الجمهورية الجزائرية الديموقراطية الشعبية

People's Democratic Republic of Algeria

وزارة التعليم العالي و البحث العلمي

Ministry Of Higher Education And Scientific Research

جامعة الشهيد حمه لخضر

University Of Echahid Hamma Lakhdar El-oued

كلية العلوم الطبيعية و الحياة

Faculty Of Natural Science And Life

قسم البيولوجيا الخلوية والجزئية

Departement Of Cellular And Molecular Biology

FINAL YEAR DISSERATATION

Submitted in parcial fulfillment of the requirements for the aword of the acadimic

Master's degree in biological sciences

Specialization: Toxicologie

THEME

Exploring the synergistic bioactivity of camaelo camaelon and bunium mauritanicumi A step toward novel Etho pharmacological Therapie

Graduted:00/00/2026

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University year :2025/2026

Dedication

I dedicate this humble fruit of my labor to the one who instilled in me a love of knowledge, the pillar of strength I lean on in my sorrow and weariness.

To the one whose feet hold paradise and who is the light of my eyes, O Lord, protect my parents, for they are your most precious gifts.

To the one whose prayers fill me with happiness and hope, my only grandmother, may God grant her a long life and bless her with health and well-being.

To the one who, after God almighty, is responsible for my achievements today and who has been like a father to me, may God reward him abundantly.

To those who strengthen me, my dear brothers and sisters.

To the one who has always stood by me, my beloved sister-in-law.

To my soulmates and companions on this journey, each by name and rank.

To everyone who has taught me a word or a skill, and who holds even a grain of love for me in their heart.

Ben ·Souhaila

Dedication

I dedicate this humble work to the dearest people in my heart,

To my beloved father, my support and the light of my eyes, who never hesitated to give me his time, effort, sacrifice, and money, and who was, after God, my strength in every path I took.

And to my dear mother, whose prayers never left me in every step I made; may Allah reward them both with the best reward.

I also dedicate it to my dear sisters and their children, to my dear brothers and their wives and children, and to my kind aunt, in appreciation of their love and constant support.

And to my companions in the university residence: Abir, Safaa, Choumissa, Malika, Marwa, Meriem El Batoul,Faiza, Dunia, and Malika,

Who shared with me the most beautiful memories and the hardest days, and who were the best companions and support.

Ben-Ahlam

Dedication

Praise be to Allah who granted me success and helped me complete this work. I dedicate the fruit of my humble effort to the dearest people to my heart...

To those whose prayers were the secret of my success, to those whose hands worked tirelessly for my comfort, to those who instilled in me hope, patience, and perseverance, my dear father and beloved mother, may Allah prolong their lives and keep them as my support and light in life.

To my dear father, who has always been an example of sacrifice and hard work, who struggled and stayed up late to provide me with strength and security, and whose support was my greatest motivation to continue this path and achieve success. May Allah protect him and keep him as a crown above my head.

And to my beloved mother, the source of tenderness and mercy, the owner of the kind heart and prayers that accompanied me in every step of my life, the one who filled my soul with hope and confidence whenever I felt tired or weak. Her presence is a blessing beyond value.

My mother and father, no matter how many words I write, I will never be able to repay you for your kindness, nor will I ever be able to describe your great place in my heart. To you both, I offer all my love, gratitude, and appreciation, and I pray that Allah protects you and keeps you as a blessing and support in my life.

To my brother Youssef, who was a source of strength and support throughout every stage, and to my brother Amine and my sister Rim, thank you for your love, constant encouragement, and for every moment you stood by my side.

To my dear fiancé, whose presence was a source of support and comfort to me, thank you for your encouraging words, your patience, and for standing beside me through difficult and happy times alike. Thank you for teaching me word skills and how to use the computer; your presence had a positive impact on helping me continue this journey.

To my dear uncle, Sharaf Eddine, who never hesitated to support me and was an example of generosity and care, I extend to him my sincere thanks and appreciation, and I also wish success and prosperity for his children.

To all my respected teachers who enlightened my path with knowledge and learning, and contributed to my academic formation, and especially to the one who truly taught me the English language, professor Yaacoub Ben Achour, you all have my deepest respect and gratitude.

To everyone who supported me, even with a kind word, and to every relative and friend who contributed to encouraging and supporting me throughout my academic journey, I dedicate this humble work as a token of love and loyalty.

Finally, I dedicate this effort to myself, who endured, worked hard, and bore all the difficulties to reach this moment. I pray that Allah makes this work the beginning of goodness and lasting success.

GH -RAOUNEK

Thanks

Praise be to Allah, by whose grace good deeds are accomplished, and who enabled us to complete this modest work. We praise Him and thank Him for what He has bestowed upon us of success, support, and patience throughout our academic journey.

We also extend our sincere thanks and gratitude to our supervisor, Dr. Wafaa Boudbia, for her valuable guidance and scientific advice which were a great help to us in completing this work. We would also like to thank the head of Laboratory (7), Professor Sultan Abdelhak, as well as Professor Khenoufa Aamer, head of Laboratory (8), for their constant cooperation without fatigue or complaint, and for their patience with us throughout the practical period.

We also do not forget the third-year undergraduate students in Applied Biochemistry, Ahmed Turki and Mohamed El Ghali Boudia, who volunteered and took charge of the process of sacrificing the mice.

Thanks are also extended to the driver of bus number (11), who transported us to the analysis

laboratory and waited for us out of concern for our safety.

We would also like to thank Mr. Ahmed Ndhir for his free formatting of the dissertation. Our sincere gratitude and appreciation to him.

♡ Heartfelt thanks to everyone who helped and encouraged us, even with a kind word ♡

ABSTRACT

This study aims to explore and evaluate the synergistic biological activity of both the chameleon (*Chamaeleo chamaeleon*) and the plant *Bunium mauritanicum*, through the use of three types of extracts: *Bunium mauritanicum* tuber extract (BME), chameleon extract (CCE), and their combined extract (CCBME). The aqueous extracts were obtained using a Soxhlet apparatus, where the extraction yields for BME, CCE, and CCBME were recorded as (16%), (21%), and (19.5%), respectively. The obtained results showed that the combined extract (CCBME) exhibited the highest activity with an IC50 = 12.73 ± 0.16 µg/mL in terms of antioxidant and anti-inflammatory activities, compared to the individual extracts. In contrast, the *Bunium mauritanicum* extract (BME) showed moderate activity with IC50 = 28.66 ± 0.06 µg/mL, while the chameleon extract (CCE) was the least effective with an IC50 value of 45.67 ± 0.07 µg/mL among all the studied extracts. All tested extracts demonstrated lower activity compared to ascorbic acid (IC50 value 2.43 ± 0.05 µg/mL), which was used as a reference compound. these results indicate that the combined formulation (CCBME) exhibits higher biological activity than the individual extracts, supporting the hypothesis that combining extracts with different biological properties can enhance biological effectiveness due to the synergistic interaction between active compounds.

•**KEY WORDS:**

BME : Bunium extract

CCE : Chameleon extract

CCBME : Combained extracts

الملخص

تهدف هذه الدراسة إلى استكشاف وتقييم النشاط الحيوي التآزري لكل من الحرباء (Chamaeleo chamaeleon) ونبات التلغودة(Bunium mauritanicum) ، وذلك من خلال استخدام ثلاثة أنواع من المستخلصات: مستخلص درنات التلغودة (BME)، ومستخلص الحربا(CCE) ، بالإضافة إلى مستخلص المزيج بينهما.(CCBME)

تم الحصول على المستخلصات المائية باستخدام جهاز سوكسليت(Soxhlet) ، حيث سُجلت مردودات الاستخلاص لكل من BME و CCEو CCBMEبقيم بلغت على التوالي: (16%)، (21%)، و.(19,5%)

أظهرت النتائج المتحصل عليها أن مستخلص المزيج (CCBME) يمتلك أعلى فعالية IC50 = 12.73 ± 0.16 µg/mL من حيث النشاط المضاد للأكسدة والمضاد للالتهابات، مقارنة بالمستخلصات الفردية. في حين سجل مستخلص التلغودة (BME)نشاطًا متوسطًاIC50 = 28.66 ± 0.06 µg/mL ، بينما كان مستخلص الحرباء (CCE) الأقل كفاءة IC50 value of 45.67 ± 0.07 µg/mLمن بين جميع المستخلصات المدروسة.

فقد أظهرت جميع المستخلصات المختبرة فعالية أقل مقارنة بحمض الأسكوربيك IC50 value 2.43 ± 0.05 µg/mL، الذي استُخدم كمركب مرجعي .وبصفة عامة، تشير هذه النتائج إلى أن التركيبة المدمجة (CCBME) تُظهر نشاطًا بيولوجيًا أعلى من المستخلصات الفردية، مما يدعم فرضية أن دمج مستخلصات ذات خصائص بيولوجية مختلفة يمكن أن يعزز الفعالية البيولوجية، وذلك نتيجة التفاعل التآزري بين المركبات الفعالة.

الكلمات المفتاحية :

BME:مستخلص الحرباء

CCE: مستخلص التلغودة

CCBME:المستخلصات المجمعة

Résumé

Cette étude vise à explorer et à évaluer l'activité biologique synergique de la fois du caméléon (*Chamaeleo chamaeleon*) et de la plante *Bunium mauritanicum*, à travers l'utilisation de trois types d'extraits : l'extrait des tubercules de *Bunium mauritanicum* (BME), l'extrait de caméléon (CCE), ainsi que leur extrait combiné (CCBME). Les extraits aqueux ont été obtenus à l'aide d'un appareil de Soxhlet, où les rendements d'extraction pour BME, CCE et CCBME ont été enregistrés respectivement à (16 %), (21 %) et (19,5 %).

Les résultats obtenus ont montré que l'extrait combiné (CCBME) présente la plus forte activité avec une IC50 = 12,73 ± 0,16 µg/mL en termes d'activités antioxydante et anti-inflammatoire, comparativement aux extraits individuels. En revanche, l'extrait de *Bunium mauritanicum* (BME) a montré une activité modérée avec une IC50 = 28,66 ± 0,06 µg/mL, tandis que l'extrait de caméléon (CCE) était le moins efficace avec une valeur d'IC50 de 45,67 ± 0,07 µg/mL parmi tous les extraits étudiés.

Tous les extraits testés ont présenté une activité inférieure à celle de l'acide ascorbique (valeur d'IC50 = 2,43 ± 0,05 µg/mL), utilisé comme composé de référence.

D'une manière générale, ces résultats indiquent que la formulation combinée (CCBME) présente une activité biologique plus élevée que les extraits individuels, ce qui soutient l'hypothèse selon laquelle la combinaison d'extraits ayant des propriétés biologiques différentes peut améliorer l'efficacité biologique, en raison de l'interaction synergique entre les composés actifs

MOTS-Clés :

BME : Extrait de caméléon

CCE : Extait de bunium

CCBME : Extrait combinés

.

Abbreviation's list

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

BME : *Bunium mauritanicum*

BTU:Benzylthiouracil

C: control

CAT: Catalase

CCE: Chamaeleo chamaeleon extract

CCBME: Combined extracts

CO2: carbon dioxide

CN: Negative control

CP: Positive control

CT: Creatinine

DPPH: 2,2'-Diphenyl-1-picrylhydrazyl

ECL: Electrochemiluminescence

EDTA: Ethylenediaminetetraacetic acid

FBS: fasting blood sugar

$FeCl_3$: Ferric chloride test

GRA: Granulocytes

GSH: Glutathione

GLDH: Glutamate dehydrogenase

GOT: Glutamate oxaloacetate transaminase

HCL: Hydrochloric acid

HGB: Hemoglobin

H2O: Water

H_2O_2: Hydrogen peroxide

LEVO: Levothyroxine

LYM: Lymphocytes

MCV: Mean corpuscular volume

MDA: Malondialdehyde

MDH: Malate dehydrogenase

Nh4: into ammonia

O2: Oxygen

OD: Optical density

OH: Hydroxyl.

PBS: Phosphate-buffered saline

PH:Potential of hydrogen

PLT: Platelets

RBC: Red blood cell

SOD: Superoxide dismutase

T3: triiodothyronine

T4: thyroxine

TCA: Trichloroacetic acid

TSH: Thyroid Stimulating Hormone

TF: thyroid follicle

UA: Uric Acid

WHO: World Health Organization

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Summary

Introduction

Introduction

In the past, humans relied on medicinal plants and animals to treat various diseases by collecting them and using them directly on affected areas, or after drying and grinding them (Bouhazza et al., 2020). This type of treatment constituted a common traditional practice and was considered a relatively safe alternative therapy, as it did not cause additional illnesses. Its use continued despite the major developments witnessed in modern pharmaceutical and chemical industries . Medicinal plants and animals are defined as natural organisms containing bioactive chemical compounds known as active constituents, which possess therapeutic and preventive properties used in the prevention and treatment of diseases. These compounds include numerous secondary metabolites such as alkaloids, flavonoids, terpenes, tannins, and saponins, whose nature and concentration vary according to species and surrounding environmental conditions. These active compounds are distributed in different plant parts such as leaves, roots, bark, flowers, fruits, and seeds, and may also be found in certain animal tissues or secretions used therapeutically. Their quantity and quality are influenced by several factors, including growth stage, climate, soil type, harvesting methods, and storage conditions. These compounds are extracted using various techniques such as hydroalcoholic, alcoholic, or aqueous extraction, in addition to specialized equipment such as the Soxhlet apparatus to obtain extracts with specific biological activity. Despite the great importance of medicinal plants and animals in traditional and modern medicine as major sources of many drugs, they may contain toxic compounds or cause side effects if misused or administered in excessive doses, which requires a precise understanding of their chemical and biological properties to ensure their safety and efficacy (Limonier,2018). Among the medicinal plants used in Algeria is Bunium mauritanicum, locally known as “Talghouda,” which is considered one of the traditional plants used in folk medicine, particularly in the treatment of thyroid disorders, although scientific studies concerning it remain limited (Chentouh et al., 2018). Thyroid diseases represent an important health concern because of the serious complications and negative effects they may have on quality of life if thyroid hormone disorders are not properly diagnosed or treated (Dehmane et al., 2022). In the same context, the Mediterranean chameleon, Chamaeleo chamaeleon, is traditionally used in folk medicine in the Algerian Sahara for the treatment of several diseases, including tonsillitis, thyroid disorders, and chronic cough. Given the therapeutic importance attributed to both Talghouda and

the chameleon in traditional practices, the present study aims to explore and evaluate the synergistic biological activity of both Chamaeleo chamaeleon and Bunium mauritanicum.

First part

Bibliographic part

Chapter I

Chamaeleo chamaeleon

I.1 Generality

The nomenclature of chameleons originates from the Greek term khamaileôn, a compound word literally meaning “lion that crawls on the ground.” These vertebrate organisms belong to the class Reptilia, a designation derived from the Latin reptilis, meaning “crawling.” Chameleons are classified within the order Squamata, from the Latin squama, meaning “scale,” and belong to the suborder Sauria, derived from the Greek root sauros, meaning “lizard.” At the family level, they are placed within the family Chameleonidae, also referred to as Chameleontidae or Chamaeleonidae. This family, which predominantly includes arboreal lizards, was first described by Constantine Samuel Rafinesque in 1815 (Bourdain, 2006).

The family Chameleonidae is considered one of the most distinctive vertebrate groups worldwide. Chameleons are among the most easily recognizable vertebrates due to their remarkable morphological diversity, as well as their unique characteristics that clearly distinguish them from other reptiles (Bickel and Losos, 2002). This family is currently divided into two main subfamilies: Chamaeleoninae and Brookesiinae. It includes more than 200 scientifically recognized species and subspecies distributed across approximately eleven genera. Chameleons exhibit a high degree of ecological adaptability, occupying habitats ranging from hot desert sand dunes, where body temperatures may exceed 39°C, to alpine mountainous regions above 3500 meters, where temperatures drop below freezing (Anderson, 2013).

Chameleons are diurnal animals. They move over short distances within their habitats to scan and explore their surroundings in search of prey, using a behavioral strategy known as “foraging,” which is characterized by limited movement and constant vigilance against potential predators. The diet of European chameleon species varies seasonally throughout the year; however, it mainly consists of bees, wasps, and grasshoppers. Chameleons capture their prey using a long, projectile tongue during spring, summer, and autumn (Jeroen et al., 2016). They are found across a wide range of thermal zones and environmental conditions, including hot and arid deserts, tropical rainforests, Mediterranean climates, and high-altitude mountainous regions (Tolley and Herrel, 2013).

Chameleons exhibit considerable morphological diversity depending on the species, with total body length ranging from 15 to 35 cm (Bourdain, 2006). They possess laterally compressed bodies, which give them a slender appearance and facilitate movement along branches while making them difficult to detect from above or below (Tolley and Burger, 2007). They also have prehensile tails and specialized feet adapted for grasping branches, functioning in a pincer-like manner. Coloration in chameleons varies according to multiple factors, including individual differences, body temperature, social conditions (such as pregnancy or stress), and camouflage requirements (Jeroen et al., 2016). Furthermore, chameleons possess a highly modified skull and a ballistic tongue used for prey capture (Diaz et al., 2018). One of their most notable features is the independent mobility of the eyes (Jeroen et al., 2016), which provides nearly 360-degree vision, allowing them to observe their surroundings in almost all directions—except directly behind the head—without moving their bodies (Tolley et al., 2009).

I.2 Definition

The common chameleon (*Chamaeleo chamaeleon*) is defined as a species of reptile belonging to the order Squamata and the family Chamaeleonidae, characterized by its well-known ability to modify skin color and patterns. Scientific literature indicates that agricultural lands and natural forests in the Mediterranean basin represent primary habitats for this arboreal species (Hodar et al., 2000). Chameleons belong to the group of slow-moving, diurnal lizards that are evolutionarily adapted to arboreal life (Bickel & Losos, 2002).

C. chamaeleon is classified as a medium-sized reptile, with a total body length (from the snout to the tip of the tail) ranging between 28 and 30 cm. The body is characterized by lateral compression, and the head bears a well-developed crest (casque), along with prominent eyes covered by conical eyelids, a prehensile tail, and opposable digits arranged in groups (two opposed to three) forming a pincer-like structure. The eyes possess the ability to move independently in all directions. This species also exhibits a remarkable capacity for rapid color change, attributed to a complex system of pigment-containing cells (chromatophores) within the skin. This ability is employed for camouflage and blending into the environment, where color varies according to the surrounding medium, as well as being influenced by psychological state, health status, and temperature (Cuadrado et al., 2001).

***Figure 1** : Chameleon (Chamaeleo chamaeleon) Photo: Gökhan Eren*

I.3 Taxonomy

The Chameleonidae family comprises 150 species, classified into two subfamilies: Brookesiinae and Chamaeleoninae. The subfamily Chamaeleoninae includes the genera:

Chamaeleo, Furcifer, Bradypodion, and Calumma. The Common Chameleon (*C. chamaeleon*) belongs to the genus Chamaeleo (Guillon, 2010).

- ✓ **Kingdom:** Animalia
- ✓ **Subkingdom:** Bilateria
- ✓ **Infrakingdom:** Deuterostomia
- ✓ **Phylum:** Chordata
- ✓ **Sub-Phylum:** Vertebrata
- ✓ **Infraphylum:** Gnathostomata
- ✓ **Superclass:** Tetrapoda
- ✓ **Class:** Reptilia
- ✓ **Sub-Class:** Lepidosauria
- ✓ **Order:** Squamata
- ✓ **Sub-Order:** Sauria
- ✓ **Infra-Order:** Iguania
- ✓ **Family:** Chamaeleonidae
- ✓ **Sub-Family:** Chamaeleoninae
- ✓ **Genus:** Chamaeleo
- ✓ **Species**: *Chamaeleo chamaeleon* (Linnaeus, 1758)

I.4 Distribution

The distribution range of the Mediterranean chameleon (*Chamaeleo chamaeleon* Linnaeus, 1758) is restricted to three main geographic regions. The first corresponds to the southern Mediterranean basin in Europe, specifically Spain, Portugal, Italy, and Greece. The second is North Africa, including the coastal areas of Western Sahara, Morocco, Algeria, Tunisia, Libya, and Egypt. The third extends to Southwest Asia, where the species occurs in southern Turkey, Syria, Lebanon, Palestine, Jordan, as well as Saudi Arabia and Yemen (Dimaki et al., 2015).

In Europe, the Mediterranean chameleon has also been documented in Malta, several Greek islands such as Crete, Samos, and Chios, and in the southern part of the Iberian Peninsula.

