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Phytochemical, biological activities of some plants on common diseases in the Oued Souf region

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Abstract

Phytochemical, biological activities of some plants on common diseases in the Oued Souf region

This study investigated the phytochemical composition and biological activities of *Bunium mauritanicum* tubers and *Brassica oleracea* var. *elongata*, two plants traditionally used in the Oued Souf region of Algeria. The study combined ethnobotanical surveys, in vitro phytochemical and biological assays, and in vivo experiments on rats. Both aqueous and methanolic extracts of *B. mauritanicum* tubers and *B. oleracea* leaves and seeds were employed. Key analytical methods included HPLC for phytochemical profiling, spectrophotometric assays for total phenolic and flavonoid content, antioxidant assays (DPPH, FRAP, β-carotene bleaching), antimicrobial activity tests, anti-inflammatory assays, and in vitro antidiabetic activity tests. In vivo studies utilized animal models of hypothyroidism and anemia, with subsequent biochemical, hematological, and histological analyses.

Phytochemical screening identified various bioactive compounds in both plants, including alkaloids, flavonoids, terpenoids, phenols, tannins, and saponins. HPLC analysis revealed higher concentrations of phenolic compounds in methanolic extracts compared to aqueous extracts for both plants. In *B. mauritanicum*, gallic acid and quercetin were prominent, while *B. oleracea* showed high levels of gallic acid, chlorogenic acid, and rutin. Both plant extracts demonstrated significant antioxidant activities across multiple assays. In the DPPH assay, *B. mauritanicum* aqueous extract (IC_{50} = 0.288 mg/mL) and *B. oleracea* methanolic leaf extract (IC_{50} = 0.058 mg/mL) showed the strongest activities. The FRAP assay revealed *B. mauritanicum* aqueous extract (EC_{50} = 0.0442 mg/mL) and *B. oleracea* methanolic leaf extract (EC_{50} = 0.814 mg/mL) as most effective. In the β-carotene bleaching assay, *B. oleracea* aqueous leaf extract (EC_{50} = 0.049 mg/mL) showed the highest activity.

Antimicrobial activity tests showed that *B. oleracea* extracts demonstrated broader antimicrobial activity compared to *B. mauritanicum*. The methanolic seed extract of *B. oleracea* showed significant inhibition against *Staphylococcus aureus* (18 mm), while *B. mauritanicum* extracts were most effective against *Escherichia coli* (8 mm for aqueous extract). Both plants also showed anti-inflammatory properties through protein denaturation inhibition assays, with *B. oleracea* aqueous leaf extract exhibiting the highest inhibition (94.8%), comparable to the reference drug diclofenac (96.46%).

In-vivo studies further explored the therapeutic potential of these plants. In a hypothyroidism model, treatment with *B. mauritanicum* tuber extract (200 mg/kg) improved thyroid hormone levels, reducing TSH from 2.1 to 1.9 µIU/mL and increasing T4 from 30.23 to32.70 nmol/L. In an anemia model, *B. oleracea* leaf extract treatment (200 mg/kg) improved hematological parameters, increasing RBC count from 6.14 to 6.35 x 10^6 cells/mL and hemoglobin from 8.70 to 14.60 g/dL. Both treatments also showed improvements in liver and kidney function markers and histological profiles, indicating protective effects against organ damage.

This comprehensive study provides scientific support for the traditional uses of *B. mauritanicum* and *B. oleracea var. elongata* in the Oued Souf region. The results suggest that *B. mauritanicum* may have potential in managing hypothyroidism, while *B. oleracea* shows promise for treating anemia. The identification of bioactive compounds and demonstration of various biological activities highlight the potential of these plants as sources of therapeutic agents.

Keywords: Phytochemical study; *Bunium mauritanicum*; *Brassica oleracea* var. *elongate*; Ethnopharmacology; Biological activities; Hypothyroidism; Anemia.

Abstract in Arabic (الملخص)

دراسة فيتوكيميائية والنشاطية البيولوجية لبعض النباتات المستعملة لعلاج الأمراض الشائعة في منطقة واد سوف

تناولت هذه الأطروحة دراسة التركيب الكيميائي النباتي والأنشطة البيولوجية لدرنات *Bunium mauritanicum* وبذور وأوراق *Brassica oleracea* var. *elongata*، وهما نباتان يستخدمان تقليديًا في منطقة وادي سوف بالجزائر. جمعت الدراسة بين المسوحات الإثنوباتية والتحاليل الكيميائية النباتية والبيولوجية في المختبر، والتجارب على الفئران في الجسم الحي. تم استخدام المستخلصات المائية والميثانولية لدرنات*B. mauritanicum* وأوراق وبذور *B. oleracea*. شملت الطرق التحليلية الرئيسية الكروماتوغرافيا السائلة عالية الاداء HPLC ، التحاليل الطيفية لقياس المحتوى الكلي للفينولات والفلافونويدات، اختبارات النشاط المضاد للأكسدة(DPPH, FRAP, β-carotene bleaching) ، اختبارات النشاط المضاد للميكروبات، اختبارمضادات الالتهاب، واختبارات النشاط المضاد لمرض السكري في المختبر. استخدمت الدراسات في الجسم الحي نماذج حيوانية لقصور الغدة الدرقية وفقر الدم، مع إجراء تحاليل كيميائية حيوية، هرمونية ونسيجية.

حدد الكشف الكيميائي النباتي مركبات نشطة بيولوجيًا متنوعة في كلا النباتين، بما في ذلك القلويدات، الفلافونويدات ، التربينات ، الفينولات ، التانينات والصابونينات. كشف تحليل HPLC عن تركيزات عالية من المركبات الفينولية في المستخلصات الميثانولية مقارنة بالمستخلصات المائية لكلا النباتين. ففي*B. mauritanicum* ، كان تركيز حمض الغاليك والكيرسيتين معتبرا، بينما أظهر *B. oleracea* مستويات عالية من حمض الغاليك، حمض الكلوروجينيك والروتين.

أظهرت مستخلصات كلا النباتين أنشطة مضادة للأكسدة معتبرة عبر اختبارات متعددة. ففي اختباركسح الجذر الحر DPPH، أظهر المستخلص المائي*لـ* *B. mauritanicum* (IC_{50} = 0.288 ملغ/مل (والمستخلص الميثانولي لأوراق *B. oleracea* IC_{50} = 0.058 ملغ/مل). كشف اختبار FRAP أن المستخلص المائي*لـ* *B. mauritanicum* (EC_{50} = 0.0442ملغ/مل (والمستخلص الميثانولي لأوراق *B. oleracea* (EC_{50} = 0.814 ملغ/مل هما الأكثر فعالية. في اختبار تبييضβ-carotene ، أظهر المستخلص المائي لأوراق *B. oleracea* (EC_{50} = 0.049 ملغ/مل (أعلى نشاط. أظهرت اختبارات النشاط المضاد للميكروبات أن مستخلصات *B. oleracea* أظهرت نشاطًا مضادًا للميكروبات أوسع نطاقًا مقارنة بـ *B. mauritanicum*. حيث أبدى المستخلص الميثانولي لبذور *B. oleracea* تثبيطًا كبيرًا ضد السلالة *Staphylococcus aureus*(بمنطقة تثبيط 18 مم)، بينما كانت مستخلصات*B. mauritanicum* أكثر فعالية ضد *Escherichia coli*(بمنطقة تثبيط 8 مم للمستخلص المائي). أظهر كلا النباتين أيضًا خصائص مضادة للالتهابات من خلال اختبارات تثبيط تمسخ البروتين، حيث أظهر المستخلص المائي لأوراق *B. oleracea* أعلى تثبيط (94.8٪)، وهي قيمة معتبرة متقاربة مع قيمة الدواء المرجعي ديكلوفيناك96.46) ٪.

أما الدراسات في الجسم الحي فكشفت عن الإمكانات العلاجية للنباتين. في نموذج قصور الغدة الدرقية، أدى العلاج بمستخلص درنات*B. mauritanicum* (200 ملغ/كغ) إلى تحسين مستويات هرمونات الغدة الدرقية، مما أدى إلى خفض TSHمن 2.1 إلى 1.9 ميكرو وحدة دولية/ملل وزيادة T4 من 30.23 إلى 32.70 نانومول/لتر. أما في نموذج فقر الدم، أدى العلاج بمستخلص أوراق *B. oleracea* (200 ملغ/كغ) إلى تحسين المعايير الدموية، مما زاد عدد خلايا الدم الحمراء من 6.14 إلى 6.35 × 10^6 خلية/مل والهيموغلوبين من 8.70 إلى 14.60 غ/ديسيلتر. أظهر كلا العلاجين أيضًا تحسينات في علامات والملامح النسيجية وكذلك في ظائف الكبد والكلى ، مما يشير إلى تأثيرات وقائية معتبرة ضد تلف الأعضاء.

توفر هذه الدراسة الشاملة دعمًا علميًا للاستخدامات التقليدية لـ*B. mauritanicum* و *B. oleracea var. elongata*في منطقة وادي سوف. تشير النتائج إلى أن*B. mauritanicum* قد يكون له إمكانات علاجية لقصور الغدة الدرقية، بينما يُظهر *B. oleracea* نتائج واعدة لعلاج فقر الدم. كما يسلط تحديد المركبات النشطة بيولوجيًا وإثبات الأنشطة البيولوجية المتنوعة الضوء على الإمكانات العلاجية للنباتين.

الكلمات المفتاحية: *Bunium mauritanicum*؛ *Brassica oleracea* var. *elongat* ؛ الطب الشعبي؛ التحليل الكيميائي النباتي؛ الأنشطة البيولوجية؛ قصور الغدة الدرقية؛ فقر الدم؛ منطقة واد سوف.

Abstract in French (Résumé)

Phytochimie et l'activités biologiques de quelque plantes sur les maladies courantes dans la région de l'Oued Souf

Cette étude a examine la composition phytochimique et les activités biologiques des tubercules *de Bunium mauritanicum* et de *Brassica oleracea* var. *elongata*, deux plantes traditionnellement utilisées dans la région d'Oued Souf en Algérie. L'étude a combiné des enquêtes ethnobotaniques, des analyses phytochimiques et biologiques *in vitro*, et des expériences *in vivo* sur les rats. Des extraits aqueux et méthanoliques des tubercules de *B. mauritanicum* et des feuilles et graines de *B. oleracea* ont été utilisés. Les principales méthodes analytiques comprenaient la HPLC pour le profilage phytochimique, des essais spectrophotométriques pour la teneur en composés phénoliques totaux et en flavonoïdes, des tests d'activité antioxydante (DPPH, FRAP, blanchiment du β-carotène), des tests d'activité antimicrobienne, des essais anti-inflammatoires et des tests d'activité antidiabétique in vitro. Les études *in vivo* ont utilisé des modèles animaux d'hypothyroïdie et d'anémie, avec des analyses biochimiques, hématologiques et histologiques subséquentes.

Le criblage phytochimique a identifié divers composés bioactifs dans les deux plantes, notamment des alcaloïdes, flavonoïdes, terpénoïdes, phénols, tanins et saponines. L'analyse HPLC a révélé des concentrations plus élevées de composés phénoliques dans les extraits méthanoliques par rapport aux extraits aqueux pour les deux plantes. Dans *B. mauritanicum*, l'acide gallique et la quercétine étaient prédominants, tandis que *B. oleracea* présentait des niveaux élevés d'acide gallique, d'acide chlorogénique et de rutine.

Les deux végétaux ont démontré des activités antioxydantes significatives. Dans le test DPPH, l'extrait aqueux de *B. mauritanicum* (IC_{50} = 0,288 mg/mL) et l'extrait méthanolique des feuilles de *B. oleracea* (IC_{50} = 0,058 mg/mL) ont montré les activités les plus fortes. Le test FRAP a révélé que l'extrait aqueux de *B. mauritanicum* (EC_{50} = 0,0442 mg/mL) et l'extrait méthanolique des feuilles de *B. oleracea* (EC_{50} = 0,814 mg/mL) étaient les plus efficaces. Dans le test de blanchiment du β-carotène, l'extrait aqueux des feuilles de *B. oleracea* (EC_{50} = 0,049 mg/mL) a montré l'activité la plus élevée. Les tests d'activité antimicrobienne ont montré que les extraits de *B. oleracea* présentaient une activité antimicrobienne plus large que *B. mauritanicum*. L'extrait méthanolique des graines de *B. oleracea* a montré une inhibition significative contre *Staphylococcus aureus* (18 mm), tandis que les extraits de *B. mauritanicum* étaient les plus efficaces contre *Escherichia coli* (8 mm pour l'extrait aqueux).

Les deux plantes ont également montré des propriétés anti-inflammatoires à travers d'inhibition de la dénaturation des protéine, l'extrait aqueux des feuilles de *B. oleracea* présentant l'inhibition la plus élevée (94,8 %), comparable au médicament de référence diclofénac (96,46 %). Les études *in vivo* ont exploré davantage le potentiel thérapeutique de ces plantes. Dans un modèle d'hypothyroïdie, le traitement avec l'extrait de tubercules de *B. mauritanicum* (200 mg/kg) a amélioré les niveaux d'hormones thyroïdiennes, réduisant la TSH de 2,1 à 1,9 µUI/mL et augmentant la T4 de 30,23 à 32,70 nmol/L. Dans un modèle d'anémie, le traitement par extrait de feuilles de *B. oleracea* (200 mg/kg) a amélioré les paramètres hématologiques, augmentant le nombre de globules rouges de 6,14 à 6,35 x 10^6 cellules/mL et l'hémoglobine de 8,70 à 14,60 g/dL. Les deux traitements ont également montré des améliorations des marqueurs de fonction hépatique et rénale et des profils histologiques, indiquant des effets protecteurs contre les dommages organiques.

Cette étude complète fournit un soutien scientifique aux utilisations traditionnelles de *B. mauritanicum* et *B. oleracea* var. *elongata* dans la région d'Oued Souf. Les résultats suggèrent que *B. mauritanicum* pourrait avoir un potentiel dans la gestion de l'hypothyroïdie, tandis que *B. oleracea* montre des promesses pour le traitement de l'anémie. L'identification de composés bioactifs et la démonstration de diverses activités biologiques soulignent le potentiel de ces plantes comme sources d'agents thérapeutiques.

Mots-clés : *Bunium mauritanicum*; *Brassica oleracea* var. *elongata*; Ethnopharmacologie; Analyse phytochimique; Activités biologiques; Hypothyroidism; Anemia; La région d'Oued Souf.

Content

Liste of Abbreviation

- AAE: Ascorbic Acid Equivalents
- AAs: Ascorbic Acid
- ALP: Alkaline Phosphatase
- ALT: Alanine Transaminase
- AST: Aspartate Aminotransferase
- BHT: Butylated Hydroxytoluene
- cells/µL: Cells per Microliter
- CFU : McFarland
- dL: Deciliters
- DPPH: 2,2-diphenyl-1-picrylhydrazyl
- EC_{50}: Effective Concentration 50%
- EEM: Erythemal Effect Spectrum
- $FeCl_3$: Ferric chloride
- g/dL: Grams per Deciliter
- GAE: Gallic Acid Equivalents
- GSH: Reduced Glutathione
- IC_{50}: Half Maximal Inhibitory Concentration
- LA : leaves aqueous extract
- LM : leaves methanolic extract
- LOVO® : Levothyroxine
- MBC : minimum bactericidal concentration
- MDA: Malondialdehyde
- MIC : minimum inhibitory concentration
- mIU: Milli-International Units
- NI : Non Inhibition
- PHZ : Phenylhydrazine
- PTU : Propylthiouracil
- RBC: Red Blood Cell
- SA : Seeds aqueous extract
- SM : Seeds methanolic extract
- SPF: Sun Protection Factor
- T3: Triiodothyronine
- T4: Thyroxine
- TFC: Total Flavonoid Content
- TPC: Total Polyphenols Content
- TSH: Thyroid Stimulating Hormone
- U: Units
- ZI : Inhibition Zone

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INTRODUCTION

Introduction

Algeria's diverse flora offers significant potential for medicinal plant research and utilization. The country boasts over 4,000 vascular plant species, including many endemics, with unique phytochemical properties (Kaabèche, 2007). Ethnobotanical surveys have documented numerous medicinal plants used by local populations to treat various ailments, particularly digestive disorders. These studies highlight the rich traditional knowledge of plant-based remedies, with leaves and seeds being the most commonly used parts, often prepared as decoctions (Hammadi et al., 2015).This wealth of medicinal plants presents opportunities for both fundamental ethnobotanical research and applied studies in natural product development (Kaabèche, 2007).

The investigation of plant species for their medicinal and nutritional characteristics is a fundamental aspect of both pharmacognosy and nutritional science (Leisegang, 2021; Tomar & Sikarwar). *Bunium mauritanicum* and *Brassica oleracea var. elongata* are notable among the many different species being studied by scientists due to their unique bioactive chemicals and their significance in traditional and modern medicine, as well as in nutrition (Fadia & Khawla, 2023; Karouche et al., 2022; Montaner et al., 2023; Orlando et al., 2022; Quézel & Santa, 1962). Studying these plants not only helps protect biodiversity but also has the potential to uncover new chemicals with therapeutic benefits.

Bunium mauritanicum, also known as Talghouda, is a medicinal plant belonging to the Apiaceae family, the “Plant List” database classifies *Bunium mauritanicum* as a synonym of *Bunium bulbocastanum*, suggesting they are the same botanical species. It is native to North Africa and widely distributed in Algeria. (Quézel & Santa, 1962; Quézel et al., 2000).

The seeds of the genus *Bunium* have been used traditionally for their antispasmodic and carminative properties (Adelifar & Rezanejad, 2021). Preliminary studies have indicated that the essential oils and extracts of four species of the genus *Bunium* possess antioxidant, antimicrobial, and potentially anticancer activities (Adelifar & Rezanejad, 2021). However, the full spectrum of the phytochemical constituents and their pharmacological properties remain underexplored.

Brassica oleracea, a member of the Brassicaceae family with a global distribution absent only in Antarctica, is consumed worldwide as a vegetable, particularly the stalk and large flowering head (Branca, Argento, et al., 2012; Nagraj et al., 2020; Vargas-Rincón et al., 2013). This diverse genus includes vegetables such as cabbage, broccoli, and kale, which are

celebrated for their nutritional value, containing an abundance of vitamins and minerals. Notably, the presence of glucosinolates (GSLs) a category of secondary metabolites renowned for their distinct taste and bioactivities has been the subject of extensive research. GSLs and their hydrolysis products, such as isothiocyanates and indoles, contribute to the plant's health benefits and have been associated with a decreased risk of chronic diseases (Argento et al., 2019; Biondi et al., 2021; Branca, Ragusa, et al., 2012; Quirante-Moya et al., 2020; Ramirez et al., 2020; Soengas et al., 2021)

Brassica oleracea, renowned for its diverse health-promoting phytochemicals, such as glucosinolates, flavonoids, hydroxycinnamic acids, and a variety of vitamins, exhibits a spectrum of beneficial effects including antioxidant, anti-inflammatory, anti-cancer, hepatoprotective, anti-obesity, and anti-diabetic properties (Gaafar et al., 2020; Kamboj et al., 2023; Li et al., 2022; Nazeri et al., 2022). Specifically, sprouts of *B. oleracea* are rich in sulforaphane, a compound gaining attention for its potential to treat neurodegenerative conditions like Parkinson's and Alzheimer's diseases. The chemoprotective capabilities of these phytochemicals are currently a focal point of research, exploring their role in combatting various types of cancer and further underscoring the medicinal value of this vegetable (Gaafar et al., 2020; Kamboj et al., 2023).

The Oued Souf region boasts a rich tradition of herbal remedies. However, limited scientific research exists on the potential benefits of many locally used plants. In particular, *Brassica oleracea var. elongata*, a specific cabbage variety, and *Bunium mauritanicum* are two species with traditional medicinal applications. **Do these plants** possess unique therapeutic properties **?** This research aimed to address this knowledge gap by investigating the pharmacological potential of both *B. oleracea var. elongata* and *B. mauritanicum*. It's important to acknowledge, however, that the selection of these plants was based on a questionnaire for folk medicine in the region. While this approach sheds light on traditional practices, it may overlook species with less established cultural significance but stronger scientific backing. This research design highlights a key challenge: **How can we** leverage the wisdom of traditional medicine to guide scientific inquiry while ensuring a robust and unbiased investigation of potential medicinal plants **?** This study aimed to contribute to the exploration of lesser-known plants like *B. oleracea var. elongata* alongside *B. mauritanicum*.
In summary, the study of *Bunium mauritanicum* and *Brassica oleracea* serves multiple scientific and practical purposes, from advancing our understanding of plant biochemistry to

informing conservation and dietary practices. The present study aims to fill the gaps in the literature regarding the pharmacological potential of *B. mauritanicum* while also providing a comparative analysis with the well-established *B. oleracea*.

The main objective of this doctoral research is to study the biological activities and phytochemical constituents of two plants frequently used in traditional medicine in the El Oued region. Based on a preliminary survey before laboratory research, and using efficient analytical methods and biological tests *in vitro* and *in vivo*, this research aims to explain the active components responsible for the alleged therapeutic benefits.

The current research is divided into two main parts:

- **Part 1** is dedicated to the theoretical framework and contains detailed information about the two study plants, in two chapters.

- **Theoretical Framework**:
  - Chapter 1. *Bunium mauritanicum*
  - Chapter 2. *Brassica oleracea var. elongata*

- **Part 2** The second part: It is divided into two parts: it presents the materials and methods used, highlights the in vitro and in vivo results that we obtained and discussed, and finally we concluded our work with a conclusion appended with recommendations.

FIRST PART

Bibliographic synthesis

CHAPTER I

Bunium mauritanicum

FIRST PART

Chapiter I: Bunium mauritanicum

Bunium mauritanicum

1.1 Family background

Apiaceae family, with its 466 genera and 3800 species, is most varied in temperate climates, especially in North America and Europe (Baczyński et al., 2021; Kirici et al., 2023; Reduron, 2020). It is known for its secondary metabolites, including essential oils and non-volatile compounds, which have both culinary and medicinal uses (Pollastro & Gaeta, 2020). The family's phytochemical diversity, particularly in the subfamily Apioideae, has been studied to trace convergent evolution (Das, 2020). The family's complex morphology, anatomy, and biology, including variations at the infraspecific level, pose challenges for identification and classification (Reduron, 2020).

1.2 *Bunium* genus background

The *Bunium* genus, known for its nutritional and chemical significance, includes various species that have been extensively studied for their health benefits and culinary uses(Mohammadhosseini et al., 2021) . *Bunium persicum*, commonly known as black cumin, is rich in essential oils, fatty acids, and flavonoids, which contribute to its antioxidant, antimicrobial, and anti-inflammatory properties (Hassanzad Azar et al., 2018). The seeds of Bunium species are particularly valued for their high protein and lipid content, making them a nutritious addition to the diet. Studies have shown that the essential oils extracted from Bunium seeds contain compounds such as cuminaldehyde, terpenes, and phenolic acids, which play a crucial role in enhancing food flavor and preserving food quality (Bansal et al., 2023). Additionally, the presence of dietary fiber in Bunium seeds aids in digestive health and helps in managing cholesterol levels (Shafiee et al., 2020). Research on the chemical composition of Bunium species has highlighted their potential as functional foods, capable of providing health benefits beyond basic nutrition (Shafiee et al., 2020; Zengin et al., 2019). The incorporation of Bunium seeds into food products can thus offer both nutritional and therapeutic advantages, contributing to overall well-being (Zengin et al., 2019).

1.3 Distribution and habitat

Bunium mauritanicum, a species belonging to the Umbelliferae family, thrives in diverse regions, spanning from Iraq and Iran to the North Western Himalayas (Mounir et al., 2022; Pimenov & Leonov, 2004). While its habitat specifics, including elevation and soil composition, remain elusive in current literature, According to ongoing studies, the impacts of climate change may potentially result in a northward migration within the Yellow River Basin in China (Accogli et al., 2024; Mounir et al., 2022). The typical habitat for this species is in sub-alpine and alpine environments in the North Western Himalayas, *Bunium mauritanicum* favors habitats characterized by lower and middle mountain slopes with soil compositions ranging from loamy sand to clay loam (Shah et al., 2019) (Ahn & lee, 2007). This species exhibits a preference for heavy clay soils with diminished native and exotic species cover, along with low levels of nitrogen and phosphorus (Djamaluddin, 2018; Shah et al., 2019). Notably, in the Bunaken National Park of North Sulawesi, Indonesia, *Bunium mauritanicum*, part of the mangrove flora, thrives across various sub-habitats defined by distinct physiographic factors and significant physical processes (Djamaluddin, 2018; Shah et al., 2019). Furthermore, taxonomic complexities persist within the Bunium genus, with 128 synonymous species and 31 unresolved species. This includes the presence of seven species within the Algerian flora, of which four are regionally endemic (Quézel & Santa, 1962).

1.4 Botanical Description

Table 1:Botanical classification of *Bunium mauritanicum* (Bansal et al., 2023; Battandier & Trabut, 1888)

Classification	Rank
Kingdom	Plantae
Subkingdom	Tracheophyta
Superdivision	Spermatophyta
Division	Magnoliopsida
Class	Apiales
Order	Apiales
Family	Apiaceae
Genus	Bunium
Species	*Bunium mauritanicum*

Trabut and Marès (1907) identified Talghouda as *B. mauritanicum*, a medicinal plant belonging to the Apiaceae family.

B.mauritanicum , plant native to southern Spain and northern Africa (Fig 1.), It is prevalent in the Algerian east, with a tuberous root. Tubercule the size and appearance of a medium-sized truffle, rough, mammillated, blackish brown on the outside, white on the inside. Stem erect, fistulous, striated, branched, reaching about 60 cm in height in our cultures (Tab 2.). Radical leaves triternatisect, cauline, with narrow, linear segments, dark green. Involucre and involucel usually quinquefoliate. Calyx with acute triangular lobes, conical stylopodia, surmounted by persistent styles, valleculae with a single band (Battandier, 1888; Trabut & Marés, 1906).

Table 2. Morphological descriptionof the plant *Bunium mauritanicum.*

Plant Part	Description (Battandier, 1888; Trabut & Marés, 1906)
Root	Tuberous, resembling a medium-sized truffle, rough, mammillated, blackish brown outside, white inside.
Stem	Erect, fistulous, striated, branched, reaches about 60 centimeters in height.
Leaves	Radical leaves are triternatisect; cauline leaves have narrow, linear segments, dark green.
Involucre and Involucel	Usually, quinquefoliate.
Calyx	With acute triangular lobes.
Stylopodia	Conical, surmounted by persistent styles.
Valleculae	Contains a single band

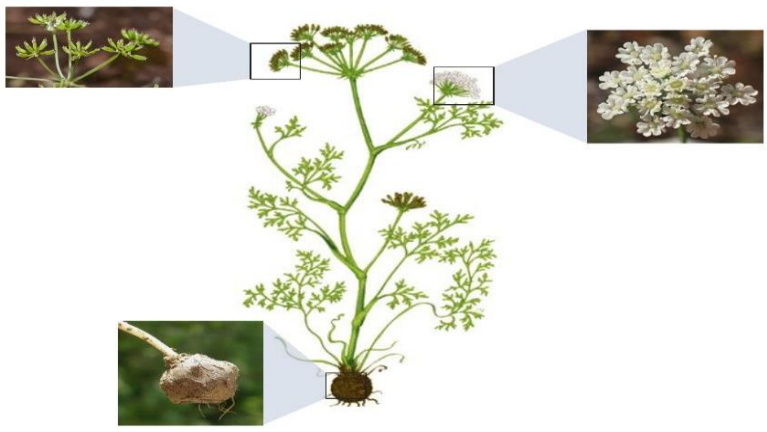


Figure 1*:Bunium mauritanicum* (Karouche et al., 2022)

1.5 Traditional Medicinal Uses

Traditional medicine practices across various cultures have utilized *Bunium mauritanicum* for centuries, particularly for treating thyroid dysfunction. The specific methods of preparation and administration may vary depending on the region. This ethnobotanical knowledge, coupled with scientific analysis of the plant's bioactive

compounds, could pave the way for the development of novel therapeutic agents (Benkhalifa & Mohamed, 2019).

The plant commonly known as "Talghouda" or groundnut is familiar to rural areas in all the Tell regions of Algeria (Benkhalifa & Mohamed, 2019; Elyebdri et al., 2017). For some, Talghouda evokes a remarkable food source, while for others, it serves as a symbol of misery, reminding them of the famine experienced during periods of scarcity, particularly during and after the Second World War, as well as during the period of the national revolution (1954-1962) (Elyebdri et al., 2017). Nowadays, it is mentioned by rare collectors but is often present among herbalists for its interest and therapeutic use. This plant hides an exceptional nutritive quality and can have a double interest for its valorization. It can be considered as a crop well adapted to mountain regions and constitutes a treasure to be explored for the treatment of goiter and thyroid dysfunction (Benkhalifa & Mohamed, 2019; Djahafi, Taïbi, et al., 2021; Elyebdri et al., 2017; Taïbi et al., 2021).

Trabut and Marès (1906) (Trabut & Marés, 1906), reported that this umbellifer, which is highly prevalent in the Tell regions, possesses a large starchy tuber that the local inhabitants harvest during times of scarcity. These tubers, once dried and lightly roasted, can be processed into a flour used for food purposes.

1.6 Nutritional Aspects

(Tab 3.) presents the evaluation of the nutritional composition of the dried tuber powder of *B. mauritanicum.*

Table 3: Displays the nutritional composition of *B. mauritanicum* (Aiouaz & Bitam, 2022b).

Composition	Powder of Talghouda (%)
Proteins	6.87
Lipids	1.59
Starch and congeners	75.79
Ash	3.34
Moisture	12.41
Dry matter	87.59
Sodium (mg/100g)	26.126
Potassium (mg/100g)	289.17
Calcium (mg/100g)	449.6

1.7 Secondary Metabolites

In an Algerian study in 2017 (Hayet et al., 2017b), they showed that the essential oil (EO) of *B. mauritanicum* contains thirty-one identified compounds (Tab 4.) by comparing their retention indices and mass spectra of each GC component with those of standards.

The systematic review on Bunium species, including *B. mauritanicum*, provides evidence of the presence and abundance of secondary metabolites in these species. The phytochemical analysis indicated the presence of essential oil in the plant, which consists of monoterpenes and sesquiterpenes. Additionally, non-volatile components such as coumarins and flavonoids were also identified (Mohammad hosseini et al., 2021).

Table 4: Secondary metabolites present in the essential oil of *B. mauritanicum*. (Hayet et al., 2017b),

Classes	Compound	RI	%	Retention Time (min)
Terpenoids	Thymol	1289	0.65	18.93
	Carvacrol	1298	0.76	19.11
	Spatuleneol	1577	4.04	26.98
	Caryophyllene oxide	1582	17.36	27.12
	Carvone	1595	0.71	27.6
	Humulene epoxide-I	1608	1.85	27.68
	10-epi-α-muurolol	1640	4.36	28.36
	β-eudesmol	1649	13.95	28.67
	α-bisabolol	1685	2.11	29.19
Phenolics	Salvial-4(14)-en-1-one	1594	2.4	27.3
	Apiol	1677	0.94	28.98
	a-pritone	1708	2.98	29.63
Sesquiterpenes	β-elemene	1389	0.28	22.08
	(E)-caryophyllene	1417	2.62	22.98
	(E)-β-farnesene	1440	0.25	23.48
	α-humulene	1452	0.31	23.84
	γ-muurolene	1478	0.67	24.29
	D-germacrene	1484	0.55	24.5
	α-muurolene	1500	0.27	24.86
	β-bisabolene	1505	0.48	24.94
	β-sesquiphellandrene	1521	0.65	25.33
	1-endo-bourbonanol	1518	2.43	25.44

	β-calacorene	1544	0.66	26
	Hedycaryol	1546	4.14	26.1
Fatty Acids & Others	Myristic acid	1720	2.4	30.53
	6,10,14-Trimethylpentadecan-2-one	1795	1.61	32.38
	Pentadecanoic acid	1820	1.8	32.66
	Palmitic acid	1984	18.39	34.87
	Linoleic acid	2173		

- RI: Retention Index, % : percentage of a particular peak area.

