflammatory and antioxidant. *Current Pharmaceutical Design, 29*(11), 883–891.

Bausa, R. K. R., David, D. B. A., Lladoc, J. N. D., Miranda, S. J. C., Sipalay, A. E. B., & Rawis, L. C. (2024). Albumin denaturation analysis for evaluating the anti-inflammatory efficacy of *Chrysopogon aciculatus*. *Lux Veritatis, 10,* 14–24. https://doi.org/10.5644/2476-5644

Belabdelli, F., et al. (2020). Chemical composition and antifungal activity of *Foeniculum vulgare* Mill. *Chemistry Africa, 3*(2), 323–328.

Belahcene, S., et al. (2023). Unveiling the chemical profiling, antioxidant and anti-inflammatory activities of Algerian *Myrtus communis* L. essential oils. *Pharmaceuticals, 16*(10), 1343.

Benabdallah, H., et al. (2022). Anti-ulcer, analgesic and antioxidant activities of aqueous extract of *Foeniculum vulgare* Mill seeds. *Turkish Journal of Agriculture – Food Science and Technology, 10*(10), 2010–2015.

Bennour, N., et al. (2020). Effect of solvent evaporation method on phenolic compounds and antioxidant activity of *Moringa oleifera* cultivated in Southern Tunisia. *South African Journal of Botany, 129*, 181–190.

Benrahou, K., et al. (2022). Inhibition of α-amylase, α-glucosidase, and lipase, intestinal glucose absorption, and antidiabetic properties by extracts of *Erodium guttatum*. *Evidence-Based Complementary and Alternative Medicine*, 2022, 5868682.

Bentahar, S., et al. (2025). Investigation of phytochemical content, antioxidant properties and antibacterial potential of Algerian *Bunium incrassatum*. *Acta Biologica Slovenica, 68*(2), 44–52.

Berman, H. M., et al. (2000). The Protein Data Bank. *Nucleic Acids Research, 28*(1), 235–242.

Bertoline, L. M., Lima, A. N., Krieger, J. E., & Teixeira, S. K. (2023). Before and after AlphaFold2: An overview of protein structure prediction. *Frontiers in Bioinformatics, 3*, 1120370.

Beyazen, A., Dessalegn, E., & Mamo, W. (2017). Phytochemical screening, antioxidant and antimicrobial activities of seeds of *Foeniculum vulgare* (Ensilal). *World Journal of Pharmaceutical Sciences*, *5*(3), 198–208.

Beyazen, A., Dessalegn, E., & Mamo, W. (2017a). Phytochemical screening and biological activities of leaf of *Foeniculum vulgare* (Ensilal). *World Journal of Agricultural Sciences, 13*(1), 1–10.

Bhattacharya, S. (2011). Are we in the polyphenols era? *Pharmacognosy Research*, *3*(2), 147.

Bittner Fialová, S., et al. (2021). Antibacterial activity of medicinal plants and their constituents in the context of skin and wound infections. *International Journal of Molecular Sciences, 22*(19), 10746.

Bonev, B., Hooper, J., & Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy, 61*(6), 1295–1300.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry, 72*(1–2), 248–254.

Calabretti, A., Mang, S. M., Becce, A., Castronuovo, D., Cardone, L., Candido, V., & Camele, I. (2021). Comparison of bioactive substances content between commercial and wild-type isolates of *Pleurotus eryngii*. *Sustainability, 13*(7), 3777.

Caponio, G. R., Difonzo, G., Troilo, M., Marcotuli, I., Gadaleta, A., Tamma, G., Gargano, M. L., & Cirlincione, F. (2025). Enhancing the nutritional and health-related properties of taralli through the use of *Pleurotus eryngii*: Focus on antioxidant and anti-inflammatory properties. *Antioxidants, 14*(5), 550.

Castaldo, L., Izzo, L., De Pascale, S., Narváez, A., Rodriguez-Carrasco, Y., & Ritieni, A. (2021). Chemical composition, in vitro bioaccessibility and antioxidant activity of polyphenolic compounds from nutraceutical fennel waste extract. *Molecules, 26*(7), 1968.

Çayan, F., Deveci, E., Tel-Çayan, G., & Duru, M. E. (2020). Identification and quantification of phenolic acid compounds of twenty-six mushrooms by HPLC–DAD. *Journal of Food Measurement and Characterization, 14*(3), 1690–1698.

Chaoua, H., Khacheba, I., Boussoussa, H., Bekhaoua, A., & Yousfi, M. (2019). A potent in vitro α-amylase inhibitory action of *Haplophyllum tuberculatum* extracts. *Current Enzyme Inhibition, 15*(3), 190–196.

Cherbal, A., Bouabdallah, M., Benhalla, M., Hireche, S., & Desdous, R. (2023). Phytochemical screening, phenolic content, and anti-inflammatory effect of *Foeniculum vulgare* seed extract. *Preventive Nutrition and Food Science, 28*(2), 141–149.

Choi, J. H., Kim, J. S., Park, J. D., & Sung, J. M. (2021). Study on antioxidant activities of *Pleurotus ostreatus*, *Pleurotus eryngii* and *Flammulina velutipes* extracts by different solvent. *Journal of the Korean Society of Food Culture, 36*(6), 622–632.

Choi, S. S., Park, H. R., & Lee, K. A. (2021). A comparative study of rutin and rutin glycoside: Antioxidant activity, anti-inflammatory effect, effect on platelet aggregation and blood coagulation. *Antioxidants, 10*(11), 1696.

Christodoulou, M. C., Orellana Palacios, J. C., Hesami, G., Jafarzadeh, S., Lorenzo, J. M., Domínguez, R., … Hadidi, M. (2022). Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals. *Antioxidants, 11*(11), 2213.

Chu, M., Khan, R. D., Zhou, Y., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. (2023). LC-ESI-QTOF-MS/MS characterization of phenolic compounds in common commercial mushrooms and their potential antioxidant activities. *Processes, 11*(6), 1711.

Cör, D., Knez, Ž., & Knez Hrnčič, M. (2018). Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of *Ganoderma lucidum* terpenoids and polysaccharides: A review. *Molecules, 23*(3), 649.

Ćorković, I., Gašo-Sokač, D., Pichler, A., Šimunović, J., & Kopjar, M. (2022). Dietary polyphenols as natural inhibitors of α-amylase and α-glucosidase. *Life, 12*(11), 1692.

Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M., & Ferreira, I. C. F. R. (2016). Biotechnological, nutritional and therapeutic uses of *Pleurotus* spp. (oyster mushroom) related with its chemical composition: A review on the past decade findings. *Trends in Food Science & Technology, 50,* 103–117.

Cui, Y. L., & Li, B. (2025). Hypoglycemic effects of edible fungus polysaccharides: A mini review. *Food & Medicine Homology, 2*(1), 9420046.

Daaloul-Jedidi, M., Soudani, A., & Messadi, L. (2017). Salinity impact on seed yield, polyphenols composition and antioxidant activity of fennel (*Foeniculum vulgare*) extracts. *Journal of New Sciences, 17,* 1–8.

Dandapat, S., & Sinha, M. P. (2015). Antioxidant and anti-inflammatory activity of *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer. *Advances in Biological Research, 9*(3), 140–145.

De Aquino, F. L. T., Da Silva, J. P., de Souza Ferro, J. N., Lagente, V., & Barreto, E. (2021). trans-Cinnamic acid, but not p-coumaric acid or methyl cinnamate, induces fibroblast migration through PKA- and p38-MAPK signalling pathways. *Journal of Tissue Viability, 30*(3), 363–371.

De Carvalho, C. C., & Caramujo, M. J. (2018). The various roles of fatty acids. *Molecules, 23*(10), 2583.

De Gioia, T., Sisto, D., Rana, G. L., & Figliuolo, G. (2005). Genetic structure of the *Pleurotus eryngii* species-complex. *Mycological Research, 109*(1), 71–80.

de Melo Macoris, J. D., Brugnari, T., Boer, C. G., Contato, A. G., Peralta, R. M., & de Souza, C. G. M. (2017). Antioxidant properties and antimicrobial potential of aqueous extract of basidioma from *Lentinus edodes*. *International Journal of Current Microbiology and Applied Sciences, 6*(9), 3757–3767.

de Oliveira Mozzaquatro, J., César, I. A., Pinheiro, A. E. B., & Caldas, E. D. (2022). Pesticide residues analysis in passion fruit and its processed products by LC–MS/MS and GC–MS/MS: Method validation, processing factors and dietary risk assessment. *Food Chemistry, 375,* 131643.

Deepalakshmi, K., & Sankaran, M. (2014). *Pleurotus ostreatus*: An oyster mushroom with nutritional and medicinal properties. *Journal of Biochemical Technology, 5*(2), 718–726.

Derbel, H., Elleuch, J., Mahfoudh, W., Michaud, P., Fendri, I., & Abdelkafi, S. (2023). In vitro antioxidant and anti-inflammatory activities of bioactive proteins and peptides from *Rhodosomonas* sp. *Applied Sciences, 13*(5), 3202.

Deveci, E., Çayan, F., Tel-Çayan, G., & Duru, M. E. (2021). Inhibitory activities of medicinal mushrooms on α-amylase and α-glucosidase enzymes related to type 2 diabetes. *South African Journal of Botany, 137*, 19–23.

Deveci, E., Tel-Çayan, G., Çayan, F., Yılmaz Altınok, B., & Aktaş, S. (2023). Chemical characterization, in vitro antioxidant, anti-cancer and enzyme inhibition activities of three edible mushroom species. *Journal of Food Measurement and Characterization, 17*(5), 5518–5533.

Devi, L. S., Ghosh, S., Das, A., Borthakur, S., & Singh, N. I. (2014). Antioxidative and free radical scavenging activity of ethanolic extract of *Lentinula edodes*. *Asian Journal of Pharmaceutical and Clinical Research, 7*(3), 195–199.

Di Napoli, M., Castagliuolo, G., Badalamenti, N., Maresca, V., Basile, A., Bruno, M., Zanfardino, A. (2022). Antimicrobial, antibiofilm, and antioxidant properties of essential oil of *Foeniculum vulgare* Mill. leaves. *Plants, 11*(24), 3573.

Di, X., Liang, X., Shen, C., Pei, Y., Wu, B., & He, Z. (2022). Carbohydrates used in polymeric systems for drug delivery: From structures to applications. *Pharmaceutics, 14*(4), 739.

Diallo, I. (2019). *Potentiels anti-oxydants et anti-inflammatoires de sporophores de Lentinula edodes (Shiitake) sous différentes conditions de culture* (Doctoral dissertation, Université Montpellier).

Diosady, L. L., Mannar, M. V., & Krishnaswamy, K. (2019). Improving the lives of millions through new double fortification of salt technology. *Maternal & Child Nutrition, 15,* e12773.

Dogan, A., Eylem, C. C., & Akduman, N. E. B. (2020). Application of green methodology to pharmaceutical analysis using eco-friendly ethanol–water mobile phases. *Microchemical Journal, 157,* 104895.

Dominguez-López, I., Pérez, M., & Lamuela-Raventós, R. M. (2024). Total (poly)phenol analysis by the Folin–Ciocalteu assay as an anti-inflammatory biomarker in biological samples. *Critical Reviews in Food Science and Nutrition, 64*(27), 10048–10054.

Dong, Q., He, D., Ni, X., Zhou, H., & Yang, H. (2019). Comparative study on phenolic compounds, triterpenoids, and antioxidant activity of *Ganoderma lucidum* affected by different drying methods. *Journal of Food Measurement and Characterization, 13*(4), 3198–3205.

Duan, L., Cheng, S., Li, L., Liu, Y., Wang, D., & Liu, G. (2021). Natural anti-inflammatory compounds as drug candidates for inflammatory bowel disease. *Frontiers in Pharmacology, 12,* 684486.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356.

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*, 177.

Elhusseiny, S. M., El-Mahdy, T. S., Awad, M. F., Elleboudy, N. S., Farag, M. M., Aboshanab, K. M., & Yassien, M. A. (2021). Antiviral, cytotoxic, and antioxidant activities of three edible agaricomycetes mushrooms: *Pleurotus columbinus, Pleurotus sajor-caju,* and *Agaricus bisporus*. *Journal of Fungi, 7*(8), 645.

Enneb, S., Drine, S., Bagues, M., Triki, T., Boussora, F., Guasmi, F., Ferchichi, A. (2020). Phytochemical profiles and nutritional composition of squash (*Cucurbita moschata* D.) from Tunisia. *South African Journal of Botany, 130,* 165–171.

Erbiai, E. H., Bouchra, B., da Silva, L. P., Lamrani, Z., Pinto, E., da Silva, J. C. E., & Maouni, A. (2021). Chemical composition and antioxidant and antimicrobial activities of *Lactarius sanguifluus,* a wild edible mushroom from northern Morocco. *Euro-Mediterranean Journal for Environmental Integration, 6*(2), 43.

Erbiai, E. H., Maouni, A., Pinto da Silva, L., Saidi, R., Legssyer, M., Lamrani, Z., & Esteves da Silva, J. C. (2023). Antioxidant properties, bioactive compounds contents, and chemical characterization of two wild edible mushroom species from Morocco: *Paralepista flaccida* and *Lepista nuda*. *Molecules, 28*(3), 1123.

Erenler, R., Atalar, M. N., Yıldız, İ., Geçer, E. N., Yıldırım, A., Demirtaş, İ., & Alma, M. H. (2023). Quantitative analysis of bioactive compounds by LC-MS/MS from *Inula graveolens*. *Bütünleyici ve Anadolu Tıbbı Dergisi, 4*(3), 3–10.

Fajar, M., Safriansyah, W., Muhajir, M. I., Maharani, R., & Supratman, U. (2024). Comparative studies of two Indonesian medicinal plants, Bidara Upas (*Merremia mammosa* Lour. Hall. f) and Adas (*Foeniculum vulgare* Miller): Antioxidant, antidiabetic, and antimicrobial activities. *Trends in Sciences, 21*(8), 7868.