Regarding Italy, sporadic sightings (vagrant records) were previously reported from Sicily; however, these records are considered to result from accidental introductions of individuals originating from North Africa. A recent taxonomic review has confirmed that the presence of C. chamaeleon in Sicily has not yet been substantiated by conclusive evidence.

In addition, recently established populations of the Mediterranean chameleon have been reported in Apulia and Calabria in southern Italy. These populations originated from relatively recent introduction events, with the Apulian population traced back to Tunisia and the Calabrian population to Palestine (Miraldo et al., 2005).

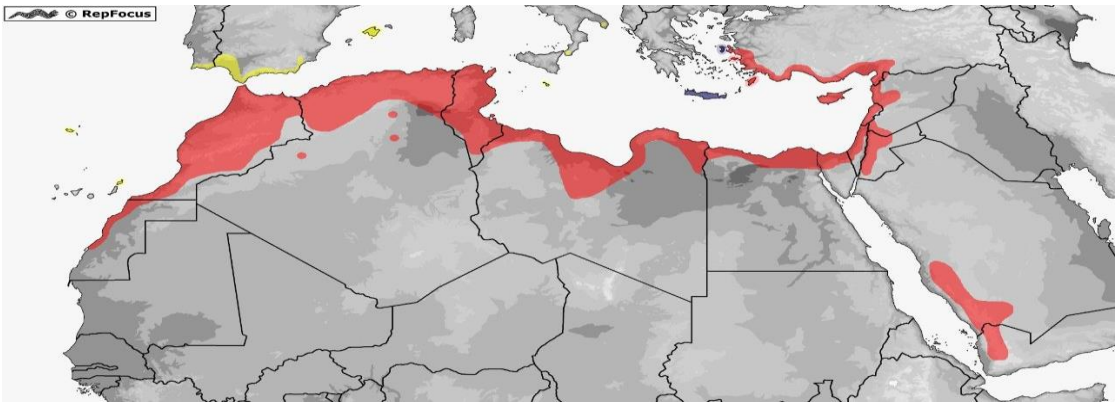

***Figure 2** : Distribution of chamaeleo chamaeleon (Mediterranean chameleon)*

(Reptilia:sauria:chamaeleonidae)

I.5 Description

The common chameleon (*Chamaeleo chamaeleon*) is regarded as a distinctive species across most of its geographic range. Its skin coloration is influenced by environmental conditions, including surrounding characteristics and temperature, and it has the ability to undergo rapid color changes, typically appearing light green at night. The species spends most of its time climbing shrubs, using its specialized tail and limbs to grasp branches, as the toes of each foot are arranged into two opposing groups that ensure a firm grip (Čerňanský, 2010).

Its body length ranges from 9 to 17 cm, excluding the tail, which measures between 12 and 14 cm. Sexual dimorphism is evident, with males being slightly larger than females. Females lay between 30 and 40 eggs in late summer, which hatch the following spring. The

common chameleon can live up to 20 years, with a recorded maximum lifespan of 22 years in captivity; however, the average lifespan generally ranges between 12 and 13 years (Sidhom et al., 2020).

I.6 Morphological particularities

Size:

The length of chameleons varies depending on the species, ranging between 15 and 35 centimeters. The common chameleon, scientifically known as Chamaeleo chamaeleon, is a medium-sized chameleon that does not exceed 30 centimeters in length, while its hatchlings measure only 15 millimeters at birth (Cuadrado and Loman, 1999).

Head:

The chameleon's head has a pyramidal shape, supported by strong bony ridges, and the occipital lobe extends to form what is known as the helmet (the helmet is the area of the head located behind the eyes) (Bourdain, 2006). The parietal crest is curved and higher than the lateral crests, which end immediately behind the temporal region (Lustig et al., 2012; Lev-Ari, 2016).

Figure 3 : *Head of Chamaeleo chamaeleon ,Photo: Gökhan Eren*

Body:

The body of *C. chamaeleon* is laterally flattened, giving it an oval shape. These reptiles are able to change the shape of their bodies thanks to their lungs and muscles, allowing the body to inflate or become flattened in the dorsoventral direction. This body configuration contributes to thermoregulation, camouflage, and communication with conspecifics; in the morning, the chameleon flattens itself as much as possible to increase its body surface area and absorb the maximum amount of solar energy. It also flattens itself while swaying behind a

branch to mimic the movement of leaves, and inflates its body to intimidate rivals (Morsy et al., 2012; Bourdain, 2006).

Limbs:

Chameleons possess unique limbs adapted to an arboreal lifestyle; their legs are long and strong, and the highly mobile hip and shoulder joints give them great flexibility (Bourdain, 2006).

Their fingers are partially fused, forming structures that function as claws, allowing a firm grip on branches. In the forelimbs, two outer fingers are fused opposite three inner fingers, while in the hind limbs, three outer fingers are fused opposite two inner fingers. All fingers bear a curved claw, which is usually white to pale yellow and may sometimes be transparent.

In addition, the scales on the palmar and plantar surfaces of the limbs, as well as those covering the ventral side of the tail tip, have a specialized structure forming adhesive pads that facilitate movement on smooth surfaces (Schleich, 1985).

***Figure 4** : Front and hind limbs of Chamaeleo chamaeleon (Original photo).*

The Tail:

The tail is solid, cylindrical, and prehensile, functioning as a fifth limb that helps the chameleon grip surrounding objects. Its length is either shorter than the body or equal to it, with a total length of 23.58 cm. In a resting position, the tail is usually curled beneath the cloaca, while it relaxes when the chameleon needs to grasp something. During terrestrial

movement, the chameleon keeps its tail raised or curved in an “S” shape (Cuadrado et al., 2003).

***Figure 5** : Tail of Chamaeleo chamaeleon (Rafi Amar)*

I.7 Anatomical Particularities

I.7.1 Cardio-Respiratory Apparatus Heart

The chameleon’s heart consists of three chambers: two atria located above a single ventricle. This ventricle is partially divided by an incomplete interventricular septum (Fouda et al., 2015), which results in the mixing of venous and arterial blood.

The heart rate plays an important role in thermoregulation in chameleons and is influenced by several factors, including:

Body temperature, as optimal cardiac muscle performance occurs at the chameleon’s preferred temperature,

Body size, with heart rate being inversely related to obesity,

Metabolic level and respiratory rate, with a decrease in heart rate during periods of apnea,

As well as sensory stimulation (Bourdain, 2006).

Blood

Blood volume in chameleons represents approximately 3% of body weight. All blood cells in reptiles are nucleated. Red blood cells have the ability to divide, and platelets can transform into red blood cells (Divers and Mader, 2005).

Granulocytes are heterogeneous and functionally equivalent to neutrophils in mammals. Basophils may account for up to 40% of the total blood cell population in reptiles (Schilliger, 2004).

Lymphocytes constitute the main type of white blood cells in the leukocyte formula, and their number varies according to several factors:

Seasons: their number decreases in winter with lower temperatures and increases in summer,

Sex: they are more numerous in females,

Nutritional status: their number decreases in cases of malnutrition,

Health status: lymphocytosis is observed in parasitic or viral diseases (Divers and Mader, 2005).

Lungs

Chameleons can breathe through both the nose and the mouth. The palate contains a pair of sinuses that allow air to pass from the nostrils to the lungs. Air inhaled through the mouth passes through the larynx and the glottis, located beneath the tongue, to reach the trachea (Bourdain, 2006).

When ambient temperature increases, both the volume of inhaled air and the respiratory rate increase. Chameleons possess a pair of lungs, the internal structure of which is divided into several chambers separated by numerous septa.

Chameleons do not have a diaphragm; therefore, respiratory movements are achieved through the contraction of striated skeletal muscles (intercostal muscles and limb muscles). When threatened, chameleons can inflate their lungs to appear larger (Perry and Duncker, 1978).

I.7.2 Skin apparatus

Scales: They cover the entire body and are keratinized thickenings produced by the epidermis. They take on various shapes (standard, conical, tubercular, etc.) depending on their location.

Skin: It consists of a superficial epidermis and a deeper dermis. The upper dermis is highly vascularized (nutrition and heat exchange), while the deep dermis contains collagen, blood vessels, nerves, and chromatophore cells. The skin is dry due to the absence of sebaceous glands (except for femoral glands). Its thickness varies according to the environment (thick in desert regions, thinner in mountainous areas).

Shedding (Moulting): This process involves the loss of dead cells from the keratinized superficial layer. It occurs more frequently during growth (every few weeks) than in adulthood (approximately every four months). Its duration ranges from a few hours to several days. Unlike snakes, chameleons continue feeding normally before shedding

I.7.3 Digestive apparatus

The digestive system of chameleons shows a limited degree of specialization. The stomach is tubular in shape and secretes digestive enzymes as well as hydrochloric acid. The intestines are short and open into the cloacal cavity (Bourdain, 2006).

Chameleons have small, conical teeth that are uniform and rudimentary, directly attached to the upper edge of the jawbone. The main function of these teeth is to grasp prey inside the oral cavity. The inner surface of the oral cavity is often pigmented. When threatened, some chameleon species expose the inside of their mouths in order to intimidate their opponents. The mouth contains mucous glands, as well as salivary glands that secrete non-sticky saliva of varying viscosity (Tolley and Herrel, 2013).

The chameleon tongue consists of an annular accelerator muscle attached to a cartilaginous projection known as the hyoid horn. This horn is in turn connected to the hyoid bone by hyoid muscles, while the hyoid bone is linked to the sternum through strong muscles. The hyoid bone is U-shaped and highly flexible (Wainwright et al., 1991).

The retractor muscles (or hyoglossus muscles) surround the accelerator muscle and attach to the underside of the tongue tip (Chen et al., 2020). The tip of the chameleon tongue is

covered with numerous epithelial glands and papillae, which adhere to the irregularities of the prey's surface like adhesive hooks (Schwenk, 1983).

The contraction of the accelerator muscle, combined with the backward movement of the hyoid horn, generates strong pressure that propels the tongue out of the mouth like a catapult. When fully extended, the tongue can reach a length equivalent to the body of the chameleon. When not in use, the tongue rests at the back of the throat, with its tip folded inward (Fouda et al., 2015).

Chameleons possess a greenish-blue liver composed of two lobes, with the left lobe being the larger one, and the gallbladder attached to it. The pancreas is yellowish in color and is located behind the stomach near the spleen, which is small and purple (Bourdain, 2006)

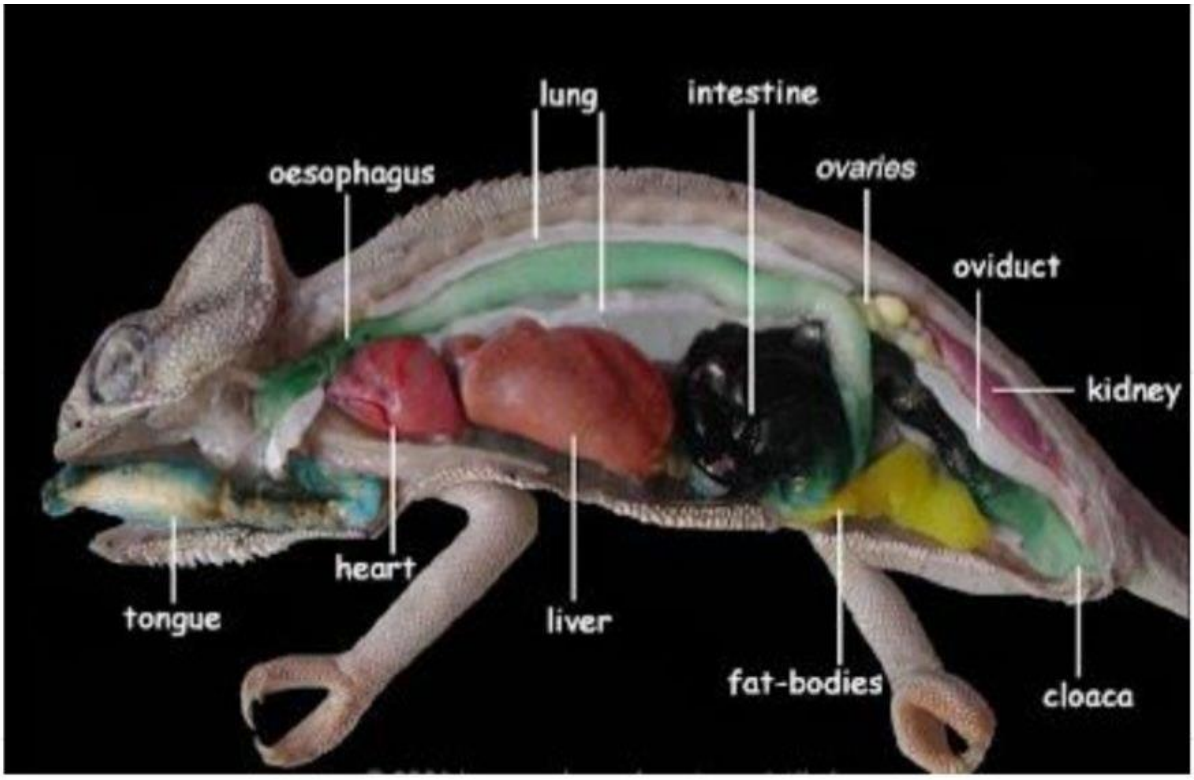

***Figure 6** : Digestive apparatus of Chamaeleo chamaeleon(Under the hood -Anatomy)*

I.7.4 Musculoskeletal system

The musculoskeletal system of the chameleon shows functional adaptations suited to an arboreal lifestyle (Higham and Jayne, 2004). The cartilaginous ribs reduce potential pain during falls, while their curved or hook-like ends ensure a strong grip on branches even under

strong wind conditions. The spine is highly flexible. In addition, the specialized muscles of the extensible tongue enable the chameleon to capture insects from a distance, while the well-developed muscles of the independently moving eyes allow it to detect both predators and prey in all directions.

Studies have also revealed a high proportion of "tonic" muscle fibres compared to "kinetic" fibres responsible for movement. The tonic fibres passively maintain a stable, energy-saving muscular state, enabling the chameleon to maintain its posture with minimal effort (Tolley and Herrel, 2013).

I.7.5 Sensory system

I.7.5.1 Nervous system

The chameleon, like other reptiles, possesses a relatively simple brain, with the brain and cerebellum together accounting for no more than 1% of the total body mass (Mader, 1996). However, the cerebral cortex is developed and includes well-defined hemispheres. The chameleon has twelve pairs of cranial nerves, including a highly developed optic nerve. The spinal cord extends along the tail, unlike in mammals.

I.7.5.2 Visual organs

The eye is the most developed sensory organ in the chameleon. The eyelids consist of small scales covering most of the eye surface, leaving only a small circular opening in front of the pupil. This provides effective protection against desiccation and injury but limits the field of vision. The chameleon expands its visual field thanks to the large laterally positioned eyeballs and the multidirectional movement of each eye independently.

Thus, it is able to perceive objects within a vertical angle of 90° and a horizontal angle of 180° (Necas, 2004). The eyes move independently, allowing the brain to receive two simultaneous visual images. In addition, the chameleon has very high visual acuity, comparable to a telephoto lens with a focal length ranging from 100 to 150 mm (Le Berre, 1995).

It can perceive colors ranging from red to violet (i.e. between 375 and 610 nm), but it cannot see either infrared or ultraviolet light (Bowmaker et al., 2005).

Figure 7 : *Visual organs of Chamaeleo chamaeleon (by Alex Hyde /science photo Libirary*

I.7.5.3 Hearing organs

The chameleon does not possess an outer ear or a tympanic membrane; however, it has a simple inner ear containing a well-developed, fluid-filled cochlea. The soft tissues and skull bones, including the well-developed pterygoid process located on both sides of the head behind the eyes, transmit sound vibrations to the inner ear. This enables the chameleon to detect low-frequency sounds, particularly in the range of 200 to 600 Hertz (Bourdain, 2006).

I.7.5.4 Olfactory and taste organs

Odours and chemical substances are detected in the chameleon through Jacobson's organ, located in the oral cavity in front of the palate. This organ, also known as the vomeronasal organ, is highly developed in reptiles compared to mammals. It consists of two cavities lined with sensory cells covered by a thin layer of mucus and is innervated by the first pair of cranial nerves. In addition, taste buds have been identified on the chameleon's tongue (Schwenk, 1985), allowing it to occasionally explore its environment by licking surfaces (Ogilvie, 1966).

I.8 Physiological characteristics

I.8.1 Thermoregulation

Chameleons, like other reptiles, are classified as ectothermic organisms, meaning that their internal body temperature is not constant and is entirely dependent on environmental conditions. Each species possesses an optimal thermal threshold known as the mean preferred temperature (T.M.P), which individuals actively attempt to achieve. When body temperature falls significantly below this optimal level, essential physiological processes such as digestion are impaired, immune efficiency decreases, renal function is compromised, and uric acid crystals may accumulate within the body (Bourdain, 2006).

To increase their body temperature, chameleons orient themselves as perpendicularly as possible to sunlight, flatten their bodies, and develop a darker skin coloration that enhances the absorption of high-energy infrared radiation. This behavior is accompanied by dilation of superficial capillaries and an increased heart rate, which together enhance heat uptake. Conversely, when internal temperature rises excessively, chameleons retreat into shaded areas, develop a lighter skin coloration, exhibit hyperventilation with an open mouth and protruded tongue, and may also seek underground shelters (Stuart-Fox & Moussalli, 2009).

The mean preferred temperature varies according to habitat (biotope), age, and physiological condition. In a study conducted on Chamaeleo chamaeleon in southwestern Spain, the average T.M.P was approximately 28°C in October and 30°C in June. Regression analyzes between T.M.P and ambient temperature (Ta) demonstrated that individuals were able to maintain a preferred body temperature of around 30°C in June, but not in October (Andrews, 2008).

chameleons exhibit greater tolerance to low temperatures than to high temperatures, and are even capable of surviving nocturnal frost in their native habitats. Under unfavorable climatic conditions, they may enter hibernation or aestivation. Hibernation occurs in high-altitude or subtropical regions during winter temperature declines, whereas aestivation occurs during periods of extreme heat or prolonged drought. In both cases, chameleons shelter beneath layers of moss, leaves, or branches as long as humidity and temperature remain unsuitable, and they completely cease all activity (Bennett, 2004).

I.8.2 Color change

The coloration of chameleon skin involves several types of specialized cells located within the dermis, whereas the epidermis is composed of transparent cells. The most superficial layer consists of iridocytes, whose cytoplasm contains suspended purine particles. Depending on their structural arrangement, these cells either absorb or do not absorb light through the Tyndall effect, thus masking the colors of deeper cellular layers to varying degrees.

Pigment-containing cells, known as chromatophores, are of several types (Goda, 2017). The most superficial chromatophores contain carotenoid pigments in their cytoplasm and include xanthophores (yellow) and erythrophores (red). In deeper layers, guanophores contain transparent guanine crystals that either reflect blue light or do not, depending on the angle of incident light. Consequently, when the layer above guanophores is composed of xanthophores, the reflected light appears green due to the combination of blue and yellow wavelengths. Finally, melanophores, which possess long dendritic extensions and contain melanin, are responsible for the dark coloration of chameleon skin (Dong et al., 2019).

Chromatophores are contractile cells capable of redistributing their pigments within the cytoplasm under the combined influence of both the central nervous system and the endocrine system (Ma et al., 2002). Among the most important hormonal regulators in chameleons are alpha-melanocyte-stimulating hormone (α-MSH) and catecholamines, particularly adrenaline (Teyssier et al., 2015). α-MSH is secreted by the pituitary gland and induces skin darkening, while adrenaline is produced by the adrenal glands and enhances skin brightness and color vividness.

In the resting state, pigments are evenly distributed within chromatophores. Light is scattered by iridocytes toward guanophores, which reflect it back toward the epidermis, resulting in bright and vivid skin coloration. Under stress conditions, such as the presence of a predator, light is redistributed into the dermal layers and xanthophores contract rapidly, leading to the simultaneous appearance of bright and dark patterns. When the chameleon is active but not threatened, melanophores contract, resulting in brighter and more vivid coloration.

Thus, a wide range of color variations becomes possible depending on the position of iridocytes in the dermis, the angle of incident light on guanophores, and the contractile state

of chromatophores. However, it is incorrect to assume that an individual chameleon is capable of expressing all possible colours, as each species possesses a specific color palette with a flexible yet limited range. The most frequently expressed colors are brown, black, yellow, and green (Zhao et al., 2019).