In their research, Algerian researchers employed conventional spectrophotometric methods and high-performance liquid chromatography (HPLC) to perform chemical screening of *Bunium mauritanicum* seeds. Their objective was to identify and quantify the secondary metabolites present in the seeds. The analysis revealed the presence of various phenolic compounds in the essential oil of *B. mauritanicum*. These include catechin, a flavanol, as well as kaempferol and quercetin, two flavonols. Additionally, hesperetin and naringenin, both flavanones, were also identified. Furthermore, the analysis identified eight phenolic acids: (chlorogenic acid, gallic acid, p-coumaric acid, caffeic acid, ferulic acid, syringic acid, sinapic acid, and ellagic acid.) (Fethi et al., 2022). In another investigation conducted in 2023, utilizing RP-HPLC analysis on various extracts of *B. incrassatum*, researchers identified a total of 12 phenolic compounds (Tab 5.). The analysis detected the presence of eight phenolic acids, namely gallic acid, caffeic acid, ellagic acid, ferulic acid, rosmarinic acid, sinapic acid, syringic acid, and vanillic acid. Furthermore, four flavonoids were identified, including catechin, hesperetin, luteolin, and quercetin.(Fethi & Djendar, 2023).

Table 5:Secondary metabolites of *B. mauritanicum* (Mohammadhosseini et al., 2021) (Fethi et al., 2022).

Chemical Family	Compounds
Phenolic Acids	Gallic Acid, Caffeic Acid, Syringic Acid, Ellagic Acid, Ferulic Acid, Rosmarinic Acid, p-Coumaric Acid, Sinapic Acid, Chlorogenic Acid, Vanillic Acid
Flavanols	Catechin
Flavonols	Quercetin, Kaempferol
Flavanones	Naringenin, Hesperetin
Coumarins	Coumarin compounds

1.8 Biological Activities

1.8.1 Antimicrobial potential

Bunium species, including *Bunium mauritanicum* (also known as *Bunium incrassatum*), possess antibacterial properties that can be attributed to their high concentration of essential oils and non-volatile metabolites, such as flavonoids and phenolic acids (Mohammadhosseini et al., 2021). These Bunium species have been shown to be effective against pathogenic bacteria, including *Escherichia coli* ,*Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Essential oils like γ-terpinene and cuminaldehyde, present in Bunium species, possess strong antibacterial effects, as evidenced by methods such as minimum inhibitory concentration (MIC) (Mohammadhosseini et al., 2021).Specifically, *Bunium mauritanicum*'s antibacterial properties can be attributed to its phenolic compounds, including flavonoids and phenolic acids, which disrupt bacterial cell wall synthesis, enzyme activity, and genetic expression, thereby inhibiting bacterial growth (Fethi et al., 2022).The bioactive compounds present in *Bunium mauritanicum* seeds, particularly its phenolic content, have been linked to potential antibacterial effects by damaging bacterial cell walls and disrupting their metabolic pathways (Amraoui et al., 2023).These findings highlight the antibacterial effectiveness of *Bunium incrassatum* and support its traditional use in treating infections (Fethi et al., 2022).

1.8.2 Anticancer potential

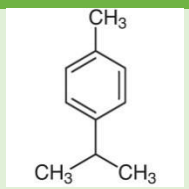
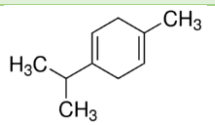
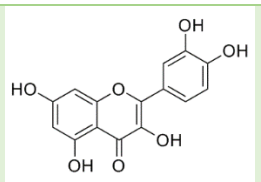
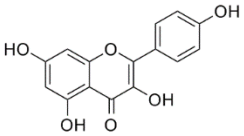
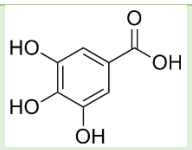
The *Bunium mauritanicum* plant exhibits a possible role in the treatment of cancerous properties due to its rich profile of bioactive compounds like phenolics and flavonoids with sesquiterpenes (Fethi et al., 2022). These compounds jointly confer the plant's ability to induce apoptosis and further inhibit the proliferation of cancer cells. In this regard, cell cycle

disruption, kinase activity inhibition, and oxidative stress relief are significant mechanisms implicated in its anticancer activity (Fethi & Djendar.,2023). Further, these plants' bioactive compounds intermission with cancer-associated signaling ways, therefore highlighting the potential of Bunium incrassated in cancer chemo-prevention and therapy (Mohammadhosseini et al., 2021). On the contrary, the specific studies about these effects are quite scarce. They, so far, tend to show a need for further probing to clarify the extent of its anticancer efficacy and the mechanism behind that (Meriem et al., 2022).

1.8.3 Antioxidant potential

The high antioxidant content of Bunium species is well-known, and *Bunium mauritanicum* is no exception (Mohammadhosseini et al., 2021). The high concentrations of phenolic chemicals, flavonoids, and terpenoids found in these plants provide them antioxidant properties. Bunium plants' polar extracts and essential oils have strong antioxidant properties because they include chemicals including cymene, γ-terpinene, and flavonoid derivatives (Fethi & DJENDAR.,2023). Scientists often use tests like the DPPH and ABTS assays to measure antioxidant activity. The phenolic component concentration of *Bunium mauritanicum* is responsible for its notable antioxidant action. Antioxidants found in Bunium plants protect cells from oxidative stress and free radicals by scavenging them, inhibiting lipid peroxidation, and chelating metal ions. Methods such as DPPH and β-carotene bleaching have verified that the plant possesses strong antioxidant properties. Bunium species include phenolic acids (chlorogenic acids, caffeic, and ellagic) and flavonoids (kaempferol and quercetin) that contribute to these effects. Crucial in avoiding cellular damage linked with chronic diseases, *Bunium mauritanicum* extracts have demonstrated free radical scavenging and oxidative damage protection capabilities. Researchers have examined the antioxidant capabilities of *Bunium mauritanicum* extracts by employing several tests, including DPPH and β-carotene/linoleic acid assays. Strong free radical neutralization was shown by the methanol extract, which had the highest DPPH scavenging potential. Because of its high phenolic content, particularly gallic acid, it has this action. How effective the extracts are as antioxidants is heavily dependent on the solvent used for extraction. *Bunium mauritanicum* and other Bunium species are attractive potential anti-aging and neurodegenerative disease preventatives due to their antioxidant characteristics (Amraoui et al., 2023; Meriem et al., 2022).

Table 6: Antioxidant compounds detected in *B. mauritanicum* according to previous studies (Amraoui et al., 2023; Fethi & DJENDAR.,2023; Fethi et al., 2022; Meriem et al., 2022; Mohammadhosseini et al., 2021)

Antioxidant	Chemical Compound Formulas	Chemical structure
Cymene	$C_{10}H_{14}$	
γ-Terpinene	$C_{10}H_{16}$	
Quercetin	$C_{15}H_{10}O_7$	
Kaempferol	$C_{15}H_{10}O_6$	
Gallic Acid	$C_7H_6O_5$	

1.8.4 Anti-inflammatory and immunomodulatory potential

Bunium mauritanicum's anti-inflammatory and immune-regulating properties are likely due to its abundance of phenolic acids, flavonoids, and essential oils. These natural compounds, such as caffeic acid, quercetin, rosmarinic acid, and luteolin, can dampen inflammation by inhibiting the production and activity of molecules that promote inflammation. These molecules include cytokines (like TNF-α and IL-6) and enzymes (like COX and LOX) .

This inhibition not only reduces the production of pro-inflammatory eicosanoids like prostaglandins and leukotrienes but also curtails the activity of nitric oxide, a potent inflammatory mediator in macrophages. The traditional use of *Bunium mauritanicum* in treating inflammatory conditions such as arthritis, bronchitis, and colitis is supported by experimental evidence demonstrating significant reduction in induced ear and paw edema in mice, highlighting its potential as a natural therapeutic agent in inflammation-related ailments.

1.8.5 Anti-hemolytic properties

In vitro study, was conducted by (Berroukeche et al., 2022) on the ability of this plant to prevent hemolysis through three methods, it showed that extracts of *Bunium mauritanicum*

demonstrated anti-hemolytic properties, indicating their potential to protect red blood cells from damage in hypotonic conditions and oxidative stress induced by hydrogen peroxide.

CHAPTER II

Brassica oleracea var. elongata

Chapiter II: *Brassica oleracea* var. elongata

1 Brassica oleracea var. *elongata*

1.1 Family background

Brassica oleracea is a species of the Brassicaceae family. The Brassicaceae family, also recognized as Cruciferae, is a prominent dicot family within the angiosperms, encompassing approximately 340 genera and 3,350 species. This family is typified by its cruciform flowers, usually featuring four petals and six stamens (with the outer two shorter than the inner four), and distinctive two-valved capsules. As one of the most extensive angiosperm families, the Brassicaceae includes a diverse array of annuals, biennials, and herbaceous perennials. It provides a significant source of edible species like vegetables, condiments, oilseeds, and fodder, contributing essential nutrients such as vitamins A, B1, B2, B6, C, E, K, and minerals like magnesium, iron, and calcium. Notably, Arabidopsis thaliana, a member of this family, serves as a critical model organism in plant molecular biology due to its well-characterized genome and suitability for genetic analysis. This model has revolutionized understanding across various domains of plant biology, including biochemistry and physiology, through the use of advanced molecular-genetic techniques. Additionally, the family is rich in secondary metabolites, including glucosinolates, which are sulfur-containing compounds known for their potential anticarcinogenic properties. The diverse wild germplasm of Brassicaceae also harbors a wealth of agronomic and economic traits, such as cytoplasmic male sterility, apomixis, and enhanced tolerance to environmental stresses, which are invaluable for crop improvement programs (Anjum et al., 2012; Raza et al., 2020; Warwick, 2011).

Brassica oleracea encompasses a wide range of cultivars, including cabbage (*var. capitata*) as the primary cultivar, along with broccoli (*var. italica*), cauliflower (*var. botrytis*), kale (*var. acephala*), kohlrabi (*var. gongylodes*), Brussels sprouts (*var. gemmifera*), among others (Fig 3.) (Ștefan & Ona, 2020).

Figure 2:The main varieties of *Brassica oleracea* (Ștefan & Ona, 2020).

1.2 Brassica genus background

The Brassica genus, encompassing a variety of species such as cabbage, broccoli, and kale, is renowned for its rich nutritional and chemical profile, which offers significant health benefits (Traka & Mithen, 2011). These vegetables are high in vitamins A, C, and K, as well as essential minerals like calcium, potassium, and iron (Kapusta-Duch et al., 2012; Traka & Mithen, 2011). The phytochemical composition of Brassica plants includes glucosinolates, which are sulfur-containing compounds that have been extensively studied for their potential anti-carcinogenic properties (Miękus et al., 2020). Upon hydrolysis, glucosinolates produce bioactive compounds such as isothiocyanates and indoles, which play a crucial role in detoxification processes and the inhibition of cancer cell growth (Das et al., 2000). Additionally, Brassica vegetables are a good source of dietary fiber, which aids in digestive health and helps regulate blood sugar levels. The antioxidant properties of Brassica species, attributed to the presence of flavonoids and carotenoids, contribute to the reduction of oxidative stress and inflammation (Cartea et al., 2010). Regular consumption of Brassica vegetables is associated with a lower risk of chronic diseases, including cardiovascular disease and certain types of cancer, highlighting their importance in a balanced diet (Cartea et al., 2010; Raiola et al., 2017).

1.3 Distribution and habitat of *Brassica oleracea*

Brassica oleracea is indigenous to the coastal regions of southern and western Europe and exhibits a preference for well-drained, nutrient-rich loams with a pH range of 6.0 to 7.5. This species is extensively cultivated across various global climates, demonstrating remarkable adaptability to diverse environmental conditions. It is particularly resilient to cooler temperatures, which enables its widespread cultivation in temperate zones. The versatility of *B. oleracea* has led to its significant role in agriculture, underpinning the production of numerous crucial vegetable crops. Its ability to thrive in a variety of habitats has facilitated its integration into different agricultural systems, thereby contributing to food security and agricultural sustainability (Falentin et al., 2023; Mabry et al., 2021; Perrino & Wagensommer, 2022).

1.4 Botanical Description

Brassica oleracea is classified within the Plantae kingdom, under the Viridiplantae subkingdom, and belongs to the Embryophyta superdivision. It is a member of the Tracheophyta division, situated within the Magnoliopsida class, which aligns with the broader order of Brassicales. As part of the Brassicaceae family, this genus Brassica represents a significant group of dicotyledonous plants. This botanical classification (Tab 7.)

Table 7:Botanical classification of *Brassica oleracea var. elongata*.**(Guo et al., 2021; Mun et al., 2015).**

Classification	Rank
Kingdom	Plantae
Class	Magnoliopsida
Order	Brassicales
Family	Brassicaceae
Genus	Brassica
Species	*Brassica oleracea var. elongata*

underscores its role in the taxonomic hierarchy, reflecting its evolutionary lineage and morphological characteristics (Tab 8.)

Table 8:Morphological description of the plant *Brassica oleracea var. elongata* (Balkaya et al., 2005; El-Esawi et al., 2012; Kennard et al., 1994).

Plant Part	Description
Root	The root system is fibrous, supporting the plant's structure and nutrient uptake.
Stem	The stem is typically short and thick, providing a strong foundation for leaf growth.
Leaves	Leaves are large, thick, and fleshy, with a waxy coating that helps in reducing water loss. Their size and color can vary significantly among different cultivars.
Involucre and Involucel	The involucre consists of bracts that surround the flower cluster, while the involucel surrounds smaller groups within the inflorescence.
Calyx	The calyx is composed of four sepals that protect the developing flower bud.
Stylopodia	Present in some cultivars, stylopodia are small, stylus-like structures found in the flower.
Valleculae	Valleculae are grooves or furrows, often present on seeds or fruits, aiding in their dispersal or attachment.

Brassica oleracea is a pivotal species in agricultural systems (Fig 4.), renowned for its diversity in vegetable crops and significant genetic resources which contribute extensively to studies on plant morphology, genome evolution, and agricultural breeding (Guo et al., 2021; Mun et al., 2015).

Figure 3:*Brassica oleracea var. elongata* (original picture. 2023).

1.5 Traditional Medicinal Uses

Brassica oleracea has been extensively utilized across various cultures for its therapeutic properties. Historically, the leaves of this vegetable have been employed as compresses to alleviate wounds, inflammation, and infections. The ingestion of Brassica

vegetables is associated with a reduced incidence of chronic ailments such as cancer, attributed to their high concentrations of vitamins, minerals, and antioxidants. In traditional healthcare practices, cabbage leaves are commonly used to mitigate symptoms of breast engorgement during lactation and to diminish pain and swelling. This historical usage underscores the plant's medicinal significance and supports its potential in natural health remedies, reflecting its rich phytochemical profile and broad therapeutic applications (Khalid et al., 2023; Lipi R Ray et al., 2021; Sundaram et al., 2020).

1.6 Nutritional Aspects

Brassica oleracea, encompassing variants such as cabbage, kale, and broccoli, is a nutrient-dense vegetable providing substantial health benefits. This cruciferous vegetable is a rich source of essential vitamins and minerals, notably vitamins C and K, calcium, and potassium, which are integral for maintaining immune functionality, bone health, and cardiovascular stability. Its high dietary fiber content supports digestive health while its low caloric value makes it an excellent choice for weight management. The vegetable's phytochemical profile includes glucosinolates, known for their cancer-preventive properties, and a variety of other bioactive compounds that contribute to its antioxidant and anti-inflammatory effects. (Tab 9.) below summarizes the nutritional composition of *Brassica oleracea*, highlighting its low lipid content and high moisture percentage, which further underscores its role in a health-conscious diet.

Table 9:. Nutritional composition of *Brassica oleracea.vegetables* per 100g(Isabel et al., 2022).

	Energy (Kcal)	Water Content (g)	Carbohydrates (g)	Fat (g)	Fibre (g)	Protein (g)	Minerals (mg)				Vitamins	
							Ca	Fe	K	Mg	C (mg)	Folate (µg)
Broccoli	34	89.3	6.6	0.37	2.6	2.8	47	0.7	316	21	89.2	63
Brussels Sprouts	43	86	9	0.3	3.8	3.4	42	1.4	389	23	85	61
Cabbage	25	92.2	5.8	0.1	2.5	1.3	40	0.5	170	12	36.6	43
Cauliflower	25	92	5	0.3	2	1.9	22	0.4	299	15	48.2	57
Kale	49	84	8.8	0.9	3.6	4.3	150	1.5	491	47	120	141
Radish	16	95.3	3.4	0.1	1.6	0.7	25	0.3	233	10	14.8	25
Turnip	28	91.9	6.4	0.1	1.8	1.2	30	0.3	191	11	21	15

The data in the table reflect *Brassica oleracea*'s nutritional efficiency, particularly in providing high levels of vital nutrients in low-calorie servings, making it an essential component of dietary regimens aimed at fostering health and preventing nutritional deficiencies.

The data in the table reflect *Brassica oleracea*'s nutritional efficiency, particularly in providing high levels of vital nutrients in low-calorie servings, making it an essential component of dietary regimens aimed at fostering health and preventing nutritional deficiencies.

1.7 Secondary metabolites of *Brassica oleracea*

Brassica oleracea, including varieties such as broccoli and cauliflower, is rich in secondary metabolites, particularly glucosinolates and phenolic compounds (Jeon et al., 2021; Yeo et al., 2021). These metabolites play a crucial role in the plant's defense mechanisms and contribute to the health benefits of these vegetables. The major chemical families of secondary metabolites in *Brassica oleracea* include phenolic acids, flavanols, flavonols, flavanones, and coumarins. Phenolic compounds such as rutin, epicatechin, and p-coumaric acid are predominant, with their concentrations significantly influenced by various environmental factors, including light quality (Jeon et al., 2021; Zhao et al., 2020). For instance, exposure to blue LED light has been shown to enhance the accumulation of individual phenolic compounds, with rutin reaching up to 1135.77 µg/g DW and epicatechin up to 743.60 µg/g DW(Sathasivam et al., 2023). Additionally, glucosinolates such as 4-hydroxyglucobrassicin, glucoerucin, and 4-methoxyglucobrassicin also show significant variation under different light treatments, indicating a strong environmental modulation of these bioactive compounds. The total glucosinolate content can vary from 27.01 to 28.69 µg/g DW, with blue LED treatment achieving the highest levels (Sathasivam et al., 2023). These findings suggest that optimizing growth conditions, particularly light quality, can significantly enhance the beneficial secondary metabolite profile of *Brassica oleracea*, making it an important consideration for both agricultural practices and nutritional strategies (Tab 10.) (Jeon et al., 2021; Sathasivam et al., 2023).

Table 10.Secondary Metabolites of *Brassica oleracea* (Jeon et al., 2021; Saeedi et al., 2021; Yeo et al., 2021)

Chemical Family	Compounds
Phenolic Acids	Rutin, Epicatechin, p-Coumaric acid
Flavanols	Catechin, Quercetin
Flavonols	Kaempferol, Myricetin
Flavanones	Naringenin, Hesperetin
Coumarins	Umbelliferone, Scopoletin
Glucosinolates	4-Hydroxyglucobrassicin, Glucoerucin
	4-Methoxyglucobrassicin, Glucoraphanin

1.8 Biological Activities

1.8.1 Antimicrobial Potential

The biological antimicrobial potential of *B. oleracea* is substantial due to its rich phytochemical composition, particularly glucosinolates and phenolic compounds. in previous studies, bioactive *Punica granatum L. Peel* extracts were used to enhance the growth and nutritive values of B. oleracea. The extracts were evaluated for their phytochemical profiles, antibacterial potential, and antioxidant activities (Dawoud et al., 2023). Both aqueous and ethanol extracts demonstrated significant antibacterial activity attributed to its high phenolic content, including 58.95% Eugenol (Al Talebi, 2023).The antibacterial activities against pathogens like *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* were notably improved, although some reduction in effectiveness was observed against specific strains (Favela-González et al., 2020).

Further studies utilizing green synthesis of silver nanoparticles (AgNPs) from *B. oleracea* extracts confirmed the presence of potent antimicrobial properties. These AgNPs exhibited broad-spectrum antibacterial activity, with inhibition zones ranging from 9 to 14 mm against bacteria such as Bacteroides fragilis and Staphylococcus epidermidis. The minimum inhibitory concentration (MIC) of the synthesized BO-AgNPs further supported their efficacy, with low MIC values indicating strong antibacterial potential (Ansar et al., 2020).

The following table summarizes the antimicrobial activities observed in *B. oleracea* varieties treated with PoPe extracts and BO-AgNPs (Tab 11.)

Table 11:Summary of antimicrobial activities observed in *B. oleracea*. (Ansar et al., 2020; Favela-González et al., 2020):

Types of bacteria	Inhibition Zone (mm)	MIC (µg/ml)
Staphylococcus epidermidis	14.33 ± 0.57	6.25
Enterococcus faecalis	11.16 ± 0.28	12.5
Proteus mirabilis	11.33 ± 0.57	25
Pseudomonas aeruginosa	12.0 ± 0.20	3.1
Escherichia coli	10.0 ± 1.0	12.5
Klebsiella pneumoniae	10.0 ± 0.50	25
Bacteroides fragilis	9.43 ± 0.40	50

1.8.2 Anticancer Potential

B. oleracea has demonstrated significant anticancer potential, largely attributed to its rich composition of polyphenolic compounds and glucosinolates. Previous studies have shown that the ethanolic crude extract and biosynthesized copper nanoparticles (CuNPs) from *B. oleracea var acephala* exhibit strong antiproliferative activity against cervical cancer HeLa cell lines (Sundaram et al., 2020). The ethanolic extract and CuNPs inhibited HeLa cell proliferation in a dose-dependent manner, with increased concentrations correlating with decreased cancer cell viability and increased cytotoxicity (Sundaram et al., 2020). These findings suggest that the polyphenolic content, particularly flavonoids and phenolic acids, along with the antioxidant properties of *B. oleracea*, contribute to its anticancer effects. Additionally, the presence of indole-3-carbinol in Brassica vegetables aids in DNA repair and inhibits the growth of cancer cells (Lučić et al., 2023; Sundaram et al., 2020).

The following table summarizes (Tab 12.) the anticancer activities observed in various studies involving *B. oleracea* extracts and CuNPs:

Table 12: Summary of anticancer activities of *Brassica oleracea* extracts and copper nanoparticles (CuNPs) against various cancer cell lines. (Ansar et al., 2020; Lučić et al., 2023; Sundaram et al.,2020)

Sample Type	Cell Line	IC_{50} Value (µg/ml)	Observed Effect
Ethanolic Crude Extract	HeLa	170.66	Decreased cell viability, increased cytotoxicity
Copper Nanoparticles (CuNPs)	HeLa	119.08	Enhanced antiproliferative activity
Ethanolic Crude Extract	MCF-7/SCs	69.47	Induced apoptotic cell death
Copper Nanoparticles (CuNPs)	MDA-MB-231/IR	-	Inhibited migration and invasion, reduced GPx1 expression

1.8.3 Anti-inflammatory and Immunomodulatory Potential

Brassica oleracea has demonstrated substantial anti-inflammatory and immunomodulatory properties, primarily due to its high content of glucosinolates, phenolic compounds, and flavonoids. These bioactive constituents can modulate inflammatory pathways and reduce the production of pro-inflammatory cytokines, thereby supporting the immune system and managing inflammatory diseases such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) (Cicio et al., 2023). Previous studies have shown that

extracts from *B. oleracea* can inhibit the NF-κB signaling pathway, reducing the secretion of inflammatory mediators like IL-1β, TNF-α, and prostaglandin E2 (PGE2) (Cicio et al., 2023; Hamed et al., 2021). Additionally, *B. oleracea* extracts have been found to enhance the activity of antioxidant enzymes, thereby mitigating oxidative stress and further contributing to their anti-inflammatory effects. The combination of these mechanisms suggests that *B. oleracea* can be a valuable adjunct in the treatment of chronic inflammatory diseases and immune system support (Shahbazi et al., 2021). The following table summarizes (Tab 13.) the anti-inflammatory and immunomodulatory activities of *B. oleracea*

Table 13:Summary of Anti-inflammatory and immunomodulatory activities of *Brassica oleracea*. (Alotaibi et al., 2021; *Shahbazi* et al., 2021).

Bioactive Compound	Mechanism of Action	Observed Effect
Glucosinolates	Modulation of NF-κB signaling	Reduced secretion of IL-1β, TNF-α, and PGE2
Phenolic Compounds	Antioxidant activity	Mitigation of oxidative stress
Flavonoids	Inhibition of inflammatory cytokines	Decreased levels of IL-6 and TNF-α
Sulfur-containing Compounds	Maintenance of intestinal barrier integrity	Improved gut health and reduced inflammation

These findings support the potential of *B. oleracea* as a functional food ingredient for anti-inflammatory and immunomodulatory applications, providing a natural and effective strategy for managing chronic inflammatory conditions.

1.8.4Antioxidant Potential

Brassica oleracea exhibits significant antioxidant potential due to its rich composition of phenolic compounds, flavonoids, and glucosinolates. These compounds have been shown to possess strong free radical scavenging activity, contributing to the plant's overall antioxidant capacity. In studies, ethanolic extracts of *B. oleracea* demonstrated higher amounts of phenolic compounds and greater antioxidant activity compared to other solvents. For instance, the hydroxyl radical scavenging activity of ethanolic crude extracts and copper nanoparticles synthesized from B. oleracea var. acephala was found to be in the range of 81-92% at a concentration of 10 µg/mL. This indicates the plant's potent ability to inhibit lipid and protein oxidation, thereby protecting tissues from oxidative stress (Miranda et al., 2024). The presence of enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) in the extracts further supports their role in mitigating

oxidative damage and promoting tissue repair (Miranda et al., 2024; Velasco et al., 2021). The following table summarizes (Tab 14.) the antioxidant activities of *B. oleracea* extracts

Table 14: Summary of antioxidant activities of different *Brassica oleracea extracts*. (Rahman et al., 2022; Velasco et al., 2021).

Extract Type	Antioxidant Activity (IC_{50} µg/mL)	Observed Effect
Methanolic Dry Extract	90 ± 2.52	Highest antioxidant activity
Ethanolic Crude Extract	170.66	Significant free radical scavenging activity
Copper Nanoparticles (CNP)	119.08	Enhanced antioxidant potential compared to crude extract

These findings underscore the potential of *B. oleracea* as a source of natural antioxidants, offering protective effects against oxidative stress and contributing to the prevention and management of various health conditions.

1.8.5 Anti-hemolytic Properties

Brassica oleracea, has shown significant anti-hemolytic properties, attributed to its bioactive compounds. These properties help protect red blood cells from hemolysis, a process where red blood cells rupture and release their contents into the bloodstream, which can lead to severe complications. Studies have demonstrated that *Brassica oleracea* extract contains various antioxidants, such as phenolic compounds, flavonoids, and isothiocyanates, which contribute to its protective effects (Tab 15.)

Table 15: Anti-Hemolytic activity of *Brassica oleracea* methanolic extract at various concentrations. (Bhushan et al., 2021; Gharari et al., 2022).

Concentration (µg/ml)	% Hemolysis (with Triton-X)	% Hemolysis (without Triton-X)
50	31.36 ± 3.92	13.28 ± 0.59
100	25.26 ± 3.25	10.16 ± 0.03
200	19.05 ± 3.11	7.78 ± 0.75
400	17.19 ± 3.04	8.52 ± 1.00
800	15.24 ± 1.47	8.49 ± 1.41
1000	13.50 ± 1.47	3.92 ± 0.65

These compounds can inhibit oxidative stress and enhance the integrity of red blood cell membranes, preventing their breakdown. In experiments, *Brassica oleracea* extracts significantly reduced hemolysis induced by oxidative agents, confirming its potential as a natural anti-hemolytic agent (Gharari et al., 2022). This property is particularly beneficial in conditions where oxidative stress is a contributing factor, providing a natural therapeutic option to support blood health.

SECOND PART

Experimental Part.

CHAPTER I

Materials and Methods

SECOND PART

CHAPTER I: Materials and Methods

Materials

1.1 Chemicals

Sodium Chloride (Nacl), Chloroform, Comassie Blue, Phosphoric Acid (H_3PO_4), Bovine SerumAlbumin (BSA), Gallic Acid, Trichloroacetic Acid (TCA), ButylatedHydroxytoluene (BHT), Chloride Hydrogen HCl, Tris, Salicylicacid, DTNB (5-5'-dithiobis2-nitrobenzoic acid), hydrogenperoxide (H_2O_2), magnesium (Mg), Fehling liquor, sulfuricacid. Aluminum chloride ($AlCl_3$), Dipotassium phosphate (K_2HPO_4), Monopotassium phosphate (KH_2PO_4), Potassium nitrite KNO_2), Magnesium sulfate ($MgSO_4$), Potassium chloride (KCl), Sacharosse, Glucose, Glycerol. Prolabo (USA) supplied aluminum chloride ($AlCl_3$), ferric chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), and trichloroacetic acid. Biochem chemopharma Co. (Cosne-Courssur-Loire, France). supplied Folin–Ciocalteu reagent, Folin–Denis reagent, and hydrogen peroxide. Methanol (99.7% GC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide ($K_4Fe(CN)_6$), ascorbic acid, gallic acid, quercetin, vanillin, p-coumaric acid, vanillic acid, chlorogenic acid, rutin, naringenin, thiobarbituric acid (98%), sulfuric acid, and other chemicals, reagents, and organic solvents were purchased from Sigma-Aldrich (Burlington, MA, USA), (HiMedia, India), All the medium of microbiology assay such as Muller-Hinton broth, Muller Hinton agar, Sabouraud dextrose broth, and Sabouraud dextrose agar from Institute of Pastor, Alger's, Algeria.

1.2 Plant material

Aerial parts (leaves and seeds) of *Brassica oleracea var. elongata* (Brassicaceae) were collected in April 2022 from the Hassi Khelifa region (33°33'50.6"N, 7°0'19.3"E) in El-Oued, Southeast Algeria. Harvested leaves were air-dried away from direct sunlight and stored in sealed containers at room temperature until needed for experiments. Seeds were partially ground and used immediately for experimental procedures.

The tubers of *Bunium mauritanicum* (Apiaceae) were collected from N'Gaous region in Batana (East Algeria) between November 2022 and January 2023. The tubers were dried in the shade and ground into a fine powder using a laboratory mill. The plant was washed thoroughly and dried at room temperature for 20 days under conditions that protected it from moisture, light, dust, and dirt, while ensuring adequate ventilation. After drying, the tubers were crushed, and the powder was stored in a dark-colored glass container.

These plant species were identified taxonomically using botanical references: “Flore et végétation du Sahara” (Ozenda, 2004), “Nouvelle flore de l'Algérie et des régions désertiques méridionales” (Quézel & Santa, 1962), and the African Plants Database. The identification was confirmed by Pr. Chouikh Atef (Proffesseur & Head of Laboratory Biology, Environment and Health, El Oued University, Algeria) and Pr. Youcef Halis (Centre of Scientific and Technical Research on Arid Regions, Touggourt, Algeria).

Table 16*:* Site geography, collection dates, and altitude of studied plants.