Farid, A., Kamel, D., Montaser, S. A., Ahmed, M. M., El Amir, M., & El Amir, A. (2020). Synergetic role of senna and fennel extracts as antioxidant, anti-inflammatory and anti-mutagenic agents in irradiated human blood lymphocyte cultures. *Journal of Radiation Research and Applied Sciences, 13*(1), 191–199.

Farooq, M. U., Mumtaz, M. W., Mukhtar, H., Rashid, U., Akhtar, M. T., Raza, S. A., & Nadeem, M. (2020). UHPLC-QTOF-MS/MS based phytochemical characterization and anti-hyperglycemic prospective of hydro-ethanolic leaf extract of *Butea monosperma*. *Scientific Reports, 10*, 3530.

Ferhat, Z., & Yessad, M. (2018). Huiles essentielles de *Laurus nobilis* L. et *Foeniculum vulgare* Mill.: Composition chimique et activités antimicrobienne et antioxydante. *Algerian Annals of Agronomy, 30*(2), 19–30.

Forouzanfar, F., Sahranavard, T., Tsatsakis, A., Iranshahi, M., & Rezaee, R. (2025). Rutin: A pain-relieving flavonoid. *Inflammopharmacology, 1–13*.

Fouda, K., & Mohamed, R. S. (2025). In silico and in vivo studies reveal the potential preventive impact of *Cuminum cyminum* and *Foeniculum vulgare* essential oil nanocapsules against depression-like states in mice. *Scientia Pharmaceutica, 93*(3), 37.

Gąsecka, M., Mleczek, M., Siwulski, M., & Niedzielski, P. (2016). Phenolic composition and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus eryngii* enriched with selenium and zinc. *European Food Research and Technology, 242*(5), 723–732.

Gashaw, G., Fassil, A., & Redi, F. (2020). Evaluation of the antibacterial activity of *Pleurotus* spp. cultivated on different agricultural wastes. *International Journal of Microbiology, 2020*, 9312489.

Gharib, M. A. A., Elhassaneen, Y. A. E. E., & Radwan, H. (2022). Nutrients and nutraceuticals content and in vitro biological activities of *Ganoderma lucidum*. *Alexandria Science Exchange Journal, 43*(2), 301–316.

Ghosh, S., Dewanjee, D., & Acharya, K. (2022). Exploring therapeutic efficacy of infusion and decoction of two wild edible mushrooms. *Kavaka, 58*(1), 11–14.

Giriwono, P. E., Iskandriati, D., Tan, C. P., & Andarwulan, N. (2020). In vitro anti-inflammatory, DPPH scavenging and FRAP activities of *Sargassum cristaefolium*. *Food Research International, 137*, 109702.

Goldsworthy, G. J., Mordue, W., & Guthkelch, J. (1972). Studies on insect adipokinetic hormones. *General and Comparative Endocrinology, 18*(3), 545–551.

Goudjil, S., Boussekine, S., Goudjil, H., Yilmaz, M. A., Ola, M. S., & Cakir, O. (2024). Investigation of Algerian *Crataegus monogyna* Jacq phenolic compounds (LC-ESI-MS/MS analysis, antioxidant activity, and enzyme inhibition) and their potential implications for food and nutraceutical applications. *Antioxidants, 13*(11), 1350.

Griffiths, H. R., Gao, D., & Pararasa, C. (2017). Redox regulation in metabolic programming and inflammation. *Redox Biology, 12*, 50–57.

Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes, 11*(8), 2248.

Hadji, M., Bendif, H., Hadji, T., Dehimi, K., Smaili, T., Farouk, K. F., … Garzoli, S. (2025). Chemical profiling via LC-ESI-MS/MS and functional bioactivities of *Foeniculum vulgare* subsp. *capillaceum*. *Journal of Food Science, 90*(7), e70436.

Hait, M., Bhardwaj, A. K., & Kashyap, N. K. (2025). Nutritional composition and value of edible mushroom. In *Bioactive compounds in edible mushrooms: Sustainability and health applications* (pp. 435–482). Springer Nature Switzerland.

Hamada Saoud, D., Hadjadj, S., Bencheikh, S. E., Goudjil, M. B., Bouafia, A., Ladjel, S., & Menaa, F. (2024). Phytochemical screening of aerial organs of wild fennel essential oils from southeast Algeria: Chemical composition, antioxidant, and antimicrobial activities. *Biomass Conversion and Biorefinery, 14*(14), 16257–16271.

Hamza, A., Zouari, N., Zouari, S., Jdir, H., Zaidi, S., Gtari, M., & Neffati, M. (2016). Nutraceutical potential, antioxidant and antibacterial activities of *Terfezia boudieri*, a wild edible desert truffle from Tunisia arid zone. *Arabian Journal of Chemistry, 9*(3), 383–389.

Han, V. X., Patel, S., Jones, H. F., Nielsen, T. C., Mohammad, S. S., Hofer, M. J., … Dale, R. C. (2021). Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: A systematic review. *Translational Psychiatry, 11*(1), 71.

Han, Y., Tang, X., Zhang, Y., Hu, X., & Ren, L. J. (2021). The current status of biotechnological production and application of ergothioneine. *Critical Reviews in Biotechnology, 41*(4), 580–593.

Hao, Y., Kang, J., Guo, X., Yang, R., Chen, Y., Li, J., & Shi, L. (2021). Comparison of nutritional compositions and essential oil profiles of dill and two fennel cultivars. *Foods, 10*(8), 1784.

Harvanová, G., Duranková, S., & Bernasovská, J. (2023). The role of cytokines and chemokines in the inflammatory response. *Alergologia Polska – Polish Journal of Allergology, 10*(3), 210–219.

Hayes, M. (2020). Measuring protein content in food: An overview of methods. *Foods, 9*(10), 1340.

He, M. M., Smith, A. S., Oslob, J. D., Flanagan, W. M., Braisted, A. C., Whitty, A., … Cunningham, B. C. (2005). Small-molecule inhibition of TNF-α. *Science, 310*(5750), 1022–1025.

Hikmawanti, N. P. E., Fatmawati, S., & Asri, A. W. (2021). The effect of ethanol concentrations as the extraction solvent on antioxidant activity of katuk (*Sauropus androgynus* (L.) Merr.) leaves extracts. *IOP Conference Series: Earth and Environmental Science, 755*(1), 012060. https://doi.org/10.1088/1755-1315/755/1/012060

Hoa, H. T., Wang, C. L., & Wang, C. H. (2015). Effects of different substrates on growth, yield, and nutritional composition of oyster mushrooms. *Mycobiology, 43*(4), 423–434.

Hong, Y., Shi, S., & Zhao, R. (2025). The potential and mechanism of chlorogenic acid in alleviating intestinal inflammation. *Scientia Agricultura Sinica, 58*(3), 600–616.

Hossain, T. J. (2024). Methods for screening and evaluation of antimicrobial activity: A review. *European Journal of Microbiology and Immunology, 14*(2), 97–115.

Huang, W., Kim, J. S., & Chung, H. Y. (2011). Antioxidant activity and phenolic content in shiitake mycelial exudates. *Natural Product Communications, 6*(6), 1934578X1100600622.

Hussain, G., Zhang, L., Rasul, A., Anwar, H., Sohail, M. U., Razzaq, A., … Sun, T. (2018). Role of flavonoids in Alzheimer’s and Parkinson’s diseases. *Molecules, 23*(4), 814.

Hussen, E. M., & Endalew, S. A. (2023). In vitro antioxidant and free radical scavenging activities of *Vernonia amygdalina*. *BMC Complementary Medicine and Therapies, 23*(1), 146.

Irshad, A., Tahir, A., Sharif, S., Khalid, A., Ali, S., Naz, A., … Ameen, A. (2023). Nutritional and biochemical composition of selected *Pleurotus* species. *BioMed Research International, 2023,* 8150909.

Jan, H., Shah, M., Andleeb, A., Faisal, S., Khattak, A., Rizwan, M., … Abbasi, B. H. (2021). Green synthesis of ZnO nanoparticles using *Aquilegia pubiflora* leaf extract. *Oxidative Medicine and Cellular Longevity, 2021*, 4786227.

Jayakumar, T., Thomas, P. A., Sheu, J. R., & Geraldine, P. (2011). Antioxidant effects of *Pleurotus ostreatus*. *Food Research International, 44*(4), 851–861.

Jung, H. J., Kim, S. J., Song, Y. S., Park, E. H., & Lim, C. J. (2010). Antiangiogenic and anti-inflammatory effects of morin. *Planta Medica, 76*(3), 273–275.

Kalleli, F., Bettaieb Rebey, I., Wannes, W. A., Boughalleb, F., Hammami, M., Saidani Tounsi, M., & M’hamdi, M. (2019). Chemical composition and antioxidant potential of essential oil and methanol extract from Tunisian and French fennel (*Foeniculum vulgare* Mill.) seeds. *Journal of Food Biochemistry, 43*(8), e12935.

Kandasamy, S., Chinnappan, S., Thangaswamy, S., Balakrishnan, S., & Khalifa, A. Y. (2020). Assessment of antioxidant, antibacterial activities and bioactive compounds of the wild edible mushroom *Pleurotus sajor-caju*. *International Journal of Peptide Research and Therapeutics, 26*(3), 1575–1581.

Karakas, F. P., Turker, A. U., & Bozat, B. G. (2023). Phenolic content, antibacterial and antioxidant potential of several edible Agaricomycetes mushrooms sold in public bazaars in Bolu, Turkey. *International Journal of Medicinal Mushrooms, 25*(1), 45–56.

Karamat, M., Asim, M., Hafeez, J., Bilal, A., Sami, A., Zia, S., & Hussain, F. (2024). Structural characterization, antioxidant, antidiabetic and antimicrobial activities of *Trachyspermum ammi* and *Foeniculum vulgare*. *Pakistan Journal of Science, 76*(2), 314–323.

Kashtoh, H., & Baek, K. H. (2023). New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects. *Plants, 12*(16), 2944.

Kaur, J., & Kaur, R. (2022). p-Coumaric acid: A naturally occurring chemical with potential therapeutic applications. *Current Organic Chemistry, 26*(14), 1333–1349.

Kaur, N., Kumar, V., Nayak, S. K., Wadhwa, P., Kaur, P., & Sahu, S. K. (2021). Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical Biology & Drug Design, 98*(4), 539–560.

Kaveh, R., Naghmachi, M., Motaghi, M. M., Amirmahani, F., & Danaei, M. (2023). Antibacterial and antioxidant activities and anticancer effects of fennel seeds (*Foeniculum vulgare*) against lung cancer cells. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 93*(2), 311–316.

Kebaïli, F. F. (2022). *Bioévaluation du champignon sauvage Ganoderma lucidum d'Algérie : Étude des propriétés nutritionnelles et activités antioxydantes et thérapeutiques* (Doctoral dissertation, Université Frères Mentouri Constantine 1).

Khammassi, M., Mighri, H., Mansour, M. B., Amri, I., Jamoussi, B., & Khaldi, A. (2022). Metabolite profiling and potential antioxidant activity of sixteen fennel (*Foeniculum vulgare* Mill.) populations growing wild in Tunisia. *South African Journal of Botany, 148*, 407–414.

Khan, I., Ullah, N., Zha, L., Bai, Y., Khan, A., Zhao, T., … Zhang, C. (2019). Alteration of gut microbiota in inflammatory bowel disease (IBD): Cause or consequence? *Pathogens, 8*(3), 126.

Khan, M. A., & Tania, M. (2012). Nutritional and medicinal importance of *Pleurotus* mushrooms: An overview. *Food Reviews International, 28*(3), 313–329.

Khatua, S., Paul, S., & Acharya, K. (2013). Mushroom as a potential source of new generation antioxidants: A review. *Research Journal of Pharmacy and Technology, 6*(5), 496–505.

Khirallah, S. M., Ramadan, H. M., Shawky, A., Qahl, S. H., Baty, R. S., Alqadri, N., … Saied, E. M. (2022). Development of novel 1,3-disubstituted-2-thiohydantoin analogues with potent anti-inflammatory activity. *Molecules, 27*(19), 6271.

Kiehlbauch, J. A., Hannett, G. E., Salfinger, M., Archinal, W., Monserrat, C., & Carlyn, C. (2000). Use of CLSI guidelines for disk diffusion susceptibility testing in New York State laboratories. *Journal of Clinical Microbiology, 38*(9), 3341–3348.

Kim, B. H., Choi, D., Piao, Y. L., Park, S. S., Lee, M. K., Cha, W. S., … Cho, H. (2012). Comparative study on antioxidant and nitrite scavenging activity of fruiting body and mycelium extract from *Pleurotus ferulae*. *Korean Journal of Chemical Engineering, 29*(10), 1393–1402.

Kim, D. H., Park, Y. H., Lee, J. S., Jeong, H. I., Lee, K. W., & Kang, T. H. (2020). Anti-obesity effect of DKB-117 through inhibition of pancreatic lipase and α-amylase activity. *Nutrients, 12*(10), 3053.

Kim, E. A., Kang, N., Heo, S. Y., Oh, J. Y., Lee, S. H., Cha, S. H., … Heo, S. J. (2023). Antioxidant, antiviral, and anti-inflammatory activities of lutein-enriched extract of *Tetraselmis* species. *Marine Drugs, 21*(7), 369.

Kim, J. H., Jang, M. J., & Park, Y. J. (2023). In vitro α-amylase, α-glucosidase, pancreatic lipase, and xanthine oxidase inhibitory activity of *Agaricus bisporus* extracts. *Mycobiology, 51*(1), 60–66.

Kim, N., Park, J. D., Choi, Y. S., Lee, M., & Sung, J. M. (2020). Antioxidant activity of *Pleurotus ostreatus* and *Pleurotus eryngii* hot water extracts by drying methods. *The Korean Journal of Food and Nutrition, 33*(1), 64–73.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., … Bolton, E. E. (2019). PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Research, 47*(D1), D1102–D1109.