Figure 8 :*The shap of the Chamaeleo chamaeleon Inside the egg(website Canvaschams)*

Figure 9 :*The egg of chameleon(Petr Nacas)*

I.9 Reproduction

The onset of the sexual season is triggered by climatic changes. Chameleons are generally solitary throughout the year, except during the breeding period, which extends from mid-July to the end of September. The receptive female displays bright coloration patterns and remains calm in the presence of the male. The male approaches the female and climbs onto her back (Andrews and Donoghue, 2004), and upon contact, one of the hemipenes becomes everted.

Copulation lasts approximately ten minutes, during which the female is relatively passive, and the interaction typically ends with the male being displaced.

Females exhibit signs of pregnancy through a characteristic coloration, often orange, and become more aggressive toward other females (Keren-Rotem et al., 2016). Feeding and drinking activity increases during the early stages of pregnancy, while it is almost completely reduced in the days preceding parturition. Females lay eggs after an incubation period of approximately two months. The female descends to the ground and excavates a burrow in loose soil at the base of a shrub, reaching a depth of approximately one and a half times her body length. She lays between 10 and 40 eggs measuring 17 x 11 mm, covers them, and then returns to her arboreal habitat.

Incubation duration varies depending on temperature and soil moisture, but typically ranges from 200 to 290 days, with embryonic development being suspended during winter. In May,hatching occurs over a period of one to two weeks, and the hatchlings measure approximately 35 mm snout-to-vent length (Andrews et al., 2008).

I.10 Use in traditional medicine

Since ancient times, humans have utilized animals for drug production, disease treatment, and wound healing. Numerous studies have provided substantial evidence for the therapeutic use of fauna in various human societies (Santos et al., 2019). Reptiles are among the most commonly used animal groups in traditional medicine, with Alves et al. (2007) reporting a total of 165 reptile species used in folk medicine worldwide.

The findings of Ogoanah and Omijie (2017) indicated that chameleons were the most commonly used species (12%) among animal species in Benin City, Nigeria. Similarly, a study by Osunsina et al. (2012) conducted in villages within buffer zones around certain national parks in Nigeria reported that chameleon meat is traditionally used in the treatment of chest pain and cough

Chapter II

Bunium mauritanicum

II.1 Definition of phytotherapy

The term Phytotherapy is of Greek origin, composed of the words phuton (plant) and therapy (treatment). It is defined as a therapeutic approach based on the medicinal properties of plants and their extracts, aimed at treating diseases, preventing them, and promoting health.

Phytotherapy focuses on treating the patient's overall condition (terrain) as well as the symptoms of the disease, adopting a holistic approach to understand the origin of symptoms and prevent their occurrence. Only plants with proven medicinal efficacy are used in this field.

The plant parts selected are those richest in active principles, which may include the whole plant, leaves, stems, branches, flowering tops, bark, roots, fruits, or flowers, used either fresh or dried. Preparation methods are chosen according to the plant part used, the nature of the active principle (hydrophilic or lipophilic), and the type of patient. For example, a young child should not be treated with a mother tinture containing a high alcohol content (Nelly, 2013).

Phytotherapy can also be considered an allopathic discipline aimed at preventing functional disorders and/or treating certain pathological conditions using plants, plant parts, or plant-based preparation (Bensalek, 2018).

It can be classified into three types of practices (Bensalek, 2018):

• A traditional practice, sometimes very ancient, based on empirical knowledge of plant properties.

• A scientific practice based on advances and evidence, focusing on the extraction of active principles from plants.

• A prophylactic practice, used since ancient times.

The use of plants in daily life, especially in cooking such as garlic, thyme, ginger, and green tea represents a form of phytotherapy. A balanced diet rich in active compounds can be considered a form of prophylactic phytotherapy.·

II.2 Types of phytotherapy

According to Strang (2006), phytotherapy includes different types

Aromatherapy (Aromatherapy)

It is defined as a therapy based on the use of aromatic substances (essential oils) secreted by many plants. These are complex substances, generally applied through the skin.

- Gemmotherapy (Gemmotherapy)

It is based on the use of alcoholic extracts prepared from young plant tissues, such as buds and roots.

- Herbalism (Herboristerie)

It is one of the oldest and most traditional forms of therapy, relying on the use of fresh or dried plants, either whole or in parts such as bark, fruits, or flowers. Preparation methods are simple and often water-based, including decoction, infusion, and maceration. These preparations are also available in modern forms such as capsules containing powdered dried plants.

- Homeopathy (Homeopathie)

It relies largely, but not exclusively, on plants. Approximately three-quarters of the active principles are of plant origin, while the remainder are of animal and mineral origin.

- Pharmaceutical Phytotherapy (Phytotherapy medication)

It involves the use of plant-derived products obtained through extraction processes, then diluted in ethyl alcohol or other solvents. These extracts are standardized in sufficient quantities to ensure a sustained and rapid effect, and are presented in various pharmaceutical forms such as syrups, drops, capsules, and lyophilized preparations.

II.3 Advantages

Phytotherapy (Phytothérapie) presents several advantages that explain its increasing use (Pasdeloup,2019):

- At the level of public health: phytotherapy helps reduce iatrogenic effects (iatrogénie) in general, and does not lead to drug dependence requiring withdrawal upon discontinuation of treatment.
- At the ecological and environmental level: plants are taken from nature and return to it after their metabolization (métabolisation) within the body, unlike drugs from the chemical industry, which may lead to the accumulation of potentially toxic pharmaceutical substances in the environment.
- At the economic level: phytotherapy products are generally less expensive compared to those of conventional medicine, especially herbal teas (tisanes); however, they are not reimbursed by social security.

II.4 Disadvantages

Phytotherapy is considered a form of natural therapy; however, it cannot necessarily be regarded as a gentle medicine as widely believed (Pasdeloup, 2019). The lack of reliable scientific evidence limits the validation of its effectiveness, as most claims regarding its therapeutic effects rely on practitioners' opinions without sufficient scientific verification. Moreover, diagnosis in this field is often imprecise, as it frequently depends on non-objective methods such as smell and observation of symptoms, with the absence of standardized tests to assess efficacy, in addition to the recourse in some practices to consulting spirits and ancestors within certain religious contexts. Furthermore, dosage determination is arbitrary and imprecise, and preparation methods often lack the necessary hygienic conditions (Sofowora, 2010).

II.5 General information on the Studied Plant (*Bunium mauritanicum*)

II.5.1 The Apiaceae family

The Apiaceae family belongs to the class Magnoliopsida (Dicotyledons) and is particularly characterized by its typical inflorescence, the umbel (ombelle), which is why it is also known as Umbelliferae (Dou et Guedda, 2020). Its structure resembles an umbrella or a tent (Bruneton, 2009).

This family includes approximately 450 genera and 3780 species (UGA "Université Grenoble Alpes"), distributed across most regions of the world, especially in temperate areas, and is relatively rare in tropical regions (Thiviya et al., 2022).

Plants of the Apiaceae family are mainly herbaceous annuals in temperate regions (fenouil, coriandre, cumin, aneth, fenouil doux), biennials (carotte, persil, panais, céleri), or perennials (angélique, livèche, fenouil amer) (Bouti, 2022). They are mostly herbaceous plants, sometimes shrubby, and highly homogeneous (Filliat, 2012), and are classified according to characteristics related to inflorescence and fruit structure (Djebablah & Henni, 2019).

• Leaves: alternate, compound, and rarely simple. Petioles are often expanded at the base and sheathe the stem. The stem is usually hollow.

• Flowers: arranged in simple or compound umbels, with small bracts called involucelles at the base. They consist of 5 petals, 5 stamens, and a bicarpellary ovary.

• Fruits: formed of 2 mericarps attached to a central axis, each mericarp having two faces: a commissural (flat) face and a dorsal (convex) face. The dorsal face usually bears 5 ribs separated by containing 4 vallecules short secretory canals (vittae).

The fruit is generally a schizocarp, containing two seeds (Aéimovié, 2017).

This is a large family of about 3000 species distributed across most regions of the world. It is botanically very homogeneous and characterized by its umbel inflorescence (Ombelliferae) (Bakri & Dilmi, 2021). In Algeria, 55 genera and 117 species have been recorded, including 24 endemic species (Quezel & Santa, 1963). The family mainly consists of herbaceous plants, either annual, biennial, or mostly perennial (Lebouazid & Meziani, 2021).

Figure 10 :*Geographical distribution of the Apiaceae family worldwide (Hattab et al., 2022).*

II.5.2 Bunium genus

The genus Bunium (family Apiaceae) comprises approximately 50 to 100 species worldwide and is widely distributed across several regions of the world (Lefhal et al., 2017). These morphological characteristics make Bunium species highly similar to those of the genus Curcuma. In fact, the genera Bunium and Curcuma are taxonomically related and are classified as useful herbs and aromatic plants, which typically grow in temperate, warm, dry, arid, and semi-arid climates, and are usually found on mountain slopes (Mohammad Hosseini et al., 2021).

Species of this genus are characterized by their aromatic and medicinal properties; Their seeds and essential oils are widely used in food and medicine (Lefhal, 2014).

In Algeria, the genus Bunium is represented by several species, including *Bunium mauritanicum*, in addition to other names and species documented in the study by Dou and Guedda (2020).

✓B. incrassatum is a widespread and common species found in agricultural fields.

✓B. fontanesii has the synonym B. mauritanicum.

✓B. chaberti, endemic to Lalla Khedidja in the Djurdjura Mountains.

✓B. elatum, very rare and endemic to the Bibans.

✓B. crassifolium, also very rare and endemic to El-Kala; B. macua, very rare in Zaccar and Bou Maâd.

✓B. alpinum grows under cedar trees of the Tell Atlas (Algiers region, Kabylie, and Aurès).

II.5.3 Species *Bunium mauritanicum*

This plant is known in rural areas of mountainous regions in Algeria and is widely used by herbal practitioners due to its multiple medicinal properties. It also exhibits high nutritional value and a diversity of benefits, making it a crop of considerable economic and nutritional importance. It is well adapted to mountainous environments and is traditionally valued in the treatment of goiter and related disorders (Boumediou & Addoun, 2017).

According to Trabut and Mares (1907), the plant known as "telghouda" is originally attributed to Bunium incrassatum and *Bunium mauritanicum*. The authors indicate that it is a widely distributed mountain plant, whose large starchy tubers were historically collected by local populations during periods of famine as an alternative food source. After drying, these tubes can be ground into flour used for nutrition. In contrast, the fresh tuber contains pungent aromatic compounds that may cause intestinal and nervous disorders upon consumption (Ben Khalifa & Toumi, 2019).

II.6 Classification

Table 1:*Scientific classification of Bunium mauritanicum (Cronquist, 1981)*

Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopida
Order	Apiales
Family	Apiaceae
Genus	Bunium
Species	Mauritanicum

II.7 Description of the species *Bunium mauritanicum*

Bunium mauritanicum . is a medicinal plant of significant therapeutic importance and belongs to the Apiaceae family. It contains a variety of bioactive compounds with multiple biological properties (Karouche et al., 2022). It is a perennial wild herbaceous plant with an umbelliferous growth pattern, characterized by umbel inflorescences with a diameter ranging from 5 to 7 cm. Its stems are thin, weakly developed, and grooved, especially in the upper part, and are aromatic. The leaves are alternate and two to three times divided into narrow strips with an overall triangular shape. The underground part consists of a brown, nearly spherical tuber, measuring 1 to 2 cm in diameter, brown on the outside and white on the inside. The flowers are small, white, and arranged in umbel inflorescences, with a flowering period extending from March to July (Adoui et al., 2022).

II.8 Geographical distribution of *Bunium mauritanicum*

Bunium mauritanicum L. is considered a widely distributed species, with records of its occurrence in northern England, Italy, Sicily, the Balkan Peninsula, and Spain, where it occurs naturally within the European region.

In North Africa, this species is naturally distributed in Morocco, Tunisia, and Algeria. It occupies a variety of habitats, including open vegetation areas and the edges of maquis (Mediterranean shrublands), as well as calcareous, clay-calcareous, and rocky soils. It is also relatively common in rude environments.

In Algeria, its distribution is mainly concentrated in the eastern regions, particularly in the Wilaya of Oum El Bouaghi, where it is considered very common (Adoui et al., 2022). It is also found in El Kala, and is reported as endemic to Lalla Khedidja in the Djurdjura Mountains within the Tell Atlas (Algiers, Kabylie, and Aurès) (Ben Khalifa & Toumi, 2019).

II.9 Chemical composition

The seeds of B. mauritanicum are composed of coumarin, β-sitosterol, sucrose, as well as oleic acid (Boulahbel et al., 2017)

***Table 2**:Chemical composition of the tuber of Bunium mauritanicum (Abderahmane, 2018)*

Type of compounds	Quantity (%)
Water	15.66
Ash	5.50
Nitrogen-containing compounds	7.00
Fat	1.34
Starch and related compounds	63.12
Cellulose	6.40
Undosed materials	0.98

II.10 Therapeutic uses of *Bunium mauritanicum*

The tuber of this plant is utilized for the extraction of edible flour (Benkhalifa & Toumi, 2019). It is also employed in various industrial fields, including cosmetics, pharmaceuticals, and the agri-food industry, due to its richness in a wide range of bioactive compounds such as coumarins, phenolic acids, terpenes, flavonoids, alkaloids, tannins, and lignins. These compounds exhibit potential biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Karouche et al., 2022).

Traditional uses:

Within the framework of traditional local medicine, the dried and powdered tubers of this plant are considered to possess astringent and antidiarrheal properties, and are also used in the treatment of inflammatory hemorrhoids. In addition, the plant is used to alleviate symptoms of bronchitis and cough. An extracted oil is also employed to relieve intestinal gas and stomach pain. In the mountainous regions of Algeria, it is further used in the treatment of hypothyroidism and angina pectoris, with a traditional belief in its effectiveness in breaking kidney stones and treating certain tumors. On the other hand, local populations collect the tubers, then dry and grind them using traditional mills to obtain a flour that is mixed with barley and consumed in the form of flatbreads. It is worth noting that the fresh tuber contains pungent aromatic compounds that may cause intestinal and nervous disorders upon consumption (Rajem, 2013).

Second part

Experimental part

Chapter I

Methods &Meterial

III.1 Preparation of samples

III.1.1. Bunium mauritanicum

Samples of the *Bunium mauritanicum* plant were obtained from herbalists in Ouargla, then they were thoroughly cleaned to remove impurities and dust. After that, the samples were left to dry at room temperature away from direct light for a period of 10 to 15 days to preserve the active compounds. After complete drying, the samples were ground using a hand mill to obtain a fine and homogeneous powder.

Figure 11 :*A picture of the Bunium mauritanicum herb.*

III.1.2. C.Chamealeon

The chamealeon specimens were obtained by hunting them in the Algerian Sahara, specifically in the Ouargla region, and then slaughtered in the laboratory. After evisceration and washing, the chameleons were dried whole using the traditional method, filling their body cavities with coarse salt. They were then placed on a piece of cardboard to dry. The drying conditions required protecting the specimens from direct sunlight, so they were placed in the shade in a well-ventilated area at a temperature between 35 and 40 degrees Celsius for one week. After drying, the specimens were thoroughly washed with distilled water and then dried again by

exposing them to sunlight for one day. Using a hand mill, the specimens were ground into a powder. The ground material was preserved for later analysis.

Figure 12 :*A picture for preparation of a chameleon sample*

III.2 Extraction

III.2.1 Aqueous extract

III.2.1.1 Chameleon extract

We utilized distilled water as the solvent, where placed 600 ml in a round-bottom flask of Soxhlet apparatus. We then took 20 grams of *C. chamaeleon* powder and placed it in the cartridge then in the chamber of the Soxhlet extractor. Approximately 10 cycles, we produced a solution that contained polar compounds more. To obtaine the dry extract, the prepared solution is next incubated in the oven at 50°C for a period of a longue time (more than a day).

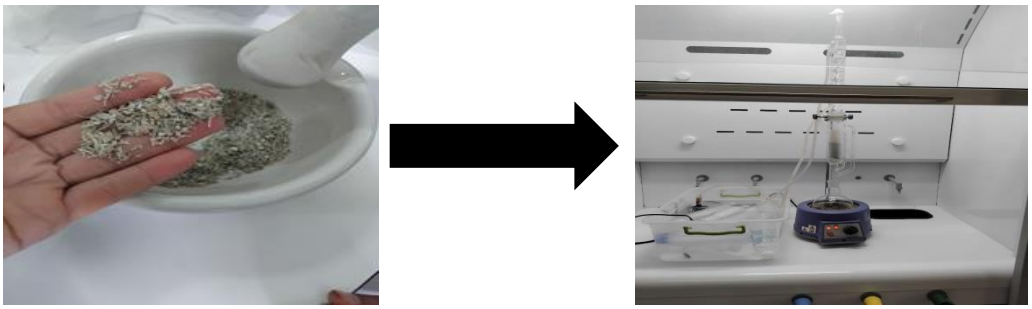

Figure 13 :*Process of extracting a chameleon solution*

III.2.1.2 Bunium extract

We utilized distilled water as the solvent, where placed 600 ml in a round-bottom flask of Soxhlet apparatus. We then took 20 grams of *Bunium mauritanicum* powder and placed it in the cartridge then in the chamber of the Soxhlet extractor. Approximately 10 cycles, we produced a solution that contained polar compounds more. To obtaine the dry extract, the prepared solution is next incubated in the oven at

50°C for a period of a longue time (more than a day).

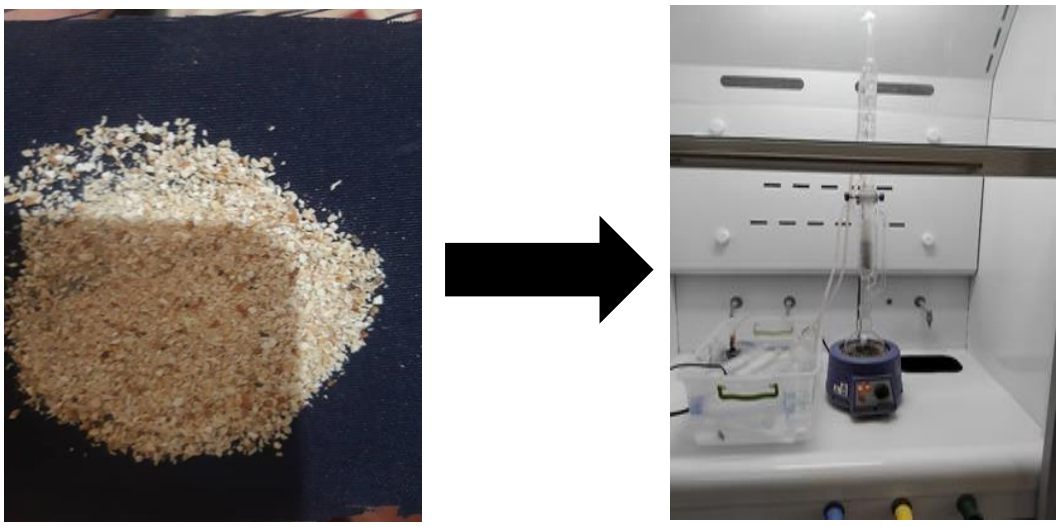

Figure 14 :*Process of extracting a Bunium solution*

III.2.1.3 Combained extracts

We utilized distilled water as the solvent, where placed 600 ml in a round-bottom flask of Soxhlet apparatus. We then took 10 grams of *Bunium mauritanicum* powder and 10 grams of *C. chamaeleon* powder and placed it in the cartridge then in the chamber of the Soxhlet extractor. Approximately 10 cycles, we produced a solution that contained polar compounds more. To obtaine the dry extract, the prepared -solution is next incubated in the oven at 50°C for a period of a longue time (more than a day).

III.3 In vitro study

III.3.1 Antioxidant activity

III.3.1.1 Free radical scavenging activity, DPPH assay

The antioxidant activity of *Bunium mauritanicum* extract, *Chamaeleo chamaeleon* extract, their mixture, and the standard antioxidant ascorbic acid was evaluated using the DPPH assay. This method assesses the ability of the tested compounds to scavenge the DPPH• free radical, as monitored by spectrophotometry. The reaction is characterized by a color change from violet (DPPH•) to yellow (DPPH-H), and the decrease in absorbance is measured at 517 nm. (Bougandoura et Bendimerad, 2013).