Plant	Geographic Coordinates	Region	Collection Dates	Altitude
Brassica oleracea var. elongata	33°33'50.6"N, 7°0'19.3"E	El Oued, Hassi Khelifa	April 2022	35 m
B. mauritanicum	35° 33′ 43″ N, 5° 36′ 39″E	Batna, N'Gaous	November 2022	770 m

1.3 Animal materiel

In this study, 60 male Wistar rats, aged 10 weeks and weighing approximately 180 ± 5 g, their parents were obtained from the Pasteur Institute of Algiers. The rats were housed in the animal facility of the Molecular and Cellular Biology Department at El-Oued University, Algeria. The housing conditions were maintained at 22.27±0.15°C and a relative humidity of 72±1.62%. The rats had free access to standard rat food and tap water throughout the study. They were adapted to an inverse 12/12 hour light/dark cycle.

1.4 Microbial strains

The antimicrobial activity of plant materials was evaluated using laboratory reference strains (American Type Culture Collection ‘‘ATCC’’ for bacteria and yeast), obtained from Institute of Pastor Algeria, Alger’s: Gram-positive bacteria: *Staphylococcus aureus* ATCC 25932, Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, and the yeast *Candida albicans* ATCC 10231.

Methods

1.1 Survey methodology

Please note: This survey is completely anonymous.

Section 1: General Information

1. Age:
   - 18-30
   - 31-50
   - 51-65
   - Over 65
2. Gender:
   - Male
   - Female
3. Occupation:
   - General Public
   - Herbalist/Traditional Healer
   - Seller of Natural Remedies

Section 2: Traditional Medicine Use

1. Do you use traditional medicine to treat any health problems?
   - Yes
   - No
2. If yes, please describe the specific condition you are treating and the traditional remedies you have used, including the plants or ingredients involved, their preparation method, and dosage. (Please use the table below) .

Health condition or disease	Plant/Ingredient	Preparation Method	Dosage	Unsuitable for	Restrictions on Use	Region of Plants
☐ Type 2 Diabetes ☐ Anemia (Nutritional-Deficiency) ☐ Hypothyroidism ☐ Osteoarthritis ☐ Low Blood Pressure				Infants Children Adults Pregnant Women Breastfeeding Other..... It does not have any side effects for all categories, according to experience		

3. Have you observed any side effects from using these traditional remedies?
   - Yes
   - No
4. If yes, please describe the side effects you experienced.

1.2 In vitro study

1.2.1 Determination of nutrient and energy values

Given the importance of wild herbs in traditional medicine and in the human diet the animal that consumes it. Therefore, its nature and properties must be investigated of these herbs. This analysis aims to study the chemical composition of two plants. The nutritional value of dry plant matter was determined by determining the following criteria :

1.2.2 Moisture Content

- **Reason for Measurement**: Moisture content affects the shelf life, stability, and quality of food products. High moisture content can lead to microbial growth and spoilage.
- **Detection Method**: The sample is dried at 105°C until a constant weight is achieved. The loss in weight is calculated as the moisture content. The method worked based on what was mentioned in the ISO 1442:1997 manual (ISO, 1997).

1.2.3 Mineral Content

- **Reason for Measurement**: Mineral content provides essential nutrients vital for various bodily functions. Knowing the mineral content helps in nutritional labeling and dietary planning.
- **Detection Method**: The sample is ashed in a muffle furnace at 550°C until all organic matter is burnt. The remaining ash, which represents the mineral content, is weighed. The method worked based on what was mentioned in the ISO 5984:2002 manual (Standardization, 2002).

1.2.4 Protein Content

- **Reason for Measurement**: Proteins are crucial for developing, repairing, and maintaining body tissues. Measuring protein content is important for evaluating the nutritional value of food.
- **Detection Method**: The sample is digested with sulfuric acid, converting nitrogen to ammonium sulfate. This is then distilled and titrated to measure nitrogen content, which is converted to protein using a specific factor. The method worked based on what was mentioned in the ISO 5983-2:2009 manual (ISO, 1997).

1.2.5 Fat Content

- **Reason for Measurement**: Fat contributes to the energy content of food and is important for flavor, texture, and satiety. Measuring fat content helps in nutritional assessment and labeling.

- **Detection Method**: The sample is extracted with a solvent (hexane or petroleum ether) in a Soxhlet apparatus. The solvent is evaporated, and the remaining residue is weighed as the fat content. The method worked based on what was mentioned in the ISO 6492:1999 manual (Standardization, 1999).

1.2.6 Carbohydrate Content

- **Reason for Measurement**: Carbohydrates are a primary energy source. Measuring carbohydrate content is essential for understanding the energy contribution and nutritional balance of food.
- **Detection Method**: Carbohydrate content is calculated by subtracting the sum of moisture, protein, fat, and ash content from 100%. The method worked based on what was mentioned in the ISO 2171:2007 manual (Standard, 2007).

1.2.7 Calcium Content

- **Reason for Measurement**: Calcium is essential for bone health, muscle function, and nerve signaling. Determining calcium content helps in assessing the dietary intake of this vital mineral.
- **Detection Method**: The sample is digested in acid and analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), which measures the light emitted by excited calcium atoms. The method worked based on what was mentioned in the ISO 11885:2007 manual.

1.2.8 Magnesium Content

- **Reason for Measurement**: Magnesium is important for muscle and nerve function, blood sugar control, and bone health. Measuring magnesium content helps in evaluating its nutritional contribution.
- **Detection Method**: Similar to calcium, the sample is digested in acid and analyzed using ICP-OES to measure the light emitted by excited magnesium atoms. The method worked based on what was mentioned in the ISO 11885:2007 manual (Guide, 2007).

1.2.9 Sodium Content

- **Reason for Measurement**: Sodium is crucial for fluid balance, nerve transmission, and muscle function. Assessing sodium content is important for managing dietary intake, especially for individuals with hypertension.
- **Detection Method**: The sample is digested in acid and analyzed using ICP-OES to measure the light emitted by excited sodium atoms. The method worked based on what was mentioned in the ISO 11885:2007 manual.

1.2.10 Iron Content

- **Reason for Measurement**: Iron is essential for oxygen transport in the blood and various metabolic processes. Measuring iron content helps in preventing and managing iron-deficiency anemia.
- **Detection Method**: The sample is digested in acid and analyzed using ICP-OES to measure the light emitted by excited iron atoms. The method worked based on what was mentioned in the ISO 11885:2007 manual.

1.2.11 Zinc Content

- **Reason for Measurement**: Zinc is important for immune function, wound healing, and DNA synthesis. Determining zinc content is essential for nutritional evaluation.
- **Detection Method**: The sample is digested in acid and analyzed using ICP-OES to measure the light emitted by excited zinc atoms. The method worked on the basis of what was mentioned in the ISO 11885:2007 manual (Ciornea et al., 2021).

1.2.12 Energy Value

- **Reason for Measurement**: Energy value indicates the caloric content of food, which is important for dietary planning and weight management.
- **Detection Method**: Energy value is calculated using Atwater factors: 4 kcal/g for proteins and carbohydrates, and 9 kcal/g for fats. The total energy is the sum of these values based on measured contents. The method worked based on what was mentioned in the ISO 1871:2009 manual (Standardization, 2009).

❖ the nutritional composition and mineral content measured in the percentage of each nutrient (%)and parts per million(ppm).

$$\text{Equation 1: } Nutrient\ (\%) = \frac{Mass\ of\ the\ nutrient\ (in\ grams)}{Total\ mass\ of\ the\ substance\ (in\ grams)} \times 100$$

$$\text{Equation 2: } ppm = \frac{Mass\ of\ solute\ (in\ grams)}{Total\ mass\ of\ solution\ (in\ grams)} \times 100$$

1.2.13 Preparation of the extracts of plant material

The phytochemical study and biological tests were conducted using the methanolic and aqueous extracts. The methanolic extraction process involved immersing 50 g of *B. oleracea* or *B. mauritanicum* tubers in 500 mL of methanol for 24 hours. For the aqueous extract, 50 g of dried tubers was placed in 500 mL of water for 24 hours. Subsequently, all the extracts underwent additional filtration through Whatman filter paper N°1. The filtered extract was then concentrated using a rotavapor (Buchi R-200, Switzerland) at a temperature of 55°C.

Figure 4:Dried tubers of *B. mauritanicum* (Original Picture).

Figure 5:Dried leaves (A) and seeds (B) of *Brassica oleracea var. elongata* (Original Picture).

The yield of the extracts was determined using the following formula (Harborne, 1998) by Chouikh et al. 2015 (Chouikh *et al*., 2015) :

Equation 3:Yield (%) = (W1 / W2) x 100)

where:

- W_1 = weight of the extract dried (g).
- W_2 = weight of the plant starting material (g).

1.2.14 Chemical screening

To identify the major classes of compounds (tannins, saponins, flavonoids, alkaloids, phenols, reducing sugars, and terpenoids) present in the extracts of leaves and seeds of *B. oleracea* and *B. mauritanicum* tubers, a confirmatory qualitative phytochemical screening was performed using standard protocols (Kumar et al., 2020).

- **Phenols**

Introduce 5 ml of extract in a test tube and drops few of natural 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds.

- **Flavonoids**

In a test tube, introduce 5ml of extract, 5ml of diluted ammoniac and 1ml of H_2SO_4. The appearance of a yellow color indicates the presence of flavonoids.

- **Alkaloids**

1 ml of aqueous extract were treated with a few drops of hydrochloric acid then 1–3 drops of Wagner reagent were added. The appearance of brown precipitate reveals the presence of alkaloids in the sample.

- **Tannins**

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride ($FeCl_3$). The presence of tannins was indicated by a greenish or bluish-blackish coloration.

- **Terpenoids**

The formation of a reddish-brown color indicates the presence of terpenoids, through the addition of chloroform (2ml) and concentrated sulfuric acid (3 ml) to 5 ml of plant extract.

- **Reducing compound**

Add Fehling's liquor (1ml of reagent A and 1ml of reagent B) to the extract and incubate the whole in a boiling water bath, the appearance of a brick-red precipitate indicates the presence of reducing sugars.

- **Saponins**

In a test tube, introduce 5ml of extract, mixed with 5ml of distilled and with vigorous manual agitation. The formation of a steady foam indicates the presence of saponins.

- **Steroids**

For 1ml of plant extract, add 0.5ml of acetic acid solution, followed by 0.5ml of concentrated H_2SO_4. If the solution does not give any green color, it proves the presence of unsaturated steroids. In a second tube, the same volume of H_2SO_4 was added. The presence of the red color indicates the presence of steroid derivatives.

1.2.15 Determination of total polyphenols

The amount of total polyphenol was determined using Folin–Ciocalteu reagent (Obanda et al., 1997), according to a procedure described by Singleton and Rossi (Singleton & Rossi, 1965). Briefly, 0.2 mL of extract (0.2 mg of dry extract dissolved in 1 mL of methanol) was mixed with 1.8 mL of de-ionized water, was mixed with 1 mL of Folin–Ciocalteu reagent (10%), and 0.8 mL of 7.5% of sodium carbonate in a test tube. The development of blue color was monitored at 760 nm after 30 min. The TPC was quantified from the gallic acid calibration curve (0 to 120 µg/mL, R^2 = 0.9981), expressed as milligrams gallic acid equivalent (GAE) per g of dry weight of extract.

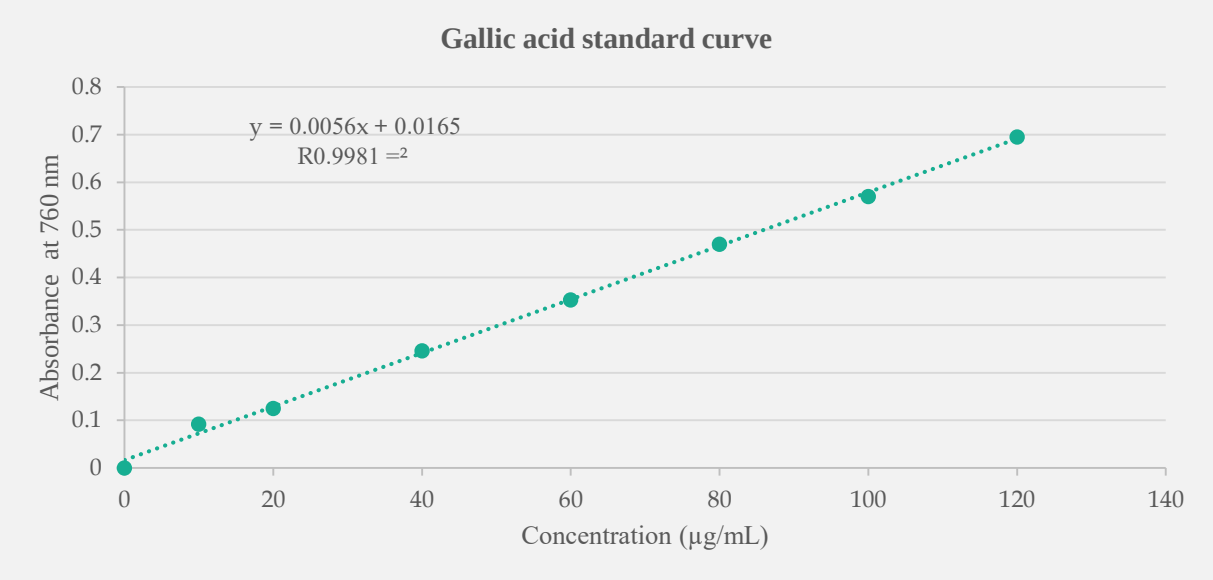


Figure 6: Standard curve of gallic acid for determination of total phenolic content

1.2.16 Determination of total flavonoids

The total flavonoïds content was determined by a colorimetric method as described in the literature (Singleton et al., 1999). 0.5 mL of extracts (0.2 mg of dry extract dissolved in 1 ml of H_2O) was added in 0.5 mL of 2% aluminium chloride ($AlCl_3$). After incubation at room temperature for 5 min, the absorbance of the mixture was measured at 430 nm. The TFC was quantified from the quercetin calibration curve (0 to 120 µg/mL, R^2 = 0.9978) expressed as milligrams of quercetin equivalents per gram (QEq/g) per g of dry weight of extract.

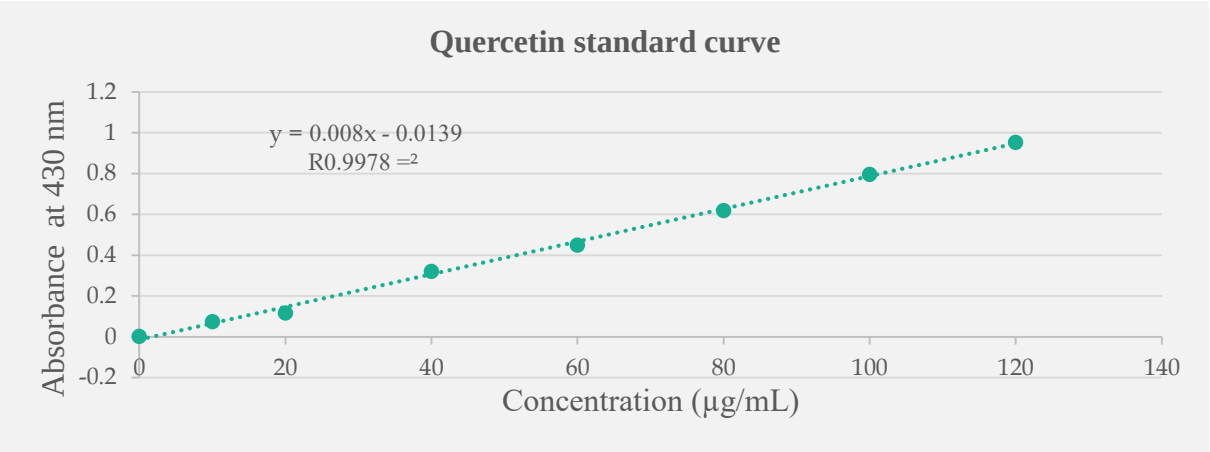


Figure 7: Standard curve of quercetin for estimating flavonoid content.

1.2.17 HPLC fractionation and analysis (RP-HPLC Analysis)

High-Performance Liquid Chromatography (HPLC) was employed using a UV-Vis Shimadzu LC 20 AL instrument with a universal injector (Hamilton, 25 µL) and a Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 µm) analytical column. A UV-VIS detector SPD 20A (Shimadzu) was used for the detection of phenolic compounds in the raw extract. The chromatographic separation process was performed using a gradient elution method with a mobile phase consisting of a mixture of acetonitrile and 0.1% acetic acid. Reverse phase chromatography analyses were executed using non-polar aliphatic components. A flow rate of 1 mL/min was maintained, and an injection volume of 0.45 µL was used. Absorbance was monitored at a wavelength of 268 nm, with both the sample and standard phases injected in volumes of 20 µL. Specific compound identification was accomplished by comparing their retention times and UV absorption spectra with those of established standards (Singleton et al., 1999).

1.2.18 Biological activities

1.2.18.1 Antioxidant Activity

1.2.18.1.1 DPPH• free radical scavenging assay

Using the methode outlined by (Cheng et al., 2006) , the potential radical-scavenging activity of *B. mauritanicum* extracts was assessed against DPPH free radicals. 1 mL of different concentrations of each extract was added to 1 mL of DPPH• solution (0.1× 10 mol), the mixture was left in the dark for 30 minutes at room temperature. At 517 nm, the absorbance of the solutions was measured. The test utilized Ascorbic acid as standards (Annexe 2).

Equation 4 :%The Percent inhibition (PI) = [(Abs c - Abs s) / Abs c) × 100[

- Abs s: absorbance of the sample or standard solution.

- Abs c: absorbance of control solution.

The radical scavenging potential of DPPH was estimated using the equation below to derive the IC_{50} values.

1.2.18.1.2 Ferric Reducing Antioxidant Power Assay (FRAP)

The Reducing power assay was prepared following the method of Benzi and Strin with some modifications (Benzie & Strain, 1999). To both extracts of *B. mauritanicum* and *B. oleracea* , phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide water solution (2.5 mL, K_3 [Fe $(CN)_6$]) were added at different quantities (mg/mL). A 10% aqueous solution of trichloracetic acid (2.5 mL) was added to the mixture, and it was incubated for 20 minutes at 50°C before being centrifuged for 10 minutes at 3000 rpm. At a wavelength of 700 nm, the absorbance was measured after mixing freshly prepared $FeCl_3$ (0.5 mL, 0.1%) solution with 2.5 mL of filtered water and the supernatant. As a positive control, ascorbic acid was used (Annexe 3)

1.2.18.1.3 β-Carotene Bleaching (BCB) assay

The β-carotene/linoleic acid bleaching assay was performed following the method described by Miraliakbari and Shahidi, with slight modification (Miraliakbari & Shahidi, 2008). To make a linoleic acid and beta-carotene stock solution. 400 µL of Tween 40 with 45 µL of linoleic acid, and 10 mL of chloroform were used to dissolve two milligrams of beta-carotene. The vacuum was used to extract the chloroform, and the residue was mixed with 100 mL of clean, aerated water. After the sample was prepared in distilled water at different concentrations (mg/mL), it was placed into separate test tubes and then filled with the aforementioned combination. After the sample was introduced to each tube, the zero-time absorbance at 470 nm was measured using a spectrophotometer. After that, the tubes were incubated for 50°C a hot water bath. The absorbance levels were measured again at two hours later at 470 nm. As the use of gallic acid served as a positive control (Annexe 4). A blank devoid of b-carotene was produced for background subtraction . To determine the antioxidant activity, the following equation was
applied:

Equation 5: Pourcentage of antioxidant activity

$$\text{Pourcentage of antioxidant activity}\% = 1 - \frac{Abc_{\text{control(120min)}} - Abc_{\text{sample(0min)}}}{Abc_{\text{control(120min)}} - Abc_{\text{control(120min)}}} \times 100$$

1.2.18.1.4 Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) was used to determine the total antioxidant activity of the different extracts of *B. mauritanicum* tubers and *B. oleracea*. (TAC) was determined as per the reported method (Prieto et al., 1999; Singleton & Rossi, 1965).

The assay based on the capacity of the extract to reduce Mo (VI)-Mo (V) and then form a green phosphate/Mo (V) complex at an acidic pH. The reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was combined with 0.3 mL of the extract and an aliquot of the extract. The mixture underwent a 90-minute incubation period at 95°C in a tube. At 695 nm, the absorbance of the solution relative to a blank was measured with a spectrophotometer. The mg of ascorbic acid equivalents per gram of excess were computed using the standard graph.

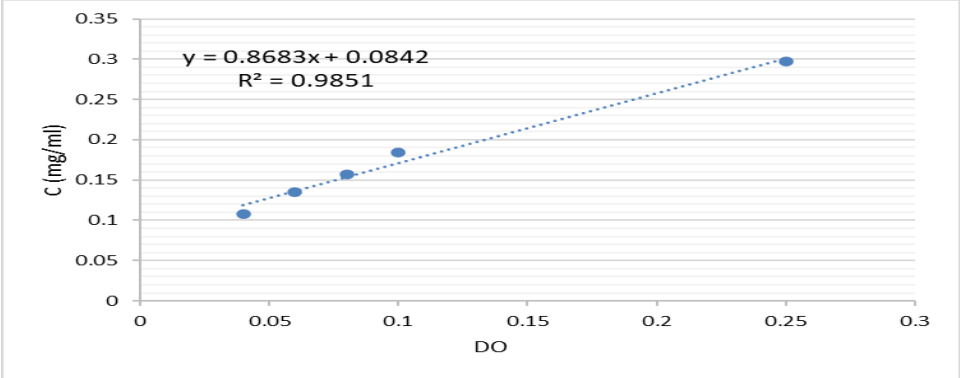


Figure 8:Calibration curve of ascorbic acid for total antioxidant capacity (TAC).

1.2.18.1.5 Determination of anti-hemolysis activity

Determination of anti-hemolysis activity by hydroxyl radical scavenging assay (HRS), the efficacy of the extracts in protecting red blood cells (RBCs) from demolition by exogenous oxidants (H_2O_2), was inspected by spectrophotometric procedure as described previously by Yang, et *al* (Yang et al., 2005). The extract (2 mL) was mixed with 40 µl of a human red blood cell (RBC) suspension for 5 minutes at 37°C. The mixture was incubated for one hour at 37°C after 40µL of 3×10^{-4}M H_2O_2, 40µL of 8×10^{-4}M $FeCl_3$, and 40µL of 5×10^{-4}M ascorbic acid were added, respectively, after incubation. The mixture centrifuged for five minutes at 700 rpm. The following equation used to calculate the hemolysis percentage based on the absorbance measured at 540 nm.

Equation 6:% of Hemolysis = [Abs Control / Abs Sample]×100

1.2.18.1.6 Sun Protection Factor (SPF) assays

The effectiveness of UV protection assessed through laboratory analysis of sunscreen products, measuring their Sun Protection Factor (SPF) values using a UV-Vis

spectrophotometer. This scientific method allows the evaluation of various experimental sunscreens for their ability to safeguard against harmful UV rays.

Mansur et al. (1986) (Mansur et al., 1986), devised a straightforward mathematical equation that replaces the *in vitro* method proposed by Sayre et al. (1979) (Sayre et al., 1979), employing UV spectrophotometry as follows:

Equation 7: $SPF = CF \times \sum EE(\lambda) \times I(\lambda) \times Abs(\lambda)$

Where: **EE(λ)**: erythemal effect spectrum; **I(λ)**: solar intensity spectrum; **Abs(λ)**: absorbance of the sunscreen product; **CF**: correction factor (= 10). The correction factor was determined so that a standard sunscreen formulation containing 8% homosalate yielded an SPF value of 4, as measured by UV spectrophotometry (Mansur et al., 1986).

Table 1 displays the normalized product function utilized in SPF calculation, based on the constants determined by Sayre et al.

Table 17: Normalized product function used in the calculation of SPF. (Sayre et al., 1979)

Wavelength (nm)	290	295	300	305	310	315	320	Total
EEM x I (normalized)	0.0150	0.0817	0.2874	0.3278	0.1864	0.0839	0.0180	1

EEM: erythemal effect spectrum; **I**: solar intensityspectrum.

According to the European recommendation in 2006 (Verheugen, 2006), sunscreen products should possess an SPF value of at least 6 and not exceed 50 . Furthermore, the SPF value should be accompanied by a qualitative description denoting the level of protection, categorized as low, medium, high, or very high protection (Tab. 17).

Table 18: The category of sunscreen products protection factor (Verheugen, 2006).

Protection Level	SPF Value
Low protection	6, 10
Medium protection	15, 20, 25
High protection	30, 40
Very high protection	50+

1.2.19 anti–inflammatory activity

In this present method evaluated by Chandra *et al.*(Chandra et al., 2012) the objective was to assess the in vitro anti-inflammatory activity of extracts from *B. mauritanicum and Brassica oleracea var. elongata* by examining their ability to inhibit the denaturation of egg albumin. The crude extracts concentrations were prepared ranging from 0.1 to 1 mg/mL in a reaction mixture consisting of 0.2 mL of fresh hen's egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 6.8), and 0.2 to 2 mL of the respective extract concentrations.

Negative controls were set up using a similar volume of double-distilled water and DMSO. The reaction mixtures were then incubated at 37°C ± 2°C in an incubator for 30 minutes and subsequently heated at 75°C ± 2°C for 10 minutes to induce denaturation. After cooling, the absorbance of each sample was measured at 660 nm using the vehicle as a blank.

Diclofenac sodium was used as a reference. The percentage protection from denaturation (% of PD) is calculated by using the following formula:

Equation 8 :%The percentage protection from denaturation = [(Abs C - Abs S) / Abs C] × 100

- Abs S: Absorbance of the test sample.
- Abs C: Absorbance of the control.

1.2.20 Antimicrobial activity

- **Wells diffusion method**

The disk diffusion method is the gold standard for confirming the susceptibility of bacteria. Standardized disk diffusion was introduced by Bauer and Kirby's experiments in 1959 (Bauer et al., 1959), Presently, the Clinical and Laboratory Standards Institute (CLSI) has issued multiple accepted and approved standards for bacteria and yeast testing (Barry, 2007). This study used the widely used method for evaluating the antimicrobial activity of plant or microbial extracts is the well-agar diffusion method. This method follows a procedure similar to the disc spread method, in which the volume of microbial inoculum is spread across the entire surface of the agar plate (Silva et al., 2013).

The antibacterial activity of the compound was assessed using the well diffusion method (WD) against four bacterial strains: *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. The compound was prepared at concentrations of 5 mg/mL and 10 mg/mL in DMSO. Mueller-Hinton agar was used as the growth medium. The bacterial strains were cultured on agar plates and incubated at 37°C for 24 hours. To ensure a consistent inoculum size, the bacterial cultures were mixed with 0.9% NaCl solution and adjusted to a turbidity equivalent to McFarland 0.5 standard ($10^{\wedge 8}$ CFU/mL).

Subsequently, the agar was poured into Petri dishes, and the bacterial suspension was swabbed evenly onto the agar surface. Wells with a diameter of 6 mm were created in the agar, and 50 µL of the compound at the respective concentrations were added to each well. The plates were then incubated at 37°C for 24 hours. The experiment was performed in triplicate to ensure accuracy and reproducibility. The inhibition Zone observed around the

wells indicated the antibacterial activity of the different extracts of *B. oleracea* against the tested bacterial strains.

- **MIC and MBC methods**

The broth microdilution assay is a standardized method for determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts against a variety of bacteria and yeast strains. The assay is performed by the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI) (Qaiyumi, 2007; Weinstein, 2018) (Wayne, 2010) (PA, 2002).

First, the bacterial and yeast suspensions are prepared. For bacteria, the strains are cultured on Mueller-Hinton agar (MHA) and then inoculated into cation-adjusted Mueller-Hinton broth (MHB). The cultures are incubated until visibly turbid and then diluted to a turbidity corresponding to 0.5 McFarland (1.5×10^8 CFU/mL) using BioMerieux *DensiCHEK Plus* for *VITEK* 2 Systems. For yeast, the strain is cultured on Sabouraud dextrose broth (SDB) and then diluted to a concentration of 10^6 CFU/mL.

Next, the plant extract solution is prepared by dissolving the extract in dimethyl sulfoxide (DMSO) to a concentration of 80 mg/mL. The solution is then homogenized by vortexing for 1 minute. The microtiter plate is set up by adding 50 µL of the plant extract solution to each well, the highest concentration incorporated into the plate is 40 mg/ml and the lowest achieved through double serial dilution is 1.25 mg/ml. Then, 50 µL of the bacterial or yeast suspension is added to each well. A growth control (no antibiotic, no xenobiotic) and a sterile control (MHB only) are included for all isolates (Schwalbe et al., 2007).

The microtiter plate is incubated at 37°C for 18-24 hours for bacteria and 48 hours for yeast. After incubation, the MIC is determined as the lowest concentration of plant extract that inhibits the growth of the bacteria or yeast. The MBC is determined by plating 10 µL from each well displaying no visible bacterial growth onto Sabouraud dextrose agar for yeast and MHA for bacteria. The extract with the lowest concentration that exhibits no bacterial growth (with 99% precision) is reported as the MBC concentration (Wayne, 2010).

After incubation for 24 h at 37 °C, resazurin (0.020 %) was added to all wells (20 µl per well), and further incubated for 72 min for the observation of colour change. On completion of the incubation, columns with no colour change (blue/purple) were scored as above the MIC value of plant extract that inhibits the growth of the bacteria or yeast (Elshikh et al., 2016) with some modification. The MBC is determined by plating 10 µL from each well displaying no visible bacterial growth onto Sabouraud dextrose agar for yeast and MHA for bacteria. The extract with the lowest concentration that exhibits no bacterial growth (with 99% precision) is reported as the MBC concentration (Wayne, 2010).

1.2.21 Antidiabetic activity

Commercial baker's yeast (*Saccharomyces cerevisiae*) Saf-Instant® 125g was washed by repeated centrifugation (3.000 × g, 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (40 mg – 5 mg) were added to 1 mL of glucose solution (25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension, vortex and further incubated at 37 °C for 30, 60 and 120 min. After that, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant (Burger et al., 1959; Cirillo, 1962).

The percent increase in glucose uptake by yeast cells was calculated using the following formula.

***Equation 9**:Increase in glucose uptake %= [(Abs control – Abs sample) /Abs control] × 100*

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. Absorbance was measured at 540 nm.

1.3 In vivo study

- **Animal Ethics**

All experimental procedures,used in this study as well as rat care and handling, adhered to the guidelines provided by the local ethics committee.The study is registered under the reference 06/2023 (Annexe 14).

- **Acute toxicity studies**

LD50 value of single different doses of the extract
via oral gavage were performed up till the dose
of 500 mg/kg bd. wt. Observing the rat mortality and

behavior were estimated for two weeks and the number of rats in this experiments n=6. Acute toxicity studies were preformed according to the method described by (Hu et al., 2012)

- **Preparation and Administration of Experimental Plants**

The experimental plants were administered as follows: TBM (tubers of *B. mauritanicum*), LBO (leaves of *Brassica oleracea var. elongata*), and SBO (seeds of *Brassica oleracea var.*) were dried, ground into a powder, mixed with water, and given to the animals in known concentrations.

1.3.1 Experimental Design

The experimental procedure lasted for 8 weeks. Initially, 60 male rats were housed in well-ventilated cages under hygienic conditions and fed a basal diet for one week for adaptation. The basal diet was prepared according to (Raiola et al., 2017). Measurements of weight, blood parameters, thyroid function tests, and histological sections were taken after two weeks. Blood was drawn two weeks after the induction of hypothyroidism and anemia to confirm the induced occurrence of each of these diseases and to note the change in the vital parameters. Blood was drawn for hematologic, biochemical, and hormonal analyses at the end of the experiment. The rats were then divided into nine groups, each consisting of six rats, as follows:

- **Induction of Hypothyroidism**

Hypothyroidism was induced in 24 rats using Propylthiouracil (PTU) at a dose of 10 mg/kg body weight via intraperitoneal injection for 15 days, following the method described by (Şener et al., 2006).