Kongkla, K., & Poeaim, S. (2016). Total phenolic content and antioxidant activity of crude methanolic extract from five *Pleurotus* species. *International Journal of Agricultural Technology, 12*(7.1), 1455–1462.

Konsue, N., Ketnawa, S., & Qin, S. (2025). Indigenous wild edible mushrooms: Unveiling the chemical compositions and health impacts. *Foods, 14*(13), 2331.

Kooti, W., Moradi, M., Ali-Akbari, S., Sharafi-Ahvazi, N., Asadi-Samani, M., & Ashtary-Larky, D. (2015). Therapeutic and pharmacological potential of *Foeniculum vulgare* Mill: A review. *Journal of HerbMed Pharmacology, 4*(1), 1–9.

Krishna, K. V., Murugan, J. M., Khan, H., Kumar, M., Veeramanikandan, V., Hatamleh, A. A., … Balaji, P. (2023). Therapeutic potential of edible *Pleurotus* mushrooms for oxidative stress and diabetes management. *Journal of King Saud University – Science, 35*(9), 102926.

Krüzselyi, D., Kovács, D., & Vetter, J. (2016). Chemical analysis of king oyster mushroom (*Pleurotus eryngii*) fruit bodies. *Acta Alimentaria, 45*(1), 20–27.

Laila, A., Alyensi, F., & Pertiwi, R. (2025). Effect of natural antioxidant supplementation of rose cider (*Rosa damascena*) on oxidative stress in high-risk pregnant women. *Journal of Health and Nutrition Research, 4*(2), 633–640.

Lang, Y., Gao, N., Zang, Z., Meng, X., Lin, Y., Yang, S., … Li, B. (2024). Classification and antioxidant assays of polyphenols: A review. *Journal of Future Foods, 4*(3), 193–204.

Lau, K. Q., Sabran, M. R., & Shafie, S. R. (2021). Utilization of vegetable and fruit by-products as functional ingredients and food. *Frontiers in Nutrition, 8,* 661693.

Lee, S. A., Park, B. R., Moon, S. M., Han, S. H., & Kim, C. S. (2020). Anti-inflammatory potential of *Trifolium pratense* L. leaf extract in LPS-stimulated RAW 264.7 cells and in a rat model of carrageenan-induced inflammation. *Archives of Physiology and Biochemistry, 126*(1), 74–81.

Lee, Y. L., Huang, G. W., Liang, Z. C., & Mau, J. L. (2007). Antioxidant properties of three extracts from *Pleurotus citrinopileatus*. *LWT – Food Science and Technology, 40*(5), 823–833.

Lekouaghet, A., Boutefnouchet, A., Bensuici, C., Gali, L., Ghenaiet, K., & Tichati, L. (2020). In vitro evaluation of antioxidant and anti-inflammatory activities of hydroalcoholic extract and fractions from *Leuzea conifera* L. roots. *South African Journal of Botany, 132,* 103–107.

Lezoul, N. E. H., Belkadi, M., Habibi, F., & Guillén, F. (2020). Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. *Molecules, 25*(20), 4672.

Li, B., Cheng, Z., Sun, X., Si, X., Gong, E., Wang, Y., … Meng, X. (2020). *Lonicera caerulea* L. polyphenols alleviate oxidative stress-induced intestinal imbalance and liver injury via Nrf2/HO-1/NQO1 and MAPK pathways. *Molecular Nutrition & Food Research, 64*(10), 1901315.

Li, S., & Shah, N. P. (2013). Effects of heat treatments on phenolic profiles and antioxidant activities of *Pleurotus eryngii* extracts. *Journal of Food Science, 78*(8), C1122–C1129.

Li, Y., Lin, H., Ma, M., Li, L., Cai, M., Zhou, N., … Cao, J. (2009). Semen quality of 1346 healthy men: Results from Chongqing, China. *Human Reproduction, 24*(2), 459–469.

Liao, J., Wang, Q., Wu, F., & Huang, Z. (2022). In silico methods for identification of potential active sites of therapeutic targets. *Molecules, 27*(20), 7103.

Lin, J. T., Liu, C. W., Chen, Y. C., Hu, C. C., Juang, L. D., Shiesh, C. C., & Yang, D. J. (2014). Chemical composition, antioxidant and anti-inflammatory properties of ethanolic extracts from *Pleurotus eryngii* fruiting bodies harvested at different times. *LWT – Food Science and Technology, 55*(1), 374–382.

Liu, S., Jia, F., Xie, X., Kang, S., Feng, M., Xiong, X., … Chen, C. (2025). Composition analysis and in vitro antioxidant, α-amylase, and lipase inhibitory activities of mushrooms from Qinling Mountains. *Chemistry & Biodiversity, e00904*.

Liu, X., Zhou, B., Lin, R., Jia, L., Deng, P., Fan, K., … Zhang, J. (2010). Extraction and antioxidant activities of intracellular polysaccharides from *Pleurotus* sp. mycelium. *International Journal of Biological Macromolecules, 47*(2), 116–119.

Loucif, K., Benabdallah, H., Benchikh, F., Mehlous, S., Souici, C. B., & Amira, S. (2020). Total phenolics, DPPH radical scavenging and β-carotene bleaching activities of *Ammoides atlantica*. *Journal of Drug Delivery and Therapeutics, 10*(3-s), 196–198.

Ma, K. C., Schenck, E. J., Pabon, M. A., & Choi, A. M. (2018). Role of danger signals in critical illness pathogenesis. *American Journal of Respiratory and Critical Care Medicine, 197*(3), 300–309.

Macwan, D., & Patel, H. V. (2023). Inhibitory effect of bioassay-guided fractionation of mushroom (*Pleurotus ostreatus*) extract on fructose-induced glycated hemoglobin and aggregation in vitro. *Pharmacognosy Research, 15,* 776–784.

Maftoun, P., Johari, H., Soltani, M., Malik, R., & Zalina, N. (2015). The edible mushroom *Pleurotus* spp.: Biodiversity and nutritional values. *International Journal of Biotechnology for Wellness Industries, 4*(2), 67–83.

Magalhães, L. M., Segundo, M. A., Reis, S., & Lima, J. L. F. C. (2008). Methodological aspects of in vitro antioxidant evaluation. *Analytica Chimica Acta, 613*(1), 1–19.

Majdoub, N., el-Guendouz, S., Rezgui, M., Carlier, J., Costa, C., Kaab, L. B. B., & Miguel, M. G. (2017). Growth, phenolics and biological activities of Apiaceae species under zinc treatment. *Industrial Crops and Products, 109*, 627–636.

Mariga, A. M., Pei, F., Yang, W. J., Zhao, L. Y., Shao, Y. N., Mugambi, D. K., & Hu, Q. H. (2014). Immunopotentiation of *Pleurotus eryngii*. *Journal of Ethnopharmacology, 153*(3), 604–614.

Marinov, V., & Valcheva-Kuzmanova, S. (2015). Pharmacological activities of anethole: A review. *Scripta Scientifica Pharmaceutica, 2*(2), 14–19.

Marrelli, M., Amodeo, V., Viscardi, F., De Luca, M., Statti, G., & Conforti, F. (2020). Essential oils of *Foeniculum vulgare* subsp. *piperitum* and anti-arthritic potential. *Chemistry & Biodiversity, 17*(11), e2000388.

Mbarga, A. J. M., Omgba, C. B. E., Djessissem, R., & Sop, M. M. K. (2020). Alpha-amylase inhibition and antioxidant potential of oyster mushrooms. *Journal of the Cameroon Academy of Sciences, 15*(3), 151–162.

Md Noh, M. F., Gunasegavan, R. D. N., Khalid, N. M., Balasubramaniam, V., Mustar, S., & Abd Rashed, A. (2020). Nutrient analysis techniques for food databases. *Molecules, 25*(19), 4567.

Mechchate, H., Es-Safi, I., Louba, A., et al. (2021). Antidiabetic activity of *Withania frutescens* extract. *Molecules, 26*(2), 293.

Mehra, N., Tamta, G., & Nand, V. (2021). Nutritional and pharmacological attributes of *Foeniculum vulgare*. *Journal of Pharmacognosy and Phytochemistry, 10*(2), 1255–1263.

Mehra, N., Tamta, G., & Nand, V. (2023). *Foeniculum vulgare*: Anti-diabetic and anti-inflammatory activity. *Indian Journal of Natural Products and Resources, 14*(3), 372–383.

Mehra, N., Tamta, G., Nand, V., & Singh, J. P. (2022). Nutritional and antibacterial profiling of fennel seeds. *Journal of Food Processing and Preservation, 46*(8), e16763.

Mendonça, J. D. S., Guimarães, R. D. C. A., Zorgetto-Pinheiro, V. A., et al. (2022). Methods for antioxidant evaluation: A review. *Molecules, 27*(11), 3563.

Modaressi, M., Shahsavari, R., Ahmadi, F., Rahimi-Nasrabadi, M., Abiri, R., Mikaeli, A., & Batoli, H. (2013). The evaluation of antibacterial, antifungal and antioxidant activity of methanolic extract of *Mindium laevigatum* (Vent.) Rech. F. from central part of Iran. *Jundishapur Journal of Natural Pharmaceutical Products, 8*(1), 34–40.

Moradi, H., Farshadfar, M., Shirvani, H., Soltani, M., & Gholipour, M. (2020). Investigation of the antibacterial effect of fennel and propolis extract on food microorganisms. *Honeybee Science Journal, 11*(20) , 34–40.

Morant-Ferrando, B., Jimenez-Blasco, D., Alonso-Batan, P., Agulla, J., Lapresa, R., García-Rodríguez, D., … Bolaños, J. P. (2023). Fatty acid oxidation organizes mitochondrial supercomplexes to sustain astrocytic ROS and cognition. *Nature Metabolism, 5*(8), 1290–1302.

Mostafa, G., Nahid, J., Alireza, D., & Ebrahim, S. S. (2021). Effect of *Foeniculum vulgare* aqueous and alcoholic seed extract against zoonotic cutaneous leishmaniasis. *Ethiopian Journal of Health Sciences, 31*(2) , 401–408.

Moumen, B. E., Bouzoubaa, A., Drioiche, A., Eddahmouny, M., Al Kamaly, O., Shahat, A. A., … Zair, T. (2025). Unveiling the chemical composition, antioxidant, and antimicrobial potentials of *Foeniculum vulgare* Mill: A combined in vitro and in silico approach. *International Journal of Molecular Sciences, 26*(10), 4499.

Mousa, T. H., Al-Obaidi, Z. M. J., & Alkhafaji, S. L. (2021). Molecular docking studies and evaluation of the anti-inflammatory activity of ibuprofen–tranexamic acid codrug. *Latin American Journal of Pharmacy, 40*(Special Issue), 128–134.

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences, 22*(7), 3380.

Nardini, M. (2023). An overview of bioactive phenolic molecules and antioxidant properties of beer: Emerging trends. *Molecules, 28*(7), 3221.

Navoda, H., & Anupama, D. D. (2022). Evaluation of antifungal plant extracts against cereal and legume seed-borne pathogens for effective management. *Studies in Fungi, 7*(1), 1–11.

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences, 56*, e17129.

Nerdy, N., & Manurung, K. (2018). Spectrophotometric method for antioxidant activity test and total phenolic determination of red dragon fruit leaves and white dragon fruit leaves. *Rasayan Journal of Chemistry, 11*(3), 1183–1192.

Noreen, S., Tufail, T., Ain, H. B., & Awuchi, C. G. (2023). Pharmacological, nutraceutical, functional and therapeutic properties of fennel (*Foeniculum vulgare*). *International Journal of Food Properties, 26*(1), 915–927.

Noreen, S., Tufail, T., Ain, H. B., Ali, A., Aadil, R. M., Nemat, A., & Manzoor, M. F. (2023). Antioxidant activity and phytochemical analysis of fennel seeds and flaxseed. *Food Science & Nutrition, 11*(3), 1309–1317.

Noreen, S., Tufail, T., Ain, H., Khalid, W., Hanif, A., Ali, B., … Abeed, A. H. (2024). Assessment of antioxidant activities of flaxseed (*Linum usitatissimum* L.) and fennel seed (*Foeniculum vulgare* Mill.) extracts. *Polish Journal of Environmental Studies, 33*(3), 3359.

Nurcholis, W., Putri, D. N. S. B., Husnawati, H., Aisyah, S. I., & Priosoeryanto, B. P. (2021). Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. *Annals of Agricultural Sciences, 66*(1), 58–62.

Obumnaeme, N., Nnodim, J., & Edward, U. (2021). Relevance of inflammatory mediators in medical laboratory diagnosis. *International Journal of Chemical and Life Sciences, 10*(11), 1–21.

Oh, W. Y., Ambigaipalan, P., & Shahidi, F. (2021). Quercetin and its ester derivatives inhibit oxidation of food, LDL and DNA. *Food Chemistry, 364*, 130394.

Omoboyowa, D. A., Singh, G., Fatoki, J. O., & Oyeneyin, O. E. (2023). Computational investigation of phytochemicals from *Abrus precatorius* seeds as modulators of peroxisome proliferator-activated receptor gamma (PPARγ). *Journal of Biomolecular Structure and Dynamics, 41*(12), 5568–5582.

Orhan, I., & Üstün, O. (2011). Determination of total phenol content, antioxidant activity and acetylcholinesterase inhibition in selected mushrooms from Turkey. *Journal of Food Composition and Analysis, 24*(3), 386–390.

Othman, A., Ismail, A., Ghani, N. A., & Adenan, I. (2007). Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry, 100*(4), 1523–1530.

Ozen, T., Darcan, C., Aktop, O., & Turkekul, I. (2011). Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey. *Combinatorial Chemistry & High Throughput Screening, 14*(2), 72–84.