The DPPH (1,1-diphenyl-2-picrylhydrazyl) solution was prepared by dissolving 4 mg of DPPH• in 100 mL of methanol. Then, 1 mL of each extract of *Chamaeleo chamaeleon* and *Bunium mauritanicum*, as well as their mixture, at concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/mL, or ascorbic acid as a standard at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08,0.09 and 0.1 mg/mL, was added to 1 mL of the previously prepared DPPH• solution.

The reaction mixture was immediately shaken after preparation and incubated in the dark for 30 minutes at room temperature to allow the reaction to proceed. The absorbance was then measured at 517 nm against the reference blank. (Talbi et al., 2015).

$$\textbf{I\%DPPH radical Scavenging} = [(A0 - A1)/A0 \times 100]$$

- I%: percentage of inhibition, A0: absorbance of the control, A1: absorbance of the sample

The IC_{50} (50% radical inhibition of the extract) was calculated from the linear equation relating concentration to the percentage of inhibition. The lower the IC_{50} value, the higher the antioxidant capacity.

III.3.1.2 Reducing Power assay

According to the method of Karagozler et al. (2008), with some modifications, 500 µL of extracts at different concentrations (0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8,0,9,and 10 mg/mL) were mixed with 1.25 mL of phosphate buffer solution (0.1 M, pH 6.6) and 1.25 mL of 1% potassium ferricyanide solution ($K_3Fe(CN)_6$). The mixture was incubated in a water bath for 20 minutes at 50°C. After cooling, 1.25 mL of 10% trichloroacetic acid (TCA) was added to stop the reaction, and the mixture was centrifuged at 3000 rpm for 5 minutes. Then, 1.25 mL of the upper layer (supernatant) was mixed with 1.25 mL of distilled water, followed by the addition of 250 µL of 0.1% ferric chloride ($FeCl_3$) solution. The absorbance was measured at 700 nm using an optical spectrophotometer.

III.3.2 Sun Protection Factor (SPF) Test

According to Rudrappa et al. (2023), with some modifications, the effectiveness of extracts (CCE), (MBE), and (CCBME) in protecting against solar radiation was evaluated by determining the Sun Protection Factor (SPF) using spectrophotometry. The aqueous extract was prepared at a concentration of 1 mg/ml, and spectroscopic measurements were performed at wavelengths between 290 and 320 nm, with a 5 nm interval. Avène commercial sunscreen was used as a positive control to validate the methodology and facilitate comparison of SPF values. The SPF was calculated according to the following equation: Spectrophotometric.

$$SPF = CF \times \sum_{290}^{320} \times EE(\lambda) \times I(\lambda) \times DO(\lambda)$$

Where: CF is the correction factor (10). EE is the erythemal effect of radiation at wavelength (λ) nm. I is the solar intensity spectrum at wavelength (λ) nm. DO(λ) is the spectrophotometric absorbance values at the wavelength. (Rudrappa et al., 2023).

III.3.3 Anti-inflammatory activity

Bovine protein was selected as a biological model to evaluate anti-inflammatory activity.

A 1% bovine protein solution was prepared, and 500 µL of the bovine protein solution was mixed

with 250 µL of the studied extract. The mixture was then incubated in a water bath at 37°C for 20 minutes, after which the solution was cooled.

Subsequently, 500 µL of distilled water was added to the mixture, and it was re-incubated in a water bath at 70°C for 5 minutes. Finally, the absorbance was measured using a spectrophotometer at a wavelength of 660 nm..(BOUDEBIA, 2023**).**

III.3.4 Hemolysis assay

Vinjamuri et al., (2015) indicates that the following procedures were used to carry out the hemolysis test, where we collected 5 ml of blood from normal subjects in EDTA tubes to prevent coagulation, and then centrifuged it for 10 minutes at 1000 rpm. Next, fully eliminated the white buffy surface and the plasma. Following which, for five minutes, wash the erythrocyte three more times with 1X PBS (phosphate-buffered saline, pH 7.4). The cleaned blood cells are kept at 4°C and utilized for no more than six hours. 50 µl of 10 dilutions of the erythrocyte suspension (100 ml of erythrocyte suspension: 900 ml of 1X PBS) were combined with 100 µl of the extract at various concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/ml), a negative control of 100 µl of 1X PBS and a positive control of 100 µl of SDS were employed. The mixture was combined, and it was then incubated for 60 minutes in a water bath at 37°C, Then add 850 µL of 1X PBS until the volume of the mixture reaches 1 mL. Finally, it was centrifuged for 3 minutes at 300 rpm. Late, using a spectrophotometer to measure the absorbance of hemoglobin in the supernatant at 540 nm, the percentage of hemolysis was estimated using the formula below:

$$\textbf{inhibtion} = 100 - \left(\frac{sample}{control}\right) \times 100$$

III.4 In vivo assays

III.4.1 Animal treatment

The study was in vivo using a total of 30 adult male Albino Wister rats, with an average weight of 106.4±9.33g , It was obtained from a rat farm in the commune of Guemar, El Oued, Province These rats were subjected to a conditioning period for a period of 7 days to animal house conditions at a temperature of 25 °C, 35% humidity, and a photoperiod of 12 hours of light

and 12 hours of darkness. They were treated according to the guidelines stipulated in the Guide for the Care and Use of Experimental Animals **(Albus, 2012)**. Polypropylene cages with a size of "50 x 35 x 20" divided into five rats in each cage with free access to water and food. The cages are lined with sawdust and cleaned daily until the end of the experiment and they were fed standard food according to **Southon *et al*., (1984)** they were given tap water to drink throughout the experiment.

III.4.2 Experimental process

Group 01;

(Cont): As a group as a control, they were given normal drinking water and fed only standard food during the experimental period.

Group 02:

(BTU): All individuals of this group were fed only standard food and given turbid drinking water cloudy with Basdène(Benzylthiouracile) at a rate of 2mg/1kg for 30 days.

Group 03 :

(BTU + Levo): All individuals in this group were fed exclusively with a standard diet and received drinking water supplemented with Benzylthiouracil (BTU) at a concentration of 2 mg/kg for 21 days.

Afterwards, they received drinking water containing Levothyroxine sodium at a concentration of 1.5 mg/kg for 7 consecutive days.

Group 04:

(BTU + *C. chamaeleon* ext): All individuals of this group were fed only standard food and given turbid drinking water cloudy with Benzylthiouracile at a rate of 2mg/kg for 21 days. They were then injected intragastricly with the aqueous extract of the *c. chameleon* for seven days at a rate of 1ml.

Group 05:

(BTU + *Bunium mauritanicum* ext): All individuals of this group were fed only standard food and given turbid drinking water cloudy with Benzylthiouracile at a rate of 2mg/kg for 21 days. They were then injected intragastricly with the aqueous extract of the *Bunium mauritanicum* for seven days at a rate of 1ml.

Group 06:

(BTU + mixture[*Bunium mauritanicum* ext +*c. chameleon* ext]): All individuals of this group were fed only standard food and given turbid drinking water cloudy with Benzylthiouracile at a rate of 2mg/kg for 21 days. They were then injected intragastricly with the aqueous extract of the mixture for seven days at a rate of 1ml.

III.5 Sacrifice of rats and collection of blood and organs

The rats are anesthetized with chloroform (94%) after 16 hours of fasting and are sacrificed. The blood sample is taken at the time of sacrifice in EDTA tubes for hematological analyzes dry tubes for biochemical analyzes. After the dissection, the liver, kidneys, and Thyroid gland are carefully removed, rinsed with distilled water then with 0.9% NaCl and then weighed. Some of the organs are used for the preparation of the homogenate and the other is kept for histological sections. The homogenates of the organs are used for the determination of the parameters of oxidative stress (malondialdehyde.

(MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT))

III.6 Methods of blood analysis

III.6.1 Hematological parameters assay

The hematological parameters (leucocytes (WBC), lymphocytes (LYM), granulocytes)GRA), erythrocytes (RBC), hemoglobin (HGB), the mean corpuscular volume (MCV ،(and platelets (PLT)) are determined by the Coulter method using the Medonic type auto hematology analyzer specific to Complete Blood Count (CBC).

III.6.2 Biochemical parameters assay

III.6.1.1 Blood glucose

Blood glucose Beta-D-glucose in the plasma is oxidized by glucose oxidase to D-glucono-1,5-lactone with the production of hydrogen peroxide; the lactone is subsequently slowly hydrolyzed to Dgluconic acid. A peroxidase enzyme subsequently converts the hydrogen peroxide created to oxygen and water. Following this, oxygen reacts with an oxygen acceptor like, converting it into a colored molecule whose concentration may be determined colorimetrically (Trinder, 1969).

Glucose＋glucose oxidase＋O_2→Gluconic Acid＋H_2O_2

H_2O_2＋Peroxidas＋oxygen acceptor(colourless)→H.O＋Oxidised acceptor(**coloured)**

III.6.2,2Thyroid hormones

Thyroid hormone (free triiodothyronine (FT3) free thyroxin (FT4) ultra-sensitive thyroidstimulating hormone (TSH)) concentrations in serum were estimated by cobas e411 analyzer, a completely automated immunological tester that applies the electrochemiluminescence (ECL) method) Marwaha et al., 2008.

III.6.2.3 Reactive protein

Concentration of C-reactive protein in serum was determined by turbidimetric method on a COBAS INTEGRA 400 analyzer. Where, this efficient monospecific antibody-based equilibrium turbidimetric immunoassay for blood C-reactive protein uses Polyethylene glycol-6000 to speed up and enhance the immunoprecipitation reaction and Tween-20 surfactant to reduce and stabilize the sample blank values (Otsuji et al., 1982).

III.6.2.4 Aspartate transaminase

In accordance with an enzymatic kinetic, the spectrophotometer measured the AST activity. In order to produce glutamate and oxaloacetate, aspartate aminotransferase (AST), also known as glutamate oxaloacetate-transaminase (GOT), catalyzes the transfer of an amino group from

aspartate to -ketoglutarate. Malate dehydrogenase (MDH) and NADH converts oxaloacetate to malate. The rate of reduction in NADH content is closely correlated with the asparatateaminotransferase activity in the sample. At a wavelength of =340 nm, reading is done via spectrophotometry (Schumann et al., 2010).

III.6.2.5 Alanine transaminase

In accordance with an enzymatic kinetic, the spectrophotometer measured the AST activity. In order to produce glutamate and oxaloacetate, aspartate aminotransferase (AST), also known as glutamate oxaloacetate-transaminase (GOT), catalyzes the transfer of an amino group from aspartate to -ketoglutarate. Malate dehydrogenase (MDH) and NADH converts oxaloacetate to malate. The rate of reduction in NADH content is closely correlated with the asparatateaminotransferase activity in the sample. At a wavelength of =340 nm, reading is done via spectrophotometry (Schumann et al., 2010)

III.6.2.6 Urea

Urease catalyzes the hemolysis of urea, present in the sample, into ammonia ($NH4^+$) and carbon dioxide (CO_2). The ammonia formed is incorporated into α-ketoglutarate by the action of glutamate dehydrogenase (GLDH) with parallel oxidation of NADH to NAD. The decrease in NADH concentration in the method is proportional to the concentration of urea in the sample tested (Kaplan, 1984).

III.6.2.7 Creatinine

The dosage is determined by how sodium picrate and creatinine interact. Alkaline picrate and creatinine interact to generate a red complex. The measurement interval was established to avoid interference from other serum components. The amount of creatinine present in the sample directly correlates with the intensity of the color those results (Peake and Whiting, 2006). Ur

III.7 Determination of oxidative stress parameters

III.7 .1 Preparation of homogenates

One gram of tissue (liver, kidney, and testis) from each rat in the different groups studied was used. After grinding and homogenizing the tissues in TBS (50 mM Tris, 150 mM NaCl, pH 7.4),

the cell suspension is centrifuged at 3000 rpm for 15 minutes. Then the supernatant obtained is kept on ice for the assays of the oxidative stress parameters.

III.7.2 Determination of protein level

The Bradford method was used to spectrophotometrically measure the protein content of the supernatants using bovine serum albumin as a reference (Bradford, 1976). 100 mg of Coomassie Brilliant Blue were dissolved in 50 ml of ethanol to make the Bradford Reagent, which was then agitated vigorously for two hours with an agitator. Next, incorporate 850 ml of water and 100 ml of phosphoric acid (to obtain 1L of solution). Whatman filter paper was used to filter the Reagent. Where, 40 µl of the homogenate and the standard were added to 2ml of Bradford Reagent in tubes in the dark. After five minutes of incubation, the absorbance at 595 nm was measured. Protein concentrations are assessed by comparison to a standard range of bovine serum albumin carried out under identical conditions

III.7.3 Determination of Malondialdehyde level

Malondialdehyde (MDA) level determination was carried out using the Quintanilha, (1981) method. Aliquots containing 1 ml of diluted homogenate mixed with 20 µl of 2% (w/v) ethanolic solution of BHT received 2 ml of MDA reagent (15 % (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid, and 0.25 N hydrochloric acid). The mixture was placed in a boiling water bath for 15 minutes, cooled, and then the precipitate was separated by centrifugation. The absorbance was then determined at 532 nm. MDA concentration was given as nmol of MDA/mg protein.

$$\text{MDA}=\frac{0D\ sample\times 10^{6}}{1\times 1.56\times 10^{5}\times mg\ prot}$$

Where: OD: Optical density at 532 nm, 1.56 x105 M-1 cm-1 :Molar extinction coefficient of the MDA, DF: Dilution factor, 1: Optical path length.

The GSH concentration determination Based on the development of a yellow color when DTNB (5,5′-Dithiobis-(2-nitrobenzoic acid ((is added to compounds containing sulfhydryl groups. The mixture of 0.8 ml tissue homogenate and 0.2 ml 0.25% sulphosalicylic acid was

agitated, and then chilled at 4 °C for 15 minutes. The mixes were then centrifuged for 5 minutes at 1000 rpm. A final mixture of 0.5 ml of supernatant, 1 ml of TBS (pH 7.4 ،(and 0.025 ml of 0.01 M DTNB was created. The absorbance at 412 nm was measured after 5 minutes. The amountof total GSH was calculated as nmol GSH/mg protein) Weckbecker and Cory, 1988.(

$$\text{GSH}(\frac{nmol}{mgprot}) = \frac{0D \times 10^{6} \times 1.525}{13100 \times 0.8 \times 0.5 \times DF \times mg\ prot}$$

With: 1.525 :Total volume of solutions used in the GSH assay at the level of supernatant 0.5) ml, supernatant +1ml Tris + 0.025ml DTNB), 13100 M-1: Molar extinction coefficient of -SH group at 412 nm, 0.8: Volume of the homogenate, 0.5: Volume of the supernatant, DF: Dilution factor.

III.7.4 Determination of Superoxide dismutase activity

The method of assaying SOD activity using NBT by the superoxide anion ($O_2^{\cdot}$), is used as a basis for detecting the presence of SOD) Beauchamp and Fridovich, 1971). Of which, mix 500µL of EDTA-Met, 900µL of Phosphate Buffer (pH = 7.8), 25µL of Sample, and 50µL of NBT .Then put the mixture in a water bath for 5min at 25°C and then add 25 µL of Riboflavin to the mixture then put the mixture in the light for 20 min, the same steps for the control with 0µL of the Sample replaced by the addition of25 µL of Buffer phosphate. the absorbance is read at λ=560 nm.

$$\mathbf{I\%} = \frac{\mathbf{0D_C \times 0D_S}}{\mathbf{0D_C}} \mathbf{\times 100}$$

With: I%: % inhibition of NBT reduction by SOD, ODC :Optical density of the control, ODS: Optical density of the sample .And: The 50% inhibition is equal to 1 unit of enzyme. 50% inhibition = 1 unit of SOD, so the antioxidant activity of the enzyme equals SOD units / mg of pro

III.7.5 Determination of Catalase activity

Catalase activity was determined using Aebi, (1984) methodology. The reaction was initiated by mixing 20 ml of supernatant with 780 ml of phosphate buffer) KH2PO4, 0.1 M; pH 7.5) and 200 µl of H2O2 (0.030 M). The drop in absorbance at 240 nm was observed every 30 seconds for

2 minutes in order to track the breakdown of H2O2. International units per minute and per gram of protein) IU/min/g of protein) were used to express the enzymatic activity.

$$\text{Catalase}\left(\text{(UI/min)}/\text{g}\right)=\frac{(2.3033/T)\times(logA1/A2)}{DF\times g\ prot}$$

Where: A1 :Absorbance at the first minute, A2: Absorbance at the second minut Time interval in minutes

III.8 Histopathological study

For the histological study, the fragments of the organs of all batches are taken. They must be removed quickly to avoid autolysis, which takes place after a few moments of the death of the animal. After rinsing the samples with distilled water then with 0.9% NaCl, they are immediately immersed in a fixative (10% formaldehyde) (Leclerc-Mercier et al., 2016). The technique used includes the following steps: placing these tissue samples in special cassettes with turned walls to allow the passage of liquids. Then dehydrating the samples at using an automatic device which allows the automatic and progressive passage of the samples in ethanol baths of increasing concentration (70%, 95% and 100%). and after that the parts are then immersed in baths of liquid paraffin. The tissues being maintained and soaked in paraffin, then comes the embedding stage which consists of including the impregnated tissue in a block of paraffin which, by solidifying, will allow its cutting. This operation uses ―socalled inclusion ‖devices closing a reservoir of paraffin maintained in the liquid state by a heating system, a small tap and a refrigerated metal plate to obtain the rapid solidification of the paraffin block containing the tissue. The production of thin sections of a few microns (5µm on average) is possible thanks to special devices called ―Microtomes‖. These sections are spread on microscope slides ‹smoothed and fixed on the slide by the use of heated gelatinous water. and for staining, the technique used is that with Hematoxylin-Eosin or (HemateinEosin) (Elmalti et al., 2007); which requires the presence of acid alcohol (100 ml of %70 ethyl alcohol + 50 ml of HCl acid), ammoniacal water (100 ml of

distilled water + 2 ml Ammonia) and Eosin solution (100ml Eosin 3% aqueous solution, 125 ml %95ethyl alcohol, 375 ml distilled water and 2 drops of acetic acid). The staining follows the following steps: deparaffinization and hydration of the slides with tap water then rinsing with distilled water. Then are placed in a Harris Hematoxylin bath (15 minutes) which colors the basophilic structures) nuclei) purplish blue. The blades are immersed in acid alcohol (1 to 2 dives ؛(then in a tap water bath with verification of the differentiation under the microscope. Bet in an ammoniacal water bath, then in an Eosin bath (15 seconds to 2 minutes) which colors the acidophilic structures (cytoplasm) pink. All of these baths are separated by tap water washes. Finally, Observation under the microscope: the preparations of which were then dried and then observed under an optical microscope and photographed using a camera (BremondGignac et al ،. 2004).

III.9 Statistical analysis

The statistical evaluation of the results is carried out by the student T test ؛which is based on the comparison between two means. The results are given in the form of means of six repetitions for each group (± standard deviations .(For this, we used the software of SPSS (version 26) and EXCEL (version 2010 ،(the significance is determined by the value $\alpha=0.05$, If $P > \alpha$, there are significant differences between the means and we reject the hypothesis of equalit

ChapterII

Results & Dicussion

IV Results

IV.1 Extraction yield: In this study, the extraction yield results showed a clear difference among the three extracts, and this variation may be attributed to the nature of the compounds present in each extract. The CCE extract recorded the highest yield at 21%, as the chameleon contains organic compounds that may be more easily extractable in an aqueous medium compared to the compounds present in Tlaghouda. The BME extract showed an extraction yield of 16%, while the mixed extract (CCBME) recorded an intermediate yield of 19.5%, which may indicate interactions between the compounds of both the plant and animal sources.

CCE	**21%**
BME	**16%**
CCBME	**19,5%**

IV.2 In vitro assays of extracts

IV.2.1 Antioxidant activity

IV.2.1.1 DPPH radical scavenging activity

The antioxidant potential of the investigated extracts was evaluated using the DPPH assay radical scavenging assay through determination of their IC50 values, which represent the concentration required to inhibit 50% of DPPH free radicals. The obtained results demonstrated significant differences in antioxidant capacity among the tested samples. The combined extract (CCBME) exhibited remarkably stronger radical scavenging activity (IC50 = 12.73 ± 0.16 µg/mL) compared with the individual extracts, indicating a possible synergistic interaction between the bioactive constituents of *Chamaeleo chamaeleon* and *Bunium mauritanicum*. In contrast, the CCE extract showed the weakest antioxidant activity among the tested extracts with an IC50 value of 45.67 ± 0.07 µg/mL, whereas BME demonstrated moderate antioxidant potential (IC50 = 28.66 ± 0.06 µg/mL). Ascorbic acid, used as the reference antioxidant standard, displayed the highest scavenging efficiency with the lowest IC50 value (2.43 ± 0.05 µg/mL). These findings suggest that the combined extract possesses enhanced free radical scavenging

activity and may constitute a promising natural source of antioxidant compounds with potential pharmacological and therapeutic applications.