Groups:

- **Group A1 (Control):** Fed a basal diet (standard rat food and water).
- **Group A2 (Hypothyroidism Control):** Induced hypothyroidism without further treatment.
- **Group A3:** Induced hypothyroidism, treated with TBM (200 mg/kg body weight) via oral gavage for 4 weeks.
- **Group A4:** Induced hypothyroidism, treated with Levothyroxine (2.5µg/kg/Day).

Table 19: Steps for Inducing Hypothyroidism and Subsequent Procedures.

Step	Description	Duration	Details
1	Animal Adaptation	1 week	Basal diet feeding for all rats
2	Induction of Hypothyroidism	15 days	PTU injection (10 mg/kg Bwt, IP)
3	Clinical Signs or blood collection	2 weeks after induction	For diagnostic of hypothyroidism
4	Treatment Period	4 weeks	Oral gavage with plant or medications
5	Fasting Period	Overnight	Before sacrificing
6	Blood Collection	Post-sacrifice	For hematologic, biochemical, and hormonal analyses
7	Organ Dissection	Post-sacrifice	Removal and sectioning of kidneys, liver, and spleen

Induction of Anemia

Anemia was induced in 30 rats using Phenylhydrazine (PHZ) at a dose of 40 mg/kg body weight via intraperitoneal injection, twice a week for 2 weeks.

Groups:

- **Group B1 (Control):** Fed a basal diet (standard rat food and water).
- **Group B2 (Anemic Control):** Induced anemia without further treatment.
- **Group B3:** Induced anemia, treated with LBO (200 mg/kg body weight) via oral gavage for 4 weeks.
- **Group B4:** Induced anemia, treated with SBO (200 mg/kg body weight) via oral gavage for 4 weeks.
- **Group B5:** Induced anemia, treated with B-Feron® (50mg/kg boody weight),(Vidal)

Table 20: Steps for Inducing Anemia and Subsequent Procedures.

Step	Description	Duration	Details
1	Animal Adaptation	1 week	Basal diet feeding for all rats
2	Induction of Anemia	2 weeks	PHZ injection (40 mg/kg Bwt, IP, twice a week)
3	Clinical Signs	2 weeks after induction	For diagnostic of anemia
4	Treatment Period	4 weeks	Oral gavage with plant or medications
5	Fasting Period	Overnight	Before sacrificing
6	Blood Collection	Post-sacrifice	For hematologic and biochemical analyses
7	Organ Dissection	Post-sacrifice	Removal and sectioning of kidneys, liver, and spleen

Sacrifice, blood and tissues collection

After 12 hours of fasting, these animals were sacrificed under slight anesthesia by chloroform (94%) by inhalation; blood samples were collected during the slaughter of animals into EDTA tube to carried FNS and dry tubes. The serum was obtained by centrifugation for 10 min at 3000 tour/min and used for biochemical analysis assays; blood sugar level measured during rat's slaughter using glucometer. Then the kidneys, liver, and spleen were isolated from these animals and washed in normal saline. A piece of thekidneys, liver, and spleen of each group of these animals were takenand placed in 10% formaldehyde for histological analysis.

- **Hematological parameters**

The determination of hematological parameters performed using fully Auto Blood CellCounter (ERMA) commercial reagent kits from Biomeghreb (Tunisia) using manualanalyzer.

- **Biochemical parameters analysis**

Serum lipid levels were determined using the commercial kit from spinreact, Spain (ref: cholesterol-20111, triglyceride-20131). And for enzymes are also measured by the use of commercial kits (Spinreat_ ref GOT-20042, GPT-20046).

- **Histopathological study of kidneys, liver, and spleen tissues**

After rats sacrificed, kidneys, liver, and spleen tissues were removed and immersed in fixative (solution 36% formaldehyde) intel the time of slices preparation. Whish dehydrated in ascending graded series of ethanol, cleaned with toluene, immersed in paraffin, and colored with hematoxylin and eosin. Histopathological evaluation was performed , by light microscope with H&E staining. (Dark arrow):

Figure 9:Organ tissue inclusion stage (Original Picture).

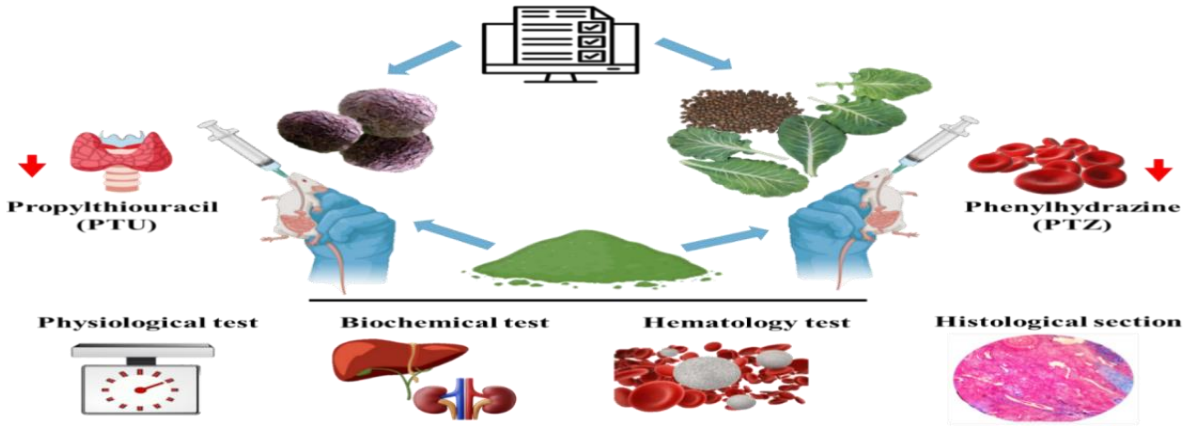


Figure 10: **.**Summary diagram of the experimental protocol (Original Picture).

1.4 Statistical analysis

All assays were performed in triplicate and results are expressed as mean ± SEM. *In vitro* assay data were analyzed using GraphPad Prism 8.0.2 software for Windows to determine the EC_{50} and IC_{50} values. The significance level was set at $\alpha = 0.05$ and the threshold for significance was defined as $p < 0.05$. P-values are displayed in figures as follows: ***, $p < 0.001$; **, $p < 0.01$; and ns, $p > 0.05$.

CHAPTER II

Results and Discussion

CHAPTER II: Results and Discussion

Results

1.1 Survey results

This survey was conducted on 105 randomly selected individuals from various social classes in the El Oued region of Algeria, from February to June 2022. The primary objective was to investigate traditional medical practices and identify commonly used herbal remedies and their applications in treating prevalent diseases.

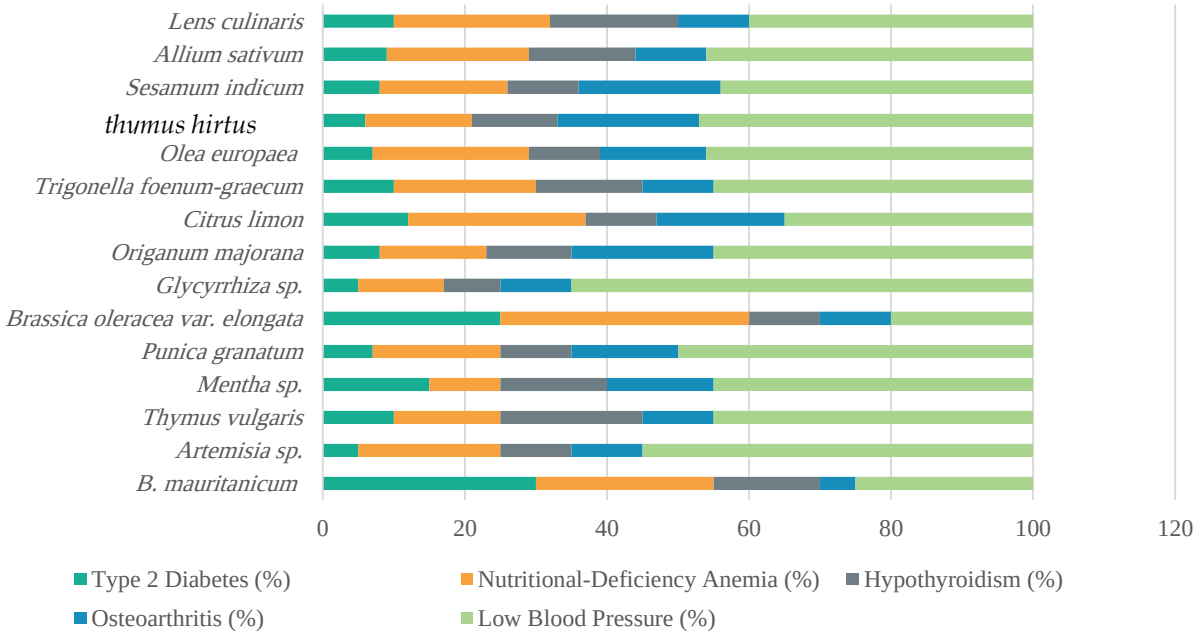


Figure 11: Graphical representation showing the medicinal uses of different types of medicinal plants according to the results of the survey.

Provided valuable insights into traditional medical practices and commonly used herbal remedies. The most common diseases identified in the region, which were determined based on a field survey involving the database of medical analysis laboratories and consultations with several private doctors, include Type 2 diabetes, nutritional deficiency anemia, hypothyroidism, osteoarthritis, and low blood pressure. Among the medicinal plants, *B. mauritanicum tubers*, *Brassica oleracea var. elongata*, and *Citrus limon* were frequently mentioned for their widespread applications. *B. mauritanicum tubers* were notably used for Type 2 diabetes (30%) and nutritional-deficiency anemia (25%). In contrast, *Brassica oleracea var. elongata* was primarily used for nutritional-deficiency anemia (35%) and Type

2 diabetes (25%). The diversity in the use of these plants reflects the local population's belief in their efficacy for multiple ailments, highlighting the cultural significance and traditional knowledge embedded in their medicinal practices. The survey results underscore the importance of these plants in managing prevalent health conditions within the community.

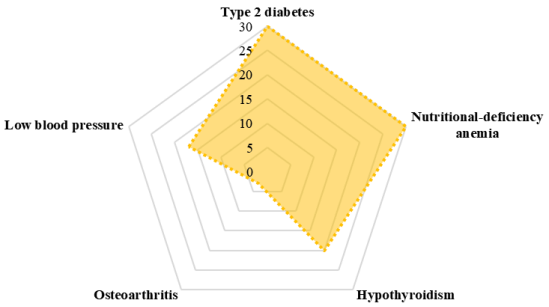


Figure 12:Radar chart showing the medicinal uses of Bunium mauritanicum tubers in the El Oued area, according to the results of the survey.

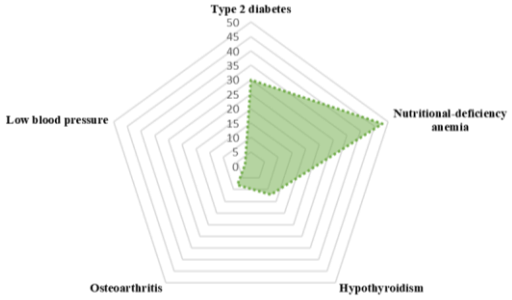


Figure 13: Radar chart showing the medicinal uses of *Brassica oleracea* var. elongata in the El Oued area, according to the results of the survey

- The survey results indicate distinct medicinal uses for *Bunium mauritanicum* tubers and *Brassica oleracea var. elongata* in the El Oued area. *Bunium mauritanicum* tubers are predominantly used for managing Type 2 diabetes (30%) and nutritional deficiency anemia (25%), highlighting their significant role in treating these prevalent conditions. Additionally, they are utilized for hypothyroidism (15%), low blood pressure (25%), and to a lesser extent, osteoarthritis (5%). On the other hand, *Brassica oleracea var. elongata* is primarily used for nutritional-deficiency anemia (35%), reflecting its importance in addressing this common health issue. It is also employed for Type 2 diabetes (25%), hypothyroidism (10%), osteoarthritis (10%), and low blood pressure (20%). These findings underscore the diverse applications of these plants in traditional medicine, demonstrating their perceived efficacy in treating a range of ailments as believed by the local population Based on the survey results and the observed scarcity of laboratory research studies on the use of *Bunium mauritanicum* tubers and *Brassica oleracea* var. *elongata* in the region for treating the most common diseases, these plants were selected for further in vitro and in vivo research. This selection aims to fill the existing knowledge gap, document the traditional uses scientifically, and verify their biological and therapeutic effectiveness. Such studies are crucial for validating the medicinal properties of these plants and potentially integrating them into modern therapeutic practices.

1.2 In vitro study

1.2.1 Nutrient and energy values

This method enabled precise determination of the proportions of protein, fat, carbohydrates, minerals, and moisture in these plant sources (Tab. 21). It is a fundamental approach in nutritional analysis, essential for comprehensively understanding the dietary and biological characteristics of these botanical specimens. The nutritional composition of *B. mauritanicum* tubers, *B. oleracea* leaves, and seeds was evaluated by calculating the percentage of each nutrient The mineral content of nutritional composition *B. mauritanicum* tubers, *B. oleracea* leaves, and seeds was analyzed for calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), and zinc (Zn) concentrations (Tab. 21), measured in parts per million (ppm)

Table 21:Nutritional Composition of *B. mauritanicum tubers, and B. oleracea l var. elongata* eaves and seeds.

Parameter	*B. mauritanicum* tubers	*B. oleracea* seeds	*B. oleracea* leaves
Moisture (%)	7.00	3.49	6.91
Mineral (%)	3.85	4.20	26.05
Proteins (%)	7.58	29.53	23.17
Fat (%)	11.53	33.04	12.47
Carbohydrate (%)	66.20	11.65	7.87
Calcium (Ca) ppm	4785.20± 0.344	0.80± 0.173	5.31± 0.134
Magnesium (Mg) ppm	967.27± 0.098	0.11± 0.034	0.46± 0.265
Sodium (Na) ppm	525.72± 0.332	0.05± 0.341	1.10± 0.341
Iron (Fe) ppm	96.89± 0.108	114.13± 0.772	386.20± 0.188
Zinc (Zn) ppm	23.39± 0.083	53.53± 0.111	47.45± 0.112

The findings of a comparative analysis conducted on the nutritional composition of *B. mauritanicum* tubers, *B. oleracea var. elongata* seeds, and leaves (Tab.21) reveal distinct nutritional profiles across these plant samples. *B. mauritanicum* tubers exhibited a notably high carbohydrate content (66.20%), suggesting a significant source of dietary energy derived from sugars and starches. In contrast, *B. oleracea* seeds stood out for their substantial levels of protein (29.53%) and fat (33.04%), indicating their potential as rich sources of essential macronutrients. However, it was B. oleracea leaves that displayed exceptional mineral richness, particularly in sels minéraux (26.05%) and calcium content (5.31 ppm), highlighting their potential dietary importance for mineral supplementation. When comparing the amount of minerals across the samples, *B. oleracea* leaves showed significantly higher concentrations

of iron (386.20 ppm) and zinc (47.45 ppm) compared to *B. mauritanicum* tubers and seeds. *B. mauritanicum* tubers, on the other hand, exhibited higher levels of magnesium (967.27 ppm) relative to *Brassica oleracea var. elongata* seeds and leaves. These variations underscore the diverse mineral compositions of these plant species, emphasizing their potential contributions to dietary intake and nutritional strategies.

-The (Tab. 22) presents the energy values of *B. mauritanicum* tubers, *B. oleracea* seeds, and leaves, measured in kilojoules per 100 grams (Kj/100g) and kilocalories per 100 grams (Kcal/100g).

Table 22: Energy values of *B. mauritanicum tubers, and B. oleracea var. elongata* leaves and seeds.

Parameter	*B. mauritanicum* tubers	*B. oleracea* seeds	*B. oleracea* leaves
Kj/100g	1680.97± 0.012	1737.24± 0.072	691.65± 0.015
Kcal/100g	398.91± 0.133	416.88± 0.133	164.05± 0.344

Among the samples, *Brassica oleracea var. elongata* seeds exhibited the highest energy values, with 1737.24 Kj/100g and 416.88 Kcal/100g, indicating their dense energy content compared to the other samples. *B. mauritanicum tubers* followed closely with 1680.97 Kj/100g and 398.91 Kcal/100g, highlighting their significant energy contribution primarily from carbohydrates. Conversely, *B. oleracea* leaves showed the lowest energy values, with 691.65 Kj/100g and 164.05 Kcal/100g, suggesting a lower calorific content relative to the tubers and seeds.

1.2.2 Chemical screening

Phytochemical analysis of the aqueous and methanolic extracts of *B. mauritanicum* tubers revealed the presence of various secondary metabolites, including alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, steroids, and reducing sugars, in varying concentrations. This indicates the richness of this plant part in effective secondary metabolic substances. (Tab. 23) summarizes the secondary metabolites found in the aqueous and methanolic extracts of *B. mauritanicum* tubers.

Table 23: Preliminary phytochemical screening of *B. mauritanicum tubers* extracts, using chemical test methods.

Secondary Metabolite		AqE	MeE
Alkaloids	**Observation**	White precipitate	No
	Results	+	-
Flavonoids	**Observation**	Intense red	
	Results	+++	+++

Terpenoids	Observation	Purple ring	
	Results	+	+
Phenols	**Observation**	Dark blue-green	
	Results	+++	+++
Tannins	**Observation**	Dark -green color	
	Results	++	+
Saponins	**Observation**	Forms foam in all tubes	
	Results	+++	++
Reducing sugars	**Observation**	Brown	
	Results	+++	+++
Steroids	**Observation**	Red	
	Results	+	+

No : no observed reaction. (+++ High concentration, ++ moderate concentration, + low concentration), -: Absence or not detected; (AqE: aqueous extract, MeE: methanolic extract).

The methanolic extract (MeE) of *B. mauritanicum* tubers (Tab. 23) demonstrates a richer content of secondary metabolites compared to the aqueous extract (AqE). For example, the MeE contains a high concentration of flavonoids, phenols, and reducing sugars, whereas the AqE also shows high concentrations of flavonoids, phenols, saponins, and reducing sugars.

(Tab. 24**)** summarizes the findings of the initial phytochemical analysis conducted on methanolic and aqueous extracts derived from the leaves and seeds of *B. oleracea*. The screening process aimed to detect the presence of various secondary metabolites, including alkaloids, flavonoids, terpenoids, phenols, tannins, saponins, and reducing sugars in these extracts.

Table 24:Preliminary phytochemical screaning of *Brassica oleracea var. elongata* leaves and seeds extracts, using chemical test methods.

Secondary metabolites	Sample	LM	LA	SM	SA
Alkaloids	Observation	White precipitate			
	Results	+	+	+	+
Flavonoïds	Observation	Intense Red	Red-Pink	Intense Red	Pink
	Results	+++	++	+++	+
Terpenoids	Observation	Purple ring			
	Results	+	+	+	+
Phenols	Observation	Dark Blue-Green	Green	Dark Blue-Green	Pale Green
	Results	+++	++	+++	+
Tannins	Observation	Black color	Bluish-Green color	Black color	Bluish-Green color
	Results	+	-	+	-
Saponins	Observation	-	++	-	+
	Results	No	Forms foam in all tubes	No	Forms foam in all tubes
Reducing sugars	Observation	Brown	Brick-Red	Brick-Red	Brick-Red
	Results	++	+	+	+

*No : no observed reaction. +: presence (+++ High concentration, ++ moderate concentration, + low concentration), -: Absence or not detected; (LA: leaves aq

the methanolic extract of leaves (LM) and seeds (SM) of *Brassica oleracea var. elongata* are richer in secondary metabolites compared to their respective aqueous extracts (LA and SA) **(**Tab. 24**)**. For instance, both LM and SM show a high concentration of flavonoids and phenols, while the aqueous extracts display lower concentrations: LA has moderate and SA has low amounts of these metabolites. Additionally, alkaloids and terpenoids are consistently present in all extracts, but tannins are only detected in methanolic extracts (LM and SM) and absent in aqueous extracts (LA and SA).

1.2.3 Phytochemical Study

1.2.3.1 Yield , Total polyphenols and flavonoids contents

The (Tab. 25) shows the yield and polyphenol and flavonoids contents of aqueous and methanolic extracts of *B. mauritanicum* tubers. The yield percentage indicates the amount of extract obtained from the plant material. In this case, aqueous extract (AqE) has a slightly higher yield (15.08%) compared to the methanolic extract (MeE) (12.23%) , suggesting that methanol is more efficient in extracting compounds from the tubers because it was the richest one in polyphenols and flavonoids. Moving on to the content of polyphenols and flavonoids, the results (Tab.25) indicate that the methanolic extract of *B. mauritanicum* tubers. has a higher concentration of both polyphenols (0.509 ± 0.141 mg of GAEq/g of extract) and flavonoids (0.097 ± 0.030 mg QEq/g of extract) compared to the aqueous extract(polyphenols: 0.352 ± 0.112 mg of GAEq/g of extract; flavonoids: 0.078 ± 0.023 mg QEq/g of extract).

The study investigated the total phenols and flavonoids content in different Brassica oleracea var. elongata leaf and seed extracts (Tab. 25). Methanolic extracts generally exhibited higher phenols and flavonoids levels compared to aqueous extracts. Methanolic seed extract had the highest phenols content (4.399±0.14 mg GAEq/g Ex), while the aqueous seed extract showed slightly lower phenols content (2.160±0.13 mg GAEq/g Ex). Similar trends were observed for flavonoids, with the methanolic seed extract (0.432±0.15 mg QEq/g Ex) surpassing the aqueous seed extract (1.241±0.17 mg QEq/g Ex). Methanolic leaf extract exhibited higher phenols content (3.462±0.17 mg GAEq/g) compared to the aqueous leaf extract (2.015±0.2 mg GAEq/g Ex), but lower flavonoids content (0.671 ± 0.05 mg QEq/g Ex) compared to the aqueous leaf extract (1.399 ± 0.17 mg QEq/g Ex).

Table 25: Yield Polyphenols and flavonoids contents in of *B. mauritanicum and Brassica oleracea var. elongata* extracts.

B. mauritanicum		**AqE**		**MeE**	
	Yield (%)	15.08%		12.23%	
	Polyphenols (mg of GAEq/g of extract)	0.352 ± 0.112		0.509 ± 0.141	
	Flavonoïds (mg QEq/g of extract)	0.078 ± 0.023		0.097 ± 0.030	
B. oleracea		**LM**	**LA**	**SM**	**SA**
	Yield (%)	9.52 %	%15.45	%6.18	% 7.75
	Polyphenols (mg of GAEq/g of extract)	3.462 ± 0.161	2.015 ± 0.192	4.399 ± 0.140	2.160 ± 0.123
	Flavonoïds (mg QEq/g of extract)	0.671 ± 0.054	1.399 ± 0.174	0.432 ± 0.152	1.241 ± 0.174

- Data are expressed as means ± SD of triplicate experiments.

1.2.3.2 Phenolic compounds profile crude extracts by RP-HPLC

Table 26: Retention time and the concentration of phenolic and flavonoids compounds identified of *B. mauritanicum. and Brassica oleracea var. elongata.* extracts.

	RP-HPLC standards					Content (µg/100 mg ED)			
	N°	Compounds		Retention Time (min)	Linear equation	LM	LA	SM	SA
B. oleracea var. elongata	1	**Phenolic acid**	Gallic acid	5.29	y = 54681x	811.59	265.71	1180.20	14.60
	2		Chlorogenic acid	13.39	y = 21665x	3330.89	690.84	626.26	790.12
	3		Vanillic acid	15.53	y = 65077x	4.72	434.02	2769.18	9.09
	4		Caffeic acid	16.27	y = 84066x	N.D.	62.63	N.D.	231.84
	5	**Flavonoide**	Vanillin	21.46	y = 58930x	485.67	95.34	461.61	247.93
	6		p-Coumaric acid	23.81	y = 49495x	N.D.	N.D.	345.37	32.90
	7		Rutin	28.37	y = 28144x	745.48	27.74	6751.99	36.96
	8		Naringin	34.78	y = 19379x	2776.25	N.D.	N.D.	48.56
	1		Quercetin	45.04	y = 45378x	5512.58	N.D.	3717.39	N.D.
						AqE		MeE	
B. mauritanicum	1	**Phenolic acid**	Gallic acid	5.29	y=54681x	224.450		69.584	
	2		Chlorogenic acid	13.392	y=21665x	N.D.		30.6	
	3		Vanillic acid	15.531	y=65077x	N.D.		N.D.	
	4		Caffeic acid	16.277	y=84066x	N.D.		N.D.	
	5	**Flavonoide**	Vanillin	21.46	y=58930x	18.3		N.D.	
	6		p-Coumaric acid	23.817	y=49495x	N.D.		N.D.	
	7		Rutin	28.37	y=28144x	178.631		N.D.	
	8		Naringin	34.788	y=19379x	N.D.		N.D.	
	9		Quercetin	45.047	y=45378x	3735.49		3325.408	

- N.D. Not Detected, Where: ED : Dry Extract.

The quantitative analysis and characterization of phenolic compounds in the aqueous (AqE) and methanolic (MeE) extracts of *B. mauritanicum* using HPLC are presented in **(**Tab. 26**)**. The methanolic extract (MeE) exhibited a more complex phenolic profile with 45 peaks compared to 44 peaks in the aqueous extract (AqE). Notably, gallic acid was present in higher concentrations in both extracts, with AqE containing 224.450 µg/100 mg and MeE containing 69.584 µg/100 mg, indicating its significant contribution to the phenolic content of these extracts.

However, compounds such as chlorogenic acid, vanillic acid, caffeic acid, vanillin, p-coumaric acid, rutin, and naringin were not detected in either AqE or MeE (Figure 1). In contrast, quercetin, a well-known flavonoid with antioxidant properties, was found in MeE and AqE extract at a concentration of 3325.408 µg/100 mg.and 3735.49 µg/100 mg.respectively. This discrepancy in phenolic composition between the two extracts underscores the potential of MeE to provide valuable bioactive compounds.

The High-Performance Liquid Chromatography (HPLC) analysis **(**Tab. 26**)** revealed distinct patterns of phenolic compounds in various extracts of *B. oleracea*. Gallic acid was present in high concentrations in the methanolic leaf and seed extracts, with values of 811.59±0.02 µg/100 mg and 1180.20 µg/100 mg, respectively. Chlorogenic acid exhibited high concentrations in the methanolic seed and aqueous seed extracts, with values of 626.26±0.01µg/100 mg and 790.12±0.02 µg/100 mg, respectively.Vanillin was detected in the methanolic leaf and seed extracts, with values of 485.67±0.03 µg/100 mg and 461.61±0.01 µg/100 mg, respectively. *P*-coumaric acid was found in the methanolic seed and aqueous seed extracts, with values of 345.37±0.02 µg/100 mg and 32.90±0.02 µg/100 mg. Vanillic acid was observed solely in the methanolic leaf extract, with a value of 4.72±0.03 µg/100 mg. Naringin was detected in the methanolic leaf and aqueoud seed extracts, with values of 2776.25±0.03 µg/100 mg and 48.56±0.03 µg/100 mg, respectively. Quercetin was present exclusively in the methanolic extracts leaf and seed , with a value of 5512.58±0.02 µg/100 mg.and 3717.39 µg/100 mg respectively.

1.2.4 Biological Activities *in vitro*

1.2.4.1 Antioxidant Power Assay (DPPH, FRAP, β-Carotene/Linoleic assay, and Anti-hemolysis activities)

Table 27: Comparative Antioxidant Potency of *B. mauritanicum and Brassica oleracea var. elongata* leaves and seeds , extracts and reference compounds using DPPH, FRAP, and β-Carotene/Linoleic assays, and Anti-hemolysis activity.

	Test	Factor	AqE		MeE		AAs
B. mauritanicum	**DPPH activity**	**IC_{50} (mg/ml)**	0.288 ± 0.050 **		0.4093 ± 0.084**		0.0145 ± 0.005
	FRAP activity	**EC_{50} (mg/ml)**	0.0442 ± 0.055 **		0.152 ± 0.001 ***		0.068 ± 0.001
	β-carotene/linoleic acid assay	**IC_{50} (mg/ml)**	1.856 ± 0.051***		0.465 ± 0.058 *		0.157 ± 0.001
	TAC values	**(mg EAA/g extract)**	0.717 ± 0.032 ***		0.367 ± 0.063 *		0.011 ± 0.044
	Anti-hemolysis activity	**Hly_{50} (mg/ml)**	0.093 ± 0.066**		0.097 ± 0.075**		0.026 ± 0.053
B. oleracea			**LM**	**LA**	**SM**	**SA**	**AAs**
	DPPH activity	**IC_{50} (mg/ml)**	0.058 ± 0.001**	2.097 ± 0.08***	0.200 ± 0.001**	0.337 ± 0.008**	0.006 ± 0.001
	FRAP activity	**EC50 (mg/ml)**	0.814 ± 0.052**	3.134 ± 0.096****	0.873 ± 0.030**	1.515 ± 0.014****	0.021 ± 0.003
	β-carotene/linoleic acid assay	**IC_{50} (mg/ml)**	0.076 ± 0.001***	0.049 ± 0.001**	0.134 ± 0.009***	0.047 ± 0.001**	0.023 ± 0.001(BHT).
	TAC values	**(mg EAA/g extracts)**	0.118 ± 0.001***	0.121 ± 0.010***	0.235 ± 0.001***	0.150 ± 0.003***	0.018 ± 0.001
	Anti-hemolysis activity	**Hly50 (mg/ml)**	0.081 ± 0.033NS	0.008 ± 0.015**	0.095 ± 0.054NS	0.080 ± 0.005*	0.075 ± 0.001

- The values are expressed as mean ± standard deviation (SD). comparison with Ascorbic acid standard : * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$); *** very highly significant difference ($P < 0.001$) . Ns : no significant AAs, Ascorbic acid.

The antioxidant potential of *B. mauritanicum* extracts was evaluated through various assays including DPPH, FRAP and β-carotene/linoleic acid assay, total antioxidant capacity and Anti-hemolysis **(**Tab. 27**)**. The aqueous extract (AqE) of *B. mauritanicum* extracts displayed a notable IC_{50} value of mg/ml 0.288 ± 0.050 mg/mL in the DPPH assa and, highly significant difference compared to IC_{50} value of ascorbic acid. indicating its superior scavenging capacity.

In the β-carotene/linoleic acid assay, MeE extract demonstrated significant effects, with IC_{50} value of IC_{50} 0.465 mg/mL compeared,to IC_{50} of ascorbi cacid.0.157 mg/mL These results are in line with a previous study where the tuber extract of *B. mauritanicum* showed an IC_{50} of 0.065 mg/mL in the DPPH assay. In the FRAP test, the aqueous extract exhibited better reducing ability compared to the methanol extract and ascorbic acid, with an $\mathbf{EC_{50}}$ value of 0.0442 mg/mL highly significant difference. Additionally, the phosphomolybdate assay showed that AqE had the highest total antioxidant capacity (TAC) value, followed by MeE and.showed very highly significant difference compared to (TAC) value of ascorbic acid used as a reference.