Pacifico, S., Galasso, S., Piccolella, S., Kretschmer, N., Pan, S. P., Nocera, P., … Monaco, P. (2018). Winter wild fennel leaves as a source of anti-inflammatory and antioxidant polyphenols. *Arabian Journal of Chemistry, 11*(4), 513–524.

Pagliari, S., Forcella, M., Lonati, E., Sacco, G., Romaniello, F., Rovellini, P., … Bruni, I. (2023). Antioxidant and anti-inflammatory effect of cinnamon (*Cinnamomum verum* J. Presl) bark extract after in vitro digestion simulation. *Foods, 12*(3), 452.

Palukuri, Y. K., & Subramaneyaan, M. (2022). Inhibitory effect of *Foeniculum vulgare* leaf extract on alpha-glucosidase and alpha-amylase activity. *Acta Scientific Medical Sciences, 6*(1), 26-31.

Panda, M. K., Das, S. K., Mohapatra, S., Debata, P. R., Tayung, K., & Thatoi, H. (2021). Mycochemical composition, bioactivities, and phylogenetic placement of three wild edible *Russula* species from Northern Odisha, India. *Plant Biosystems, 155*(5), 1041–1055.

Pentu, N., Azhakesan, A., & Kumar, P. K. (2025). In silico molecular docking and ADME/T studies of flavonol compounds against selected proteins involved in inflammation mechanism. *Journal of Applied Pharmaceutical Research, 13*(1), 95–111.

Pereira, S. C., Oliveira, P. F., Oliveira, S. R., Pereira, M. D. L., & Alves, M. G. (2021). Impact of environmental and lifestyle use of chromium on male fertility: Focus on antioxidant activity and oxidative stress. *Antioxidants, 10*(9), 1365.

Petraglia, T., Latronico, T., Fanigliulo, A., Crescenzi, A., Liuzzi, G. M., & Rossano, R. (2023). Antioxidant activity of polysaccharides from the edible mushroom *Pleurotus eryngii*. *Molecules, 28*(5), 2176.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry, 30*(1), 11–26.Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of Zanthoxylum armatum DC. *The Scientific World Journal*, *2020*(1), 8780704.

Pifferi, C., Fuentes, R., & Fernández-Tejada, A. (2021). Natural and synthetic carbohydrate-based vaccine adjuvants and their mechanisms of action. *Nature Reviews Chemistry, 5*(3), 197–216.

Ponnampalam, E. N., Kiani, A., Santhiravel, S., Holman, B. W., Lauridsen, C., & Dunshea, F. R. (2022). The importance of dietary antioxidants on oxidative stress, meat and milk production, and their preservative aspects in farm animals. *Animals, 12*(23), 3279.

Prabu, M., & Kumuthakalavalli, R. (2014). In vitro and in vivo anti-inflammatory activity of methanolic extract of *Calocybe indica*. *World Journal of Pharmaceutical Sciences, 3,* 776–783.

Pragasam, S. J., Venkatesan, V., & Rasool, M. (2013). Immunomodulatory and anti-inflammatory effect of p-coumaric acid in experimental inflammation in rats. *Inflammation, 36*(1), 169–176.

Puri, L., Hu, Y., & Naterer, G. (2024). Critical review of ash content and composition in biomass pyrolysis. *Frontiers in Fuels, 2,* 1378361.

Putra, N. R., Fajriah, S., Qomariyah, L., Dewi, A. S., Rizkiyah, D. N., Irianto, I., … Arya, N. N. (2024). Emerging extraction methods and health applications of *Ulva lactuca*. *South African Journal of Chemical Engineering, 47*(1), 233–245.

Raharjo, D., & Haryoto. (2019). Antioxidant activity of mangrove *Sonneratia caseolaris* using FRAP method. In *Proceedings of ISETH (International Summit on Science, Technology, and Humanity)* (pp. 623–629).

Raman, J., Jang, K. Y., Oh, Y. L., Oh, M., Im, J. H., Lakshmanan, H., & Sabaratnam, V. (2021). Cultivation and nutritional value of *Pleurotus* spp.: An overview. *Mycobiology, 49*(1), 1–14.

Randhawa, K., & Shri, R. (2018). Comparison of antioxidant and anticholinesterase activities of selected *Pleurotus* species (Agaricomycetes) from India. *International Journal of Medicinal Mushrooms*, *20*(8), 739–748.

Rao, Y., & Xiang, B. (2009). Determination of ash in Chinese herbal medicine by NIR spectroscopy. *Yakugaku Zasshi, 129*(7), 881–886.

Razliqi, R. N., Ahangarpour, A., Mard, S. A., & Khorsandi, L. (2023). Gentisic acid protects against diabetic nephropathy via Nrf2 pathway. *Chemico-Biological Interactions, 380,* 110507.

Rebey, I. B., Wannes, W. A., Kaab, S. B., Bourgou, S., Tounsi, M. S., Ksouri, R., & Fauconnier, M. L. (2019). Bioactive compounds in *Pimpinella anisum*. *Scientia Horticulturae, 246,* 453–461.

Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Nutritional value of cultivated mushrooms. *Food and Chemical Toxicology, 50*(2), 191–197.

Rekowski, A., Langenkämper, G., Dier, M., Wimmer, M. A., Scherf, K. A., & Zörb, C. (2021). Bradford assay for wheat proteins. *Cereal Chemistry, 98*(5), 1059–1065.

Rodríguez De Luna, S. L., Ramírez-Garza, R. E., & Serna Saldívar, S. O. (2020). Green extraction of flavonoids. *The Scientific World Journal, 2020*, 6792069.

Romojaro, A., Botella, M. Á., Obón, C., & Pretel, M. T. (2013). Wild edible plants as functional foods. *International Journal of Food Sciences and Nutrition, 64*(8), 944–952.

Rondeau, J. M., Ramage, P., Zurini, M., & Gram, H. (2015). Mechanism of canakinumab (IL-1β antibody). *mAbs, 7*(6), 1151–1160.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., … Islam, M. R. (2022). Flavonoids and therapeutic applications. *BioMed Research International, 2022,* 5445291.

Rugolo, M., Spréa, R. M., Dias, M. I., Pires, T. C., Añibarro-Ortega, M., Barroetaveña, C., … Barros, L. (2022). Wild edible mushrooms of Patagonia. *Foods, 11*(21), 3516.

Ryu, H. S., Kim, S. H., Jeon, M. H., & Choi, H. Y. (2018). Antioxidant and anticancer effects of mushroom extracts. *The Korean Journal of Food and Nutrition, 31*(6), 911–918.

Saber, J. I., & Eshra, D. H. (2019). Fennel seeds as preservative and functional food. *Alexandria Science Exchange Journal, 40,* 406–414.

Sadowska-Bartosz, I., & Bartosz, G. (2022). Antioxidant capacity evaluation methods. *Processes, 10*(10), 2031.

Saleem, A., Akhtar, M. F., Sharif, A., Akhtar, B., Siddique, R., Ashraf, G. M., … Alharthy, S. A. (2022). Phenolic acids therapeutic potential. *Molecules, 27*(21), 7286.

Saleem, A., Saleem, M., & Akhtar, M. F. (2020). *Moringa oleifera* pharmacological properties. *South African Journal of Botany, 128*, 246–256.

Santoro, V., Rosa, E., Donadio, G., Polito, F., De Feo, V., & De Tommasi, N. (2024). Valorization of fennel waste. *Scientific Reports, 14*(1), 31287.

Santoso, M., Ong, L. L., Aijijiyah, N. P., Wati, F. A., Azminah, A., Annuur, R. M., … Judeh, Z. M. (2022). Synthesis, α-glucosidase inhibition, α-amylase inhibition, and molecular docking studies of 3,3-di(indolyl)indolin-2-ones. *Heliyon, 8*(3), e09045.

Saquib, S. A., AlQahtani, N. A., Ahmad, I., Arora, S., Asif, S. M., Javali, M. A., & Nisar, N. (2021). Herbal synergy with antibiotics. *Journal of Infection in Developing Countries, 15*(11), 1685–1693.

Sayah, K., El Omari, N., Kharbach, M., Bouyahya, A., Kamal, R., Marmouzi, I., … Faouzi, M. E. A. (2020). Fennel extracts chemical profile and bioactivity. *Advances in Pharmacological and Pharmaceutical Sciences, 2020*, 8852570.

Senapati, M. R., & Behera, P. C. (2023). Novel extraction conditions for phytochemicals. In *Recent Frontiers of Phytochemicals* (pp. 27-61). Elsevier.

Shahat, A. A., Ibrahim, A. Y., Hendawy, S. F., Omer, E. A., Hammouda, F. M., Abdel-Rahman, F. H., & Saleh, M. A. (2011). Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. *Molecules, 16*(2), 1366-1377.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods*, *18*, 757-781.

Shalihah, A., Christianty, F. M., & Fajrin, F. A. (2021). Anti inflammatory Activity of the Ethanol Extract of Cinnamon (Cinnamomum burmannii) Bark using Membrane Stabilization Method and Protein Denaturation. *Indonesian Journal of Pharmaceutical Science and Technology*, *1*, 9-14.

Sharif, S., Mustafa, G., Munir, H., Weaver, C. M., Jamil, Y., & Shahid, M. (2016). Proximate composition and micronutrient mineral profile of wild Ganoderma lucidum and four commercial exotic mushrooms by ICP-OES and LIBS. *J. Food Nutr. Res*, *4*(11), 703-708.

Sharma, A., Tirpude, N. V., Kumari, M., & Padwad, Y. (2021). Rutin prevents inflammation-associated colon damage via inhibiting the p38/MAPKAPK2 and PI3K/Akt/GSK3β/NF-κB signalling axes and enhancing splenic Tregs in DSS-induced murine chronic colitis. *Food & function*, *12*(18), 8492-8506.

Shcherbik, N., & Pestov, D. G. (2019). The impact of oxidative stress on ribosomes: From injury to regulation. *Cells*, *8*(11), 1379.

Shi, L., Zhao, W., Yang, Z., Subbiah, V., & Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. *Environmental Science and Pollution Research*, *29*(54), 81112-81129.

Shibko, S., Koivistoinen, P., Tratnyek, C. A., Newhall, A. R., & Friedman, L. (1967). A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid, and glycogen from a single rat liver homogenate or from a subcellular fraction. *Analytical Biochemistry*, *19*(3), 514–528.

Shraim, A. M., Ahmed, T. A., Rahman, M. M., & Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT, 150*, 111932.

Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., ... & Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature reviews Molecular cell biology*, *23*(7), 499-515.

Silva, H., & Lopes, N. M. F. (2020). Cardiovascular effects of caffeic acid and its derivatives: a comprehensive review. *Frontiers in physiology*, *11*, 595516.

Singh, P., Vishwakarma, S. P., & Singh, R. L. (2013). Evaluation of antioxidant, oxidative DNA damage protective and antimicrobial activities of Foeniculum vulgare plant. *J Med Plant Res*, *7*(35), 2551-2563.

Singh, R., Kaur, N., Shri, R., Singh, A. P., & Dhingra, G. S. (2020). Proximate composition and element contents of selected species of Ganoderma with reference to dietary intakes. *Environmental monitoring and assessment*, *192*(5), 270.

Singhal, S. S., Singh, S. P., Singhal, P., Horne, D., Singhal, J., & Awasthi, S. (2015). Antioxidant role of glutathione S-transferases: 4-Hydroxynonenal, a key molecule in stress-mediated signaling. *Toxicology and applied pharmacology*, *289*(3), 361-370.

Sirimanne, M. A., & Liyanage, G. S. (2024). Potential utilization of paddy straw and coconut coir for the cultivation of Pleurotus eous: effects on nutritional composition and antioxidant capacity. *Brazilian Journal of Development*, *10*(11), e74476-e74476.

Sknepnek, A., Miletić, D., Stupar, A., Salević-Jelić, A., Nedović, V., & Cvetanovic Kljakic, A. (2025). Natural Solutions for Diabetes: The Therapeutic Potential of Plants and Mushrooms. Frontiers in Nutrition, 12, 1511049.

Skoryk, O. D., & Horila, M. V. (2023). Oxidative stress and disruption of the antioxidant defense system as triggers of diseases. *Regulatory Mechanisms in Biosystems*, *14*(4), 665-672.

Sova, M. (2012). Antioxidant and antimicrobial activities of cinnamic acid derivatives. *Mini reviews in medicinal chemistry*, *12*(8), 749-767.

Srinivas, U. S., Tan, B. W., Vellayappan, B. A., & Jeyasekharan, A. D. (2019). ROS and the DNA damage response in cancer. *Redox biology*, *25*, 101084.

Stastny, J., Marsik, P., Tauchen, J., Bozik, M., Mascellani, A., Havlik, J., ... & Kloucek, P. (2022). Antioxidant and anti-inflammatory activity of five medicinal mushrooms of the genus Pleurotus. *Antioxidants*, *11*(8), 1569.

Stojkovic, D., Smiljkovic, M., Ciric, A., Glamoclija, J., Van Griensven, L., Ferreira, I. C., & Sokovic, M. (2019). An insight into antidiabetic properties of six medicinal and edible mushrooms: Inhibition of α-amylase and α-glucosidase linked to type-2 diabetes. *South African Journal of Botany*, *120*, 100-103.

Subramanian, S. P., & Prasath, G. S. (2014). Antidiabetic and antidyslipidemic nature of trigonelline, a major alkaloid of fenugreek seeds studied in high-fat-fed and low-dose streptozotocin-induced experimental diabetic rats. *Biomedicine & Preventive Nutrition*, *4*(4), 475-480.

Sud, M., Fahy, E., Cotter, D., Brown, A., Dennis, E. A., Glass, C. K., … Subramaniam, S. (2007). LIPID MAPS structure database (LMSD). *Nucleic Acids Research, 35*(suppl_1), D527–D532.