***Table 3**:IC50 values of CCE, BME, CCBME, and Ascorbic Acid determined by the DPPH assay*

Sample	IC50 (µg/mL)
CCE	45.67±0.07
BME	28.66±0.06
CCBME	12.73±0.16
Ascorbic Acid	2.43±0.05

IV.2.1.2 Reducing power

The reducing power assay was further applied to evaluate and compare the electron-donating capacity of extracts derived from *Chamaeleo chamaeleon* and *Bunium mauritanicum*, as well as their combined formulation (CCBME). This assay is a widely accepted method for assessing antioxidant potential based on the reduction of ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, which reflects the ability of bioactive compounds to act as reductants and terminate oxidative chain reactions. The EC50 value serves as an inverse indicator of antioxidant strength, where lower values correspond to higher reducing efficiency.

The results demonstrated a marked variation in reducing power among the tested extracts. The combined extract (CCBME) exhibited the most pronounced reducing activity, with an EC50 value of 134.33 ± 10.16 µg/mL. This relatively lower EC50 value, compared with the individual extracts, suggests a possible synergistic interaction between the bioactive constituents present in *Chamaeleo chamaeleon* and *Bunium mauritanicum*. Such synergy may enhance electron transfer capacity through complementary phytochemical profiles, including phenolic compounds, alkaloids, and other redox-active secondary metabolites.

In comparison, the BME extract demonstrated moderate reducing power activity with an EC50 value of 248.45 ± 42.56 µg/mL, indicating a lower electron-donating ability relative to the

combined extract. This suggests that while *Bunium mauritanicum* contains bioactive compounds capable of redox activity, their overall efficiency may be enhanced when combined with other phytochemical systems.

The CCE extract exhibited the weakest reducing potential among the tested samples, with a significantly higher EC50 value of 467.88 ± 32.87 µg/mL. This indicates a comparatively limited ability to donate electrons and reduce ferric ions, possibly reflecting a lower concentration or reduced accessibility of redox-active compounds in this extract.

Nonetheless, all tested extracts showed substantially lower reducing power compared to the standard antioxidant ascorbic acid, which exhibited an extremely high reducing efficiency with an EC50 value of 1.19 ± 0.75 µg/mL. This stark contrast underscores the exceptional redox capacity of ascorbic acid and provides a reference point for evaluating natural antioxidant systems.

On the whole, the findings highlight that the combined formulation (CCBME) demonstrates superior reducing power compared to the individual extracts, supporting the hypothesis that combining biologically distinct extracts may enhance antioxidant performance through synergistic interactions. These results also emphasize the potential of natural extracts as alternative sources of redox-active compounds with applications in pharmacological, nutraceutical, and oxidative stress-related research.

***Table 4**:EC50 values of CCE, BME, and CCBME extracts determined by reducing power assay.*

Sample	EC50 (µg/mL)
CCE	467.88±32.87
BME	248.45±42.56
CCBME	134.33±10.16
Ascorbic Acid	1.19±0.75

IV.2.2 Anti-inflammatory activity

The inhibition of bovine serum albumin (BSA) denaturation is a well-established in vitro model used to evaluate the anti-inflammatory potential of bioactive compounds. This assay is based on the principle that inflammatory processes often involve protein denaturation, during which native protein structures lose their conformational integrity, exposing hidden epitopes that can trigger autoimmune and inflammatory responses. Therefore, any substance capable of preventing or reducing protein denaturation is considered to possess membrane-stabilizing and anti-inflammatory properties.

In the present results, the IC_{50} values demonstrate a clear variation in anti-inflammatory efficacy among the tested samples. The extract of *Chamaeleo chamaeleon* (CCE) exhibited an IC_{50} value of 735.42 ± 8.77 µg/mL, indicating a moderate but relatively weak ability to inhibit albumin denaturation compared to the other tested samples. This suggests that its phytochemical composition contains bioactive compounds capable of stabilizing protein structure, although at a lower potency or concentration.

The extract of *Bunium mauritanicum* (BME) showed improved activity, with an IC_{50} value of 677.56 ± 10.56 µg/mL. This enhancement reflects a higher presence or better efficacy of anti-inflammatory constituents, likely including phenolic compounds, flavonoids, or volatile aromatic metabolites known to interact with protein structures and prevent thermal denaturation. These molecules may exert their effects through hydrogen bonding, hydrophobic interactions, and antioxidant-mediated stabilization mechanisms.

Interestingly, the combined extract (CCBME), representing the synergistic formulation of CCE and BME, demonstrated a further improvement in activity with an IC_{50} value of 587.43 ± 9.59 µg/mL. This reduction in IC_{50} indicates a stronger inhibitory effect on BSA denaturation and suggests a synergistic interaction between the phytochemical profiles of both plants. The enhanced activity may arise from complementary mechanisms, where different classes of bioactive compounds act cooperatively to stabilize protein structure more effectively than either extract alone.

For comparison, ascorbic acid, used as a standard reference compound, exhibited the strongest activity with an IC_{50} value of 476.31 ± 8.52 µg/mL. Ascorbic Acid is well known for its potent antioxidant properties, which contribute to its ability to prevent oxidative and structural protein damage. Although the plant extracts did not surpass the standard, the relatively close activity of the combined extract highlights its promising pharmacological potential.

Generally, these findings suggest that both individual and combined extracts possess measurable anti-inflammatory activity through the stabilization of protein structures against denaturation. The observed synergistic effect in the combined extract indicates that phytochemical interactions between *Chamaeleo chamaeleon* and *Bunium mauritanicum* may enhance their biological efficacy, supporting their potential use in the development of natural anti-inflammatory agents.

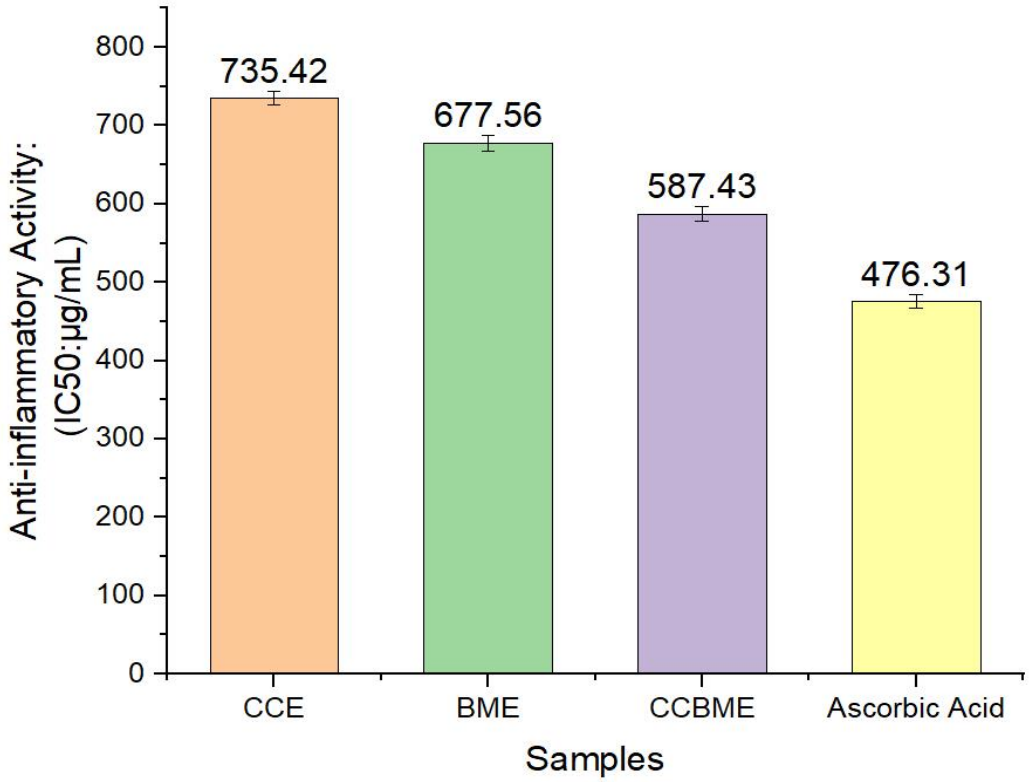

Figure 15:*Synergistic anti-inflammatory potential of Chamaeleo chamaeleon and Bunium mauritanicum extracts evaluated by BSA protein denaturation assay.*

IV.2.3 Photoprotective activity

The in vitro determination of sun protection factor (SPF) provides a quantitative estimation of the ability of natural and synthetic samples to absorb or block ultraviolet (UV) radiation, particularly within the UVB range responsible for erythema and photo-induced skin damage. In this context, higher SPF values correspond to stronger photoprotective efficacy and greater potential for application in sunscreen and cosmeceutical formulations.

The extract of *Chamaeleo chamaeleon* (CCE) exhibited an SPF value of 22.04 ± 2.17, indicating a moderate level of photoprotection. This activity suggests that CCE contains a notable amount of UV-absorbing secondary metabolites capable of absorbing radiation in the UV region. These compounds may act by dissipating UV energy through resonance stabilization and reducing the penetration of harmful radiation into deeper skin layers.

Similarly, the extract of *Bunium mauritanicum* (BME) showed a slightly higher SPF value of 24.35 ± 2.51. This improvement indicates a more efficient photoprotective profile compared to CCE, which may be attributed to differences in phytochemical composition, particularly the presence of aromatic essential oils, coumarins, and antioxidant-rich phenolic constituents. These molecules are known to contribute to both UV absorption and oxidative stress mitigation, thereby providing a dual protective mechanism against photodamage.

Remarkably, the combined extract (CCBME) demonstrated a significantly enhanced SPF value of 34.65 ± 1.12, representing a strong photoprotective effect among the tested natural samples. This marked increase suggests a synergistic interaction between the phytochemical profiles of CCE and BME. Such synergy may result in broader UV absorption spectra, improved molecular diversity of UV-filtering compounds, and enhanced stabilization of excited electronic states following UV exposure. The relatively low standard deviation also indicates consistent and stable photoprotective performance of the combined formulation.

In comparison, the commercial sunscreen Avène (Avene®) exhibited the highest SPF value of 50.04 ± 2.35, confirming very strong and standardized photoprotective efficacy. This level of protection is expected, as commercial sunscreens are formulated with optimized combinations of organic and inorganic UV filters designed to provide broad-spectrum protection against both UVA and UVB radiation. The significantly higher SPF of Avene® highlights the difference between purified, industrially engineered formulations and crude or semi-purified plant extracts.

Collectively, the first dataset demonstrates that while individual extracts exhibit moderate photoprotective activity, their combination (CCBME) significantly enhances UV protection,

supporting the concept of synergistic phytochemical interactions as a promising strategy in natural sunscreen development.

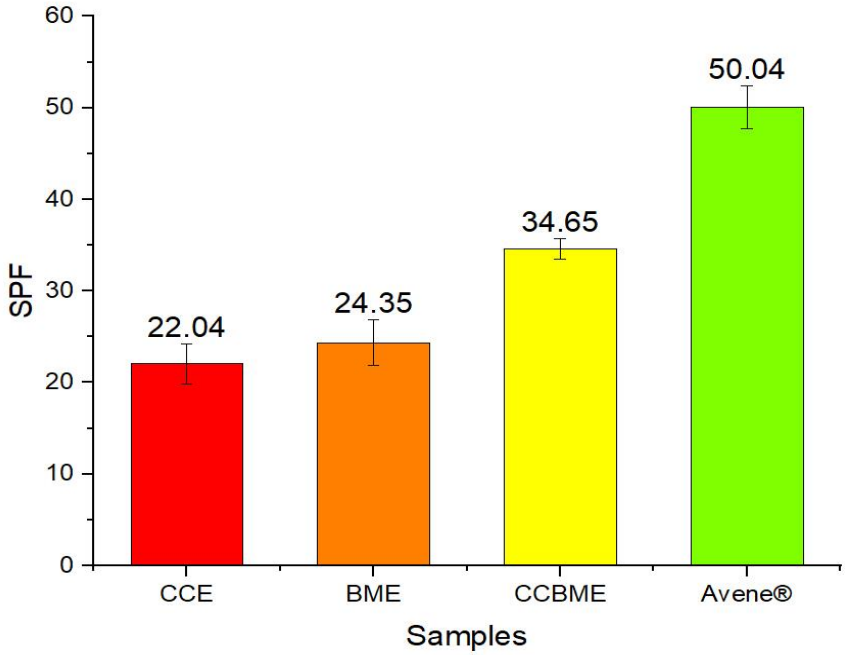

Figure 16 :*Photoprotective Activity of CCE, BME, CCBME and Avene®*

IV.2.4 Hemolytic activity

The hemolytic activity assay is a widely used method to evaluate the biocompatibility and membrane-stabilizing potential of bioactive extracts by measuring their ability to induce or prevent the lysis of red blood cells (RBCs). Low hemolysis percentages indicate strong erythrocyte membrane protection and good safety profiles, while higher values reflect membrane destabilization and cytotoxic potential.

In the present results, the extract of Chamaeleo chamaeleon (CCE) shows a clear dose-dependent increase in hemolytic activity, ranging from 5.53% at 10 µg/mL to 20.55% at 100 µg/mL. At lower concentrations, CCE demonstrates good hemocompatibility; however, at higher concentrations, hemolysis approaches the upper safety threshold, suggesting moderate membrane

interaction likely due to the increasing availability of bioactive compounds capable of interacting with lipid bilayers.

The extract of Bunium mauritanicum (BME) exhibits consistently lower hemolytic values compared to CCE, ranging from 4.02% to 11.33%. This indicates a better membrane-stabilizing profile and improved biocompatibility, suggesting that BME contains fewer or less aggressive membrane-disrupting constituents. Even at the highest concentration tested, BME remains within or close to the safe hemolysis range, highlighting its relatively favorable safety profile.

Notably, the combined extract (CCBME) demonstrates the lowest hemolytic activity across all concentrations, with values ranging from 2.43% to 5.23%. This strong reduction in hemolysis indicates a pronounced synergistic membrane-protective effect, where the combination of constituants from both extracts sources enhances erythrocyte membrane stability. The consistently low values across the concentration range suggest that the combined formulation not only maintains but improves biocompatibility, making it the most promising among the tested natural samples.

In contrast, the positive control Sodium dodecyl sulfate (SDS) exhibits very high hemolytic activity, increasing from 25.45% to 62.12% with concentration. This strong dose-dependent increase confirms its well-known role as a potent membrane-disrupting agent that solubilizes lipid bilayers, leading to extensive erythrocyte lysis.

Taken together, the results demonstrate a clear pattern of dose-dependent but safe hemolytic behavior for the plant extracts, with CCBME showing the highest level of erythrocyte membrane protection, followed by BME and then CCE. The marked contrast with SDS highlights the strong biocompatibility of the natural extracts, particularly the synergistic formulation, which appears to offer enhanced membrane stability and minimal cytotoxic risk.

***Table 5**:Concentration-dependent hemolytic activity and red blood cell membrane stability of CCE, BME, and CCBME compared with Sodium Dodecyl Sulfate (SDS)*

C (µg/mL)	% Hemolysis of			
	CCE	**BME**	**CCBME**	**SDS**
10	5.53	4.02	2.43	25.45
20	8.37	6.81	2.76	40.12
50	10.42	8.72	3.21	44.56
80	12.61	9.81	4.22	50.87
100	20.55	11.33	5.23	62.12

IV.3 In vivo study of extracts

IV.3.1 Weight Gain Index

The effects of benzylthiouracil-induced hypothyroidism and subsequent treatments with *Chamaeleo chamaeleon* extract, *Bunium mauritanicum* extract, their combination, and levothyroxine on the weight gain index of experimental rats are presented in Table 06. The obtained findings demonstrated marked alterations in body weight progression among the different experimental groups, reflecting the metabolic disturbances associated with hypothyroidism and the therapeutic efficacy of the administered treatments.

Prior to treatment, animals belonging to the hypothyroid group (CN) exhibited a significant increase in the weight gain index (13.9 ± 0.8) compared with the control group (10.3 ± 0.4) ($p < 0.05$). This elevation indicates the successful induction of hypothyroidism by benzylthiouracil administration, which is commonly associated with reduced basal metabolic rate, impaired lipid and carbohydrate metabolism, fluid retention, and decreased energy expenditure. Similar increases were also observed in the treatment groups before therapeutic intervention, including the levothyroxine-treated group (CP; 14.4 ± 0.3), the *Chamaeleo chamaeleon* extract-treated group (CCE; 13.6 ± 0.8), the *Bunium mauritanicum* extract-treated group (BME; 15.1 ± 0.9), and the combined extract-treated group (CCBME; 13.5 ± 0.1). The highest elevation was recorded in the BME group, which showed a highly significant increase compared with the control group (p

< 0.001). These findings confirm the establishment of a pronounced hypothyroid state in all intoxicated groups prior to treatment administration.

Following treatment, the untreated hypothyroid group (CN) maintained a significantly elevated weight gain index (14.6 ± 0.2) relative to the control group (10.8 ± 0.7), indicating the persistence of metabolic dysfunction and reduced catabolic activity associated with thyroid hormone deficiency. The continuous increase in body weight observed in this group may be attributed to diminished thermogenesis, lipid accumulation, and impaired mitochondrial oxidative metabolism, all characteristic features of hypothyroidism.

In contrast, administration of levothyroxine markedly reduced the weight gain index in the CP group to 9.6 ± 0.8, demonstrating a significant improvement compared with the untreated hypothyroid group. This normalization reflects the restoration of thyroid hormone homeostasis and metabolic activity induced by hormone replacement therapy. Levothyroxine treatment likely enhanced cellular oxygen consumption, stimulated lipolysis, and increased basal energy expenditure, thereby reversing hypothyroidism-associated weight accumulation.

Similarly, treatment with *Chamaeleo chamaeleon* extract produced a notable reduction in body weight gain, with the CCE group recording a value of 11.1 ± 0.5 after treatment. Although slightly higher than the control value, the reduction remained statistically significant compared with the CN group, suggesting that the animal extract exerted a beneficial modulatory effect on metabolic disturbances induced by benzylthiouracil. This improvement may be related to the presence of bioactive compounds capable of enhancing endocrine regulation, antioxidant defense mechanisms, and energy metabolism.

The group treated with *Bunium mauritanicum* extract (BME) also exhibited a substantial decline in the weight gain index following treatment, reaching 10.3 ± 0.4, a value remarkably close to that of the normal control group. This finding indicates a strong corrective effect of the plant extract on hypothyroidism-associated metabolic imbalance. The observed activity may be attributed to the richness of *Bunium mauritanicum* in phenolic compounds, flavonoids, and

antioxidant constituents known to improve metabolic efficiency and reduce oxidative stress-mediated endocrine dysfunction.

Interestingly, the combined treatment group (CCBME) displayed the most pronounced improvement among all extract-treated groups, with a post-treatment value of 9.1 ± 0.2. This reduction was even lower than that observed in the levothyroxine-treated group, suggesting a potential synergistic interaction between *Chamaeleo chamaeleon* and *Bunium mauritanicum* extracts. The combined administration may have enhanced metabolic restoration through complementary mechanisms involving antioxidant protection, endocrine modulation, improved mitochondrial function, and regulation of lipid metabolism.

***Table 6**:Weight Gain Index of different experimental groups.*

	Weight Gain Index	
	Before treatment	**After treatment**
Control	10.3±0.4	10.8±0.7
CN	13.9±0.8[a]	14.6±0.2 [a]
CP	14.4±0.3[b NS]	9.6±0.8 [NS*]
CCE	13.6±0.8[a NS]	11.1±0.5 [NS *]
BME	15.1±0.9[c NS]	10.3±0.4 [NS *]
CCBME	13.5±0.1 [b NS]	9.1±0.2 [NS *]

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3.2 Organ weight index

The effects of benzylthiouracil-induced hypothyroidism and the different therapeutic treatments on liver and kidney weight indices are presented in Table 07. Organ weight index is an important physiological and toxicological parameter commonly used to evaluate metabolic alterations, tissue hypertrophy, or potential organ damage induced by pathological conditions or administered treatments.