In terms of anti-hemolysis activity measured by the HRS method, AqE and MeE demonstrated values of Hly_{50} = 0.093 mg/mL and 0.097 mg/mL, respectively, while ascorbic acid exhibited a lower value of 0.026 mg/mL and.showed highly significant difference compared to ascorbic acid

The antioxidant activity of various *B. oleracea var. elongata* extracts was assessed using DPPH, *β*-Carotene bleaching method, Anti-hemolysis, FRAP, and TAC assays **(**Tab. 27**)**. The methanolic leaf extract exhibited the strongest antioxidant activity in the DPPH assay ($IC_{50:}$ 0.058±0.001 mg/mL and very highly significant difference compared to ascorbic acid), while the aqueous leaf extract showed the highest activity in the *β*-Carotene bleaching method : (IC_{50} 0.049±0.001 mg/mL). The aqueous leaf extract demonstrated the strongest anti-hemolysis activity (Hly_{50}: 0.008±0.015 mg/mL), while the methanolic leaf extract had the highest reducing power in the FRAP assay (EC_{50}: 0.814±0.052 mg/mL and very highly significant difference compared to ascorbic acid). In the TAC assay, the methanolic leaf extract exhibited the strongest total antioxidant capacity (IC_{50}: 55.274±0.007 mg/mL).

β-Carotene bleaching method: The aqueous extracts of leaves (LA) and seeds (SA) showed the highest antioxidant activity with EC_{50} values of 0.049±0.001 mg/mL and 0.047±0.001 mg/mL, respectively. The methanolic extract of leaves (LM) had an EC50 value of 0.076±0.001 mg/mL, while the methanolic extract of seeds (SM) had an EC_{50} value of 0.134±0.009 mg/mL.

ascorbic acid used as a reference, had an EC_{50} value of 0.023±0.001 mg/mL.and very highly significant difference ($P < 0.001$) compared to EC_{50} value of of ascorbic acid .

1.2.4.2 Sun Protection Factor SPF assay

The Sun Protection Factor (SPF) is a critical measure for evaluating the effectiveness of sunscreens in preventing sunburn and skin damage. SPFs are classified into low (SPF < 12), moderate (SPF = 12–30), and high (SPF ≥ 30) protection categories. The study's findings indicate that compared to the commercial sunscreen Avene® (SPF 41 ± 4), the efficacy of the extracts varies significantly difference. *B. oleracea* extracts show high SPF values **(**Tab. 28**)**, with the methanolic extract (SM) at 45.58 ± 10 and significant difference ($P < 0.05$) compared to sunscreen Avene®. indicating strong sun protection, while the aqueous extract (LA) has moderate protection at 14.77 ± 8. In contrast, *B. mauritanicum* extracts have low SPF values, with the aqueous (AqE) and methanolic (MeE) extracts at 4.994 ± 0.037 and 4.312 ± 0.033, respectively, indicating minimal sun protection. This analysis highlights that while *B. oleracea* extracts, particularly the methanolic extract, can offer strong sun protection, *B. mauritanicum* extracts provide significantly lower protection compared to the commercial product.

Table 28: Measured SPF values of *B. mauritanicum and B. oleracea var. elongata* extracts and commercial sunscreens *(*Avene®).

	SPF values				
B. oleracea	LM	LA	SM	SA	*Avene®*
	38.05 ±6*	14.77 ±8**	45.58 ±10*	27.95 ±5**	41±4
B. mauritanicum	AqE		MeE		*Avene®*
	4.994±0.037***		4.312±0.033***		41±4

- The values are expressed as mean ± standard deviation (SD). comparison with *Avene®* standard : * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$); *** very highly significant difference ($P < 0.001$) Ns : no significant .

1.2.4.3 In vitro anti-inflammatory activity

The results of anti-anflamatory activity of *B. mauritanicum* and *B. oleracea var. elongata* extracts seeds and leavs showed the figures 14 and 15 :

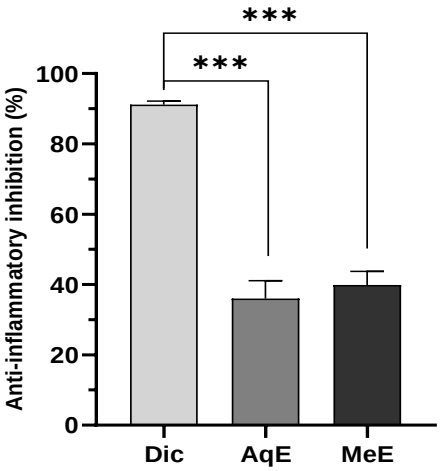


Figure 14: Anti-inflammatory inhibition percentages of tubers extract of *B. mauritanicum*

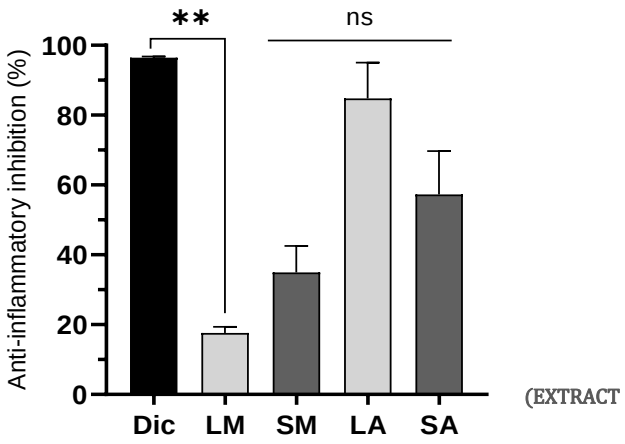


Figure 15: Anti-inflammatory potential of aqueous a nd methanol extracts of leaves and seeds *B. oleracea*.

- the values are expressed as mean ± standard deviation (SD). comparison with diclofenac standard : ** highly significant difference (P < 0.01); *** very highly significant difference (P < 0.001) Ns : no significant .-.(LA: leaves aqueous extract, LM: leaves methanolic extract, SA: Seeds aqueous extract, SM: Seeds methanolic extract)

The anti-inflammatory activity of *B. mauritanicum* was evaluated using its aqueous extract (AqE) and methanolic extract (MeE) compared to the reference compound Diclofenac (Dic), as depicted in **(**Fig. 14**)**. The results reveal that AqE extract exhibits an anti-inflammatory inhibition of 32.12%, while MeE demonstrates a slightly higher inhibition of 35.33%. In contrast, Diclofenac shows a significantly higher anti-inflammatory inhibition of 92.13%,(very highly significant

Compqred to AqE and MeEe extracts.) indicating its superior efficacy in reducing inflammation compared to the extracts. This data suggests that while both extracts of *B. mauritanicum* have measurable anti-inflammatory effects, they are markedly less effective than Diclofenac.

-The anti-inflammatory activity of *B. oleracea* extracts, particularly in the aqueous leaf extract, was notably high (94.8%) compared to the methanolic extract of leaves (17.6%). **(**Fig. 15**)**. This indicates the potential of these extracts in reducing inflammation, with the choice of solvent for extraction influencing the potency of anti-inflammatory compounds present. The

aqueous leaf extract exhibited the highest inhibition percentage (94.8%), closely matching that of the reference compound diclofenac (96.46%)%) and showed that there**(** aqueous leaf extract**.)** no signifcant differences compared to diclofenac standard.. the methanolic extract of leaves showed very highly significant difference .compeared to reference compound diclofenac

1.2.4.4 Antimicrobial activity

Results of antibacterial activity of tubers extract of *B. mauritanicum* and the value of inhibition Zone appair in the following formes

- **Wells diffusion method**

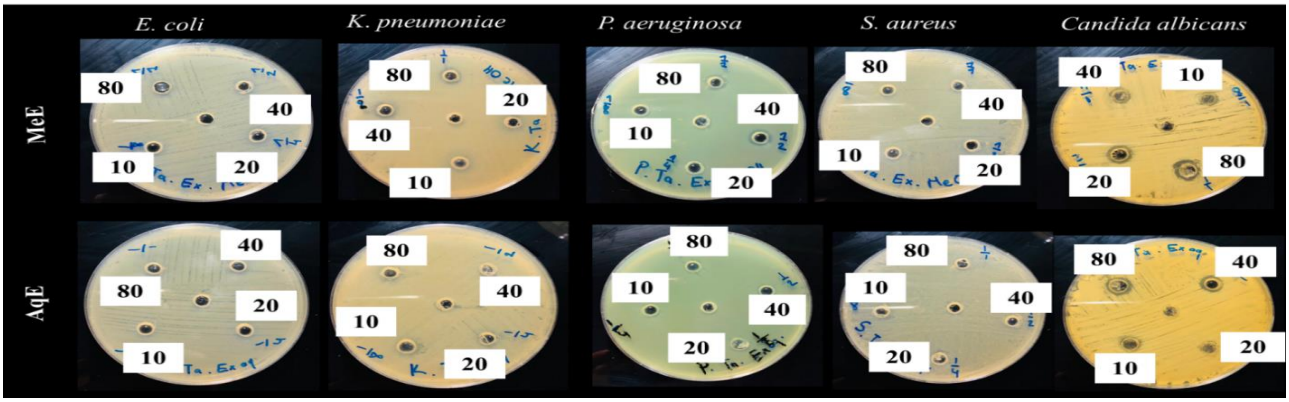


Figure 16*:*Pictures of results of antibacterial activity of tubers extract of *B. mauritanicum.*

Table 29*:*Results of antibacterial activity of of tubers extract of *B. mauritanicum.*

	Inhibition Zone								
Strains	**ZI of AqE (mm)**				**ZI of MeE (mm)**				**ZI of (CIP -5)**
***Escherichia coli* ATCC 25922**	**8**	**7**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**17**
***Pseudomonas aeruginosa* ATCC 27853**	**7**	**7**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**22**
***Klebsiella pneumoniae* ATCC 13883**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**20**
***Staphylococcus aureus* ATCC 25932**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**14**
	Anti-candidal activity								
***Candida albicans* ATCC 10231**	**11**	**9**	**7**	**NI**	**10**	**8**	**NI**	**NI**	**17**

- concentrations of extractes ascending to the table (80.40.20.10.mg/ml)* **ZI**. Inhibition Zone et Ciprofloxacin comme antibiotique ZI of (CIP -5: 50 ug/ml)

- The antibacterial activity of *B. mauritanicum* tubers' aqueous (AqE) and methanolic (MeE) extracts was evaluated against various bacterial and fungal strains, as shown in **(**Fig. 16**)**. The inhibition Zone (ZI) results indicate that both extracts exhibit some degree of antibacterial

activity. Against Gram-negative bacteria, *Escherichia coli* showed a ZI = 8 mm and 7 mm respectively with AqE extract ,at the concentrations 80.and 40 mg/ml. while *Pseudomonas aeruginosa* showed a ZI = 7 mm with AqE extracts,at 80.and 40 mg/ml concentrations, respectively *Klebsiella pneumoniae* and *Staphylococcus aureus* (Gram-positive) showed no inhibition (NI) with either extract.and methanolic (MeE)extract of *B. mauritanicum* tubers' showed no inhibition (NI) for these strains.

- Additionally, the anti-candidal activity against *Candida albicans* revealed ZIs = 11 mm and 9 mm for AqE and 10 mm also 8 mm for MeE,extracts *B. mauritanicum* tubers' respectively at 80.and 40 mg/ml concentrations. **(**Tab. 29**)**. The results indicate that the extracts demonstrate greater activity against *Candida albicans* than against any bacterial strain, and there is no significant difference in activity between Gram-positive and Gram-negative bacteria as both showed limited or no inhibition.

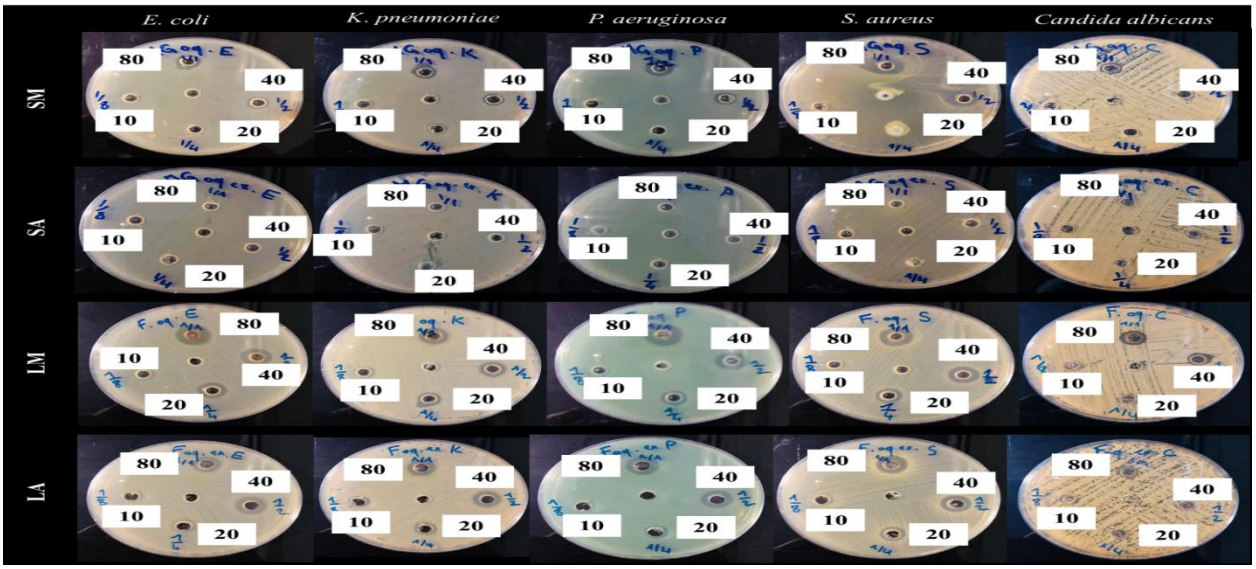


Figure 17:Pictures of results of antibacterial activity of aqueous and methanol extracts of leaves and seeds of *B. olerace.*

Table 30:Results of antibacterial activity of tubers extract of aqueous and methanol extracts of seeds of *B. oleracea. var. elongata*

	Inhibition Zone								
Strains used	**ZI of SM (mm)**				**ZI of SA (mm)**				**ZI of (CIP -5)**
***Escherichia coli* ATCC 25922**	**10**	**7**	**17**	**NI**	**NI**	**NI**	**NI**	**NI**	**17**
***Klebsiella pneumoniae* ATCC 13883**	**10**	**7**	**22**	**NI**	**NI**	**NI**	**NI**	**NI**	**22**
***Pseudomonas aeruginosa* ATCC 27853**	**12**	**8**	**20**	**NI**	**NI**	**NI**	**NI**	**NI**	**20**
***Staphylococcus aureus* ATCC 25932**	**18**	**14**	**14**	**NI**	**NI**	**NI**	**NI**	**NI**	**14**

	Anti-candidal activity								
Candida albicans ATCC 10231	**7**	**NI**	**17**	**NI**	**NI**	**NI**	**NI**	**NI**	**17**

➢ concentrations of extractes ascending to the table (80.40.20.10.mg/ml)* **ZI**. Inhibition Zone.

The antibacterial activity of the aqueous (SA and LA) and methanolic (SM and LM) extracts of B. oleracea leaves and seeds was evaluated against various bacterial and fungal strains, as shown in **(**Tab. 30, 31**)**. For the methanolic extract of seeds (SM) in **(**Tab. 30**)**, significant antibacterial activity was observed, with zones of inhibition (ZI) = 10 mm against Escherichia coli, 10 mm against *Klebsiella pneumoniae*, 12 mm against *Pseudomonas aeruginosa*, and 18 mm against Staphylococcus aureus at the concentration 80mg/ml for everyone. The aqueous extract of seeds (SA) showed no inhibition (NI) for these strains. The anti-candidal activity showed that ZI = 7 mm at 80mg/ml concentration.for the methanolic extract of seeds,also no inhibition for the aqueous extract.

Table 31:Results of antibacterial activity of of tubers extract of aqueous and methanol extracts of leaves *B. oleracea. var. elongata*

	Inhibition Zone							
Strains used	**ZI of LM (mm)**				**ZI of LA (mm)**			
***Escherichia coli* ATCC 25922**	**12**	**13**	**NI**	**NI**	**17**	**15**	**13**	**NI**
***Pseudomonas aeruginosa* ATCC 27853**	**12**	**12**	**8**	**NI**	**12**	**10**	**8**	**NI**
***Staphylococcus aureus* ATCC 25932**	**14**	**14**	**7**	**NI**	**17**	**15**	**10**	**NI**
Klebsiella pneumoniae ATCC 13883	**15**	**13**	**9**	**NI**	**16**	**14**	**10**	**NI**
	Anti-candidal activity							
Candida albicans ATCC 10231	**NI**	**NI**	**NI**	**NI**	**10**	**8**	**NI**	**NI**

➢ concentrations of extractes ascending to the table (80.40.20.10.mg/ml)* **ZI**. Inhibition Zone.

In **(**Tab. 31**)**, the methanolic extract of leaves (LM) displayed notable antibacterial activity, with ZIs = 12 mm against *Escherichia coli*, 12 mm against *Pseudomonas aeruginosa*, 14 mm against *Staphylococcus aureus*, and 15 mm against *Klebsiella pneumoniae*. at 80mg/ml concentration for everyone. The aqueous extract of leaves (LA) also showed antibacterial activity, with ZIs = 17 mm against *Escherichia coli*, 12 mm against *Pseudomonas aeruginosa*, 17 mm against *Staphylococcus aureus*, and 16 mm against *Klebsiella pneumonia* at 80 mg/ml concentration the.methanolic. extracts of leaves showed anti-candidal activity against *Candida albicans* ZIs of 10 mm.at 80mg/ml concentration in contrast The aqueous showed no inhibition.

Overall, the methanolic extracts (SM and LM) of *B. oleracea* demonstrated greater antibacterial activity compared to the aqueous extracts (SA and LA), particularly against Gram-positive bacteria such as *Staphylococcus aureus*. The activity was also higher against Gram-negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae*, indicating broad-spectrum antibacterial properties.

- **MIC and MBC methods**

The methanolic extract of *B. mauritanicum* tubers and Seeds and leaves of *B. oleracea B. oleracea var. elongata* had higher antimicrobial activity than the aqueous extract against all bacterial and fungal strains tested. The MIC and MBC values of the methanolic extract were lower than those of the aqueous extract for all the strains tested, except for *Candida albicans* .the results shown in (Tab. 32).

Table 32:MIC, MBC and MBC/MIC ratio of tubers extracts of *B. mauritanicum* aqueous and methanol also leaves and seed extracts of *B. oleracea. var. elongata*

	Bacteria strains (n = 3)	AqE			MeE		
		MIC value (mg/ml)	MBC value (mg/ml)	MBC/MIC ratio	MIC value (mg/ml)	MBC value (mg/ml)	MBC/MIC ratio
A. mauritanicum	***Escherichia coli* ATCC 25922**	10	20	2	2.5	10	4
	***Klebsiella pneumoniae* ATCC 13883**	10	20	2	2.5	10	4
	***Pseudomonas aeruginosa* ATCC 27853**	>40	>40	NA	20	20	1
	***Staphylococcus aureus* ATCC 25932**	5	10	2	5	10	2
	***Candida albicans* ATCC 10231**	10	10	1	5	5	1
B. oleracea var. elongata			**LA**			**LM**	
	***Escherichia coli* ATCC 25922**	20	20	1	5	10	2
	***Klebsiella pneumoniae* ATCC 13883**	20	40	2	5	10	2
	***Pseudomonas aeruginosa* ATCC 27853**	>40	>40	NA	20	40	2
	***Staphylococcus aureus* ATCC 25932**	10	10	1	10	10	1
	***Candida albicans* ATCC 10231**	>40	>40	NA	>40	>40	NA
			SA			**SM**	
	***Escherichia coli* ATCC 25922**	20	40	2	20	20	1
	***Klebsiella pneumoniae* ATCC 13883**	40	>40	NA	20	20	1
	***Pseudomonas aeruginosa* ATCC 27853**	40	>40	NA	40	40	1
	***Staphylococcus aureus* ATCC 25932**	20	20	1	10	10	1
	***Candida albicans* ATCC 10231**	>40	>40	NA	>40	>40	NA

- NA; not available.(LA: leaves aqueous extracts, LM: leaves methanolic extracts, SA: Seeds aqueous extract,s SM: Seeds methanolic extracts)of B. oleracea var. elongata, *MIC (Minimum Inhibitory Concentration) and* MBC (Minimum Bactericidal Concentration) .

The table (Tab. 32) demonstrates that the methanolic extract of *B. mauritanicum* tubers exhibited greater antimicrobial activity compared to the aqueous extract against all tested bacteria and fungi. This is evident by the lower MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of methanolic extract for most strains. For example, against *Escherichia coli*, the MIC and MBC of methanolic extract values were 2.5 mg/ml and 10 mg/ml, respectively, compared to 10 mg/ml and 20 mg/ml for the aqueous extract and against. ***Staphylococcus aureus***, the MIC and MBC of the methanolic extract were 5 mg/ml and 10 mg/ml, respectively, in the same values for the aqueous extract of *B. mauritanicum* tubers and anticandidal activity against against ***Candida albicans***. MIC and MBC values of: methanolic extract 5 mg/ ml,

However, the effectiveness seems to vary depending on the bacterial strain. Both extracts showed activity against *Candida albicans*, with a MIC and MBC =10 mg/ml for both aqueous and 5 mg/ml of methanolic extracts from *B. mauritanicum*. *Pseudomonas aeruginosa* appeared to be resistant to the aqueous extracts of both *B. mauritanicum* and *B. oleracea*, with MIC values exceeding 40 mg/ml (considered not active). MIC and MBC values of: Seeds methanolic extract of *B. oleracea* for most strains For example, against ***Escherichia coli***,were 20 mg/ml and 20 mg/ml, respectively, compared to 20 mg/ml and 40 mg/ml of seeds aqueous extract.and for: leaves aqueous extract against ***Klebsiella pneumoniae*** , 20 mg/ml and 40 mg/ml, respectively, compared to 5 mg/ml and 10 mg/ml for the Seeds methanolic extact .and, against ***Candida albicans***. MIC and MBC values of: Seeds and leaves methanolic ***or*** aqueous extract >40 mg/ml (considered not active).

Overall, the results suggest that the methanolic extract of *B. mauritanicum* tubers possesses broader and more potent antimicrobial properties against the tested bacteria and fungi compared to the aqueous extract. The effectiveness can be categorized as both inhibitory and lethal. Lower MIC values indicate that the extract inhibits bacterial growth at lower concentrations, while the MBC values corresponding to the MIC suggest the extract can also kill the bacteria at some concentrations.

1.2.4.5 Antidiabetic Activity

The **(**Fig. 18**)** presents data on the in vitro antidiabetic activity of *Bunium mauritanicum* tuber extracts using a yeast cell model. The results depict the percentage of glucose uptake by yeast cells mediated by the extracts at different concentrations (40 mg/ml and 20 mg/ml) and incubation times (30, 60, and 120 minutes).

Unfortunately, a complete analysis is limited by the absence of control groups (without extracts) and baseline values (glucose uptake at minute T0). However, some observations can

be made. Both aqueous (AqE) and methanolic (MeE) extracts appear to increase glucose uptake by yeast cells compared to the control group (presumably without extract) over time.

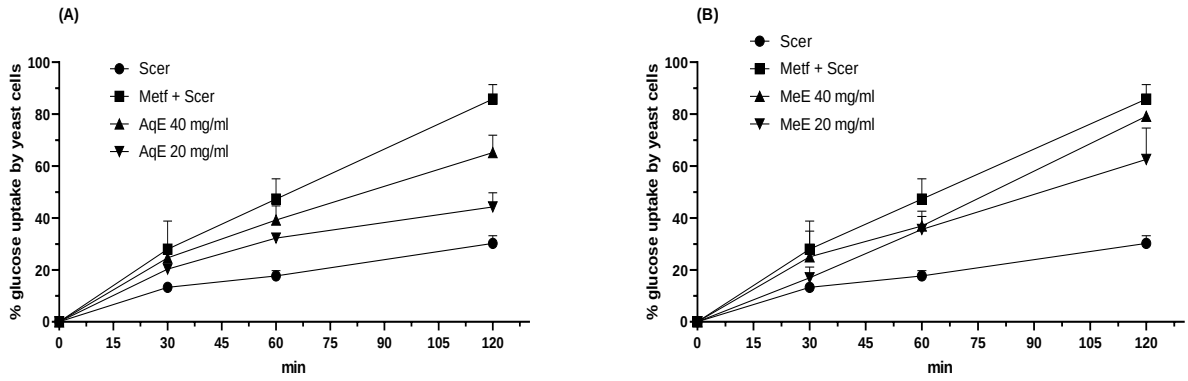


Figure 18: *In vitro* antidiabetic activity of tubers extracts of *Bunium mauritanicum* using a yeast cell model represented % glucose uptake by yeast cells mediated by the *Bunium mauritanicum* extracts according to time; Metf = Metformin; (A) for AqE = aqueous extract; (B) for MeE = methanolic extract; *Scer* = *Saccharomyces cerevisiae*

The methanolic extract (MeE) might show a slightly higher glucose uptake compared to the aqueous extract (AqE) at some concentrations and time points. For example, at 60 minutes with 40 mg/ml concentration, MeE shows a 33.2% uptake compared to AqE**'s** 22.2% uptake

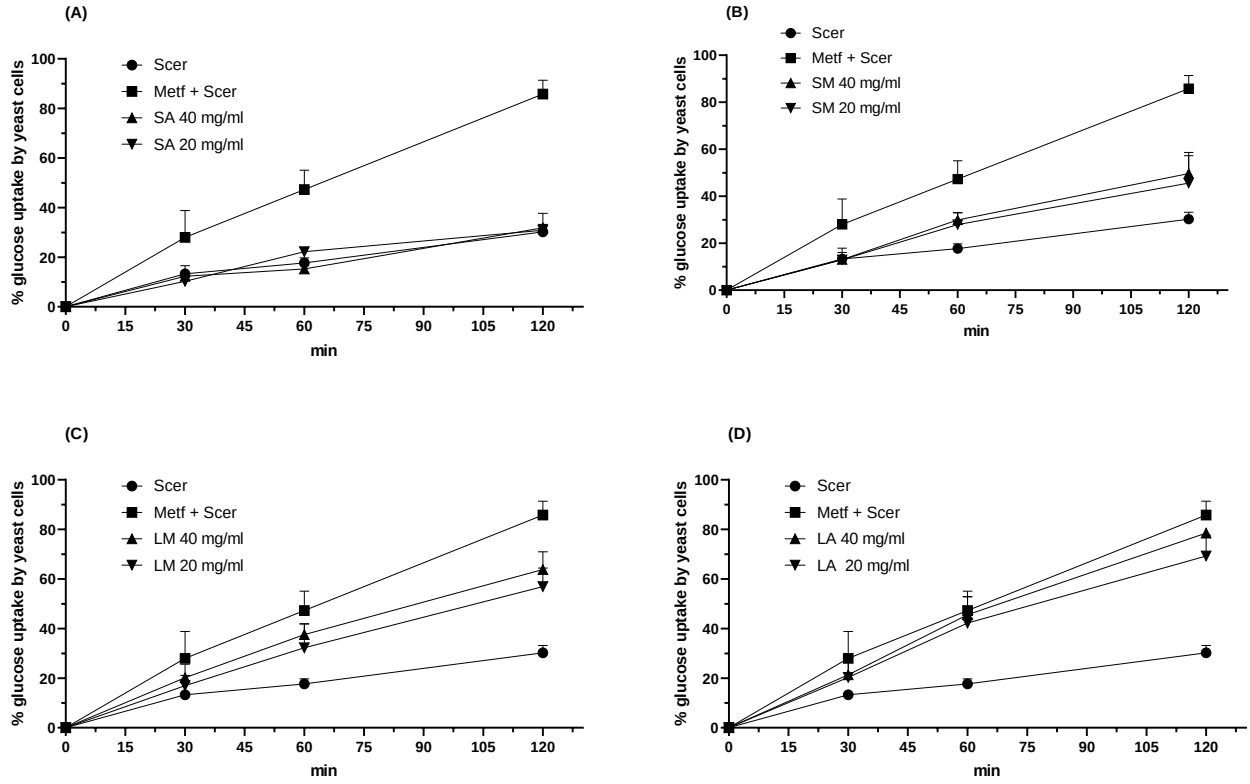


Figure 19:*In vitro* antidiabetic activity of extracts of *B. oleracea var. elongata* using a yeast cell model represented % glucose uptake by yeast cells mediated by the *B. oleracea var. elongata* extracts according to time; Metf = Metformin; (A) for: Seed extract aqueous ; (B) for:seed extract methanolic ; (C) for:leaf extract methanolic (D) for:leaf extract aqueous . *Scer* = *Saccharomyces cerevisiae*

Analyzing the in vitro antidiabetic potential of *Brassica oleracea var. elongata* extracts (Fig. 19) reveals as potential stimulatory effect on glucose uptake by yeast cells compared to a presumed control group. Across all extracts (seeds aqueous - SA, seeds methanolic - SM, leaves methanolic - LM, leaves aqueous - LA), an increase in glucose uptake is observed over time. The methanolic leaf extract (LM) exhibits the most significant increase, particularly at later time points (60 and 120 minutes), although a single most effective extract is not conclusively shown. Seed extracts (SA and SM) generally show lower glucose uptake compared to leaf extracts.

1.3 In vivo study

1.3.1 Acute toxicity results

oral gavage of different single dose of Bunium mauritanicu and *Brassica oleracea* var. elongata used on extracts showed no toxicity signs detected when given to male rats up to the dose 500 mg /kg.bd.wt. No mortality was recorded after twenty four hrs and during two weeks after giving the extract. Accordingly the extract is considered safe and secure when supplemented to rats

1.3.2 Hormonal analysis

Table 33 showed results of effect of *B. mauritanicum tubers* extract and /Levothyroxine sodium administration on serum thyroid hormones(TSH- T3- T4) in mal rats in different groups

Table 33 :effect *of B. mauritanicum tubers* extract and /*Levothyroxine sodium* administration on serum thyroid hormones in mal rats in different groups

Parameters	Control	PTU	PTU+TBM	PTU+LOVO®
TSH (µIU/ml)	1.463 ± 0.01528	2.1 ± 0.2 γ	1.9 ± 0.1 *α	1.703 ± 0.07572*ns
T3 (ng/ml)	0.65 ± 0.02	0.6567 ± 0.01528 ns	0.63 ±0.01* ns	0.58 ±0.58**ß
T4 (nmol/l)	34.37 ± 0.4509	30.23 ± 0.7506 ß	32.7 ± 0.8*α	33.57± 1.079**ns

- The values are expressed as mean ± standard deviation (SD=6). comparison with PTU group: * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$); *** very highly significant difference ($P < 0.001$), comparison with Control group: α significant difference ($P < 0.05$); β highly significant difference ($P < 0.01$); γ very highly significant difference ($P < 0.001$) ns : no significant

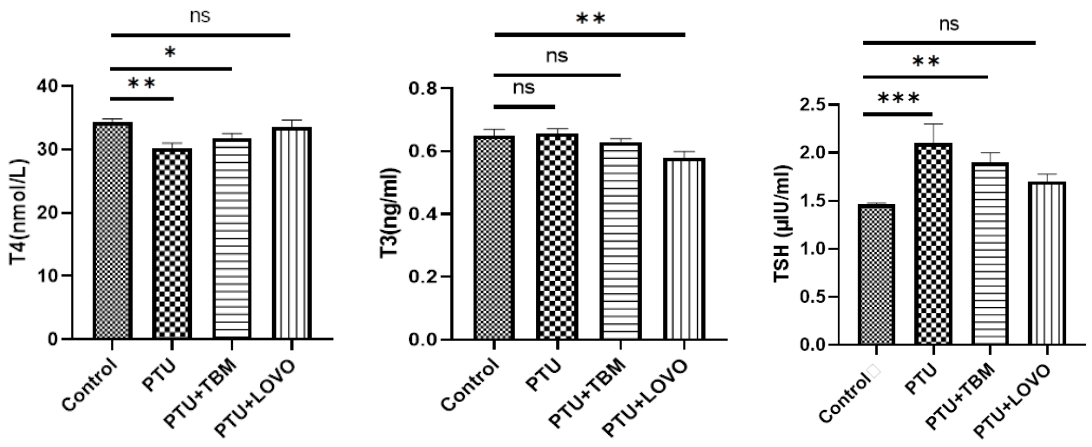


Figure 20: Thyroid hormones (TSH, T3, T4) for rats exposed to *propylthiouracil* (PTU) at doses10mg/kg and administered *B. mauritanicum tubers* (TBM) and Levothyrox® in different groups.