Sulaiman, M., Ebehairy, L., Nissapatorn, V., Rahmatullah, M., Villegas, J., Dupa, H. J., ... & Wiart, C. (2024). Antibacterial phenolic compounds from the flowering plants of Asia and the Pacific: coming to the light. *Pharmaceutical Biology*, *62*(1), 713-766.

Suleiman, W. B., Shehata, R. M., & Younis, A. M. (2022). In vitro assessment of multipotential therapeutic importance of Hericium erinaceus mushroom extracts using different solvents. *Bioresources and Bioprocessing*, *9*(1), 99.

Surana, A. R., Kumbhare, M. R., Gunjal, A. R., Goswami, S. S., & Ghuge, D. M. (2022). Chemical characterization, thrombolytic and antioxidant activity of *Hibiscus tiliaceus* L. leaves. *Natural Product Research, 36*(23), 6106–6110.

Tajini, F., Bouali, Y., & Ouerghi, A. (2020). Etude de la qualité nutritionnelle de fruit de Phœnix dactylifera L.: mesure des paramètres biochimiques. *Revue Nature et Technologie, 12*(2), 39-49.

Tamboli, E., Bhatnagar, A., & Mishra, A. (2018). Alpha-amylase inhibitors from mycelium of an oyster mushroom. *Preparative Biochemistry and Biotechnology, 48*(8), 693-699.

Telci, I., Demirtas, I., & Sahin, A. (2009). Variation in plant properties and essential oil composition of sweet fennel (*Foeniculum vulgare* Mill.) fruits during stages of maturity. *Industrial Crops and Products, 30*(1), 126–130.

Teniou, S., Bensegueni, A., Hybertson, B. M., Gao, B., Bose, S. K., McCord, J. M., & Hininger-Favier, I. (2022). Biodriven investigation of the wild edible mushroom *Pleurotus eryngii* revealing unique properties as functional food. *Journal of Functional Foods, 89,* 104965.

Torres-Martínez, B. D. M., Vargas-Sánchez, R. D., Torrescano-Urrutia, G. R., Esqueda, M., Rodríguez-Carpena, J. G., Fernández-López, J., & Sánchez-Escalante, A. (2022). *Pleurotus* genus as a potential ingredient for meat products. *Foods, 11*(6), 779.

Toumi, M. E., Rebai, R., Kebaili, F. F., Meriane, I., Achouri, Z. A., Lahneche, M. A., & Necib, Y. (2025). Molecular, phylogenetic, and chemical characterization and in vitro evaluation of the antioxidant and cytotoxicity potential of *Cyclocybe cylindracea* strain TMES42 (Agaricomycetes) from Algeria. *International Journal of Medicinal Mushrooms, 27*(10), 43-59.

Touny, A. A., Venkataraman, B., Almarzooqi, S., Patil, R. B., Bhongade, B. A., Harjaček, M., & Subramanya, S. B. (2025). Molecular docking, dynamics, and preclinical studies reveal morin hydrate as a potent PPARγ and Nrf2 agonist that mitigates colon inflammation. *Pharmacology Research & Perspectives, 13*(4), e70132.

Triwardhani, A., Nugraha, A. P., Hadianto, L., Yudianto, D. O., Kumala, D., Rosari, F. S., & Joestandari, F. (2023). Molecular docking of marumoside, rutin, and quercetin in *Moringa oleifera* to bone remodeling biomarkers: An in silico study. *Journal of International Dental and Medical Research, 16*(3), 995–1003.

Tumilaar, S. G., Hardianto, A., Dohi, H., & Kurnia, D. (2024). A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *Journal of Chemistry, 2024,* 5594386.

Twinomuhwezi, H., Awuchi, C. G., & Rachael, M. (2020). Comparative study of the proximate composition and functional properties of composite flours of amaranth, rice, millet, and soybean. *American Journal of Food Science and Nutrition, 6*(1), 6–19.

Udeh, A. S., Ezebialu, C. U., Eze, E. A., & Engwa, G. A. (2021). Antibacterial and antioxidant activity of different extracts of some wild medicinal mushrooms from Nigeria. *International Journal of Medicinal Mushrooms, 23*(10), 83-95.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms, 9*(10), 2041.

Veljović, S., Veljović, M., Nikićević, N., Despotović, S., Radulović, S., Nikšić, M., & Filipović, L. (2017). Chemical composition, antiproliferative and antioxidant activity of differently processed *Ganoderma lucidum* ethanol extracts. *Journal of Food Science and Technology, 54*(5), 1312–1320.

Vella, F. M., Pignone, D., & Laratta, B. (2024). The Mediterranean species *Calendula officinalis* and *Foeniculum vulgare* as valuable source of bioactive compounds. *Molecules, 29*(15), 3594.

Venmathi Maran, B. A., Iqbal, M., Gangadaran, P., Ahn, B. C., Rao, P. V., & Shah, M. D. (2022). Hepatoprotective potential of Malaysian medicinal plants: A review on phytochemicals, oxidative stress, and antioxidant mechanisms. *Molecules, 27*(5), 1533.

Vitale, S., Colanero, S., Placidi, M., Di Emidio, G., Tatone, C., Amicarelli, F., & D'Alessandro, A. M. (2022). Phytochemistry and biological activity of medicinal plants in wound healing: An overview of current research. *Molecules, 27*(11), 3566.

Vona, R., Pallotta, L., Cappelletti, M., Severi, C., & Matarrese, P. (2021). The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. *Antioxidants, 10*(2), 201.

Wabaidur, S. M., Obbed, M. S., Alothman, Z. A., Alfaris, N. A., Badjah-Hadj-Ahmed, A. Y., Siddiqui, M. R., & Aldayel, T. S. (2020). Total phenolic acids and flavonoid contents determination in Yemeni honey of various floral sources: Folin–Ciocalteu and spectrophotometric approach. *Food Science and Technology, 40*, 647–652.

Wan, F., Zhong, R., Wang, M., Zhou, Y., Chen, Y., Yi, B., & Zhang, H. (2021). Caffeic acid supplement alleviates colonic inflammation and oxidative stress potentially through improved gut microbiota community in mice. *Frontiers in Microbiology, 12,* 784211.

Wang, D., Zhang, M., Wan, J., Liu, H., Wang, Y., Yang, R., & Zhao, Y. (2024). Enhancing digestibility and intestinal peptide release of *Pleurotus eryngii* protein: An enzymatic approach. *Journal of Fungi, 10*(12), 890.

Wang, J., An, W., Wang, Z., Zhao, Y., Han, B., Tao, H., & Wang, X. (2024). Vanillin has potent antibacterial, antioxidant, and anti-inflammatory activities in vitro and in mouse colitis induced by multidrug-resistant *Escherichia coli*. *Antioxidants, 13*(12), 1544.

Wany, A., Pandey, P., & Raja, W. (2022). Evaluation of phytochemical screening and antibacterial activity of *Foeniculum vulgare* seed extract using disk diffusion method. *Advances in Biopharmaceutical and Pharmacological Research, 4*, 7–12.

Wu, D., Jia, X., Zheng, X., Mo, Y., Teng, J., Huang, L., & Xia, N. (2023). Physicochemical and functional properties of *Pleurotus eryngii* proteins with different molecular weight. *LWT, 184*, 115102.

Yalcin, O. U., Sarikurkcu, C., Cengiz, M., Gungor, H., & Ćavar Zeljković, S. (2020). *Ganoderma carnosum* and *Ganoderma pfeifferi*: Metal concentration, phenolic content, and biological activity. *Mycologia, 112*(1), 1–8.

Yan, J., Zhu, L., Qu, Y., Qu, X., Mu, M., Zhang, M., & Sun, L. (2019). Analyses of active antioxidant polysaccharides from four edible mushrooms. *International Journal of Biological Macromolecules, 123,* 945–956.

Yang, R. L., Li, Q., & Hu, Q. P. (2020). Physicochemical properties, microstructures, nutritional components, and free amino acids of *Pleurotus eryngii* as affected by different drying methods. *Scientific Reports, 10*(1), 121.

Yildirim, N. C., Turkoglu, S., Yildirim, N. U., & Kaplan Ince, O. (2012). Antioxidant properties of wild edible mushroom *Pleurotus eryngii* collected from Tunceli province of Turkey. *Digest Journal of Nanomaterials and Biostructures, 7*(4), 1647-1654.

Ysrafil, Y., Sapiun, Z., Slamet, N. S., Mohamad, F., Hartati, H., Damiti, S. A., & Nainu, F. (2023). Anti-inflammatory activities of flavonoid derivatives. *ADMET and DMPK, 11*(3), 331–359.

Yu, E. J., Han, S. R., Kim, K. H., Park, B. R., Lim, K. O., & Oh, T. J. (2018). Antibacterial and antioxidant activity of *Pleurotus eryngii* extracts. *Indian Journal of Public Health Research & Development, 9*(12), 2298–2305.

Yuan, G., Guan, Y., Yi, H., Lai, S., Sun, Y., & Cao, S. (2021). Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. *Scientific Reports, 11*(1), 10471.

Yue, F., Zhang, J., Xu, J., Niu, T., Lü, X., & Liu, M. (2022). Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol–sulfuric acid method. *Frontiers in Nutrition, 9*, 963318.Zambari, I. F., Muhamad, N. A., & Hafid, S. R. A. (2021). A comparison of Bradford, Lowry, and UV-280 methods to determine the protein content from green and red Christia vespertilionid leaves. *International Journal of Studies, 6*(5), 508-510.

Zanini, L., Zaltron, A., Turato, E., Zamboni, R., & Sada, C. (2022). Opto-microfluidic integration of the Bradford protein assay in lithium niobate lab-on-a-chip. *Sensors, 22*(3), 1144.

Zeeshan, A., Akram, H., Qasim, S., Naseer, A., Nazar, F., & Rafique, O. (2023). The healing touch of *Foeniculum vulgare* Mill. (fennel): A review on its medicinal value and health benefits. *Journal of Health and Rehabilitation Research, 3*(2), 793–800.

Zervakis, G. I., Ntougias, S., Gargano, M. L., Besi, M. I., Polemis, E., Typas, M. A., & Venturella, G. (2014). A reappraisal of the *Pleurotus eryngii* complex: New species and taxonomic combinations based on a polyphasic approach, and an identification key to *Pleurotus* taxa associated with Apiaceae plants. *Fungal Biology, 118*(9–10), 814–834.

Zhang, B., Li, Y., Zhang, F., Linhardt, R. J., Zeng, G., & Zhang, A. (2020). Extraction, structure and bioactivities of polysaccharides from *Pleurotus eryngii*: A review. *International Journal of Biological Macromolecules, 150,* 1342–1347.

Zhang, Y., Xun, H., Gao, Q., Qi, F., Sun, J., & Tang, F. (2023). Chemical constituents of the mushroom *Dictyophora indusiata* and their anti-inflammatory activities. *Molecules*, *28*(6), 2760.

Zheng, X., Sun, H., Wu, L., Kong, X., Song, Q., & Zhu, Z. (2020). Structural characterization and inhibition of α-glucosidase by polysaccharides from fruiting bodies and mycelia of *Pleurotus eryngii*. *International Journal of Biological Macromolecules, 156*, 1512–1519.

류혜숙, 김수연, 전문희, & 최해연. (2018). 새송이버섯, 팽이버섯 열수추출물의 항산화 및 항암 활성. 한국식품영양학회지, *31*(6), 911-918.

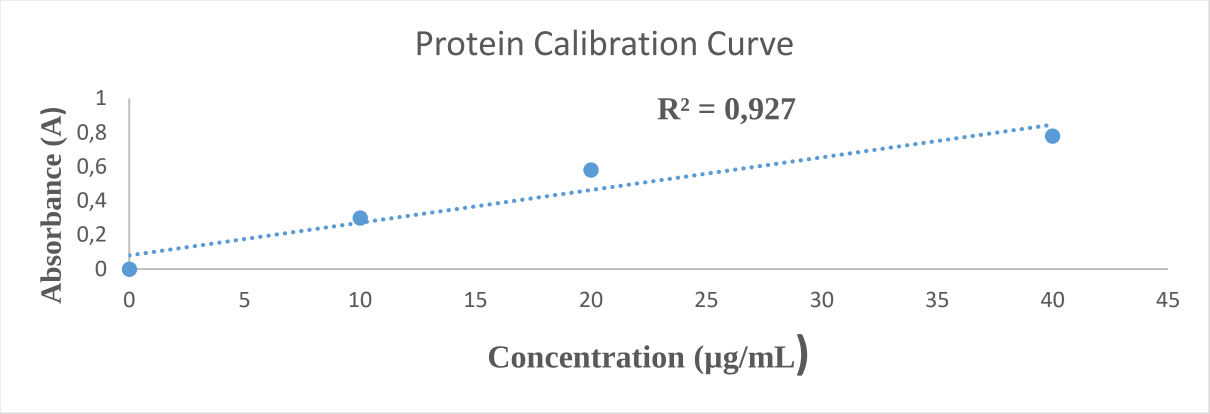
안명수, 김현정, & 서미숙. (2006). 새송이버섯 (*Pleurotus eryngii*) 부위별 추출물의 이화학적 특성. 한국식생활문화학회지, *21*(3), 297-302.