The liver weight index of the control group was 3.51 ± 0.35%, reflecting normal hepatic physiological status. In the hypothyroid group (CN), a slight increase was observed (3.64 ±

0.43%); however, this variation remained statistically non-significant compared with the control group, suggesting that benzylthiouracil administration did not induce marked hepatic enlargement or severe structural alterations. Similarly, the levothyroxine-treated group (CP) and the *Chamaeleo chamaeleon* extract-treated group (CCE) showed liver indices of 3.67 ± 0.51% and 3.71 ± 0.35%, respectively, both remaining close to the control values. In contrast, the *Bunium mauritanicum* extract-treated group (BME) exhibited a slight reduction in liver weight index (3.23 ± 0.37%), while the combined treatment group (CCBME) recorded a value of 3.55 ± 0.22%, very similar to that of the control group. Nevertheless, all differences were statistically non-significant.

Regarding kidney weight indices, the control group presented a normal value of 0.66 ± 0.07%. The hypothyroid group (CN) showed a slight non-significant increase to 0.74 ± 0.08%, indicating minimal renal alterations associated with hypothyroidism. Treatment with levothyroxine (CP), *Chamaeleo chamaeleon* extract (CCE), *Bunium mauritanicum* extract (BME), and the combined extracts (CCBME) resulted in kidney indices of 0.67 ± 0.09%, 0.64 ± 0.01%, 0.68 ± 0.04%, and 0.62 ± 0.03%, respectively. All values remained within the normal physiological range, suggesting the absence of renal hypertrophy or nephrotoxic effects.

Table 7*:Organ Weight Index of different experimental groups.*

	Organ Weight Index %	
	Liver	**Kidneys**
Control	3.51±0.35	0.66±0.07
CN	3.64±0.43 ^{NS}	0.74±0.08 ^{NS}
CP	3.67±0.51 ^{NS}	0.67±0.09 ^{NS}
CCE	3.71 ±0.35^{NS}	0.64±0.01^{NS}
BME	3.23±0.37^{NS}	0.68±0.04 ^{NS}
CCBME	3.55±0.22 ^{NS}	0.62±0.03 ^{NS}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3.3 Hematological parameters

The effects of benzylthiouracil-induced hypothyroidism and the different treatments on hematological parameters are presented in Table 08. Hematological analysis represents an important indicator of physiological status, inflammatory response, immune function, and the potential toxicological effects of experimental treatments.

The hypothyroid group (CN) showed a significant increase in white blood cell count (WBC) ($9.33 \pm 0.28 \times 10^9$/L) compared with the control group ($7.77 \pm 0.33 \times 10^9$/L), indicating the presence of an inflammatory or stress response induced by benzylthiouracil administration. Likewise, lymphocyte (LYM) and granulocyte (GRA) counts were markedly elevated in the CN group, reaching $6.31 \pm 0.28 \times 10^9$/L and $6.36 \pm 0.18 \times 10^9$/L, respectively. The increase in granulocyte count was highly significant ($p < 0.001$), suggesting activation of immune and inflammatory mechanisms associated with hypothyroidism and oxidative stress.

Treatment with levothyroxine (CP) slightly reduced lymphocyte and granulocyte levels compared with the untreated hypothyroid group; however, WBC and GRA values remained significantly higher than those of the control group. In contrast, administration of *Chamaeleo chamaeleon* extract (CCE) and *Bunium mauritanicum* extract (BME) markedly improved these alterations. Both treatments reduced WBC, LYM, and GRA counts toward normal physiological values, with significant differences compared with the CN group. The combined treatment group (CCBME) exhibited the greatest normalization effect, recording WBC, LYM, and GRA values (7.74 ± 0.56, 4.22 ± 0.12, and $3.24 \pm 0.11 \times 10^9$/L, respectively) very close to those of the control group. These findings suggest a strong synergistic anti-inflammatory and immunomodulatory effect of the combined extracts.

Regarding erythrocytic parameters, no significant variations were observed in hemoglobin concentration (HGB) or red blood cell count (RBC) among the experimental groups. Hemoglobin values ranged between 13.22 ± 0.34 and 14.43 ± 0.23 g/dL, while RBC counts remained relatively stable, indicating that hypothyroidism and the tested treatments did not induce marked anemia or erythrocyte alterations during the experimental period.

Similarly, platelet counts (PLT) showed no significant differences among the groups, although the CN group exhibited a slight increase compared with the control group. Treatment with the extracts, particularly the combined treatment, restored platelet levels toward normal values.

***Table 8**:Plasma concentration of hematological parameters of different experimental groups.*

	WBC($\times10^9$/L)	LYM($\times10^9$/L)	GRA($\times10^9$/L)	HGB (g/dL)	RBC($\times10^{12}$/L)	PLT ($\times10^9$/L)
Control	7.77±0.33	4.24±0.16	3.21±0.12	14.26±0.28	7.34±0.23	416±11.2
CN	9.33±0.28 [b]	6.31±0.28[a]	6.36±0.18 [c]	14.24±0.25[NS]	7.29±0.24[NS]	476±12.5 NS
CP	10.73±0.31[a] NS	5.43±0.22[a] NS	5.37±0.14[b]	13.34±0.32[NS]	7.27±0.21[NS]	456.3±10.4 NS
CCE	8.44±0.55 NS*	4.81±0.56 NS*	3.67±0.21 NS*	14.43±0.23[NS]	7.56±0.37 [NS]	480.7±11.5 NS
BME	8.37±0.53 NS*	4.43±0.83 NS*	3.74±0.32 NS*	13.62±0.37 NS	7.64±0.65 [NS]	465.5±13.2 NS
CCBME	7.74±0.56 NS*	4.22±0.12 NS*	3.24±0.11 NS*	13.22±0.34 NS	7.31±0.32 [NS]	423.4±11.3 NS

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3.4 Glycemia, liver and kidneys function parameters

The effects of benzylthiouracil-induced hypothyroidism and the different treatments on fasting blood sugar, hepatic biomarkers, and renal function parameters are presented in Table 09. These biochemical markers are essential indicators for evaluating metabolic disturbances and the functional integrity of the liver and kidneys under hypothyroid conditions.

Fasting blood sugar (FBS) levels showed no significant differences among the experimental groups. The control group recorded a value of 0.67 ± 0.05 g/L, while the hypothyroid group (CN) showed a slightly lower level (0.63 ± 0.08 g/L). Similarly, the treated groups, including levothyroxine (CP), *Chamaeleo chamaeleon* extract (CCE), *Bunium mauritanicum* extract (BME), and the combined treatment (CCBME), exhibited values comparable to the control

group. These findings indicate that benzylthiouracil-induced hypothyroidism did not markedly affect glucose homeostasis during the experimental period.

In contrast, significant alterations were observed in hepatic enzyme activities. The CN group exhibited a marked increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, reaching 197.6 ± 7.4 U/L and 120 ± 3.6 U/L, respectively, compared with the control group (145.5 ± 8.3 U/L and 81.21 ± 5.9 U/L). The elevation of these transaminases suggests hepatic stress and altered liver function associated with hypothyroidism and oxidative damage induced by benzylthiouracil administration.

The levothyroxine-treated group (CP) showed a further increase in AST and ALT activities, with values of 224.4 ± 7.2 U/L and 154.6 ± 2.7 U/L, respectively. These increases remained highly significant compared with the control group, indicating that levothyroxine treatment did not fully restore hepatic biochemical balance under the present experimental conditions.

Conversely, treatment with *Chamaeleo chamaeleon* extract (CCE) markedly improved liver enzyme levels. AST and ALT values decreased to 151.2 ± 5.1 U/L and 95.8 ± 9.3 U/L, respectively, approaching normal control values. Similar improvements were observed in the *Bunium mauritanicum* extract-treated group (BME), which recorded AST and ALT values of 155.6 ± 6.3 U/L and 98.1 ± 5.5 U/L. These findings suggest hepatoprotective effects of both extracts, likely mediated through their antioxidant and anti-inflammatory bioactive constituents.

Interestingly, the combined treatment group (CCBME) exhibited the greatest protective effect, with AST and ALT values of 141.2 ± 6.6 U/L and 82.3 ± 4.5 U/L, respectively, nearly identical to those of the control group. This remarkable normalization indicates a synergistic interaction between *Chamaeleo chamaeleon* and *Bunium mauritanicum* extracts in preserving hepatic integrity and reducing hypothyroidism-induced liver dysfunction.

Regarding renal biomarkers, urea and creatinine (CT) levels showed no significant differences among all experimental groups. The absence of marked alterations in these parameters suggests that benzylthiouracil-induced hypothyroidism did not cause severe renal

impairment. Furthermore, treatment with the extracts, either alone or in combination, did not induce nephrotoxic effects and maintained renal function within normal physiological limits.

***Table 9**:Glycemia, liver and kidneys function parameters of different experimental groups*

	FBS (g/L)	AST (U/L)	ALT (U/L)	Urea (g/L)	CT (mg/L)
Control	0.67±0.05	145.5±8.3	81.21±5.9	0.48±0.14	3.4±0.3
CN	0.63±0.08 NS	197.6±7.4[a]	120±3.6[b]	0.46 ±0.11[NS]	3.7±0.5[NS]
CP	0.66±0.05 NS	224.4±7.2[c**]	154.6±2.7[b**]	0.41±0.15 [NS]	3.9±0.2[NS]
CCE	0.68±0.05[NS]	151.2±5.1[NS**]	95.8±9.3 [NS **]	0.43±0.12 [NS]	3.4±0.2 [NS]
BME	0.69±0.06 NS	155.6±6.3 NS***	98.1±5.5 [NS **]	0.41±0.13 [NS]	3.1±0.3 [NS]
CCBME	0.62±0.07 NS	141.2±6.6 NS***	82.3±4.5 [NS **]	0.48±0.14 [NS]	3.2±0.5 [NS]

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3.5 Thyroid function parameters

The effects of benzylthiouracil-induced hypothyroidism and the different therapeutic treatments on thyroid hormone levels are presented in Table 10. Thyroid hormones, including triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH), are essential indicators for evaluating thyroid gland function and the severity of hypothyroidism.

The hypothyroid group (CN) exhibited a marked and highly significant reduction in T4 and T3 levels compared with the control group. T4 concentration decreased from 48.5 ± 0.8 pmol/L in the control group to 17.01 ± 1.4 pmol/L in the CN group, while T3 levels declined from 5.43 ± 0.22 pmol/L to 1.96 ± 0.24 pmol/L. These decreases confirm the successful induction of hypothyroidism by benzylthiouracil administration, which inhibits thyroid hormone synthesis and disrupts normal endocrine function. In parallel, TSH levels significantly increased in the CN group (0.898 ± 0.31 µUI/L) compared with the control group (0.471 ± 0.43 µUI/L), reflecting compensatory pituitary stimulation in response to reduced circulating thyroid hormones.

Treatment with levothyroxine (CP) partially improved thyroid hormone levels. T4 concentration increased to 23.10 ± 1.9 pmol/L, showing a significant improvement compared with the untreated hypothyroid group. However, T3 levels remained low (1.98 ± 0.80 pmol/L), indicating incomplete restoration of thyroid hormonal balance. In contrast, TSH levels markedly decreased to 0.243 ± 0.55 µUI/L, suggesting suppression of pituitary stimulation following hormone replacement therapy.

Administration of *Chamaeleo chamaeleon* extract (CCE) produced a notable recovery in thyroid function parameters. T4 and T3 levels increased to 31.8 ± 1.3 pmol/L and 3.54 ± 0.22 pmol/L, respectively, while TSH concentration declined toward normal values (0.331 ± 0.23 µUI/L). These findings indicate a beneficial effect of the animal extract in improving thyroid activity and restoring endocrine balance.

Similarly, treatment with *Bunium mauritanicum* extract (BME) significantly ameliorated thyroid dysfunction. The group exhibited T4 and T3 values of 37.5 ± 1.5 pmol/L and 3.65 ± 0.33 pmol/L, respectively, accompanied by a reduction in TSH levels to 0.376 ± 0.22 µUI/L. The improvement observed in this group may be attributed to the antioxidant and bioactive phytochemical constituents of the plant extract, which could contribute to the protection of thyroid tissue and stimulation of hormone synthesis.

Interestingly, the combined treatment group (CCBME) showed the most pronounced recovery among all treated groups. T4 and T3 concentrations reached 46.7 ± 1.6 pmol/L and 4.89 ± 0.26 pmol/L, respectively, values very close to those of the control group. In addition, TSH levels normalized almost completely (0.465 ± 0.25 µUI/L). These results strongly suggest a synergistic therapeutic effect between *Chamaeleo chamaeleon* and *Bunium mauritanicum* extracts, leading to substantial restoration of thyroid gland function and hormonal homeostasis.

***Table 10**:Thyroid function parameters of different experimental groups.*

	T4 (pmol/L)	T3 (pmol/L)	TSH (µUI/L)
Control	48.5±0.8	5.43±0.22	0.471±0.43
CN	17.01±1.4 ^{c}	1.96±0.24^{c}	0.898±0.31^{b}
CP	23.10±1.9 ^{b*}	1.98±0.80 $^{c\ NS}$	0.243±0.55^{a**}
CCE	31.8±1.3 ^{NS***}	3.54±0.22 ^{a***}	0.331±0.23 ^{NS***}
BME	37.5±1.5 ^{c**}	3.65±0.33^{a*}	0.376±0.22 ^{NS**}
CCBME	46.7±1.6 ^{NS***}	4.89±0.26 ^{a*}	0.465±0.25 ^{NS**}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3,6 Oxidative stress parameters

IV.3.6.1 Liver oxidative stress parameters

The effects of benzylthiouracil-induced hypothyroidism and the different treatments on hepatic oxidative stress markers are presented in Table 11. Oxidative stress is a key mechanism involved in thyroid dysfunction–related tissue injury and is characterized by increased lipid peroxidation and depletion of antioxidant defenses.

In the hypothyroid group (CN), a marked oxidative imbalance was observed compared with the control group. Malondialdehyde (MDA), a marker of lipid peroxidation, significantly increased to 6.76 ± 0.12 nmol/mg protein, indicating enhanced membrane lipid damage. In parallel, antioxidant defense parameters were significantly reduced, including glutathione (GSH) (7.33 ± 1.17 nmol/mg pr), superoxide dismutase (SOD) (4.33 ± 1.29 U/mg pr), and catalase (CAT) (7.12 ± 0.85 UI/min/g pr). These alterations confirm that benzylthiouracil-induced hypothyroidism induces a strong oxidative stress state in hepatic tissue.

Treatment with levothyroxine (CP) partially improved the oxidative status. MDA levels decreased compared with the CN group (5.81 ± 0.23 nmol/mg pr), while antioxidant enzymes showed moderate recovery, particularly SOD (6.11 ± 1.13 U/mg pr). However, GSH and CAT levels remained relatively low, indicating incomplete restoration of antioxidant defense mechanisms.

Administration of *Chamaeleo chamaeleon* extract (CCE) produced a noticeable improvement in oxidative stress parameters. Although MDA remained relatively elevated (6.77 ± 0.42 nmol/mg pr), antioxidant markers showed partial recovery, with increases in GSH (9.31 ± 1.42 nmol/mg pr), SOD (5.23 ± 1.23 U/mg pr), and CAT (8.25 ± 0.64 UI/min/g pr). These results suggest a protective antioxidant potential of the extract against hypothyroidism-induced oxidative damage.

Similarly, the *Bunium mauritanicum* extract-treated group (BME) demonstrated improved antioxidant status, with reduced MDA levels (5.25 ± 0.54 nmol/mg pr) and increased antioxidant enzyme activities, including GSH (9.22 ± 1.55 nmol/mg pr), SOD (6.42 ± 1.24 U/mg pr), and CAT (8.28 ± 0.45 UI/min/g pr). These findings indicate a stronger lipid peroxidation–reducing effect compared with CCE alone, suggesting potent hepatoprotective antioxidant activity.

Notably, the combined treatment group (CCBME) exhibited the most pronounced normalization of oxidative stress markers. MDA levels decreased close to control values (4.23 ± 0.23 nmol/mg pr), while antioxidant defenses were significantly restored, with GSH (10.96 ± 1.21 nmol/mg pr), SOD (8.18 ± 1.31 U/mg pr), and CAT (10.32 ± 0.67 UI/min/g pr) approaching or nearly matching control levels. These results indicate a strong synergistic antioxidant effect between *Chamaeleo chamaeleon* and *Bunium mauritanicum* extracts.

***Table 11**:Effect of CCE, BME and CCBME extracts on oxidative stress markers in liver tissue.*

	MDA (nmol/mg pr)	**GSH (nmol/mg pr)**	**SOD (U/mg pr)**	**CAT (UI/min/g pr)**
Control	4.51±0.19	11.57±1.25	8.11±1.12	10.71±0.93
CN	6.76±0.12 ^{a}	7.33±1.17 ^{c}	4.33±1.29 ^{c}	7.12±0.85 ^{c}
CP	5.81±0.23 ^{a*}	8.11±1.41 $^{c\ NS}$	6.11±1.13 ^{a**}	7.63±0.72 $^{a\ NS}$
CCE	6.77±0.42 $^{a\ NS}$	9.31±1.42 $^{a\ NS}$	5.23±1.23 ^{a*}	8.25±0.64 ^{NS**}
BME	5.25±0.54 $^{a\ NS}$	9.22±1.55 $^{a\ NS}$	6.42±1.24 ^{a*}	8.28±0.45 $^{NS\ ***}$
CCBME	4.23±0.23 ^{NS*}	10.96±1.21 ^{NS***}	8.18±1.31 ^{NS***}	10.32±0.67 ^{NS**}

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c);

Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3.6.2 Kidneys oxidative stress parameters

The effects of benzylthiouracil-induced hypothyroidism and the different treatments on renal oxidative stress parameters are presented in Table 12. Oxidative stress plays a central role in kidney dysfunction, particularly through lipid peroxidation and impairment of endogenous antioxidant defense systems.

In the hypothyroid group (CN), a clear disruption of the antioxidant system was observed compared with the control group. Although malondialdehyde (MDA) levels remained relatively unchanged (4.86 ± 0.58 nmol/mg pr), a marked and significant depletion of antioxidant defenses was recorded. Glutathione (GSH) decreased to 7.83 ± 0.57 nmol/mg pr, superoxide dismutase (SOD) to 6.93 ± 1.45 U/mg pr, and catalase (CAT) to 5.13 ± 0.45 UI/min/g pr. These findings indicate that benzylthiouracil-induced hypothyroidism primarily impaired renal antioxidant capacity rather than inducing strong lipid peroxidation.

Treatment with levothyroxine (CP) partially improved oxidative balance in kidney tissue. GSH levels increased to 9.01 ± 0.49 nmol/mg pr, while SOD (7.10 ± 1.24 U/mg pr) showed slight improvement compared with the CN group. CAT activity also increased to 7.23 ± 0.22 UI/min/g pr, indicating partial restoration of enzymatic antioxidant defense. However, MDA levels remained unchanged, suggesting limited effect on lipid peroxidation in renal tissue.

Administration of *Chamaeleo chamaeleon* extract (CCE) resulted in a noticeable enhancement of antioxidant status. GSH (9.21 ± 0.42 nmol/mg pr), SOD (7.47 ± 1.41 U/mg pr), and CAT (8.51 ± 0.24 UI/min/g pr) were all significantly improved compared with the hypothyroid group, indicating a strong renal protective effect through reinforcement of endogenous antioxidant systems. MDA levels remained stable (4.32 ± 0.43 nmol/mg pr), confirming the absence of increased lipid peroxidation.

Similarly, the *Bunium mauritanicum* extract-treated group (BME) showed further improvement in oxidative defense markers, with increased GSH (9.75 ± 0.57 nmol/mg pr), SOD (8.23 ± 1.23 U/mg pr), and CAT (9.42 ± 0.53 UI/min/g pr). These results suggest a stronger antioxidant effect compared with CCE, likely related to the rich phytochemical composition of

the plant extract. MDA levels remained comparable to control values, indicating effective protection against oxidative membrane damage.

Notably, the combined treatment group (CCBME) exhibited the most pronounced improvement, with antioxidant parameters nearly restored to normal physiological levels. GSH reached 11.33 ± 0.52 nmol/mg pr, SOD 9.72 ± 1.22 U/mg pr, and CAT 10.23 ± 0.54 UI/min/g pr, all closely matching control values. MDA remained stable (4.72 ± 0.41 nmol/mg pr), confirming strong protection against lipid peroxidation. These findings strongly suggest a synergistic antioxidant effect between *Chamaeleo chamaeleon* and *Bunium mauritanicum* extracts in renal tissue.