The evaluation of T4 (thyroxine) levels in our experimental groups revealed significant results. In the group treated with PTU (Propylthiouracil), we observed a very significant decrease in T4 levels compared to the control group, with a p value of $P < 0.01$). This observation is in line with expectations, since PTU inhibits the synthesis of thyroid hormones, thus leading to a reduction in T4 levels in a context of induced hypothyroidism.

In the PTU + TBM group, we also see a significant decrease in T4 levels, with a p-value of($P < 0.05$) . However, it is essential to note that this decrease is less pronounced than that observed in the PTU alone group. This suggests that the TBM extract exerts a positive effect on T4 synthesis, thus mitigating the negative impact of PTU. The TBM extract therefore seems to have modulatory properties that promote T4 production, which could be attributed to an improvement in thyroid function or a stimulation of thyroid hormone biosynthesis.

Finally, in the PTU + Lovo group, we do not observe any significant difference in T4 levels compared to the control group. This indicates that Lovo, a drug known to regulate T4 synthesis, is effective in stabilizing the levels of this hormone, without however inducing a significant change compared to a normal state. This could also suggest that the effect of Lovo is sufficient to compensate for the inhibition induced by PTU.

The analysis of T3 (triiodothyronine) levels in our experimental groups revealed interesting results. In the pathological group treated with PTU (Propylthiouracil) alone, we did not observe any significant difference in plasma T3 levels compared to the control group. This result suggests that, despite the inhibition of hormone synthesis induced by PTU, T3 levels remain relatively constant, which could be due to a compensatory effect of the organism or to a homeostatic regulation of thyroid hormones.

In the PTU + TBM group, the situation remains similar, with no significant difference observed in T3 levels compared to the control group. This indicates that the TBM extract does not seem to have a notable effect on T3 levels in the context of PTU-induced hypothyroidism. This lack of variation could suggest that TBM does not directly influence the conversion of T4 to T3 or that its effects are insufficient to induce a measurable change in the levels of this hormone.

In contrast, in the PTU + Lovo group, we observed a very significant decrease in T3 levels, with a p-value of $P < 0.01$). This result suggests that Lovo, a drug known to regulate thyroid function, has a direct effect on the reduction of plasma T3 levels. This decrease could be attributed to the inhibition of the conversion of T4 to T3 or to a downregulation of T3 secretion by the thyroid gland, which could be a mechanism of action of the drug.

For TSH levels in our experimental groups revealed significant results. In the group treated with PTU (Propylthiouracil), we observed a very highly significant increase in TSH levels compared to the control group, with a p value of $P < 0.001$). In the PTU + TBM group,and PTU + Lovo group, we also see a significant decrease in TSH levels compared to the control group.

1.3.3 Biochemical and hematological blood analysis.

- ***B. mauritanicum tubers***

Biochemical analysis.

The results of Biochemical analysis in rats treated in table 34.

Table 34: Biochemical analysis values in rats treated with *propylthiouracil* and administered *B. mauritanicum tubers* and control group.

Parameters	AST (UI/L)	ALAT (UI/L)	ALP (UI/L)	GGT (UI/L)	DB (µmol/L)	TB (µmol/L)	Crea (mg/L)	Urea (g/L)
Control	93.47 ± 0.52	37.5 ± 0.58	96.74 ± 0.01	5.08 ± 0.15	2.83 ± 0.02	4.05 ± 0.04	4.77 ± 0.23	0.18 ± 0.01
PTU	157.69± 2.60	53.24 ± 0.72	180.57 ± 1.04	6.54 ± 0.20	5.06 ± 0.17	10.05 ± 0.38	5.27 ± 0.42$^{\alpha}$	0.39 ± 0.06$^{\alpha}$
PTU+TBM	104.21± 2.38	37.32 ± 0.49	103.89 ± 0.61	5.35 ± 0.05	3.09 ± 0.13	4.28 ± 0.09	3.78 ± 0.23	0.23 ± 0.02
PTU+LOVO®	98.50 ± 2.50	39.00 ± 0.60	100.50 ± 1.00	5.20 ± 0.10	3.00 ± 0.15	4.10 ± 0.20	4.00 ± 0.30	0.20 ± 0.03

(TBM: tubers of *B. mauritanicum*, LOVO®: Levothyrox® (Levothyroxine sodium) a standard medication used to treat hypothyroidism., AST: aspartate aminotransferase, ALT: alanine transaminase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, DB: direct bilirubin, TB: total bilirubin, Crea: creatinine. comparison with PTU group: * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$); *** very highly significant difference ($P < 0.001$), comparison with Control group: α significant difference (P

< 0.05); β highly significant difference (P < 0.01); γ very highly significant difference (P < 0.001) . Results are expressed as mean ± SD (n = 6).

Presented in **(**Tab. 34**)** demonstrates significant changes in various parameters in rats treated with propylthiouracil (PTU) and administered *B. mauritanicum* tubers (TBM) and Levothyrox® (LOVO®). The control (PTU) group showed elevated levels of AST, ALAT, ALP, GGT, DB, TB, compared to the group control, indicating significant liver and kidney dysfunction due to hypothyroidism.and. creatinine, and urea showed significant difference compared to the group control, Treatment with TBM resulted in substantial reductions in these parameters, particularly AST, ALAT, ALP, GGT, and creatinine, suggesting hepatoprotective and renoprotective effects. Similarly, the LOVO® group showed significant improvements in these biochemical markers, indicating its efficacy as a standard treatment for hypothyroidism. Both TBM and LOVO® treatments demonstrated their potential in mitigating the adverse effects of PTU-induced hypothyroidism, with LOVO® showing slightly more pronounced effects in some parameters. These results highlight the therapeutic potential of *B. mauritanicum* tubers in managing hypothyroidism-related biochemical alterations.

Hematological analysis

The results of Hematological l analysis in rats treated in table 35

Table 35: Hematological analysis values in rats treated with propylthiouracil and administered *B. mauritanicum tubers* and control group.

Parameters	RBC (×10^6 cells ml^{-1})	Hemoglobin (g dl^{-1})	Hematocrit (%)	Platelets 10^3/mm^3	WBC (×10^3 cells ml^{-1})
Control	7.20 ± 0.10	15.80 ± 0.25	43.00 ± 0.80	470.00 ± 15.00	4.50 ± 0.15
PTU	5.90 ± 0.60 $^{\gamma}$	8.50 ± 0.35 $^{\gamma}$	31.50 ± 0.50 $^{\gamma}$	1100.00 ± 25.00 $^{\gamma}$	5.70 ± 0.25 $^{\beta}$
PTU+TBM	6.40 ± 0.20 $^{\alpha*}$	14.80 ± 0.20 $^{\alpha*}$	40.20 ± 1.00 $^{\alpha*}$	800.00 ± 20.00 $^{\beta**}$	4.90 ± 0.15 $^{\alpha*}$
PTU+LOVO®	6.90 ± 0.25 $^{\beta**}$	13.50 ± 0.30 $^{\beta**}$	37.00 ± 0.90 $^{\beta**}$	900.00 ± 22.00 $^{\beta**}$	5.00 ± 0.20 $^{\beta*}$

TBM: tubers of *B. mauritanicum*, LOVO®: Levothyrox®: (Levothyroxine sodium) a standard medication for hypothyroidism. group co.: control, RBC: Red blood cells, WBC: white blood cells. Results are expressed as mean ± SD (n = 6). comparison with PTU group: * significant difference (P < 0.05); ** highly significant difference (P < 0.01); *** very highly significant difference (P < 0.001), comparison with Control group: α significant difference (P < 0.05); β highly significant difference (P < 0.01); γ very highly significant difference (P < 0.001). Ns : no significant.

-The hematological analysis in **(**Tab. 35**)** shows significant changes in blood parameters in rats treated with propylthiouracil (PTU) and administered *B. mauritanicum* tubers (TBM) and Levothyrox® (LOVO®). The (PTU) group demonstrated substantial reductions in RBC

count, hemoglobin levels, and hematocrit percentage, very highly significant difference . alongside highly significant increases in platelet and WBC counts , compared to The control group indicating severe hematological disruptions due to hypothyroidism. Treatment with TBM resulted in marked improvements in these parameters, with increases in RBC count, hemoglobin levels, and hematocrit with a significant difference, and decreases in platelet and WBC counts, indicating a mitigative effect on PTU-induced hematological alterations. Similarly, the LOVO® group showed significant recovery, although slightly less pronounced than the TBM group. Both treatments demonstrated their potential in alleviating the adverse hematological effects of PTU, with TBM showing slightly superior efficacy. These results highlight the therapeutic potential of *B. mauritanicum* tubers in managing hypothyroidism-related hematological changes.

- ***Brassica oleracea var. elongata***
- ***Biochemical analysis.***

The results of Biochemical analysis in rats treated in table 36.

Table 36: Biochemical analysis values in rats treated with *phenylhydrazine* and administered *Brassica oleracea var. elongata* leaf and seed.and control group

Parameters	AST (UI/L)	ALAT (UI/L)	ALP (UI/L)	GGT (UI/L)	DB (µmol/L)	TB (µmol/L)	Crea (mg/L)	Urea (g/L)
Control	93.47 ± 0.52	37.5 ± 0.58	96.74 ± 0.01	5.08 ± 0.15	2.83 ± 0.02	4.05 ± 0.04	4.77 ± 0.23	0.18 ± 0.01
PHZ	157.69± 2.60	53.24 ± 0.72	180.57± 1.04	6.54 ± 0.20	5.06 ± 0.17	10.05 ± 0.38	5.27 ± 0.42$^{\alpha}$	0.39 ± 0.06$^{\alpha}$
PHZ+LBO	104.21± 2.38	37.32 ± 0.49	103.89± 0.61	5.35 ± 0.05	3.09 ± 0.13	4.28 ± 0.09	3.78 ± 0.23	0.23 ± 0.02
PHZ+SBO	131.09± 4.12	43.05 ± 1.81	130.95± 1.85	5.91 ± 0.04	4.10 ± 0.04	6.46 ± 0.26	4.93 ± 0.19	0.34 ± 0.03
PHZ+ B-Feron®	110.50± 2.70	39.20 ± 0.60	115.75± 1.00	5.50 ± 0.10	3.20 ± 0.15	4.50 ± 0.20	4.00 ± 0.30	0.25 ± 0.03

LBO: *Brassica oleracea var. elongata* leaf, SBO: *Brassica oleracea var.* seeds, PHZ: Phenylhydrazine, B-Feron®: Standard dietary supplement containing iron, B12, B9, and Vitamin C. AST: aspartate aminotransferase, ALT: alanine transaminase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, DB: direct bilirubin, TB: total bilirubin, Crea: creatinine. comparison with PHZ group: * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$); *** very highly significant difference ($P < 0.001$), comparison with Control group: α significant difference ($P < 0.05$); β highly significant difference ($P < 0.01$); γ very highly significant difference ($P < 0.001$). Results are expressed as mean ± SD (n = 6).

The biochemical analysis presented in (Tab. 36), reveals significant difference alterations in various parameters following phenylhydrazine (PHZ) treatment(creatinine, and urea) and subsequent administration of *Brassica oleracea* var. elongata leaf (LBO) and seeds (SBO). The positive control (PHZ) group exhibited elevated levels of AST, ALAT, ALP, GGT, DB,

TB,. compared to the group control and showed no significant difference . creatinine, and urea showed, significant difference compared to the group control .indicating hepatic and renal dysfunction. Treatment with LBO markedly reduced these parameters, approaching the levels observed in the group control, particularly in AST, ALAT, ALP, GGT, and creatinine, suggesting hepatoprotective and renoprotective effects. Similarly, SBO treatment significantly decreased these parameters to a lesser extent than LBO. The B-Feron® group also demonstrated substantial improvements in biochemical parameters, indicating the therapeutic potential of this standard dietary supplement. These results collectively suggest that both LBO and SBO possess protective effects against PHZ-induced biochemical alterations, with LBO showing slightly more pronounced effects.

- **Hematological analysis**

Hematological analysis treated rats with and administration *Phenylhydrazine*, with *Brassica oleracea var. elongata* plant showed the results as in table 35

Table 37: Hematological analysis values in rats treated with *Phenylhydrazine*, and administration with *Brassica oleracea var. elongata* leavs and seeds and control group.

Parameters	RBC ($\times 10^6$ cells ml^{-1})	Hemoglobin (g dl^{-1})	Hematocrit (%)	Platelets $10^3/mm^3$	WBC ($\times 10^3$ cells ml^{-1})
Control	7.10 ± 0.13	15.70 ± 0.34	42.54 ± 1.21	463.00 ± 28.78	4.40 ± 0.10
PHZ	6.14 ± 0.55 γ	8.70 ± 0.34 $^{\gamma}$	32.40 ± 0.49 $^{\gamma}$	1081.00 ± 0.58 $^{\gamma}$	5.62 ± 0.24 $^{\beta}$
PHZ+LBO	6.35 ± 0.15 $^{\alpha *}$	14.60 ± 0.19 $^{\alpha *}$	39.80 ± 1.12 $^{\alpha *}$	792.00 ± 0.58 $^{\beta **}$	4.82 ± 0.20 $^{\alpha *}$
PHZ+SBO	6.32 ± 0.20 $^{\alpha *}$	14.21 ± 0.15 $^{\alpha *}$	37.62 ± 0.55 $^{\beta *}$	781.00 ± 25.89 $^{\beta **}$	4.22 ± 0.15 $^{\gamma ***}$
PHZ+ B-Feron®	6.84 ± 0.22 $^{\beta **}$	13.30 ± 0.25 $^{\beta **}$	36.80 ± 0.84 $^{\beta **}$	894.00 ± 6.58 $^{\beta **}$	4.92 ± 0.18 $^{\beta *}$

LBO: *Brassica oleracea var. elongata* leaf, SBO: *Brassica oleracea var. elongata* seeds, PHZ: Phenylhydrazine, B-Feron®: Standard dietary supplement containing iron, B12, B9, and Vitamin C. Negative co.: control, RBC: Red blood cells, WBC: white blood cells. Results are expressed as mean ± SD (n = 6). comparison with PHZ group: * significant difference ($P < 0.05$); ** highly significant difference ($P < 0.01$); *** very highly significant difference ($P < 0.001$), comparison with Control group: α significant difference ($P < 0.05$); β highly significant difference ($P < 0.01$); γ very highly significant difference ($P < 0.001$) Ns : no significant

The hematological analysis in **(**Tab. 36**)** illustrates the effects of Phenylhydrazine (PHZ.) treatment and subsequent administration of *Brassica oleracea var. elongata* leaf (LBO) and seeds (SBO) on various blood parameters in rats. The group (.PHZ) exhibited very highly significant difference ($P < 0.001$). reductions in RBC count, hemoglobin levels, and hematocrit percentage, alongside a marked increase in platelet count (very highly significant difference.) and WBC count, (highly significant difference.) compared to the control group

indicating severe hematological disruptions due to anemia . Treatment with LBO resulted in notable improvements in these parameters, with increases in RBC count, hemoglobin levels, and hematocrit(significant difference.) , and decreases in platelet(highly significant difference.). and WBC counts,compared to The group (.PHZ) suggesting a mitigative effect on PHZ-induced hematological alterations. Similarly, the SBO group demonstrated substantial recovery, though slightly less pronounced than the LBO group. The B-Feron® group, treated with a standard dietary supplement, also showed highly significant restoration of normal hematological values, highlighting its efficacy. Overall, these results underscore the potential of *B. oleracea var. elongata* leaf and seeds in alleviating the adverse hematological effects induced by PHZ , with LBO showing a slightly superior effect.

1.3.4 Histological results

- ✓ ***B. mauritanicum tubers***

These results of histological sections of various organs the liver kidneys and spleen were viewed by light microscope with H&E staining. (Dark arrow) (x 100).and Dr.Hmidatou specialist in pathological anatomy . helped us analyze and interpret them.

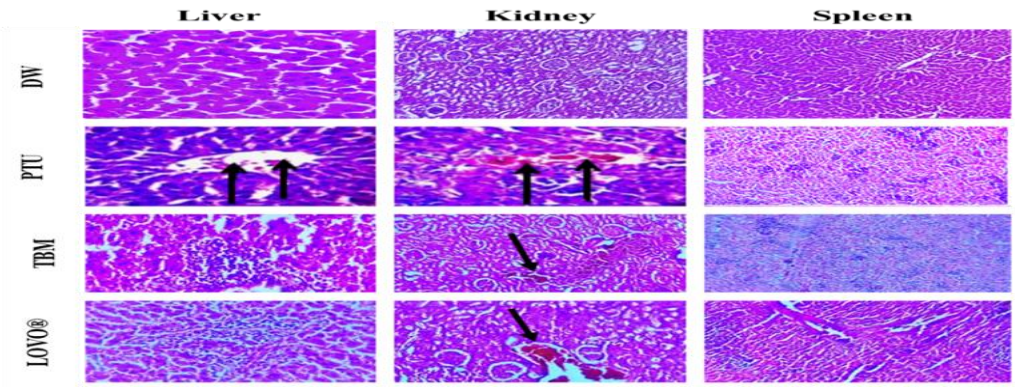


Figure 21: Histological examination of rat liver, spleen, and kidney, in the different studied groups, (control.and.hypothyroidism groups, (patient group), and patient groups treated with TBM. Levothyrox®.) by light microscope with H&E staining. (Dark arrow) (x 100)

: inflammatory cells heavily infiltrate the portal area, inflammation , nécroses (DW: distilled water, TBM: tubers of B. mauritanicum, LOVO®: Levothyrox®: Levothyroxine sodium) a standard medication for hypothyroidism) , * (**DW**) : In the distilled water group as a control group or a negative control* **PTU**: The PTU group means is that hypothyroidism group as a positive control * **TBM**: patient group treated with. B. mauritanicum tubers * **LOVO**®:patient group treated with: Levothyrox®: (Levothyroxine sodium) a standard medication for hypothyroidism.

(Fig. 21**)** The histological examination in Figure 24 provides a striking visualization of the varying degrees of tissue damage and recovery across different experimental groups. The liver, kidney, and spleen tissues stained with H&E illustrate the profound effects of

hypothyroidism induced by PTU and the therapeutic interventions applied. In the control group (DW), the tissues appear completely normal, with well-organized liver hepatocytes, intact renal structures, and healthy lymphoid follicles in the spleen. This serves as the baseline of healthy tissue architecture. In sharp contrast, the PTU group shows a devastating impact, with severe inflammatory cell infiltration in the liver's portal area (black arrows), accompanied by extensive necrosis. The kidneys exhibit interstitial nephritis, indicative of significant inflammatory damage to renal tubules, while the spleen suffers from lymphoid depletion, reflecting a compromised immune response. These findings vividly demonstrate the widespread organ damage induced by hypothyroidism, reinforcing the critical importance of thyroid hormone homeostasis in maintaining systemic health.

However, upon introducing therapeutic agents, the scenario begins to change. The TBM group treated with tubers of *B. mauritanicum* exhibits partial but noticeable recovery. In the liver, inflammation is visibly reduced, although some residual infiltration remains. The kidneys show moderate improvement, with diminished interstitial nephritis, suggesting that the bioactive compounds in *B. mauritanicum* are beginning to mitigate the inflammatory damage. Perhaps most striking is the recovery in the spleen, where lymphoid tissues show signs of restoration, pointing to the plant's potential immunomodulatory effects. These improvements, while not complete, suggest that *B. mauritanicum* possesses considerable antioxidant and anti-inflammatory properties that promote healing, though it does not achieve the full restoration of organ function.The most profound recovery is observed in the Levothyrox® (LOVO®) group, where tissues are almost entirely restored to their normal state. In the liver, the inflammatory infiltrates have nearly disappeared, and the hepatocytes exhibit a largely intact structure, suggesting that Levothyrox® effectively halts and reverses the damage caused by PTU. The kidneys, too, show remarkable improvement, with near-normal renal structures and minimal residual inflammation, indicating that Levothyrox® plays a pivotal role in preserving renal function. The spleen, which had been severely depleted in the PTU group, now displays fully

restored lymphoid follicles, highlighting the effectiveness of Levothyrox® in reestablishing immune health.

- ✓ ***Brassica oleracea var. elongata***

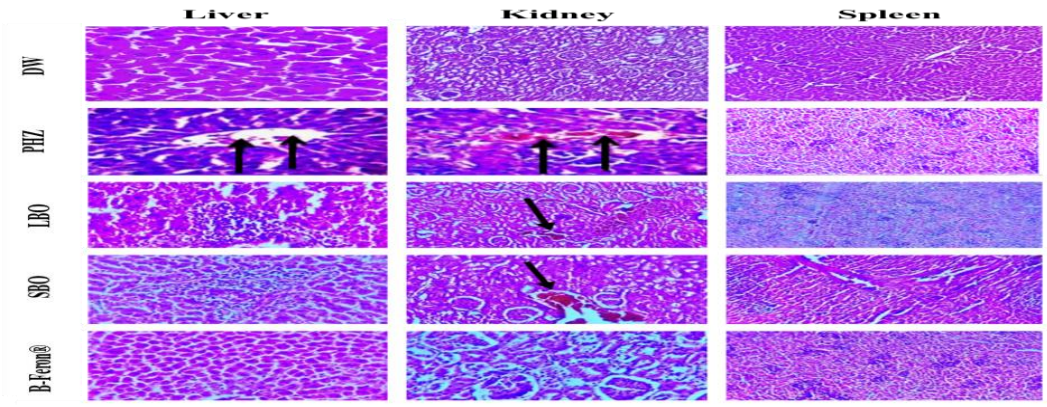


Figure 22 Histological examination of rat liver, spleen, and kidney, in the different studied groups (control and..anemic groups(patient group) and patient groups treated with LBO. SBO. B-Feron®.):, by light microscope with H&E staining. (Dark arrow) (x 100)

→ : inflammatory cells heavily infiltrate the portal area, inflammation , nécroses (DW: distilled water, LBO: Brassica oleracea var. elongata leaves, SBO: Brassica oleracea var. elongata seeds, PHZ: Phenylhydrazine, B-Feron®: Standard dietary supplement containing iron, B12, B9, and Vitamin C.)*. **(DW)** : In the distilled water group as a control group or a negative control***PHZ**:The PHZ group means is that anemic or Phenylhydrazine group as a positive control * **LBO**: patient groups treated with. Brassica oleracea var. elongata leaves * **SBO**: patient groups treated with. Brassica oleracea var. elongata seeds, ***B-Feron**®: :patient groups treated with. Standard dietary supplement containing iron, B12, B9, and Vitamin C.

(Fig. 22**)** This histological examination reveals significant differences in liver, kidney, and spleen tissue integrity, inflammation, and recovery among the treatment groups. The control group (DW) exhibited normal histological features, with intact cellular structures. In contrast, the phenylhydrazine (PHZ) group showed severe tissue damage, with extensive inflammatory cell infiltration, particularly around the liver's portal areas, kidney inflammation, and spleen structural depletion, consistent with the toxic effects of PHZ.

The group treated with *Brassica oleracea* leaf extract (LBO) demonstrated moderate recovery, with reduced inflammation and partial restoration of liver, kidney, and spleen structure, indicating its antioxidant and protective properties. The *Brassica oleracea* seed extract (SBO) group showed even better recovery, with near-normal tissue architecture in all organs, due to the higher concentrations of bioactive compounds like phenolics and glucosinolates. The B-Feron® group, receiving a dietary supplement containing iron and essential vitamins, exhibited a recovery comparable to the SBO group, highlighting the role of nutrients in restoring tissue health and combating oxidative stress and anemia

DISCUSSION

Discussion

Bunium mauritanicum, known as 'Talghouda' in Algeria, is a medicinal and culinary plant with significant antioxidant properties (Toul et al., 2022a). Its seeds contain various phenolic compounds, including flavonoids and phenolic acids, which contribute to its antioxidant activity (Toul et al., 2022a). The plant is widely used in traditional Algerian medicine, particularly In the Tiaret region (Djahafi, Taibi, et al., 2021). Studies have shown that *B. mauritanicum* extracts can affect hematological parameters and reproductive organs in female rabbits, suggesting potential estrogenic effects at certain doses (Chentouh et al., 2017). The plant's roots contain compounds such as scopoletin, scoparone, and beta-sitosterol (Chentouh et al., 2017). Despite its importance in traditional medicine, *B. mauritanicum* remains understudied, particularly regarding its phytochemical profile (Toul et al., 2022a). Further research is needed to understand its therapeutic potential fully and to develop novel strategies for drug discovery based on this endemic species (Djahafi, Taibi, et al., 2021) (Toul et al., 2022a) .

The domestication of *B. oleracea* is debated, with hypotheses suggesting either northwest European or Mediterranean origins. A study of ancient Greek and Latin texts from the 6th century BCE to the 4th century CE supports a Mediterranean domestication location and provides insights into early agricultural practices and uses of brassica vegetables (Maggioni et al., 2017).

Brassica oleracea, particularly the white cabbage variety (*var. capitata f. alba*), is a cruciferous vegetable with diverse traditional uses and pharmacological properties. It has been used to treat various ailments including diabetes, cancer, and inflammation (Lipi Rani Ray et al., 2021; Sati et al., 2023). The plant contains numerous phytochemicals such as alkaloids, flavonoids, and glucosinolates, which contribute to its medicinal effects (Lipi Rani Ray et al., 2021; Sati et al., 2023). In Turkey, *B. elongata*, a wild relative, is found in central and eastern regions, preferring unfertile soils on hillsides (Dönmez et al., 2020).

According to previous studies *Brassica oleracea*, encompassing variants such as cabbage, kale, and broccoli, is a nutrient-dense vegetable providing substantial health benefits. This cruciferous vegetable is a rich source of essential vitamins and minerals, notably vitamins C and K, calcium, Proteins and potassium (Isabel M Pires et al., 2022).and another study showed that the nutritional composition of *B. mauritanicum* proteins, lipids, iron and zinc *Bunium mauritanicum* tuber powder (Aiouaz & Bitam, 2022b) According to the

findings, these plants are nominated to be food sources of proteins, carbohydrates, minerals). As for the low lipid content in all six species, it may be attributed to the fact that wild plant leaves in general do not have an abundant content of fat content, especially their leaves, unlike their fruits (Achinewhu *et al.*, 1995; Lykke & Padonou, 2019). presents the energy values of *B. mauritanicum* tubers, *B. oleracea* seeds, and leaves,is due to the presence of carbohydrates.The difference in nutritional composition between seeds and leaves is due to the fact that seeds are storage organs and leaves are synthetic organs

The results of this study (Tab. 24) (for *B. oleracea*) align with the findings of Ayadi et al. (Ayadi et al., 2022), who performed a comprehensive investigation into the phytochemical composition of four *Brassica* species: *B. rapa*, *B. oleracea*, *B. napus*, and *B. juncea*. Employing advanced analytical methods such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), the researchers successfully identified and quantified an extensive array of bioactive compounds in the aqueous and organic extracts of Brassica seeds. These compounds encompassed volatile oils, glycosides, reducing sugars, polyphenols, phenolic acids, flavonoids, alkaloids, saponins, terpenoids, tannins, and glucosinolates, all of which were found to be present in significant quantities.

Similarly, Parikh and Khanna (Parikh & Khanna, 2014) undertook a detailed phytochemical screening of six *Brassica* species, including *B. oleracea* var. *capitata*, *B. juncea*, *B. napus*, *B. rapa*, *B. carinata*, and *B. nigra*. Employing a combination of qualitative and quantitative methods, they characterized the occurrence and relative abundances of key bioactive compounds such as alkaloids flavonoids phenols tanins saponins their findings underscore the diverse of phytochemical profiles of these Brassica species highlighting their potential as valuables sources of naturally occurring bioactive compounds .

Comparing these findings (Tab. 23) **for** *B. mauritanicum* tubers with previous research, Fadia et al. (Fadia & Khawla., 2023). also identified alkaloids, saponins, tannins, and steroids, which are consistent with the current study's results. However, the absence of flavonoids in this study contrasts with the findings of (Adlifa r& Rezanejad., 2020). . who reported their presence in *B. mauritanicum* tubers. The differences in phytochemical composition between the studies may be attributed to variations in climatic conditions, environmental growth conditions, and the application of plant materials, leading to significant differences in specific phytochemical constituents (Karimi et al., 2020). This study is the first to investigate the diverse phytochemical compounds in the aqueous and methanolic extracts of *B. mauritanicum* tubers. Further research is needed to deepen our understanding in this area.

B. mauritanicum tubers have been found to contain high levels of total phenols and flavonoids (Tab. 25), with the methanol extract showing the highest concentration (Karouche et al., 2020). This is consistent with the findings of other studies on related species, such as *Bunium pachypodum* and *B. mauritanicum*, which also showed high levels of these compounds (Nassima, 2022; Toul, 2022). The antioxidant activity of these compounds has been demonstrated in various plant species, including those from the same botanical family as *B. mauritanicum* (Guiche, 2015). These findings suggest that *B. mauritanicum* tubers may have potential health benefits due to their high phenolic and flavonoid content.

Previous studies for *Brassica oleracea*, particularly red and green cabbage, has been found to have high levels of polyphenols and flavonoids, which contribute to its antioxidant activity (Upadhyay et al., 2016). These compounds are also present in other parts of the plant, such as the stem and flower of *Brassica oleracea* var. acephala (Aydın, 2020). The extraction of polyphenols from Brassica vegetables is most effective with 60% methanol, which also enhances their antibacterial and antioxidant properties (Jaiswal et al., 2012). The methanol extract of broccoli, a variety of Brassica oleracea, has been shown to have significant free radical scavenging activity, further highlighting the potential health benefits of these compounds (Sv et al., 2012).the results shown in (Tab. 24), for *Brassica oleracea* (leavs and seeds).support the results of previous studies but the difference in the amount of these compounds between leaves and seeds is due to their metabolic activity. .also the difference in the amount of polyphenols and flavonoids estimated for both plants (*Brassica oleracea and B. mauritanicum*) is due to method of extraction and the type of solvent used in extraction.the polarity of solvent olso affects the extraction of secondary metabolites.