APPENDIX

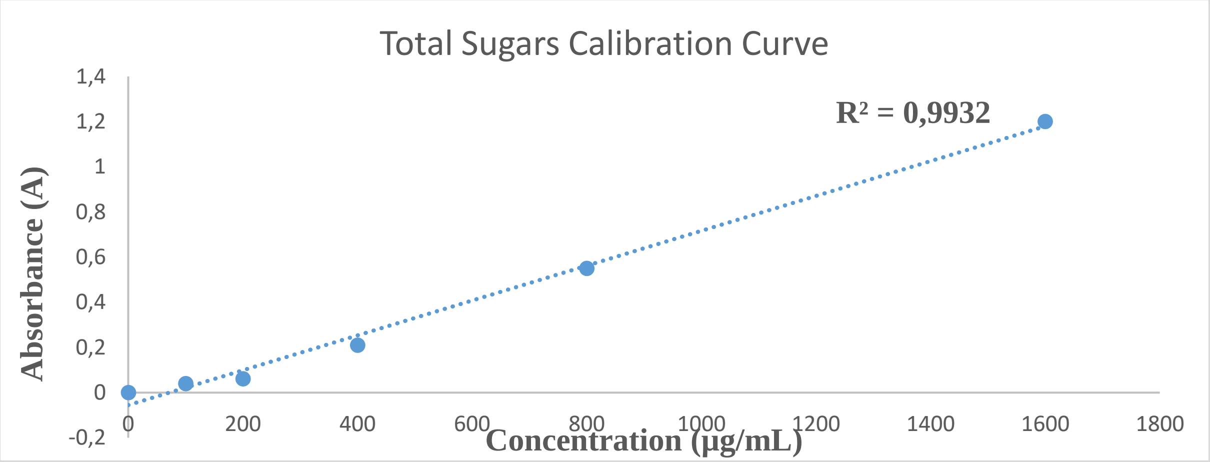
Appendices

Appendix A. Calibration Curves

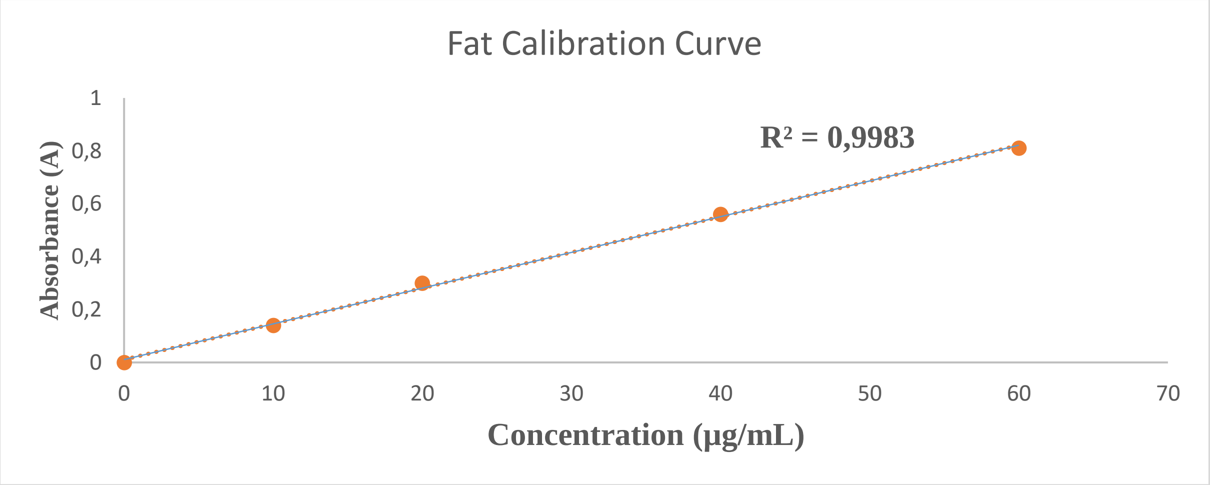
Appendix A1. Calibration curve for protein determination.



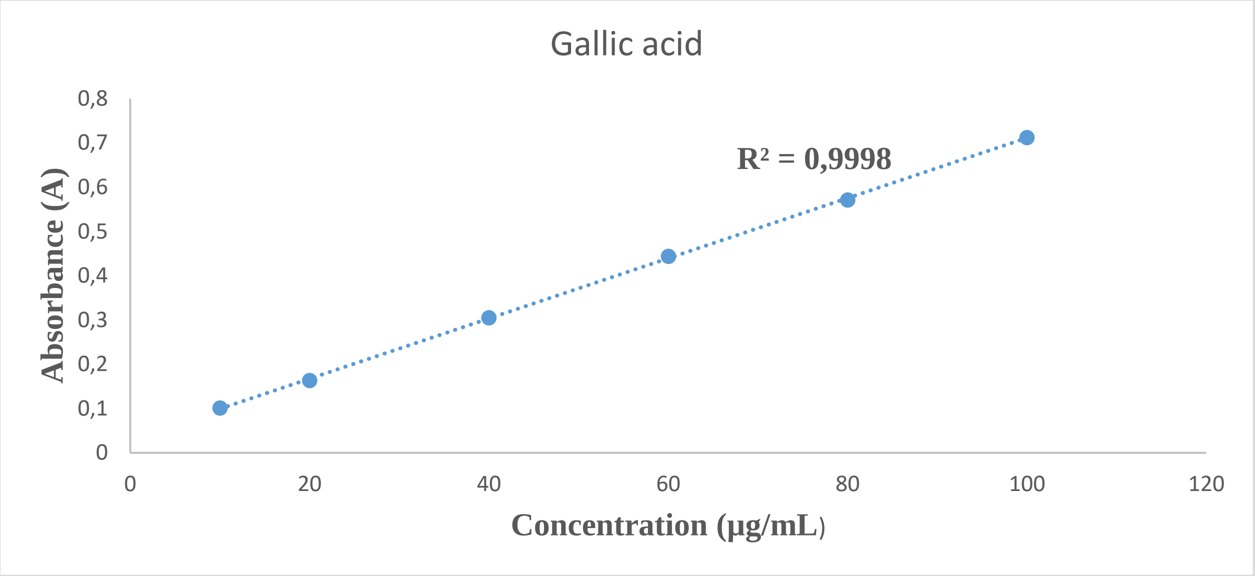
Appendix A2. Calibration curve for total sugars determination.



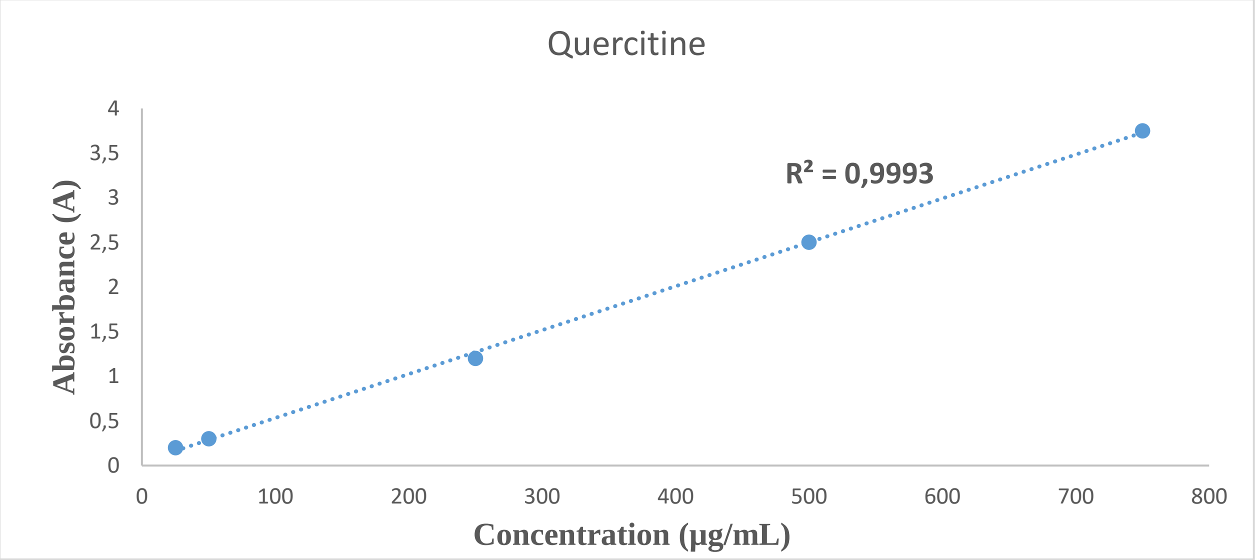
Appendix A3. Calibration curve for lipid determination.



Appendix A4. Calibration curve for total phenolic content (TPC).

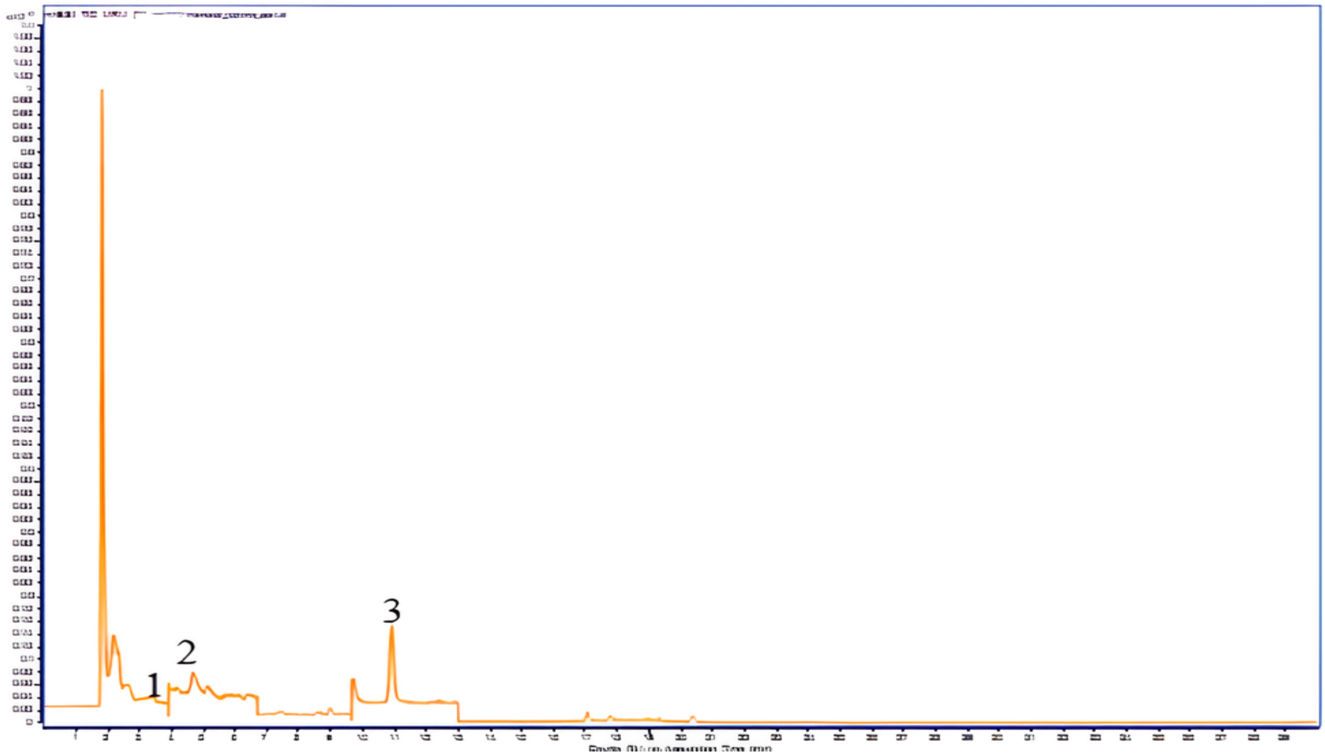


Appendix A5. Calibration curve for total flavonoid content (TFC).

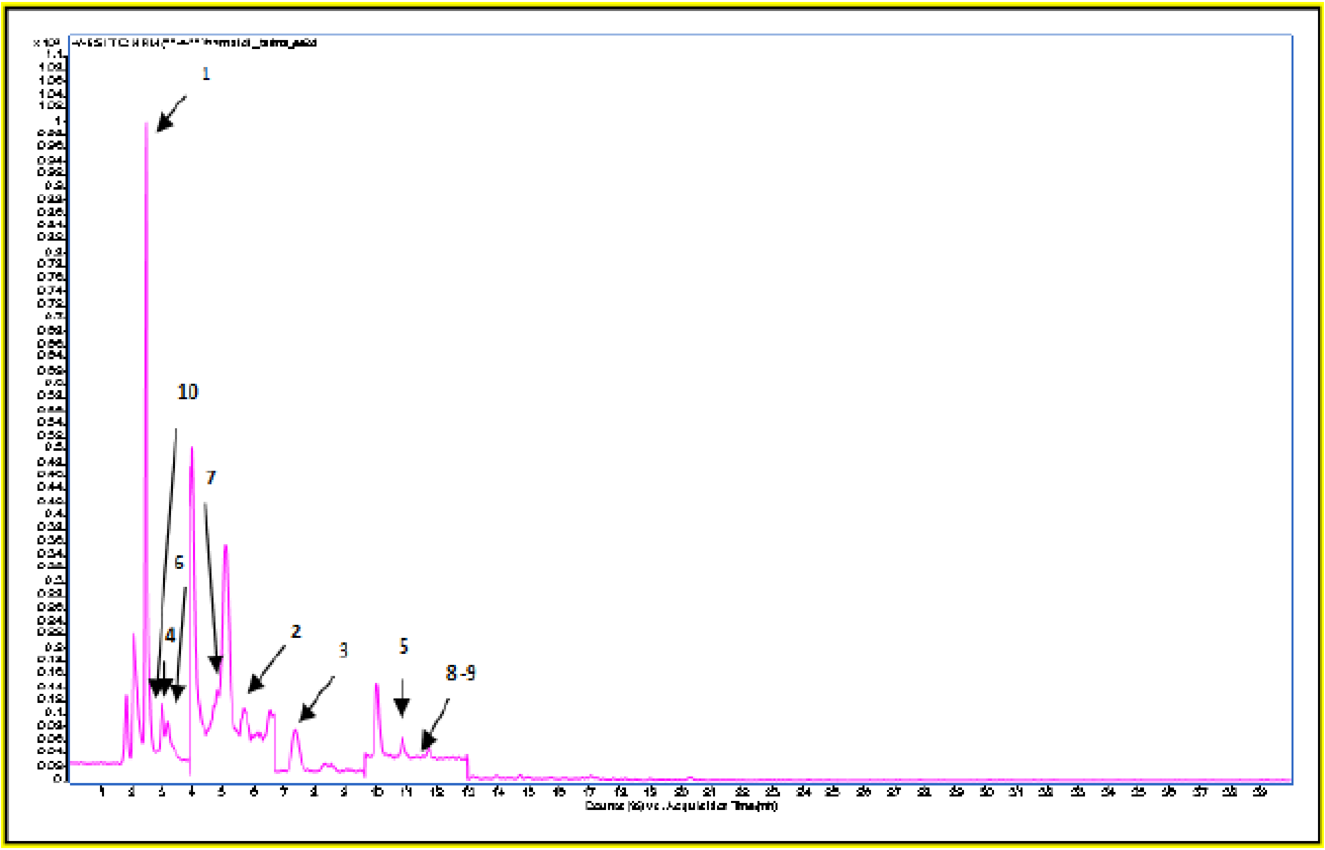


Appendix B. LC–MS/MS Analysis

Appendix B1. LC–MS/MS chromatogram of the ethanolic extract of *P. eryngii*.