***Table 12**:Effect of CCE, BME and CCBME extracts on oxidative stress markers in kidney tissue.*

	MDA (nmol/mg pr)	**GSH (nmol/mg pr)**	**SOD (U/mg pr)**	**CAT (UI/min/g pr)**
Control	4.91±0.65	11.66±0.55	9.21±1.54	10.11±0.23
CN	4.86±0.58 NS	7.83±0.57 c	6.93±1.45 c	5.13±0.45 c
CP	4.91±0.78 NS	9.01±0.49 a*	7.10±1.24 a NS	7.23±0.22 a*
CCE	4.32±0.43 NS	9.21±0.42 NS***	7.47±1.41 NS***	8.51±0.24 NS***
BME	4.42±0.35 NS	9.75±0.57 NS***	8.23±1.23 NS***	9.42±0.53 NS **
CCBME	4.72±0.41 NS	11.33±0.52 NS***	9.72±1.22 NS**	10.23±0.54 NS**

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c);
Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

IV.3.7 Histopathological studies

IV.3.7.1 Thyroid Histopathology

Control (C)

The thyroid gland of the control group exhibited a normal histoarchitecture characterized by well-organized and uniformly distributed thyroid follicles. Follicles were round to oval in shape, lined by a single layer of cuboidal epithelial cells, and contained abundant, homogeneous colloid. The interfollicular connective tissue appeared normal, with no signs of congestion, inflammation, or structural disruption.

CN (Benzylthiouracil group)

The CN group showed marked histological alterations consistent with hypothyroidism. Thyroid follicles appeared irregular in shape with reduced colloid content and areas of follicular shrinkage. The follicular epithelium was frequently disrupted and appeared flattened or degenerated in several regions. Mild vascular congestion and interstitial disorganization were also observed, indicating functional suppression of thyroid activity induced by benzylthiouracil.

CP (Levothyroxine-treated group)

Levothyroxine administration induced partial restoration of thyroid structure. Follicular architecture appeared more organized compared to CN, with moderate colloid reaccumulation. However, some follicles still exhibited irregular outlines and mild epithelial flattening. Overall, the tissue showed clear signs of recovery but did not fully reach the normal histological state.

CCE (*Chamaeleo chamaeleon* extract)

The CCE-treated group displayed notable improvement in thyroid morphology. Follicles regained a more regular shape, and colloid content was moderately restored. Follicular epithelial cells appeared more intact and closer to normal cuboidal morphology. Only mild residual alterations were observed, indicating a protective and partially restorative effect of the extract.

BME (*Bunium mauritanicum* extract)

The BME group exhibited further improvement in thyroid structure. Thyroid follicles were relatively well preserved with improved colloid density and better epithelial organization compared to CN and CCE groups. The tissue architecture suggested a stronger corrective effect on thyroid histology, with minimal degenerative changes.

CCBME (Combined extracts)

The combined treatment group showed almost complete restoration of normal thyroid histology. Follicles were well defined, evenly distributed, and filled with abundant colloid. The epithelial lining appeared intact, cuboidal, and regularly arranged, closely resembling the control

group. No significant degenerative or inflammatory lesions were observed, indicating a strong synergistic protective effect.

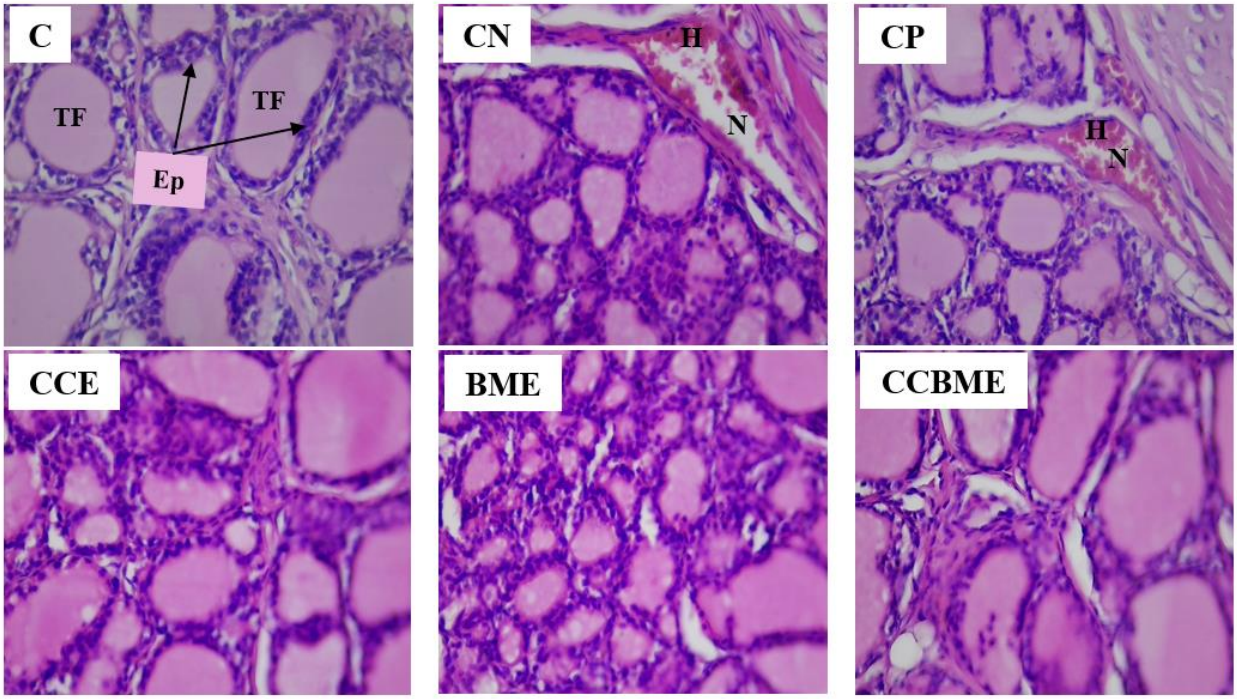

Figure 17 :*Microscopic observation of thyroid gland histological sections from different experimental groups, (C) Control group, (CN) Benzylthiouracil group, (CP) Group treated with levothyroxine, (CCE) Group treated with Chamaeleo chamaeleon extract*

IV.3.7,2 Liver Histopathology

Control (C)

The liver of control rats exhibited a normal and well-preserved histoarchitecture. Hepatic lobules were clearly defined with hepatocyte cords radiating regularly from the central vein. Hepatocytes showed normal morphology with intact cytoplasm and centrally located nuclei. The sinusoidal spaces were well organized, patent, and free from congestion or inflammatory infiltration, indicating normal liver function and structural integrity.

CN (Benzylthiouracil group)

The CN group showed marked histopathological alterations reflecting hypothyroidism-induced hepatic injury. The normal architecture was disrupted with disorganization of hepatocyte cords. Hepatocytes exhibited degenerative changes such as cytoplasmic vacuolization, swelling, and mild ballooning.

Sinusoids appeared dilated and congested, and areas of inflammatory cell infiltration were observed, particularly around portal regions. These changes suggest oxidative stress, impaired lipid metabolism, and hepatocellular dysfunction induced by benzylthiouracil treatment.

CP (Levothyroxine-treated group)

Levothyroxine administration led to partial restoration of liver structure. Hepatic cords appeared more organized compared to the CN group, and hepatocyte morphology showed improvement with reduced degeneration.

However, mild sinusoidal congestion and occasional cellular vacuolization were still present. Inflammatory infiltration was considerably reduced, indicating partial recovery of hepatic function, although complete normalization was not achieved.

CCE (*Chamaeleo chamaeleon* extract)

The CCE-treated group showed a noticeable improvement in liver histology. Hepatocyte arrangement was more regular, and cellular integrity was better preserved. Degenerative changes were reduced compared to the CN group.

Sinusoids were moderately clear with limited congestion, and inflammatory infiltration was minimal. These findings suggest a hepatoprotective effect, likely related to antioxidant and anti-inflammatory properties of the extract.

BME (*Bunium mauritanicum* extract)

The BME group exhibited further improvement in hepatic structure. Hepatocyte cords were well organized, and cells maintained normal morphology with minimal vacuolization.

Sinusoidal spaces appeared clearer and more regular, with very low levels of congestion. Inflammatory infiltration was rare, indicating a strong protective effect against hypothyroidism-induced hepatic damage and oxidative stress.

CCBME (Combined extracts)

The combined treatment group showed near-complete restoration of normal liver histology. Hepatic architecture was well preserved, with intact hepatocyte cords radiating normally from the central vein.

Hepatocytes exhibited normal morphology without signs of degeneration or vacuolization. Sinusoids were clear, and no inflammatory infiltration or necrotic areas were observed. This indicates a strong synergistic hepatoprotective effect of the combined extracts.

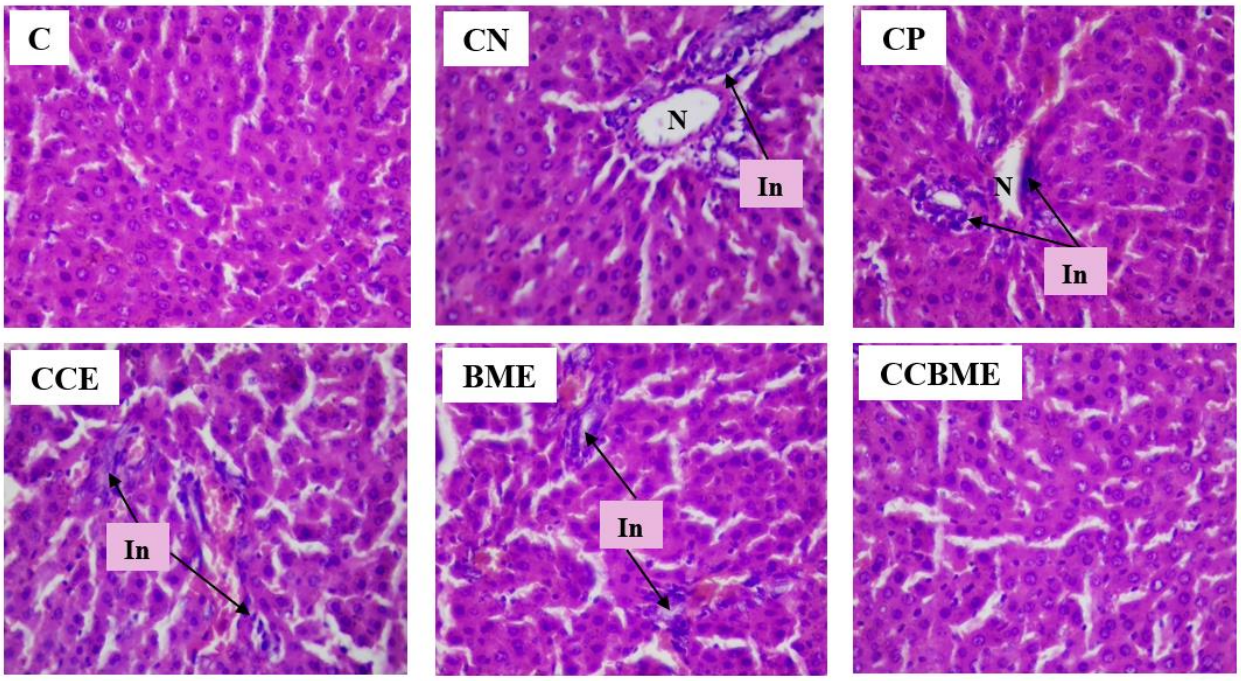

Figure 18 :*Microscopic observation of liver histological sections from different experimental groups, (C) Control group, (CN) Benzylthiouracil group, (CP) Group treated with levothyroxine, (CCE) Group treated with Chamaeleo chamaeleon extract*

IV.3.7.3 Kidney Histopathology

Control (C)

The kidney of control rats displayed normal histological features. Renal corpuscles were intact with well-defined glomeruli and Bowman's capsules. Proximal and distal tubules showed normal epithelial lining and clear lumens, with no signs of degeneration or congestion.

CN (Benzylthiouracil group)

The CN group showed mild to moderate renal alterations. Glomeruli exhibited slight congestion and focal hemorrhage, while tubular structures showed epithelial disorganization and mild swelling. Some tubules had narrowed lumens, and early signs of interstitial stress were observed. These changes indicate renal susceptibility to hypothyroidism-induced metabolic and oxidative disturbances.

CP (Levothyroxine-treated group)

The CP group exhibited mild to moderate histopathological renal changes. Glomeruli showed mild vascular congestion with focal hemorrhagic areas, whereas the renal tubules demonstrated epithelial disarray and slight cellular swelling. Several tubules presented narrowed luminal spaces, accompanied by early indications of interstitial stress. These findings suggest increased renal vulnerability to the metabolic and oxidative impairments associated with hypothyroidism.

CCE (*Chamaeleo chamaeleon* extract)

The CCE group exhibited well-preserved renal histology. Glomeruli appeared nearly normal, and tubular structures maintained their integrity with minimal degenerative changes.

Congestion was significantly reduced, and no major inflammatory infiltration was observed, suggesting a nephroprotective effect of the extract.

BME (*Bunium mauritanicum* extract)

The BME group showed further improvement in kidney histology. Glomeruli were clearly defined, and tubular epithelial cells appeared intact with preserved lumens.

Only minimal histological alterations were detected, indicating strong renal protection and stabilization of kidney tissue structure.

CCBME (Combined extracts)

The combined treatment group demonstrated almost complete restoration of normal kidney architecture. Renal corpuscles and tubular structures appeared intact and well organized.

No significant lesions, congestion, or inflammatory infiltration were observed. These results indicate a strong synergistic nephroprotective effect of the combined extracts, with near-complete histological recovery.

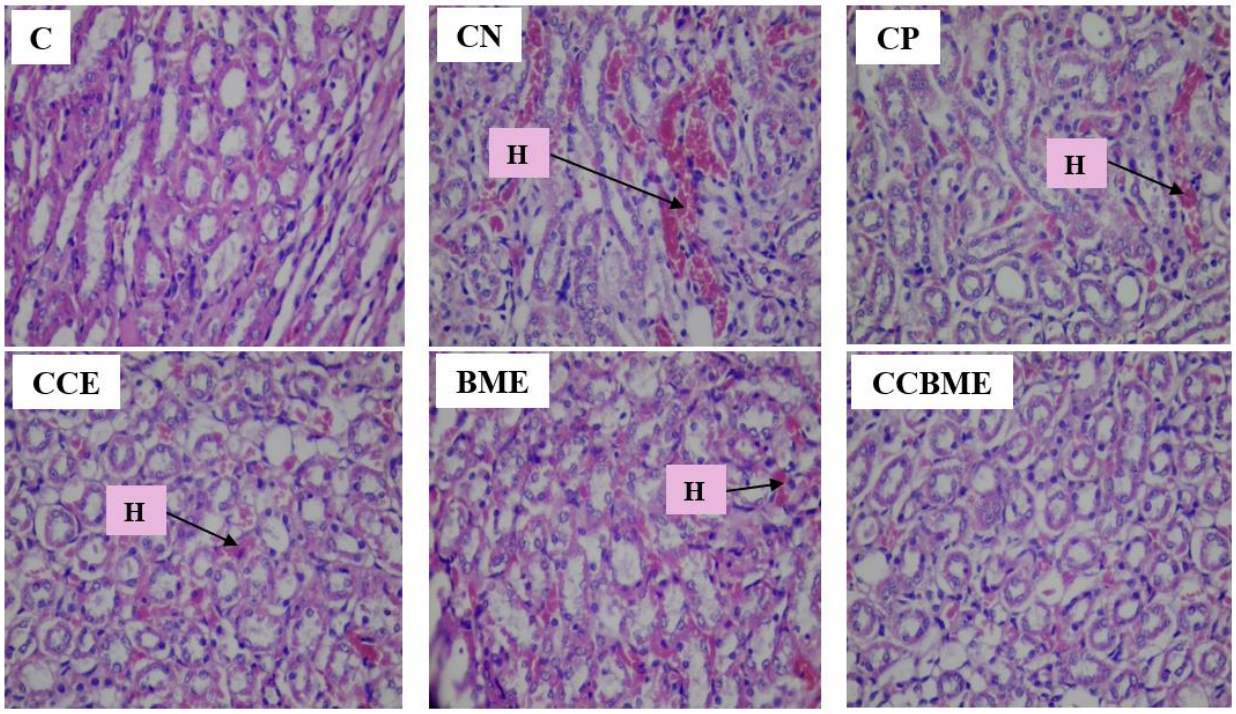

Figure 19 : *Microscopic observation of thyroid gland histological sections from different experimental groups, (C) Control group, (CN) Benzylthiouracil group, (CP) Group treated with levothyroxine, (CCE) Group treated with Chamaeleo chamaeleon extract, (BME) Grou*

IV.4 Discussion

Medicinal plants are among the most important alternative therapies that have received increasing attention in recent years, particularly with the growing awareness of the side effects caused by chemical treatments used in modern medicine. This trend has contributed to the increased reliance on herbal medicine because of its potential therapeutic benefits and relatively fewer side effects when used properly and under the supervision of specialists, which highlights the necessity of documenting these plants and strengthening the scientific knowledge related to them (Yamina, 2021).

The importance of medicinal plants is also evident in the pharmaceutical industry, as data indicate that approximately 75% of medicines are derived from plant sources, while at least 25% contain an active compound of plant origin (Adossides, 2003).

In this context, our study focused on the medicinal plants Bunium mauritanicum and Chamaeleo chamaeleon, known for their traditional use in the treatment of thyroid disorders. The thyroid gland plays a vital role in the body through the secretion of hormones that regulate essential metabolic processes within cells, thereby affecting energy expenditure, body weight, heart rate, muscle activity, mood, concentration, body temperature, and digestive functions. Hypothyroidism leads to a slowdown in metabolism and energy production. This disorder is characterized by elevated levels of thyroid-stimulating hormone (TSH) and decreased levels of triiodothyronine (T3), while the levels of T3 and T4 are closely related to TSH levels (Khemili and Bachkat, 2016).The Soxhlet method was selected for the extraction of nutrients from the chameleon (C. chamaeleon) and Bunium mauritanicum due to its low solvent consumption, high yield, and high extraction efficiency within a short period of time. However, some compounds may degrade because of heat during extraction, particularly proteins, which are converted into peptides or amino acids in the extract (Słomińska et al., 2012). Since the tissues of the chameleon (C. chamaeleon), which are of animal origin, contain many polar compounds, as does Bunium mauritanicum, aqueous extraction was used due to the high polarity of water, as mentioned by Nawaz et al. (2020), in addition to the fact that the chameleon (C. chamaeleon) contains many polar compounds, including proteins, carbohydrates, minerals, and vitamins (Park et al., 2022).

In a study conducted by Boudebia (2023) on Chamaeleo chamaeleo, the aqueous extraction yield reached 27.09%, which is higher than the yield obtained in our study (21%). This difference in extraction yield is attributed to the region and season of capture, as they are considered decisive factors in determining the yield. Furthermore, extraction duration, drying conditions, and storage period are influential factors. The results of this study also showed that the aqueous extraction yield of Bunium mauritanicum L. was approximately 16%. In a study conducted by Sharififar et al. (2010) on Bunium persicum, the aqueous extraction yield was 1.3%. In another study conducted by El Kolli et al. (2017) on B. incrassatum and B. alpinum, the extraction yield by hydrodistillation was 0.09% and 0.1%, respectively. These results are very low compared with Our findings.

The results of the study by Koura and Selmi (2020), conducted on Bunium mauritanicum, showed an aqueous extraction yield of 23.4%, which is higher than our results. These differences may be attributed to the part used in extraction, the metabolite content of each species, and the nature of the solvents used.

The combined extract (CCBME) recorded the lowest IC50 value compared to the individual extracts (CCE and BME), while ascorbic acid showed the highest antioxidant activity. The decrease in IC50 of the combined extract indicates a higher ability to scavenge free radicals, suggesting a synergistic effect between the components of the two extracts. The superior activity of ascorbic acid is attributed to its nature as a standard antioxidant characterized by high efficiency and rapid reactivity toward free radicals.

CCBME showed the lowest EC50 value compared to CCE and BME, while ascorbic acid remained the most effective. The in EC50 reflects a higher reduction electron-donating capacity of the combined extract, indicating enhanced synergy among phenolic and other bioactive compounds. Ascorbic acid exhibits very strong power reducing due to its electron-rich chemical structure.