In comparison with the results of *B. mauritanicum* extract in **(**Tab. 26**)**, a previous study using HPLC, which compared the results to those of 18 reference standards, showed several major intense peaks at a wavelength of 280 nm. Analysis of these chromatograms identified thirteen phenolic compounds: six in the methanol extract (five phenolic acids and flavanones) and seven in the petroleum ether extract (three phenolic acids, two flavonols, flavanols, and flavanones). These results are identical to those achieved in this study (Toul et al., 2022a) (Toul & Djendar, 2023). These findings were supported by (Hayet et al., 2017a), who found that the essential oils and methanolic extracts of *B. mauritanicum* demonstrated significant antioxidant activity. These studies collectively suggest that *B. mauritanicum* is a rich source of phenolic compounds with a

A range of studies have explored the phenolic composition of *Brassica oleracea* using HPLC. (Ahmed & Rao, 2014) identified Rutin, Quercetin, and Kaempferol in *Brassica oleracea* L.var capitata, while (Taveira et al., 2009) found 36 phenolic compounds in vitro shoots of *Brassica oleracea* var. costata, with the highest content and antioxidant potential in shoots grown in a specific culture medium. (Ferreres et al., 2008) characterized 37 phenolic compounds in in vitro shoots of *Brassica oleracea* L. var. costata, with a strong antioxidative capacity. (Ferreres et al., 2008) investigated the influence of fertilization on the phenolic composition of tronchuda cabbage, a variety of *Brassica oleracea*, finding that samples grown without excess fertilizers had higher phenolic content. These studies collectively highlight the diverse phenolic composition of *Brassica oleracea* and the potential impact of growth conditions on this composition.

According to previous studies . *B. mauritanicum,* a plant species used in Algerian traditional medicine, has been found to possess significant antioxidant properties. (Toul et al., 2022b) (Toul & Djendar, 2023) both identified methanol extracts as having the highest DPPH scavenging potential and inhibitory effect against β-carotene bleaching. These extracts also contained a range of phenolic compounds, including flavonoids and phenolic acids. (Berroukeche et al., 2021) further confirmed the antioxidant potential of *B. mauritanicum*, with acetonic tuber extracts showing high scavenging potential and protective effects against hemolysis. The roots of *B. mauritanicum* have been found to positively impact biological, biochemical, and histological parameters in female rats, including an increase in body and organ weight and changes in blood lipid levels (Attoui et al., 2021).these studies confirm the results obtained**(**Tab. 27**)**..

the antioxidant potential of *B. mauritanicum* extracts was evaluated through various assays including DPPH, FRAP and β-carotene/linoleic acid assay, total antioxidant capacity and Anti-hemolysis in **(**Tab. 27**)**, . The aqueous extract (AqE) displayed a notable IC_{50} value of 0.288 mg/mL in the DPPH assay, indicating its superior scavenging capacity. This finding is consistent with a previous study where the methanolic extract of the plant's leaves exhibited strong antioxidant capacity for the DPPH radical. This highlights the robust antioxidant potential of the plant extracts, particularly when compared to previous findings. (Adelifar & Rezanejad., 2020).

In the β-carotene/linoleic acid assay, both AqE and MeE demonstrated significant effects, with IC_{50} values These results .in the FRAP test, the aqueous extract exhibited better reducing ability compared to the methanol extract and ascorbic acid, . Additionally, the

phosphomolybdate assay showed that AqE had the highest total antioxidant capacity (TAC) value, followed by MeE.

These results are explained by the presence of bioactive compounds whose concentrations vary to the type of extract and this phytochemicals compounds for exemple Gallic acid Chlorogenic acid Naringin all of them possess hydroxyl groups that can readily donate hydrogen atoms to neutralize DPPH radicals,. (Gawlik et al., 2013). the differencein in the concentrations of these compounds between extracts leads to a difference in antioxidant properties

this is supported by the results of table **(**Tab.25. 26**)**

Compared to Previous studies. *Brassica oleracea*, commonly known as cabbage, has been found to possess significant antioxidant activity in various studies. (Lakshmi & VeronicaShalini, 2016) reported high levels of phenolic and flavonoid content, as well as potent antioxidant activity in the methanolic extract of *Brassica oleracea*. This was further supported by (Kl et al., 2010), who found that *Brassica oleracea* var. capitata exhibited the highest DPPH activity and reducing power among ten common vegetables. (Köksal & Gülçin, 2008) also demonstrated the antioxidant potential of cauliflower, a variety of *Brassica oleracea*, through various assays including DPPH and FRAP. (Soengas et al., 2012) further highlighted the antioxidant activity of Brassica crops, with kale and cauliflower showing the highest activity at different plant stages. These studies collectively suggest that *Brassica oleracea,* including its various varieties, possesses significant antioxidant power, as evidenced by its performance in DPPH, FRAP, and other antioxidant assays.the results obtained in the table**(**Tab. 27**)** *Brassica oleracea var. elongata* leaf and seeds, explained by ,The antioxidant activity of *B. oleracea* extracts was investigated using five different assays, each with its own unique mechanism of action. The DPPH assay measures the ability of antioxidants to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) by donating hydrogen atoms or electrons . The methanolic extract of leaves (LM) exhibited the strongest antioxidant activity in this assay, which can be attributed to the high content of phenolic compounds and flavonoids. These phytochemicals possess hydroxyl groups that can readily donate hydrogen atoms to neutralize DPPH radicals, thereby reducing their stability and purple color . (Gawlik et al., 2013)

The β-Carotene bleaching method evaluates the ability of antioxidants to prevent the oxidation of β-Carotene in the presence of linoleic acid and oxygen(Miller.,1971). The aqueous extracts of leaves and seeds showed the highest antioxidant activity in this assay, likely due to the presence of water-soluble antioxidants such as ascorbic acid and

glucosinolates. These compounds can neutralize free radicals generated during linoleic acid oxidation, thereby preventing the degradation of *β*-Carotene(Podsedek., 2007). Phenolic compounds and flavonoids can also contribute to this activity by chelating metal ions involved in the initiation of lipid peroxidation(Rice-Evans et al., 1997). The anti-hemolysis activity assesses the ability of antioxidants to protect red blood cells from oxidative damage induced by free radicals such as peroxyl radicals (Cherrada et al., 2023). The aqueous extract of leaves exhibited the strongest anti-hemolysis activity, followed by the aqueous extract of seeds and the methanolic extract of leaves. Phenolic compounds and flavonoids can prevent hemolysis by scavenging free radicals, donating hydrogen atoms to neutralize peroxyl radicals, and stabilizing the cell membrane (Carvalho et al., 2010).. The FRAP (Ferric Reducing Antioxidant Power) assay measures the ability of antioxidants to reduce ferric ions (Fe^{+3}) to ferrous ions (Fe^{+2}) under acidic conditions. The methanolic extracts of leaves and seeds showed the highest reducing power in this assay, indicating the presence of phenolic compounds and flavonoids that can donate electrons to reduce Fe^{+3}. The reducing power of these phytochemicals is related to the number and position of hydroxyl groups in their structure (Pulido et al.,2000). Lastly, the TAC (Total Antioxidant Capacity) assay evaluates the overall antioxidant potential of a sample by measuring its ability to scavenge the stable radical cation (Re, et al., 1999). In conclusion, the antioxidant activity of *B. oleracea* extracts can be attributed to the presence of phenolic compounds and flavonoids, which play a crucial role in scavenging free radicals, reducing metal ions, preventing lipid peroxidation, and protecting cells from oxidative damage. The varying antioxidant activities observed in different assays can be explained by the specific mechanisms of action and the solubility of the antioxidant compounds present in each extract.

In terms of anti-hemolysis activity measured by the HRS method, AqE and MeE of *B. mauritanicum* and *Brassica oleracea var. elongata* demonstrated . These results indicate the ability of these extracts and compounds to protect red blood cells from oxidative damage, suggesting their potential application in developing new drugs, particularly for patients undergoing treatments prone to causing hemolysis. by (Elizondo-Luevano et al., 20),

These findings underscore the value of exploring plant-based solutions for addressing oxidative stress-related diseases and highlight the potential therapeutic applications of *B. mauritanicum* extracts by (Hemmami et al., 2023; Zeghoud et al., 2022),

A range of studies have explored the sun protection factor (SPF) of various plant extracts. (Lefahal et al., 2022) found that the aerial parts of *Bunium alpinum* a species related to *Bunium incrassatum*, exhibited significant photoprotective effects, with a major compound,

Apigenin-7- O -rutinoside, showing high SPF values. Similarly, (Adawiyah, 2019) (Andrade et al., 2019) both identified potential sun protection in plant extracts, with the former determining .

The factor for Sun Protection an indicator that is required to be listed on sunscreen products is the SPF value, which shows how efficient the product is at preventing sunburns and other skin harm. Its capacity to absorb, reflect, or deflect solar radiation determines how effective it is. It can be categorized into three groups based on the value: low sun protection (SPF<12), moderate sun protection (SPF=126–30), and strong sun protection (SPF≥30). There may be sunscreen activity in plants. Antioxidants are the reason for this, (Boo, 2019) . The study's findings**(**Tab. 28**)** for *B. mauritanicum* indicated that, compared to the commercial sunscreen (Avene®), which had a very high efficacy of more than 40, the two extracts had a low efficacy as a sunscreen with an SPF value of less than 12.These results are explained by the presence of compounds containeds in the extracts that absorb ultraviolet rays

A range of studies have explored the sun protection factor (SPF) of various *Brassica oleracea* extracts. (Mazumder et al., 2018) (Sharma et al., 2020) both found that these extracts have UV protection capabilities, with the latter identifying eggplant as having the highest SPF among the vegetables tested. (Lolo et al., 2017) (Rohmah et al., 2020) specifically focused on the SPF of Portulaca oleracea and Lactuca sativa var. crispa L. extracts, respectively, with both studies finding high SPF values for these extracts. These findings suggest that *Brassica oleracea* extracts, including those from Portulaca oleracea and Lactuca sativa var. crispa L., have potential as natural sunscreen ingredients

Based on the Sun Protection Factor (SPF) assay results presented in , the table**(**Tab. 28**)** methanolic and aqueous extracts of *B. oleracea* leaves and seeds exhibit promising potential for UV protection. The methanolic seed extract demonstrated the highest SPF value at 45.58, followed by the methanolic leaf extract at 38.05. The aqueous extracts also showed notable SPF values, with the seed extract at 27.95 and the leaf extract at 14.77.

These findings suggest that the extracts contain compounds that contribute to their UV protective properties. Polyphenols, particularly flavonoids, are known to play a significant role in protecting the skin from ultraviolet radiation. Flavonoids, such as quercetin and rutin, have been reported to absorb UV light and act as natural sunscreens (Saewan et al., 2013). They can also scavenge free radicals generated by UV exposure, thus reducing oxidative stress and preventing DNA damage (Nicohls et al., 2010)..

Terpenes, another class of compounds present in *B. oleracea* extracts, have also been associated with UV protection. Certain terpenes, such as limonene and geraniol, have shown

the ability to absorb UV light and protect against UV-induced skin damage. (Kaur et al., 2010). dditionally, saponins have been reported to possess antioxidant properties and may contribute to the overall UV protective effect of the extracts (Ashok et al., 2012).

These percentages reflect the ability of these extracts and compounds to inhibit the denaturation of egg albumin. Protein denaturation is the process whereby proteins undergostructural unraveling of their secondary and tertiary conformationsas a consequence of external perturbation, such as heat, acidity,basicity, concentrated inorganic salt, or organic solvent (Islam et al., 2023). The significance of these findings lies in their potential to inform the development of plant-based anti-inflammatory remedies. While AqE and MeE exhibit anti-inflammatory properties (Fig. 17.18), Diclofenac, a known pharmaceutical anti-inflammatory agent, demonstrates substantially higher efficacy in this in vitro assay. These results emphasize the importance of understanding the anti-inflammatory potential of plant extracts, as they may serve as natural alternatives in managing chronic inflammatory conditions. Some phenolic compounds, such as flavonoids and phenolic acids, have been shown to bind to plasma proteins and protect these bonds from being broken (Kurlbaum & Högger, 2011). This may explain the potent anti-inflammatory activity of the crude extracts of *B. mauritanicum* which contains bioflavonoids such as gallic acid acid, quercetin, andrutin.Furthermore, such assessments can contribute to the broader field of precision medicine and have implications for drug development, especially in targeting molecular pathways associated with inflammation, such as NF-κB and COX-2, and regulating pro-inflammatory cytokines like TNF-α and IL-6 (Pandith et al., 2013; Van Hoyweghen et al., 2014; Xu et al., 2007).

The anti-inflammatory activity of *B. oleracea extracts* (Fig. 18) can be attributed to the presence of various phytochemical compounds, including polyphenols, flavonoids, terpenes, and saponins. These compounds play a crucial role in protecting tissues and cells from infections through several mechanisms: Polyphenols: Polyphenolic compounds, such as phenolic acids and flavonoids, possess strong antioxidant and anti-inflammatory properties. They scavenge free radicals, reduce oxidative stress, and modulate inflammatory pathways by inhibiting pro-inflammatory enzymes and cytokines (Pandey & Rizvi, 2009). Flavonoids, a subclass of polyphenols, have been shown to exhibit anti-inflammatory effects by inhibiting enzymes involved in the production of inflammatory mediators, such as cyclooxygenase (COX) and lipoxygenase (LOX). They also suppress the activation of NF-κB, a key transcription factor in the inflammatory response (Serafini et al., 2010). Terpenes and terpenoids have demonstrated anti-inflammatory properties by inhibiting the release of pro-

inflammatory cytokines, such as TNF-α and IL-1β, and reducing the production of nitric oxide (NO) and prostaglandin E2 (PGE2) (Guimarães et al., 2013). Saponins have been reported to possess anti-inflammatory effects by modulating the immune system, inhibiting the production of pro-inflammatory cytokines, and reducing the expression of adhesion molecules involved in the inflammatory process (Sparg et al., 2004). The higher anti-inflammatory activity observed in the aqueous extracts compared to the methanolic extracts suggests that the water-soluble phytochemical compounds, such as polyphenols and flavonoids, may be the primary contributors to the anti-inflammatory properties of *B. oleracea*

B. mauritanicum, a species of the *Bunium genus*, has been found to exhibit antibacterial activity in vitro. (Hayet, 2017) reported that the essential oils of *B. mauritanicum* demonstrated modest antibacterial activity against both Gram-positive and Gram-negative bacteria. This finding is consistent with the broader pharmacological potential of the *Bunium genus*, which includes significant antibacterial properties (Mohammad hosseini, 2021). Further research is needed to explore the specific mechanisms and potential applications of *B. mauritanicum*, antibacteria activity.

A series of studies have demonstrated the in vitro antibacterial activity of *B. oleracea. Begum* (2013) found that the acetone extract of *B. oleracea* exhibited the highest antimicrobial activity against a range of bacteria and fungi. (Sibi, 2013) and (Zamir , 2013) both reported significant antibacterial activity of *B. oleracea*, with the methanol extract showing distinct zones of inhibition against various bacterial strains. (Waghulde, 2018) further confirmed the antimicrobial effect of *B. oleracea*, with significant inhibition against both gram positive and gram negative bacteria. These studies collectively suggest that *B. oleracea* has potential as an antibacterial agent.and Further studies utilizing green synthesis of silver nanoparticles (AgNPs) from *B. oleracea* extracts confirmed the presence of potent antimicrobial properties. These AgNPs exhibited broad-spectrum antibacterial activity, with inhibition zones ranging from 9 to 14 mm against bacteria such as Bacteroides fragilis and Staphylococcus epidermidis. The minimum inhibitory concentration (MIC) of the synthesized BO-AgNPs further supported their efficacy, with low MIC values indicating strong antibacterial potential (Ansar et al., 2020).

These previous studies confirm and prove the results obtained in the table(30,31) The biological antimicrobial potential of *B. oleracea var. elongata*(leaves. seeds) and *B. mauritanicum* extracts is substantial due to its rich phytochemical composition, particularly

glucosinolates and phenolic compounds they have an inhibitory or killing effect on bacteria MIC and MBC.

Diabetes mellitus is a complex and diverse group of disorders characterized by chronic hyperglycemia (Delvecchio, 2023). This condition can be caused by a range of factors, including insulin hyposecretion and/or insensitivity (Mealey & Ocampo, 2007). It is now recognized that diabetes mellitus is not a single disease, but rather a syndrome with various forms and pathophysiologies (Al Homsi & Lukic, 1993). This heterogeneity underscores the need for personalized treatment approaches.

Research on *B. mauritanicum* has shown promising antidiabetic activity. (Hayet et al., 2017a)found that the essential oils and methanolic extracts of these plants exhibited significant antioxidant, anti-inflammatory, and anti-hemolytic activities. Similarly, (Berroukeche et al., 2021)reported that the acetonic and ethanolic tuber extracts of *B. mauritanicum* demonstrated strong antioxidant and anti-hemolytic properties. These findings suggest that these plants have the potential to be developed as antidiabetic agents. However, more research is needed to specifically investigate its antidiabetic effects in an animal model to support the study results further. This study investigated the antidiabetic potential of aqueous (AqE) and methanolic (MeE) extracts from the tubers of *Bunium mauritanicum* and *Brassica oleracea var. elongata* leavs and seeds using an in vitro glucose uptake assay with Saccharomyces cerevisiae (baker's yeast) as a model organism (figure 21.22) . The purpose of this assay was to evaluate the ability of the plant extracts to enhance glucose uptake by yeast cells, which serves as an indicator of their potential to regulate blood glucose levels in diabetic conditions.

The methodology involved incubating varying concentrations (5, 10, 20, and 40 mg/ml) of the extracts with a glucose solution and yeast suspension at 37°C for specified time intervals. The glucose uptake was then determined by measuring the absorbance of the supernatant at 540 nm and calculating the percentage increase in glucose uptake compared to a control without the test sample. Metformin, a standard antidiabetic drug, was used as a positive control.

The results, as shown in Figures 4 and 5, demonstrated that both AqE and MeE exhibited dose-dependent and time-dependent increases in glucose uptake by yeast cells. At the highest concentration tested (40 mg/ml), MeE showed slightly higher glucose uptake compared to AqE at 120 minutes (Figure). The glucose uptake values for the extracts were lower than that of the positive control, metformin. These findings suggest that *B.*

mauritanicum tuber extracts possess antidiabetic potential, with the methanolic extract showing marginally better activity than the aqueous extract.

Although there are no previous studies of *Bunium mauritanicum* specifically for this type of test. However, some studies have reported the antidiabetic potential of other species within the Bunium genus. For instance, demonstrated the α-glucosidase inhibitory activity of essential oils from *B. persicum* fruits, suggesting their potential use in managing type 2 diabetes (Degtjareva et al., 2009). Similarly, another study found that hydroalcoholic extracts of *B. persicum* fruits exhibited antihyperglycemic effects in streptozotocin-induced diabetic rats (Anshika et al., 2022). antidiabetic potential of *Brassica oleracea var. elongata*.results (figure 22.) shown appear similar in antidiabetic potential for the various extracts of seeds and leaves(AqE and MeE.) and this is due to their contain compounds that have an effect on Glut (glucose channels.), (Navale & Paranjape, 2016). The phenolic profile of *B. mauritanicum* tuber *Brassica oleracea var. elongata* extracts,and analyzed by RP-HPLC (Figure 17), revealed the presence of several bioactive compounds that may contribute to the observed antidiabetic activity. Gallic acid, a phenolic acid known for its antioxidant and antidiabetic properties (Huang et al., 2016), was found in both AqE and MeE. The presence of gallic acid in the extracts suggests that it may play a role in the observed glucose uptake enhancement (Patel & Goyal, 2011). Additionally, quercetin, a flavonoid with well-established antidiabetic effects (M Eid & S Haddad, 2017), was exclusively detected in MeE. The presence of quercetin in the methanolic extract may explain its slightly higher antidiabetic activity compared to the aqueous extract (Chen et al., 2014). Other compounds, such as rutin and vanillin, were also detected in the extracts and may contribute to the overall antidiabetic potential of *B. mauritanicum* tubers (Ghorbani, 2017) .

Hypothyroidism is characterized by decreased thyroid hormone production, primarily affecting T3 and T4 levels (Dias et al., 2022). The condition is diagnosed through clinical examination and laboratory tests, with primary hypothyroidism showing elevated TSH and low free T4 levels (Chaker et al., 2000). TSH, produced by the anterior pituitary, is the primary stimulus for thyroid hormone production and is regulated by the hypothalamic-pituitary axis (Pirahanchi & Jialal, 2020). In hypothyroidism, TSH plays a significant role in T3 generation from T4 in peripheral tissues. Primary hypothyroidism exhibits highe3:T4 ratios and serum T3 levels compared to central hypothyroidism, despite similarly low T4 levels (Kabadi, 1993). Treatment typically involves levothyroxine (LT4) replacement, which normalizes TSH and T4 levels. However, some patients may continue to experience persistent symptoms even after treatment, affecting their quality of life (Chaker et al., 2000) .

The results in highlight t**(**Tab.33**)**he effects of different treatments on T4 synthesis in a PTU-induced hypothyroidism model. The highly significant decrease in T4 levels in the PTU group is expected and confirms the efficacy of this compound as an inhibitory agent for thyroid hormone synthesis. This observation highlights the importance of hormonal regulation in maintaining thyroid homeostasis.The effect observed with the TBM extract is particularly intriguing. The less marked decrease in T4 levels in the PTU + TBM group indicates that this extract could play a protective or stimulating role on thyroid function. It would be relevant to further explore the mechanisms of action of the TBM extract, in particular by examining whether it promotes T4 synthesis via specific signaling pathways or by improving the availability of precursors necessary for T4 production.The lack of significant difference in T3 levels in the PTU alone and PTU + TBM groups suggests that, despite variations in thyroid hormone synthesis, T3 levels may remain relatively stable due to compensatory mechanisms. It would be interesting to explore whether other factors, such as precursor availability or enzymatic activity involved in T4 to T3 conversion, could influence these results.The effect observed with Lovo is particularly relevant, as it highlights the ability of this drug to modulate T3 levels. The significant decrease in T3 levels in the PTU + Lovo group could have important clinical implications, particularly in the management of thyroid disorders. It would be relevant to further explore the mechanisms of action of Lovo, particularly by examining its impact on the metabolic pathways of T4 to T3 conversion and on the regulation of thyroid hormone secretion.In conclusion, our results highlight the complex interactions between treatments and T3 levels in the context of PTU-induced hypothyroidism. Although TBM extract did not show a significant effect on T3 levels, the regulatory effect of Lovo deserves further investigation. These observations pave the way for future research to better understand the mechanisms of action of these agents and their clinical potential in the management of thyroid disorders.

Recent studies have explored the effects of *Bunium mauritanicum* (Talghouda) on thyroid function and related parameters in rats. (Aiouaz & Bitam, 2022a) found that Talghouda treatment improved thyroid gland repair and reactivated thyroid follicles in both hyper- and hypothyroid rats. (Attoui et al., 2021) observed that a diet supplemented with Talghouda powder led to increased body and organ weights, as well as changes in biochemical parameters**(**Tab. 34**)** and thyroid histology. (Attoui et al., 2021) reported that Talghouda extracts exhibited strong antioxidant and anti-hemolytic properties, while dietary supplementation affected body weight, biochemical markers, and organ histology. Although not specifically studying *Bunium mauritanicum*, (Idris, 2011) investigated the effects of other

plant extracts on thyroid hormones in rats, finding that Moringa oleifera extract significantly decreased thyroxine levels. These studies suggest that Talghouda and other plant-based treatments may have potential therapeutic effects on thyroid function and related physiological parameters.according to the previous studies Talghouda has demonstrated several impacts on rat physiology. Dietary supplementation with *Bunium mauritanicum* tuber powder resulted in increased body weight, blood glucose levels, triglycerides, cholesterol, and HDL levels in female rats (Attoui et al., 2021). It also demonstrated potential benefits for thyroid health, repairing and reactivating thyroid follicles in both hyper- and hypothyroid rats (Aiouaz & Bitam, 2022a). The plant's organic extracts exhibited estrogenic effects on rabbit ovaries at moderate doses, increasing primary and secondary follicles (Aiouaz & Bitam, 2022a). Additionally, *B. mauritanicum* extracts showed strong antioxidant and anti-hemolytic properties in vitro (Attoui et al., 2021), these studies suggest that *B. mauritanicum* may have potential as a functional food and traditional medicine, affecting various physiological parameters including thyroid function, lipid metabolism, and reproductive health. However, further research is needed to fully understand its mechanisms of action and potential therapeutic applications. biochemical parameters results show the therapeutic effct of TBM and Levothyrox® at levels of AST, ALAT, ALP, GGT, DB, TB, **(**Tab. 34**)** compared to the control the due to presence bioactive compounds in the TBM and Levothyrox® that have a pharmacological effect.

Studies have investigated the effects of Talghouda and thyroid dysfunction on hematological parameters in rats. Talghoud a supplementation improved thyroid tissue damage in hyper- and hypothyroid rats (Aiouaz & Bitam, 2022a) and increased body weight and various biochemical parameters (Attoui et al., 2021). Hypothyroidism and hyperthyroidism induced by propylthiouracil (PTU) and L-thyroxine, respectively, affected hematological parameters in rats(PTU) **(**Tab. 35**)**. Hyperthyroid rats showed decreased mean corpuscular hemoglobin concentration (MCHC) and platelet count compared to hypothyroid rats (Sinan & Keskin, 2016). Hypothyroidism caused anemia in rats with functional gonads, while castration reversed the effects of thyroxine deficit on the erythrogram (Gomes et al., 2004). Both hypothyroidism and hypogonadism had minimal effects on the leucogram (Gomes et al., 2004) the results obtained in. **(**Tab. 35**)** in the two groups (TBM and Lovo) compared to the control explained by, *Bunium mauritanicum* tuber powder contains the nutritional composition proteins lipids.iron .zinc which can be the reason for this therapeutic effect on the hematological and parameters(Hemoglobin concentrations, RBC,Hematocrit and patelets). These studies suggest that thyroid dysfunction and Talghouda supplementation can

influence hematological parameters in rats, with potential implications for thyroid health composition (T3, T4, TSH).. and blood composition (Hemoglobin concentrations, RBC,Hematocrit and patelets), (Aiouaz & Bitam, 2022a).

Phenylhydrazine (PHZ) is commonly used to induce experimental anemia in rats, causing a decrease in red blood cells, hemoglobin, and hematocrit (Redondo et al., 1995; Sheth et al., 2021). The severity of anemia depends on the compound's functional group, with non-splenectomized rats becoming anemic more rapidly due to splenic sequestration of damaged erythrocytes (Sheth et al., 2021). PHZ treatment leads to erythroid hyperplasia in bone marrow and extramedullary hematopoiesis in the spleen and liver (Redondo et al., 1995). It also triggers immune activation, increasing circulating leukocytes, particularly lymphocytes, and elevating serum IgG and prostaglandin E2 levels (Vedvick & Itano, 1980). The spleen enlarges during recovery in non-splenectomized rats, while splenectomized animals remain anemic until treatment cessation (Vedvick & Itano, 1980). PHZ-induced anemia affects multiple organs, including the liver, heart, spleen, and bone marrow, with the potential for recovery upon treatment with anti-anemic formulations (Sheth et al., 2021).according to the results obtained **(**Tab. 37**)** The group treated with (.PHZ) exhibited v. reductions in RBC count, hemoglobin levels, and hematocrit percentage, alongside a marked increase in platelet count and WBC count, (highly significant difference.) compared to the control group because phenylhydrasine induces anemia as a consequence of peroxidations of RBC mrmbrane lipids and this effect may be a result of the autoxidations of the drug and oxygen radicals with membrane lipids.(Orrico et al., 2023) .

The groups treated SBO and LBO showed significant improvement on hematological parameters (Hemoglobin concentrations, RBC,Hematocrit and patelets). comapared to the control this effect may be due to the nutritional value of LBO and SBO(especially the percentage of iron and zinc zich arol in hematopoiesis).the biochemical parameters results**(**Tab. 36**)** show also the therapeutic effct in the groups treated with LBO, SBO, B-Feron®: at levels of AST, ALAT, ALP GGT, creatinine, compared to the control the due to presence antioxidant compounds in the LBO and SBO protectes against oxidative stress also inflamition and that have a pharmacological effect.

Brassica oleracea varieties, including leaf cabbage and cauliflower greens, are traditional vegetables with significant nutritional value. While some varieties may be lower in protein and iron compared to Swiss chard (Mariga et al., 2012), they are generally rich in other nutrients like fiber, calcium, and vitamin C. Cauliflower greens, in particular, contain high levels of iron at 40 mg per 100g (Mohankumar & Bhavani, 2004). However, the

bioavailability of minerals in these vegetables may be limited due to anti-nutritional factors such as cyanides, tannins, and oxalates

(Vargas-Rincón et al., 2013). Iron fortification in leafy vegetables through agronomic prac.tices or breeding approaches has shown promise, with some bio-fortified varieties achieving up to 40 times higher iron content than traditional varieties (Chatterjee, 2016). Despite potential concerns about effects on packed cell volume and liver enzymes (Mohankumar & Bhavani, 2004) iron-rich leafy vegetables could play a role in addressing iron deficiency anemia, especially among vegetarians who require 1.8 times more iron than non-vegetarians (Chatterjee, 2016).

The histological results (Fig. 24.25), presented underscore the severe systemic damage caused by PTU-induced hypothyroidism, affecting multiple organs, including the liver, kidney, and spleen. The PTU group's findings, particularly the heavy inflammatory infiltrates, necrosis, and lymphoid depletion, reflect the far-reaching consequences of disrupted thyroid hormone regulation, which triggers oxidative stress, impairs cellular metabolism, and leads to inflammatory responses (Alkalby & Alzerjawi, 2013; Chan & Ng, 1995). Hypothyroidism's impact on immune function is clearly evident in the spleen's lymphoid depletion, highlighting the thyroid's critical role in immune system maintenance (Nakamura et al., 2007; Rooney et al., 2003).

The introduction of therapeutic interventions shows the potential for reversing this damage. The *B. mauritanicum* treatment demonstrates a promising ability to reduce inflammation and restore tissue integrity. The partial reduction in liver inflammation and the restoration of lymphoid tissue in the spleen suggest that *B. mauritanicum* exerts anti-inflammatory and immunomodulatory effects, likely due to its phytochemical constituents such as flavonoids, phenolic acids, and saponins (Benkhnigue et al., 2023; Boutlelis et al., 2023). These bioactive compounds are known to scavenge free radicals, inhibit inflammatory cytokines, and enhance the immune response (El-Ghazouani et al., 2024). Although *B. mauritanicum* does not achieve complete tissue recovery, its moderate effects suggest that it could be a valuable complementary therapy, especially for reducing inflammation and supporting immune function. This natural remedy offers an alternative approach, particularly for patients seeking plant-based treatments(Abd-ElGawad et al., 2022).

Levothyrox®, on the other hand, demonstrates superior therapeutic efficacy, almost completely restoring the liver, kidney, and spleen structures to their normal state. This suggests that Levothyrox® not only replaces the deficient thyroid hormone but also counteracts the biochemical disturbances caused by hypothyroidism, such as oxidative

damage, inflammation, and immune dysregulation (Balkrishna et al., 2024; Gupta & Misra, 2013). The fact that the tissues are nearly identical to those of the control group emphasizes Levothyrox®'s crucial role in reversing systemic damage, restoring organ function, and maintaining metabolic equilibrium in hypothyroid conditions (Bozbek & Şentürk, 2023).

The study clearly establishes Levothyrox® as the gold-standard treatment for hypothyroidism, with unparalleled efficacy in reversing the organ damage induced by PTU. Meanwhile, *B. mauritanicum* offers a natural, albeit less potent, alternative with potential for use as a complementary therapy. These findings highlight the importance of early and effective treatment of hypothyroidism to prevent irreversible organ damage, and suggest potential future exploration of *B. mauritanicum* for its therapeutic applications in inflammatory and immune-related conditions.

This study highlights the profound impact of phenylhydrazine (PHZ) toxicity on vital organs, primarily through oxidative stress and hemolytic, resulting in significant liver, kidney, and spleen damage. PHZ induces liver inflammation by promoting the infiltration of inflammatory cells, a hallmark of oxidative liver injury(Akara et al., 2021). This is compounded by nephrotoxicity, as evidenced by the disruption of renal tubules and interstitial inflammation, and spleen dysfunction marked by lymphoid depletion and immune compromise. These pathological effects align with the known mechanisms of PHZ, which primarily causes hemolysis and triggers systemic oxidative stress (Madhikarmi & Murthy, 2015; Obayuwana et al., 2022).