Appendix B2. LC–MS/MS chromatogram of the ethanolic extract of *F. vulgare* leaves.



Appendix C. Biological Activities

Appendix C1. Percentage inhibition of antioxidant activity of *P. eryngii* and *F. vulgare* extracts.

Assay	Sample	Extract	Concentrations (µg/mL)					
			31.25 µg/mL	**62.50 µg/mL**	**125.00 µg/mL**	**250.00 µg/mL**	**500.00 µg/mL**	**1000.00 µg/mL**
DPPH scavenging activity	***P. eryngii***	Aqueous	10,63 ± 8,26	17,64 ± 1,27	29,01 ± 3,18	40,85 ± 2,54	49,69 ± 2,41	55,23 ±2,48
		Ethanolic	1,54 ± 1,24	3,01 ± 0,73	6,53 ± 1,60	16,87 ± 1,58	24,94 ± 2,13	39,49 ± 5,58
	***F. vulgare* leaves**	Aqueous	32,53 ± 2.83	35,83 ± 1.27	43,16 ± 0.84	53,25 ± 0.73	58,20 ± 0.63	65.71 ± 0.34
		Ethanolic	35,99 ± 0.55	61,13 ± 1.55	66,64 ± 1.10	71,96 ± 1.10	77,28 ± 0.84	82,61 ± 0,85
	Standard	Ascorbic acid	43,93 ± 0.52	50,13 ± 0.28	59,08 ± 0.72	66,12 ± 0.29	71,32 ± 0.13	88,12 ± 0.69
		BHA	43,16 ± 0.84	51,23 ± 2.27	58,23 ± 2.45	62,96 ± 5.08	67,18 ± 5.88	82,51 ± 8,67
H_2O_2 scavenging activity	***P. eryngii***	Aqueous	33,97 ± 0.62	43,03 ± 0.52	44,59 ± 0.16	51,10 ± 5.35	59,38 ± 1.06	59.38 ± 2.31
		Ethanolic	32,45 ± 0.35	38,41 ± 1.18	41,23 ± 0.82	46,18 ± 0.66	48,56 ± 2.84	53.54 ± 1.2
	***F. vulgare* leaves**	Aqueous	07,05 ± 0.39	40,10 ± 2.27	46,13 ± 0.93	56,05 ± 6.37	62,85 ± 0.20	71.85 ± 1.01
		Ethanolic	12,7 ± 0.54	26,3 ± 1.56	51,36 ± 1.02	63,25 ± 1.31	73,12 ± 1.30	80.96 ± 1.01
	Standard	Ascorbic acid	44,23 ± 0.89	53,46 ± 0.77	56,90 ± 0.81	63,90 ± 0.54	71,44 ± 0.45	90,64 ± 0.22
		BHA	41,50 ± 0.19	50,00 ± 0.27	60,88 ± 0.38	63,73 ± 0.16	79,50 ± 0.23	88,15 ± 0.13
β-carotene bleaching inhibition	***P. eryngii***	Aqueous	1,64 ± 0.49	20,20 ± 3.89	30,12 ± 6.09	37,33 ± 7.49	47,68 ± 6.15	55.23 ± 2.48
		Ethanolic	2,79 ± 0.33	3,28 ± 1.72	7,64 ± 6.68	17,42 ± 1.31	32,26 ± 1.10	39.49 ± 5.58
	***F. vulgare* leaves**	Aqueous	13,42 ± 2.35	18,70 ± 3.35	39,26 ± 1.01	47,75 ± 0.57	59,17 ± 1.61	67.81 ± 1.31
		Ethanolic	29,84 ± 2.88	45,47 ± 2.75	57,24 ± 2.14	64,53 ± 1.18	73,30 ± 3.33	81.66 ± 0.65
	Standard	Ascorbic acid	52,61 ± 0.24	74,45 ± 0.23	96,43 ± 0.12	98,52 ± 0.24	99,15 ± 0.10	99,72 ± 0.11

Values are expressed as mean ± standard deviation (n = 3).

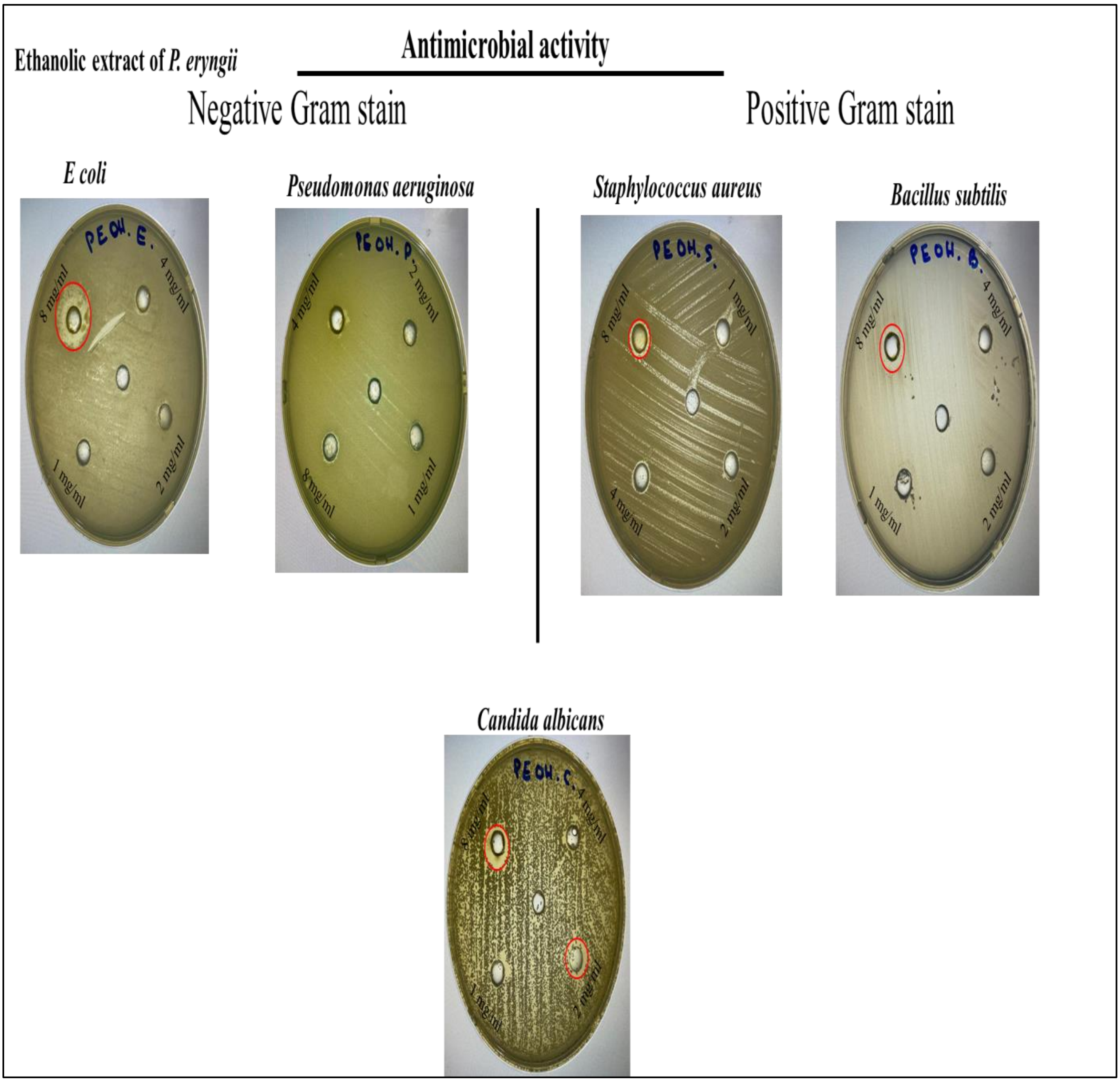
Appendix C2. Percentage inhibition of protein denaturation and α-amylase activity of *P. eryngii* and *F. vulgare* extracts.

Assay	Sample	Extract	Concentrations (µg/mL)					
			50.00 µg/mL	**100.00 µg/mL**	**250.00 µg/mL**	**500.00 µg/mL**	**1000.00 µg/mL**	**2000.00 µg/mL**
Inhibition of albumin denaturation	***P. eryngii***	Aqueous	12,24 ± 1.77	16,81 ± 2.39	37,24 ± 0.89	46,76 ± 0.46	59.59 ± 1.26	67,99 ± 0.89
		Ethanolic	1,03 ± 0.34	8,04 ± 1.62	27,58 ± 0.78	37,32 ± 1.22	49.85 ± 0.89	57,08 ± 1.38
	***F. vulgare* leaves**	Aqueous	11,06 ± 0.22	28,98 ± 0.38	43,88 ± 0.34	61,87 ± 0.34	66,00 ± 0.46	74,19 ± 0.64
		Ethanolic	34,81 ± 1.87	50,53 ± 1.32	62,80 ± 0.89	71.53 ± 1.22	77,88 ± 1.10	80,12 ± 1.02
	Standard	Diclofenac	76,62 ± 0.26	91,15 ± 0.95	95,36 ± 0.77	96,23 ± 0.46	98,74 ± 0.43	99,01 ± 0.38
-	**-**	**-**	**125.00 µg/mL**	**250.00 µg/mL**	**500.00 µg/mL**	**1000.00 µg/mL**	**1500.00 µg/mL**	**2000.00 µg/mL**
α-amylase inhibition	***P. eryngii***	Aqueous	9,83 ± 0.73	14,68 ± 2.58	18,30 ± 2.49	31,59 ± 1.85	42,22 ± 1.86	47.19 ± 3.20
		Ethanolic	12,87 ± 0.95	24,47 ± 0.90	28,17 ± 1.00	34,57 ± 0.95	38,13 ± 0.76	42.43 ± 1.07
	***F. vulgare* leaves**	Aqueous	3,15 ± 1.02	6,58 ± 2.41	16,88 ± 1.75	26,58 ± 3.32	42,52 ± 2.60	47.26 ± 1.86
		Ethanolic	7,54 ± 1.50	17,37 ± 1.15	76,3 ± 1.54	79,22 ± 3.14	80,75 ± 1.63	81.86 ± 1.20
	Standard	Acarbose	67,47 ± 2.29	80,44 ± 3.90	92,34 ± 3.67	95,62 ± 0.49	97,02 ± 1.52	97,81 ± 1.66

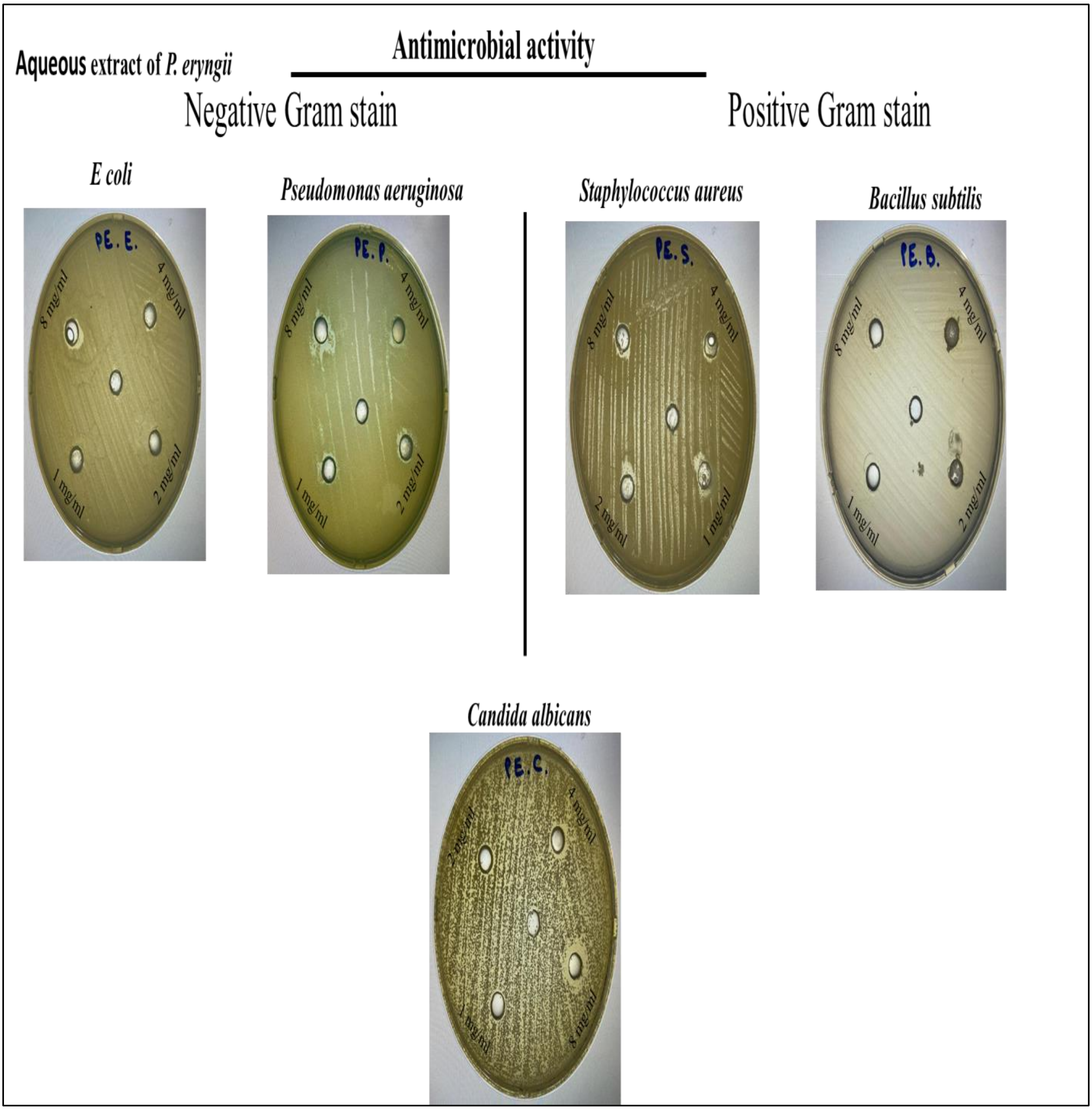
Values are expressed as mean ± standard deviation (n = 3).