CCBME recorded the lowest IC50 value in inhibiting albumin protein denaturation compared to the individual extracts, indicating its superior activity, while ascorbic acid showed the highest effectiveness. The improved activity of the combined extract suggests a synergistic interaction

between plant compounds that enhances protein stabilization and prevents denaturation. The strong activity of ascorbic acid is related to its ability to reduce oxidative stress associated with inflammation.

CCBME showed the highest SPF value compared to CCE and BME, while the commercial product Avène exhibited the highest overall value. The increase in SPF of the combined extract is due to the diversity of compounds capable of absorbing ultraviolet radiation (UV-A and UV-B), leading to a broader spectral absorption range. The superiority of Avène is linked to its standardized formulation enriched with specialized UV filters.

CCBME showed the lowest hemolysis percentage compared to CCE and BME, while SDS recorded the highest hemolytic activity. The low hemolysis observed indicates greater stability of red blood cell membranes and minimal interaction with membrane lipids. In contrast, SDS, due to its strong detergent nature, causes membrane disruption and cell lysis.

The body weight gain index decreased more significantly in the CCBME group compared to other groups, reflecting an improvement in energy metabolism and a supportive role of the combined extract in metabolic functions associated with hypothyroidism.

No significant changes were observed in liver and kidney weights among all groups, indicating the absence of toxic effects and no evidence of tissue hypertrophy or damage.

White blood cell counts increased in the CN group, while CCBME restored values close to normal, with stable Hb and RBC levels. This indicates an inflammatory response in the CN group, whereas treatment with CCBME showed an anti-inflammatory and immunomodulatory effect without affecting blood cell production.

AST and ALT levels increased in the CN group, while they decreased significantly in the CCBME group to near-control levels, with no major changes observed in kidney parameters. This reflects liver damage induced by hypothyroidism, whereas the improvement after treatment suggests a hepatoprotective effect associated with the antioxidant activity of the combined extract.

T3 and T4 levels decreased, while TSH increased in the CN group. CCBME restored these parameters to near-normal levels. This indicates that the combined extract improved thyroid activity and restored hormonal balance by reducing oxidative stress and supporting thyroid function.

MDA levels increased and antioxidant defenses decreased in the CN group, while CCBME showed the best improvement in GSH, SOD, and CAT levels with a marked reduction in MDA. This demonstrates the ability of the combined extract to reduce lipid peroxidation and enhance endogenous antioxidant defense systems, thereby protecting cells from oxidative damage.

The CN group showed clear histological deterioration, while CCBME restored the thyroid tissue architecture to a near-normal state, indicating the ability of the combined extract to re-establish tissue organization and reduce cellular damage associated with the disease condition.

Clear histological deterioration was observed in the CN group, with gradual improvement in the treated groups, and the best restoration was recorded in the CCBME group. This reflects a hepatoprotective effect associated with the ability of the combined extract to reduce oxidative stress and cellular inflammation, thus contributing to liver tissue protection and enhanced repair processes.

Slight histological changes were observed in the CN group, while the CCBME group showed an almost normal renal structure. This indicates the ability of the combined extract to protect the kidneys from metabolic disturbances associated with the disease condition, without inducing any tissue toxicity, confirming its safety and protective effect.

Conclusion

Conclusion

Traditional medicine is widely used due to its pivotal role in the treatment of many chronic and acute diseases. Given the increasing interest in medicinal plants and wild animals, studies related to the evaluation of natural products of animal and plant origin are essential, as they remain insufficiently explored, which may lead to major medical developments. Based on this, our study aims to explore the synergistic biological activity of both the chameleon Chamaeleo chamaeleon and the plant Bunium mauritanicum.

The overall results obtained, whether in vitro or in vivo assays, indicate that the combined extract (CCBME) showed a clear superiority compared to the individual extracts of both the chameleon and Bunium mauritanicum. This superiority was reflected in the enhancement of antioxidant activity, improvement of reducing power, and increase in anti-inflammatory efficacy, in addition to a notable protective activity against ultraviolet radiation, along with a high level of cellular safety (low hemolysis).

Biological studies also confirmed that the combined extract contributed to the improvement of physiological and biochemical parameters, particularly through the restoration of thyroid hormonal balance, reduction of oxidative stress markers, and enhancement of antioxidant defense systems, in addition to exhibiting hepatoprotective and nephroprotective effects. Histological analysis supported these findings, showing a clear restoration of the normal structure of the studied organs compared to untreated groups.

These results strongly support the hypothesis of a synergistic effect between the active compounds present in the chameleon extract and the Bunium mauritanicum extract, where this synergy enhances biological efficacy through complementary mechanisms of action. Accordingly, the combination of the two extracts can be considered a promising strategy to improve therapeutic efficiency compared to the use of each extract separately.

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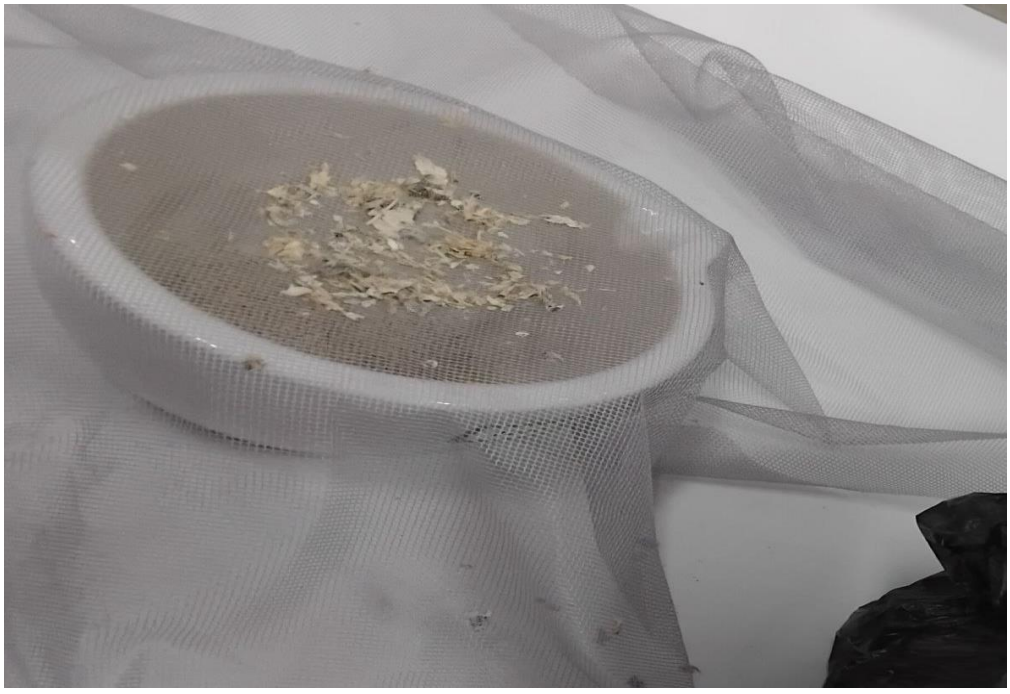
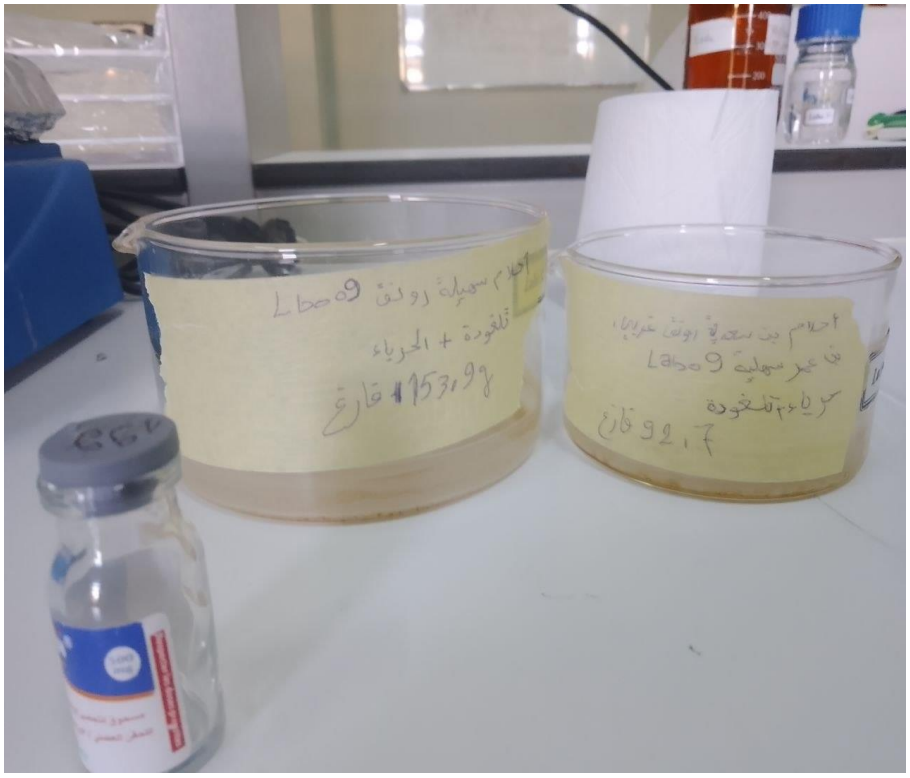
Annexes

Annexe01:*Step of drying the chameleon after washing it from salt.*

Annexe02:*Ground Talghouda.*



Annexe03: *Filtering the chameleon with a cloth after grinding it.*



Annexe04:*Filtering the chameleon with a cloth after grinding it*

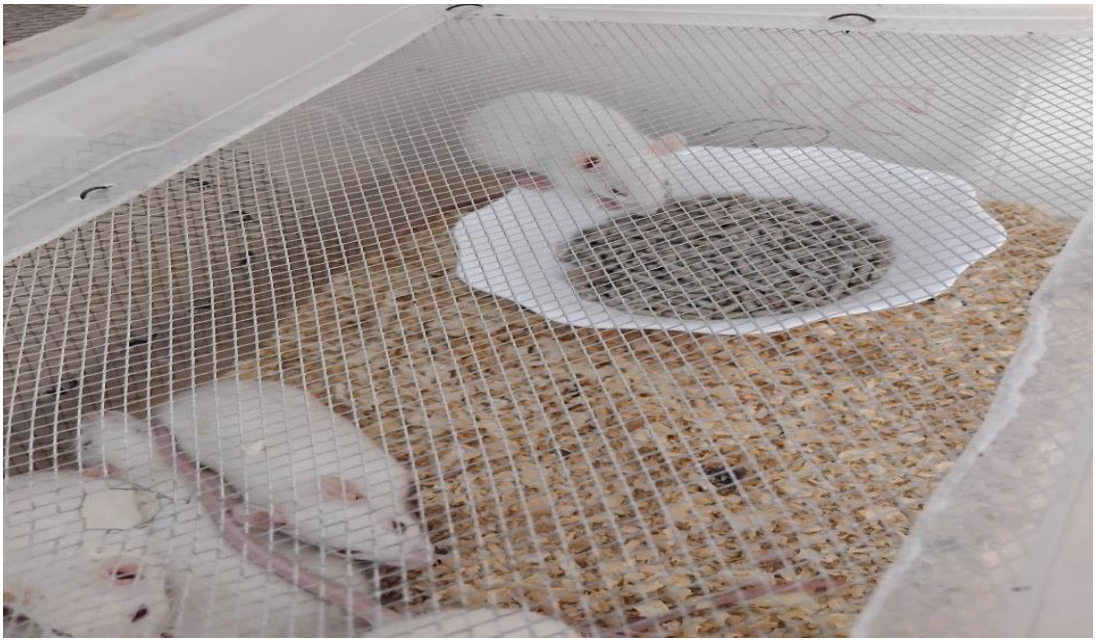
Annexe05: *Damaged solution.*

Annexe06: *Removing the dried solution from the tools used.*

Annexe07: *One of the hemolysis test steps.*

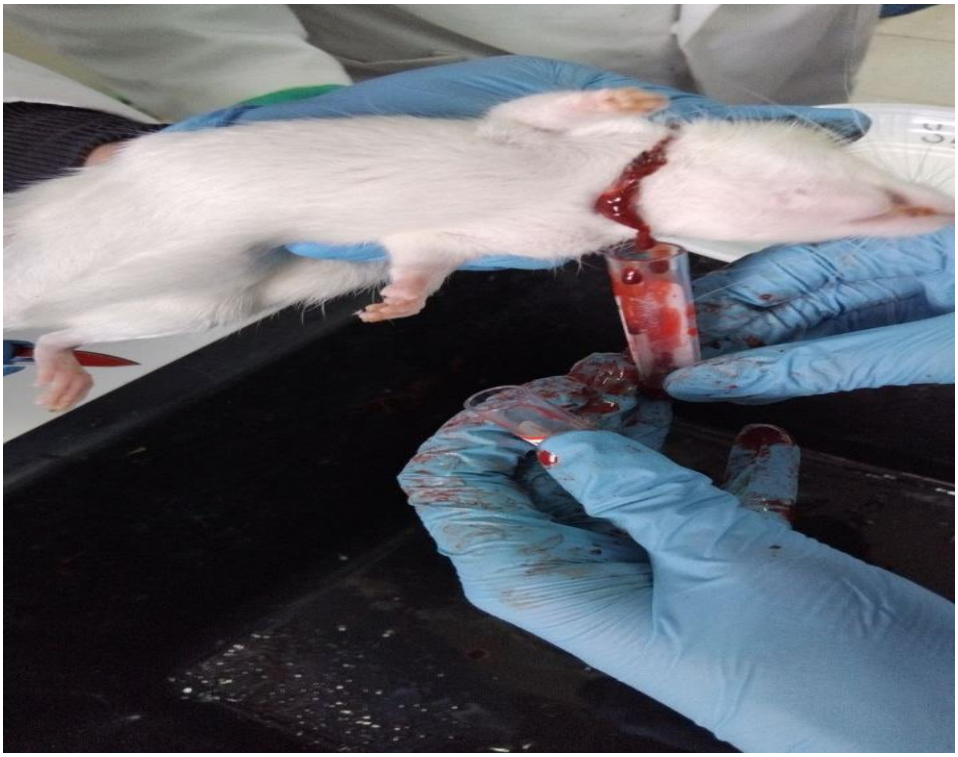
Annexe08*:Rat food.*

Annexe09: *Daily monitoring of the rats (1)*



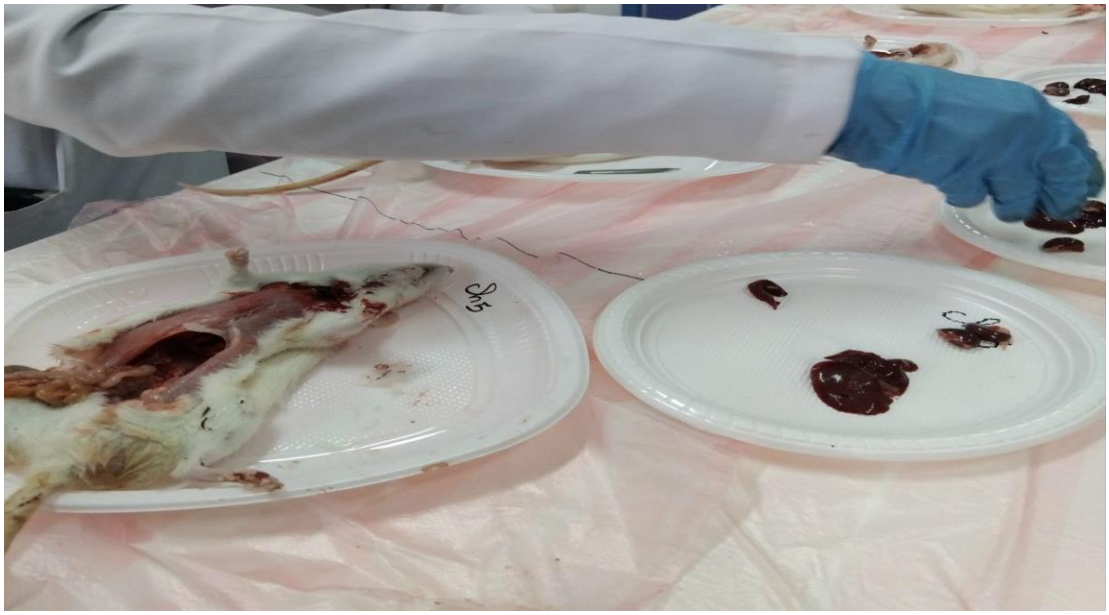
Annexe10: *Daily monitoring of the rats (2)*

Annexe11:*Administration of the prepared extract to rats.*

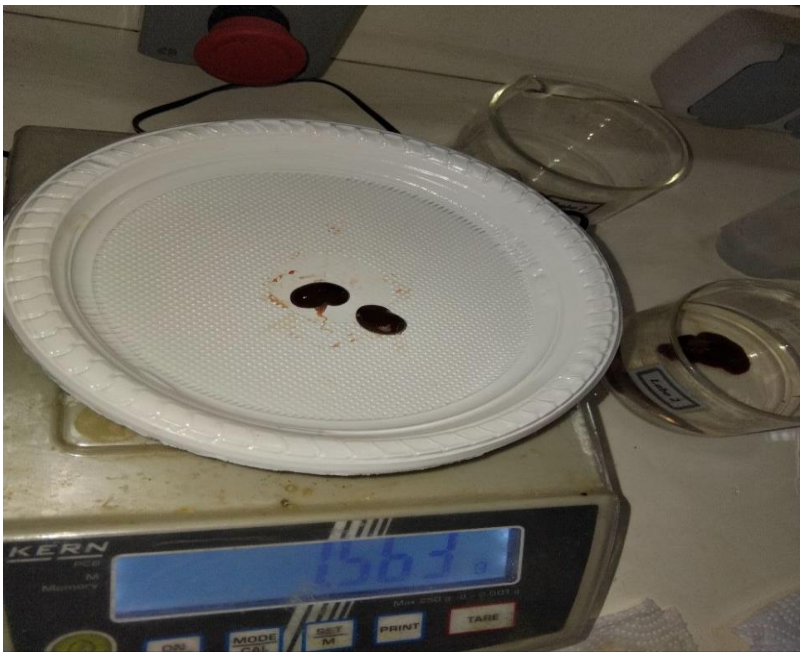


Annexe12: *Euthanasia of rats.*

Annexe13:*Arrangement of blood tubes.*

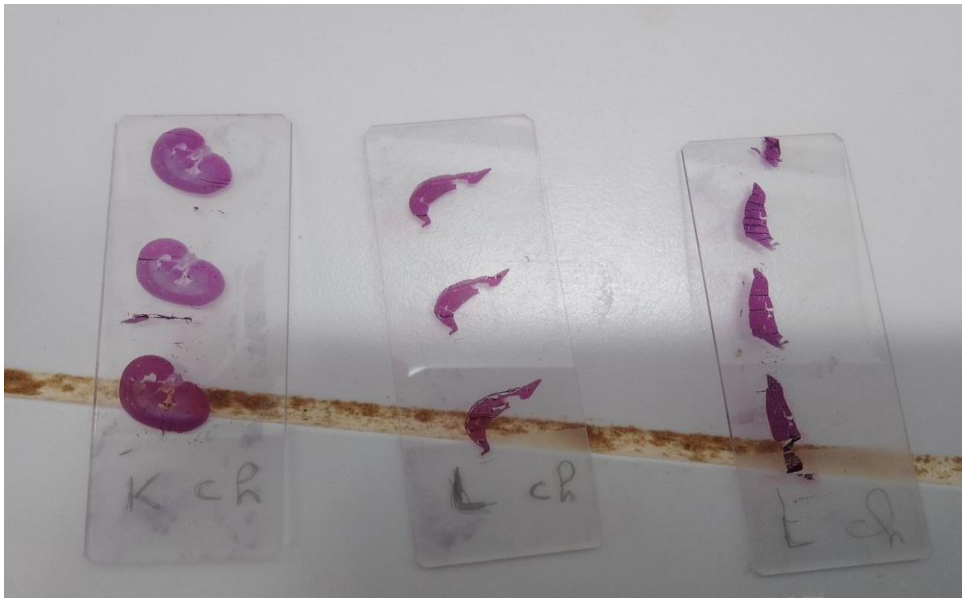


Annexe14: *Dissection of the rat and extraction of its organs.*



Annexe15: *The rat's kidneys*

Annexe16:*Trachea accompanied by the gland.*



Annexe17:*Kidney and liver tissues*