The therapeutic interventions using *Brassica oleracea* leaf and seed extracts reveal the organ-protective effects of plant-derived antioxidants. The leaf extract (LBO), rich in flavonoids and glucosinolates, demonstrated hepatoprotective and nephroprotective properties, significantly reducing inflammation and promoting moderate tissue recovery (Hamed et al., 2021; Hossain et al., 2022). However, the seed extract (SBO) exhibited superior efficacy, likely due to its higher concentration of bioactive compounds, which confer stronger antioxidant, anti-inflammatory, and cytoprotective effects, allowing for near-normal restoration of tissue architecture (Obayuwana & Obayuwana, 2022; Ray et al., 2021).

The B-Feron® supplement also showed notable recovery, underscoring the critical role of essential nutrients like iron, B12, B9, and vitamin C in mitigating the oxidative stress induced by PHZ (Zovko Koncic & Tomczyk, 2013). These nutrients are pivotal in promoting red blood cell production, reducing hemolysis, and restoring liver and kidney function. The near-complete tissue recovery in the B-Feron® group emphasizes the synergistic role of

antioxidants and micronutrients in restoring tissue integrity and function post-toxicity (Pasdaran et al., 2023).

The findings from this study suggest that combining phytotherapeutic agents like *Brassica oleracea* extracts with micronutrient supplementation could provide an effective strategy to counteract the deleterious effects of chemical-induced oxidative stress. This approach could be valuable for developing treatments for toxin-induced organ damage, with broader applications in addressing liver and kidney diseases and immune system compromise caused by various toxic insults. Future studies could explore the optimal combination of phytochemicals and micronutrients to maximize recovery and provide comprehensive protection against oxidative damage.

Conclusion

Conclusion

This thesis investigated the phytochemical composition and biological activities of Bunium mauritanicum tubers and leaves and seeds of *Brassica oleracea var. elongata*, two plants traditionally used in the Oued Souf region of Algeria. The study combined ethnobotanical surveys, *in vitro* phytochemical and biological assays, and in vivo experiments on rats to comprehensively evaluate these plants' potential therapeutic properties.

The phytochemical analysis revealed a rich profile of bioactive compounds in both plants. HPLC analysis identified significant concentrations of gallic acid, chlorogenic acid, and rutin in *B. oleracea* extracts, while *B. mauritanicum* showed high levels of gallic acid and quercetin. These findings provide a scientific basis for the plants' traditional medicinal uses and highlight their potential as sources of beneficial phytochemicals.

Antioxidant assays demonstrated the potent free radical scavenging abilities of both plants. Notably, the methanolic leaf extract of B. oleracea exhibited exceptional DPPH scavenging activity (IC_{50}: 0.058±0.001 mg/mL), surpassing even that of the standard antioxidant, ascorbic acid. The aqueous extract of *B. mauritanicum* also showed strong antioxidant potential across multiple assays, including DPPH, FRAP, and β-carotene bleaching tests. These results suggest that both plants could be valuable sources of natural antioxidants.

Antimicrobial studies revealed that B. oleracea extracts possessed broader spectrum activity compared to B. mauritanicum. The methanolic seed extract of *B. oleracea* demonstrated significant inhibition against Staphylococcus aureus (18 mm inhibition zone), while *B. mauritanicum* extracts were most effective against *Escherichia coli*. These findings indicate the potential of these plants, particularly *B. oleracea*, in combating various pathogenic microorganisms.

The *in vitro* anti-inflammatory assays showed promising results for both plants. B. oleracea aqueous leaf extract exhibited the highest protein denaturation inhibition (94.8%), comparable to the reference drug diclofenac (96.46%). This suggests that B. oleracea could be a potent natural anti-inflammatory agent, potentially useful in treating inflammatory conditions.

In vivo studies provided compelling evidence for the therapeutic potential of these plants. *B. mauritanicum* tuber extract (200 mg/kg) significantly improved thyroid hormone levels in hypothyroid rats, reducing TSH and increasing T4 levels. Meanwhile, *B. oleracea* leaf extract (200 mg/kg) showed remarkable efficacy in treating anemia, significantly improving hematological parameters including red blood cell count and hemoglobin levels.

These results validate the traditional uses of these plants and suggest their potential in managing endocrine and hematological disorders.

Histological analyses further corroborated the therapeutic effects of the plant extracts. Both *B. mauritanicum* and *B. oleracea* treatments showed significant improvements in liver, kidney, and spleen histology in rats with induced hypothyroidism and anemia. This indicates that these plant extracts not only ameliorate specific symptoms but also offer broader protective effects against organ damage associated with these conditions.

From a professional standpoint, this thesis represents a significant contribution to the field of ethnopharmacology and natural product research. The comprehensive approach, combining traditional knowledge with modern scientific techniques, provides a robust framework for evaluating medicinal plants. The identification of specific bioactive compounds and their associated activities opens new avenues for drug discovery and development. Particularly noteworthy is the potential of *B. mauritanicum* in thyroid disorders and B. oleracea in anemia treatment, which could lead to the development of novel, plant-based therapies. Future research should focus on isolating and characterizing specific bioactive compounds, exploring their mechanisms of action, and conducting human clinical trials to fully evaluate their therapeutic potential. Overall, this thesis not only advances our understanding of *B. mauritanicum* and *B. oleracea var. elongata* but also underscores the potential of ethnobotanical research in addressing global health issues.

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Annexes

Annexe 1.

_ استبيان حول الطب الشعبي في منطقة وادي سوف بالجزائر https://forms.gle/BMKFnFgtS3vb9DM89

يهدف هذا الاستبيان إلى استكشاف ممارسات الطب الشعبي في منطقة وادي سوف بالجزائر. وستكون المعلومات التي تقدمها حاسمة لفهم العلاجات العشبية المستخدمة محليًا وفوائدها الصحية المحتملة .

ملاحظة :هذا الاستبيان مجهول الهوية تمامًا.

القسم الأول: المعلومات العامة

1. العمر:
   - ○ 18-30
   - ○ 31-50
   - ○ 51-65
   - ○ أكثر من 65
2. الجنس:
   - ○ ذكر
   - ○ أنثى
3. المهنة:
   - ○ عامة الناس
   - ○ معالج أعشاب / معالج تقليدي
   - ○ بائع الأعشاب الطبيعية

القسم الثاني: استخدام الطب الشعبي

1. هل تستخدم الطب الشعبي لعلاج أي مشاكل صحية؟
   - ○ نعم
   - ○ لا
2. إذا كانت الإجابة بنعم، فيرجى تحديد المشكلة الصحية المحددة التي تعالجها والعلاجات العشبية التي استخدمتها، بما في ذلك النباتات أو

الحالة	نبات	طريقة التحضير	الجرعة	غير مناسب لـ	قيود الاستخدام	موقع تواجد النبات
☐ مرض السكري من النوع II				الرضع		
☐ فقر الدم (نقص التغذية)				الأطفال		
☐ قصور الغدة الدرقية				البالغون		
☐ هشاشة العظام				الحوامل		
☐ انخفاض ضغط الدم				المرضعة		
				اخرى		
				ليس له اي اعراض لكل الفئات جانبية حسب التجربة		

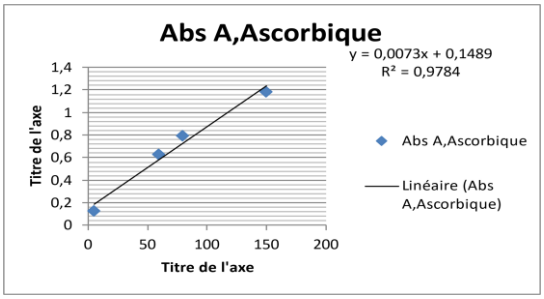
المكونات المشاركة، وطريقة تحضيرها، والجرعة.

3. هل لاحظت أي آثار جانبية من استخدام هذه العلاجات العشبية؟
   - ○ نعم
   - ○ لا
4. إذا كانت الإجابة بنعم، فيرجى وصف الآثار الجانبية التي واجهتها.

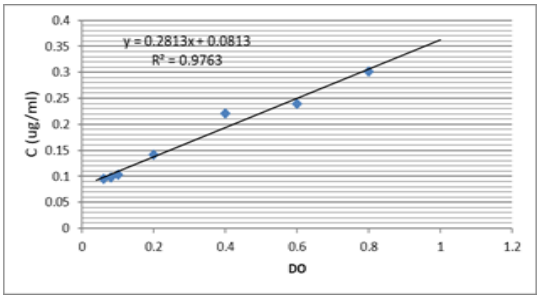
القسم الثالث: معلومات إضافية (اختياري)

1. هل هناك أي شيء آخر ترغب في مشاركته حول ممارسات الطب الشعبي في منطقة وادي سوف؟

شكرًا لك على مشاركتك !



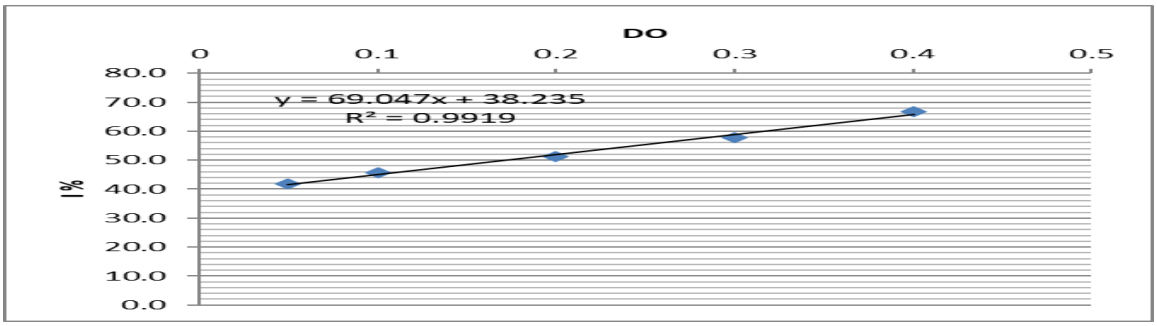
Calibration curve of ascorbic acid for DPPH assays.



Calibration curve of ascorbic acid for FRAP assays.

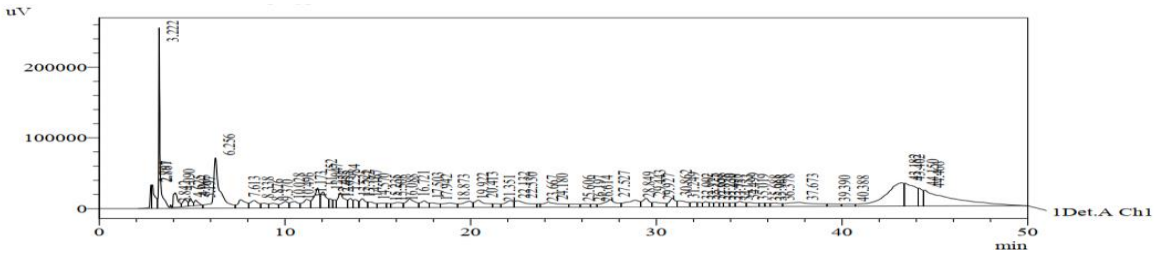
Annexe 3.

Annexe 4.



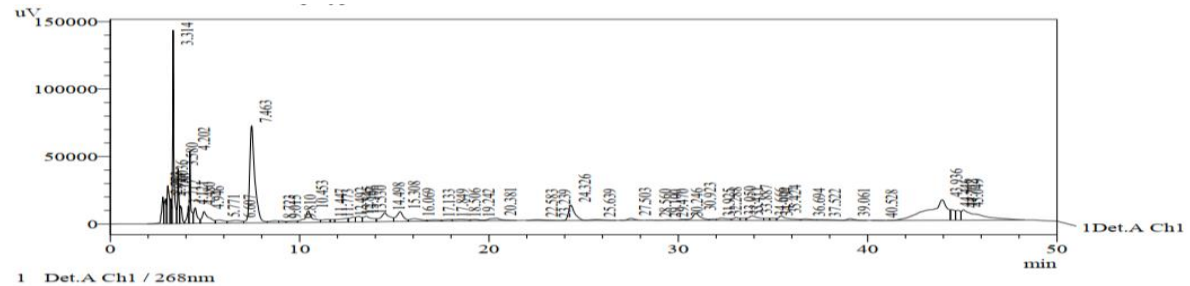
Calibration curve of gallic acid for β-Carotene Bleaching (BCB) assay..

Annexe 5.



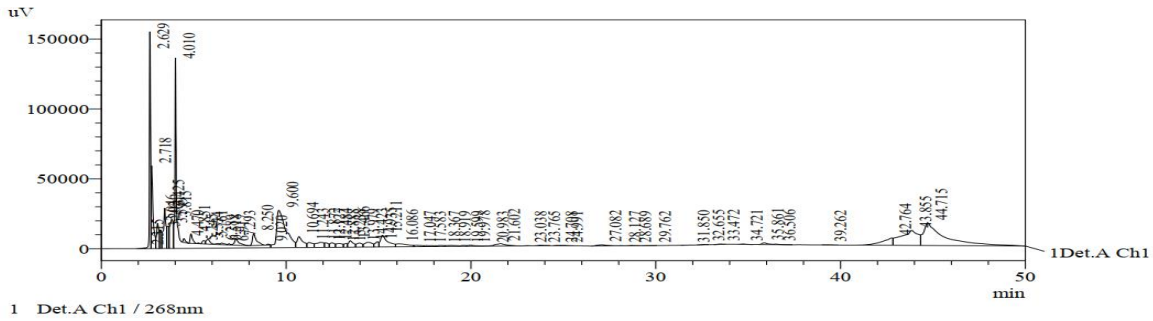
Chromatogram of leaves methanolic extract of *Brassica oleracea* var. elongata.

Annexe 6.



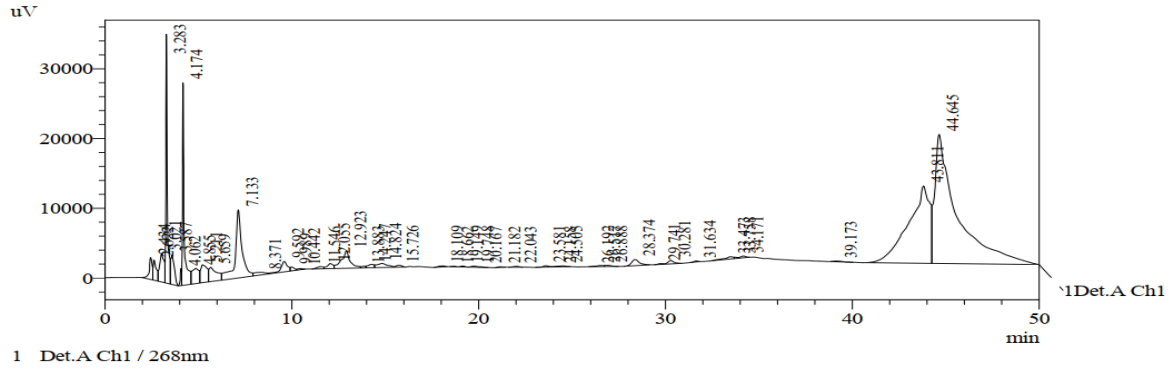
Chromatogram of seeds methanolic extract of *Brassica oleracea* var. elongata.

Annexe 7.



Chromatogram of leaves aqueous extract of *Brassica oleracea* var. elongata.

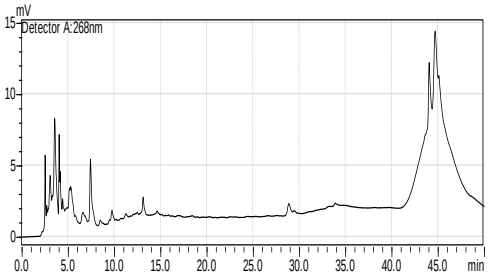
Annexe 8.



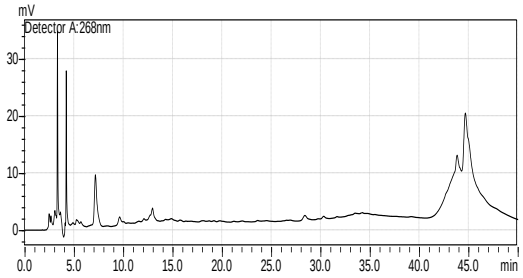
Chromatogram of seeds aqueous extract of *Brassica oleracea* var. elongata.

Annexe 9.

Figure: Steps for **Sacrifice**



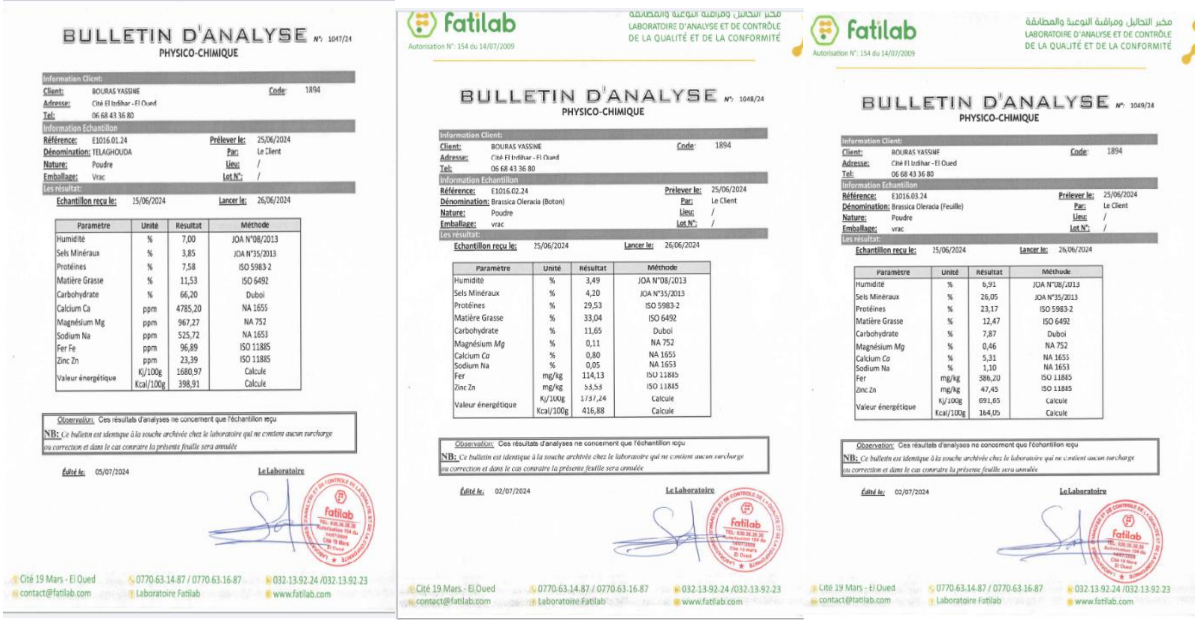
Chromatogram of Bunium mauritanicum tubers aqueous extract



Chromatogram of Bunium mauritanicum tubers methanolic extract

Annexe 10.

Annexe 11.



BULLETIN D'ANALYSE N°: 1047/24
PHYSICO-CHIMIQUE

Information Client:

Client:	BOURAS YASSINE	Code:	1894
Adresse:	Cité El Izdihar - El Oued		
Tel:	06 68 43 36 80		

Information Echantillon

Référence:	E1016.01.24	Prélever le:	25/06/2024
Dénomination:	TELAGHOUDA	Par:	Le Client
Nature:	Poudre	Lieu:	/
Emballage:	Vrac	Lot N°:	/

Les résultat:

Echantillon reçu le: 15/06/2024 Lancer le: 26/06/2024

Parametre	Unité	Résultat	Méthode
Humidité	%	7,00	JOA N°08/2013
Sels Minéraux	%	3,85	JOA N°35/2013
Protéines	%	7,58	ISO 5983-2
Matière Grasse	%	11,53	ISO 6492
Carbohydrate	%	66,20	Duboi
Calcium Ca	ppm	4785,20	NA 1655
Magnésium Mg	ppm	967,27	NA 752
Sodium Na	ppm	525,72	NA 1653
Fer Fe	ppm	96,89	ISO 11885
Zinc Zn	ppm	23,39	ISO 11885
Valeur énergétique	Kj/100g	1680,97	Calcule
	Kcal/100g	398,91	Calcule

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu
NB: *Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée*

Édité le: 05/07/2024 Le Laboratoire

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مخبر التحاليل ومراقبة النوعية والمطابقة
LABORATOIRE D'ANALYSE ET DE CONTRÔLE DE LA QUALITÉ ET DE LA CONFORMITÉ

BULLETIN D'ANALYSE N°: 1048/24
PHYSICO-CHIMIQUE

Information Client:

Client:	BOURAS YASSINE	Code:	1894
Adresse:	Cité El Izdihar - El Oued		
Tel:	06 68 43 36 80		

Information Echantillon

Référence:	E1016.02.24	Prélever le:	25/06/2024
Dénomination:	Brassica Oleracia (Boton)	Par:	Le Client
Nature:	Poudre	Lieu:	/
Emballage:	vrac	Lot N°:	/

Les résultat:

Echantillon reçu le: 25/06/2024 Lancer le: 26/06/2024

Parametre	Unité	Résultat	Méthode
Humidité	%	3,49	JOA N°08/2013
Sels Minéraux	%	4,20	JOA N°35/2013
Protéines	%	29,53	ISO 5983-2
Matière Grasse	%	33,04	ISO 6492
Carbohydrate	%	11,65	Duboi
Magnésium Mg	%	0,11	NA 752
Calcium Ca	%	0,80	NA 1655
Sodium Na	%	0,05	NA 1653
Fer	mg/kg	114,13	ISO 11885
Zinc Zn	mg/kg	53,53	ISO 11885
Valeur énergétique	Kj/100g	1737,24	Calcule
	Kcal/100g	416,88	Calcule

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu
NB: *Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée*

Édité le: 02/07/2024 Le Laboratoire

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مخبر التحاليل ومراقبة النوعية والمطابقة
LABORATOIRE D'ANALYSE ET DE CONTRÔLE DE LA QUALITÉ ET DE LA CONFORMITÉ

BULLETIN D'ANALYSE N°: 1049/24
PHYSICO-CHIMIQUE

Information Client:

Client:	BOURAS YASSINE	Code:	1894
Adresse:	Cité El Izdihar - El Oued		
Tel:	06 68 43 36 80		

Information Echantillon

Référence:	E1016.03.24	Prélever le:	25/06/2024
Dénomination:	Brassica Olerada (Feuille)	Par:	Le Client
Nature:	Poudre	Lieu:	/
Emballage:	vrac	Lot N°:	/

Les résultat:

Echantillon reçu le: 15/06/2024 Lancer le: 26/06/2024

Parametre	Unité	Résultat	Méthode
Humidité	%	6,91	JOA N°08/2013
Sels Minéraux	%	26,05	JOA N°35/2013
Protéines	%	23,17	ISO 5983-2
Matière Grasse	%	12,47	ISO 6492
Carbohydrate	%	7,87	Duboi
Magnésium Mg	%	0,46	NA 752
Calcium Ca	%	5,31	NA 1655
Sodium Na	%	1,10	NA 1653
Fer	mg/kg	386,20	ISO 11885
Zinc Zn	mg/kg	47,45	ISO 11885
Valeur énergétique	Kj/100g	691,65	Calcule
	Kcal/100g	164,05	Calcule

Observation: Ces résultats d'analyses ne concernent que l'échantillon reçu
NB: *Ce bulletin est identique à la souche archivée chez le laboratoire qui ne contient aucun surcharge ou correction et dans le cas contraire la présente feuille sera annulée*

Édité le: 02/07/2024 Le Laboratoire

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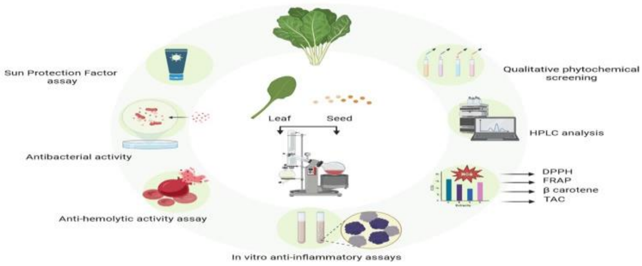
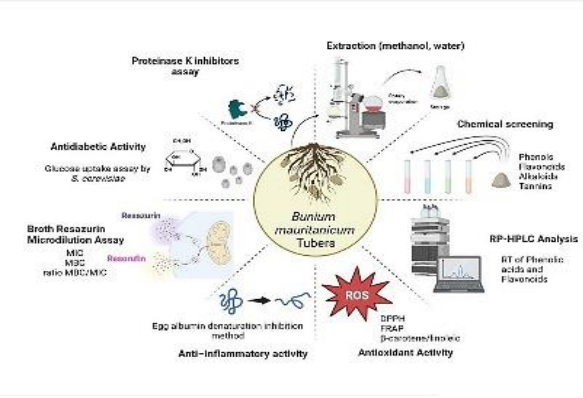
Nutritional Value Analysis

Annexe 12.



Graphycal abstract of in vitro study of *Brassica oleracea* var. elongate

Annexe 13.



Graphycal abstract of *Bunium mauritanicum tubers*

Annexe 14.

People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of El Oued

Faculty of Life and Natural Sciences

The Scientific Council **El Oued: 16/04/2023**

N°: 14/S.C/F.L.N.S/E.U/2023

CERTIFICATE OF ETHICS APPROVAL

The head of the Scientific Council admits that the experimental protocol used in the study conducted by **Yassine Bouras** and his co-authors is in accordance with the requirements and guidelines of the Local Ethical Committee. The study is registered under the reference **06/2023**.

The approval was done at scientific council meeting No. 07/2022-2023, held on 22/03/2023.

Study Title:

Phytochemical analysis and in vitro and in vivo biological activities of leaves and seeds extracts of *Brassica oleracea* var. elongata

Reference Study Number: (06/2023)

The head of the Scientific Council

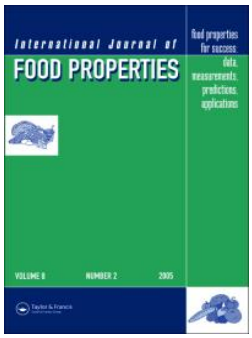
رئيس المجلس العلمي لكلية
العلوم الطبيعية والحياة
د/خزاني وشير

CERTIFICATE OF ETHICS APPROVALE

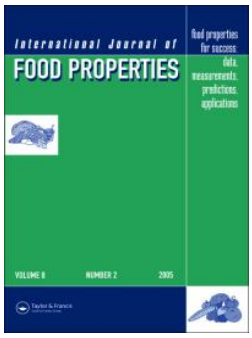
Annexe 15.

A. International Publications

Zaater, A., Serhoud, M. O., Ben Amor, I., Zeghoud, S., Hemmami, A., Rebiai, A., ... **Bouras, Y** ..& Barhoum, A. (2024). Exploring the potential of a *Ephedra alata* leaf extract: Phytochemical analysis, antioxidant activity, antibacterial properties, and green synthesis of ZnO nanoparticles for photocatalytic degradation of methylene blue. Frontiers in Chemistry, 12, 1367552. https://doi.org/10.3389/fchem.2024.1367552



Cherrada, N., Elkhalifa Chemsa, A., Gheraissa, N., Zaater, A., Benamor, B., Ghania, A., ... **Bouras, Y** & Teferi Asres, D. (2024). Antidiabetic medicinal plants from the Chenopodiaceae family: a comprehensive overview. International Journal of Food Properties, 27(1), 194-213. https://doi.org/10.1080/10942912.2023.2301576



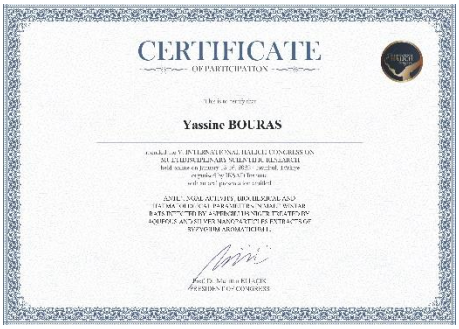
Gheraissa, N., Chemsa, A. E., Cherrada, N., Benamor, B., Erol, E., Elsharkawy, E. R., **Bouras, Y**... & Atoki, A. V. (2024). Exploring the phytochemical and biological properties of Salsola foetida: a promising wild plant from Southeastern Algeria. International Journal of Food Properties, 27(1), 584-601. https://doi.org/10.1080/10942912.2024.2339248

Bouras, Y., Chouikh, A., Cherrada, N., Gheraissa, N., Chenna, D., Chemsa, A. E., Hemmami, H., Atoki, A. V., Sawicka, B., Atanassova, M., Ahmad, S. F., Attia, S. M., & Messaoudi, M. (2024). Phytochemical profile and biological activities of *Brassica oleracea* var. *elongata* leaf and seed extracts: An in vitro study. Italian Journal of Food Science, 36(4), 193-207. https://doi.org/10.15586/ijfs.v36i4.2691

Bouras, Y., Chouikh, A., & Ben Ali, A. (2024). *Bunium mauritanicum* tubers: Phytochemical Analysis, Anti-microbial and proteinase K inhibitors properties. African Journal of Biological Sciences, 6(13), 2831-2842. https://doi.org/10.48047/AFJBS.6.13.2024.2831-2842

Abdelmalek, B., Amara Djilani, G., Ammar Touhami, L., Messaoudi, M., Kaddour, A., Assia, B., Zeid, A., Chemsa, A. E., Mekhadmi, N. E., Cherrada, N., **Bouras, Y**., & Aydi, S. (2024). Investigation of Secondary Metabolite Content and Antioxidant and Inhibitory Activities of Extracts from Parts of *Salsola richteri* L. Plant. African Journal of Biological Sciences, 6(10), 6266-6280. https://doi.org/10.48047/AFJBS.6.10.2024.6266-6280

B. International Scientific Communications



BOURAS, Y., CHOUIKH, A. Antifungal activity, biochemical and haematological parameters in male Wistar rats infected by *Aspergillus niger* treated by aqueous and silver nanoparticles extracts of *Syzygium aromaticum* L. V. International Halich Congress on Multidisciplinary Scientific Research, 15-16 January, 2023. Online (Istanbul, Turkey)..

BOURAS, Y., CHOUIKH, A. Evaluation of antioxidants activity of crude aqueous extract of *Syzygium aromaticum* plant. 1st International Conference on Agricultural Research vis-à-vis the Environment in the Saharan Context (of El Oued), 10-11 May, 2023. University of Echahid Hamma Lakhdar, El Oued, Algeria

BOURAS, Y., CHOUIKH, A. Biological Activities, and Uses of Latex from Selected Species of latex plants, A mini Review. International cukurova agriculture and veterinary congress held on february 27-28, 2023 / adana / türkiye

C. National Scientific Communications

BOURAS, Y., CHOUIKH, A. Comparative Analysis of Antioxidant, Anti-inflammatory, and Antimicrobial Properties of *Bunium mauritanicum* Tubers and *Brassica oleracea var. elongata* Extracts. BRS Day, 1st National Forum for PhD Students and Young Researchers on The Impact of Saharan Bioresources in the face of global changes, 1 December, 2022. Kasdi Merbah Ouargla University, Ouargla, Algeria.

BOURAS, Y., CHOUIKH, A., ALIA, F. The effects of the aqueous extract of *Syzygium aromaticum*, Testis and oxidative stress parameters in male Wistar rats infected by *Aspergillus niger*. 1st National Webinar on Natural Products and Bioactive Compounds, an issue of sustainable development, 11 March, 2023. Online.