Appendix D. Antimicrobial Activity

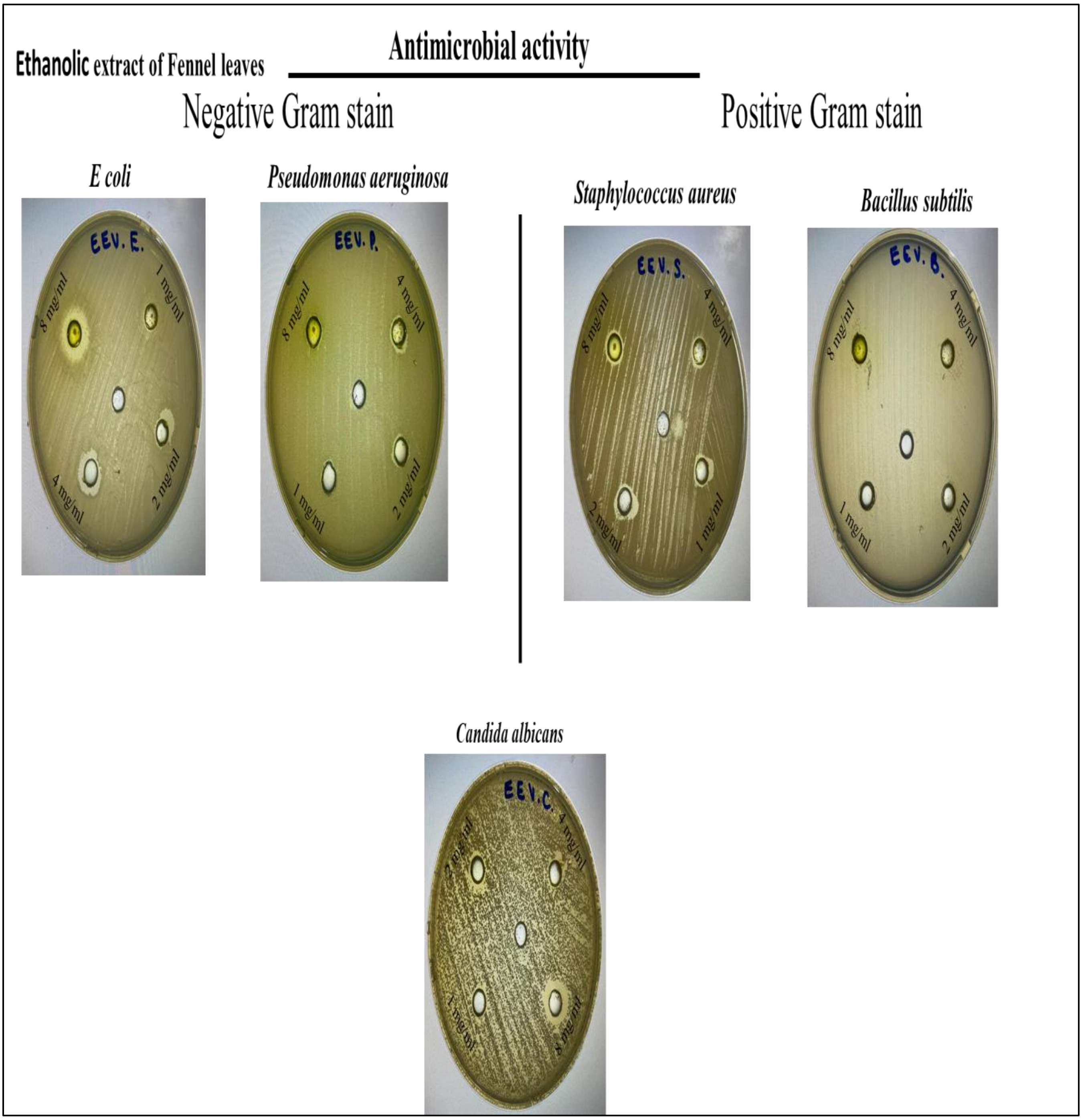
Appendix D1. Antimicrobial activity of the ethanolic extract of *P. eryngii*.



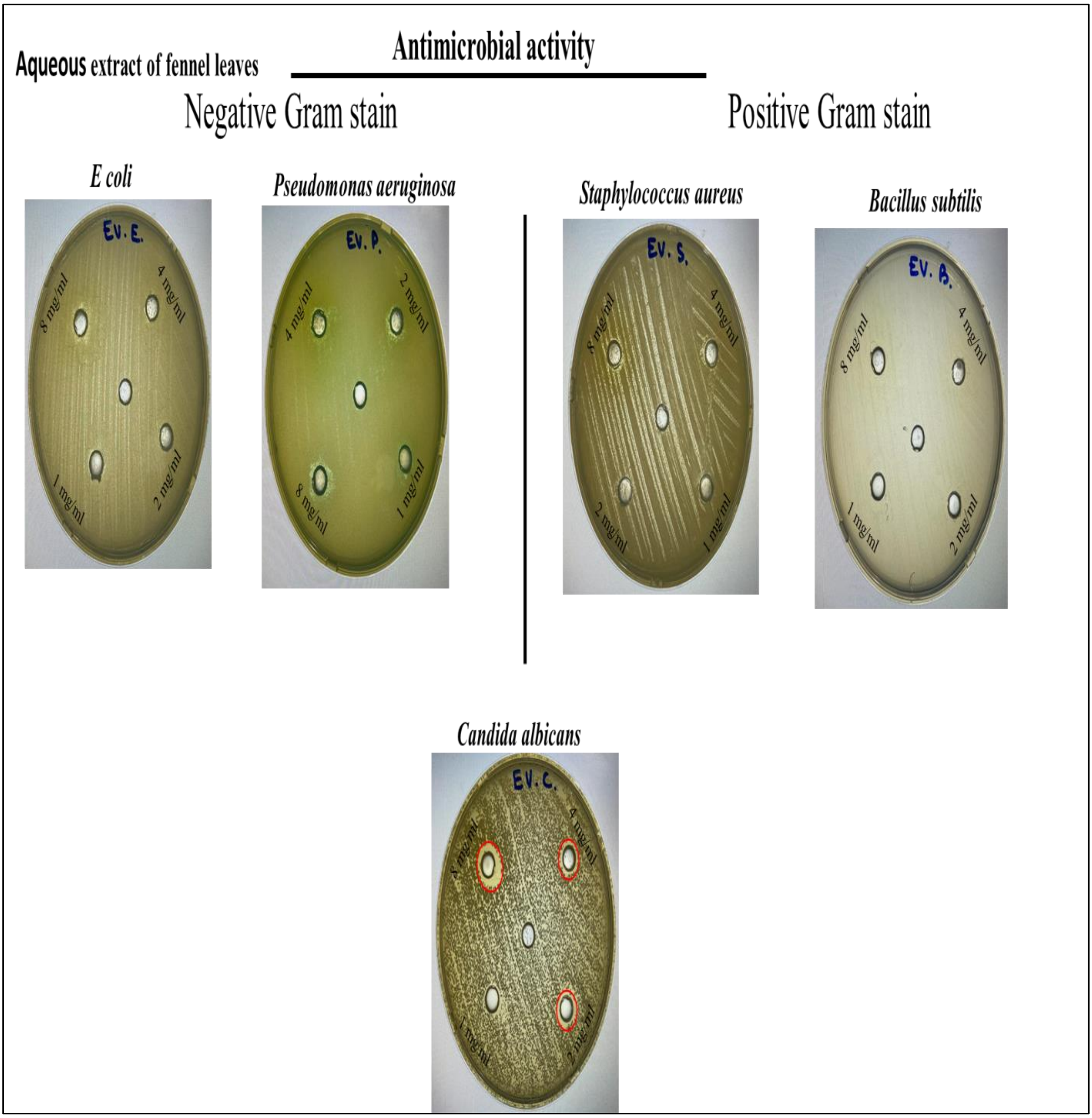
Appendix D2. Antimicrobial activity of the aqueous extract of *P. eryngii*.



Appendix D3. Antimicrobial activity of the ethanolic extract of *F. vulgare* leaves.



Appendix D4. Antimicrobial activity of the aqueous extract of *F. vulgare* leaves



PUBLICATIONS
AND
COMMUNICATIONS

A. Publications

1. **Ali Si-Larbi,** Fatiha Benahmed, Toumi Mohammed Esseddik, Boualem Benamar Aissa, Meriane Ilhem. (2025). In Vitro Antioxidant, Anti-Inflammatory Activities, and Phenolic Compounds Determination of King Oyster *Pleurotus eryngii* (Agaricomycetes) Mushroom from Algeria. International Journal of Medicinal Mushrooms, Volume 28, 2026 (1). DOI: 10.1615/IntJMedMushrooms.2025061860.

B. International communications

1. **Ali Si-Larbi**, Fatiha Benahmed, Chaimaa Khadir. (2025). Nutritional value and antioxidant potential evaluation of aqueous extract of *Agaricus bisporus* mushroom. 1ST International Seminar On Diversity, Valorization & Conservation Of Phytodiversity (1st ISDVCP'25), May 20th-22nd, Chlef, Algeria.
2. **Ali Si-Larbi,** Fatiha Benahmed, (2025). Phytochemical composition, antioxidant properties, and nutritional assessment of the hydroethanolic extract of *Agaricus bisporus*. 15th International mardin artuklu scientific researches conference, October 4-7, Mardin, TÜRKİYE.
3. **Ali Si-Larbi,** Fatiha Benahmed, (2025). Phytochemical Composition and Antioxidant Properties of the Aqueous Extract of *Foeniculum vulgare* Stems. The First International Conference on Chemistry, Material Energy & Environment (CMEE'2025), November 4-6, Batna, Algérie.
4. **Ali Si-Larbi,** Fatiha Benahmed, (2025). Phytochemical Composition and Antioxidant Properties of the Ethanolic Extract of *Pleurotus eryngii*. The Second International Conference on Chemistry Science, Materials Design and their applications ICCSM'2025, November 18-19, Chlef, Algérie.

5. Benahmed, F., Rached, W., **Si-Larbi, A.**, Medjadji B., Mehrab, E., & Kharoubi, O. (2024). Oxidative stress in *Vicia faba* L. seedlings under different abiotic stresses: Salinity, water deficit, and heavy metal stress [Poster presentation]. II. International Biological & Life Sciences Congress (BIOLIC 2024), Antalya, Turkey.

6. Benahmed, F., Rached, W., MEDJADJI B., **Si-Larbi, A.**, & Kharoubi, O. (2024, September 18-20). Therapeutic benefits of medicinal plants for treating colic diseases used by the population of Relizane [Poster presentation]. VI. International Agricultural, Biological & Life Science Conference (AGBIOL 2024), Edirne, Turkey.

7. Benahmed, F., Rached, W., Medjadji B., **SI-LARBI, A.**, & Kharoubi, O. (2024, October 31 - November 2). Mercury-induced acute nephrotoxicity in rats: Treatment with aqueous extract of *Pistacia atlantica* (Desf) [Poster presentation]. II. International Biological & Life Sciences Congress (BIOLIC 2024), Megasaray Westbeach Hotel, Antalya, Turkey.

C. National communications

1. **Si-Larbi Ali,** Benahmed Fatiha, Felouah Noura. (2025). Composition chimique et activité antifongique de l'huile essentielle de *Rosmarinus officinalis* L. Le deuxième Séminaire National de Chimie en ligne SNC'25, Mai 13-14, Batna, Algérie.
2. **Ali Si-Larbi,** Fatiha Benahmed, Hind Ahmed Ben Ali (2025)**.** Évaluation de la Valeur Nutritive et du Potentiel Antioxydant de l'Extrait Aqueux du Champignon *Agaricus campestris*. Le 2ème Symposium National de BIOLOGIE PHARMACOTOXICOLOGIE & BIOPRODUITS DES PAM (Plantes Aromatiques et Médicinales) (2SNBPT-PAM-2025), 28-29 Mai, Blida, Algérie.
3. **Ali Si-Larbi,** Fatiha Benahmed, (2025). Biological Activities of Phenolic Compounds from *Agaricus campestris*: Antioxidant and AntiInflammatory Potential. Le 2éme Séminaire National Hybride sur: Biodiversité, Santé, Environnement: Enjeux du Développement Durable (SNHBSEEDD'2025). Le 21 Octobre 2025, Mila, Algérie.
4. **Ali Si-Larbi,** Fatiha Benahmed, (2025). Eco-friendly Valorization of *Foeniculum vulgare* Stems: Bioactive Compounds and Antioxidant Potential. National Seminar on the Application of Biomaterials in the Environment (SNABE'2025), Le 28-29 Octobre 2025, Adrar, Algérie.