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Theme

Evaluation of the biological activities of *Chamaeleo chamaeleon:* A reptile used in altenative medicine in El-Oued region

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إهداء

بسم الله الرحمن الرحيم " وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِين"

من قال أنا لها نالها وإن أبت رغما عنها اتيت بها لم تكن الرحلة قصيرة ولا ينبغي لها أن تكون لم يكن الحلم قريبا ولا الطريق محفوفا بالتسهيلات لكنني فعلتها ونلتها.....

الى الذي زين اسمي بأجمل الألقاب .... الذي غرس في روحي مكارم الاخلاق، إلى فخري واعتزازي الذي رحل عن الدنيا ولم يرحل من قلبي ...روح والدي الغالي ..(رحمه الله)

الى من جعل الله الجنة تحت أقدامها واحتضنني قلبها قبل يدها وسهلت لي الشدائد بدعائها ،الى القلب الحنون والشمعة التي كانت في الظلمات ،سر قوتي ونجاحي ، ...والدتي ... .

ادعو الله أن يمد في عمركي وانت في احسن حال.

الى ضلعي الثابت وامان ايامي ..... الى ملهمي نجاحي إلى من شددت عضدي بهم فكانوا لي ينابيع ارتوي منها ...إلى خيرة ايامي وصفوتها إلى قرة عيني ....اخواني واخواتي الغاليين

وإلى زوجة أخي الغالية ... التي كانت لي أختًا قبل أن تكون من العائلة،شكرا لكلماتك التي منحتني دفئا في الايام الصعبة، وإلى صغيرتها التي منحت أيامي نكهة جميلة وبسمة دافئة. .

إلى أبناء وبنات أخواتي الأحبّة..... أنتم النور الذي يضيء قلبي،أهديكم هذه الخطوة، علّها تكون مصدر إلهام لكم يومًا

الى صديقاتي العزيزات.... رفيقات الدرب، شكرًا لوجودكنّ في كل تفاصيل هذه الرحلة ، كنتنّ وما زلتنّ نعمة لا تُقدّر بثمن

الى أساتذتي الكرام ممن لم يتوانوا في مديد العون لنا ....يا من صنعت لنا المجد، لكل من كان عونا وسندا في هذا الطريق

أهديكم هاذا الانجاز وثمرة نجاحي الذي لطالما تمنيته.

واخيرا الشكر الموصول لنفسي على الصبر والتي كانت اهلا المصاعب ها أنا اليوم اختم كل ما مررت به واتممت اول ثمراته بفضل الله....

فالحمدلله عند البدء وعند الختام راجية من الله تعالى أن ينفعني بما علمني وان يعلمني ما أجهل

✍ اكرام هارون

إهداء

إلى من كان رضاهم بركة، ودعاؤهم نورًا لا ينطفئ إلى من منحوني الحياة، وكانوا لي عونًا وسندًا في كل مراحلها...

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من كانت دائمًا بجانبي، تشاركني الحلم وتحتفل بإنجازي كأنه لها...

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ضحكاتكم، كلماتكم، حضوركم... كانت طاقة دفعتني للاستمرار في كل لحظة تعب،

لكم أقول: هذا النجاح نجاحكم أيضًا.

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وأهديه، بكل حب، لكل من صنع هذا الحلم معي.

✍ اية عيشوش

إهداء

لى والدي الغالي.....

إلى من غرست في قلبي معنى الثبات، وعلمتني أن الإرادة طريق النجاح. كنتَ الحصن الذي احتميت به، والسند الذي لم يخذلني يومًا.

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أهدي هذا التخرج عربون امتنان وفخر، لأنها كانت دائمًا الأوفى والأقوى

✍ فليون أسماء

إهداء

إلى من كانا بعد الله سبب هذا النجاح،

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✍ هبة السلام الأرقط

شكر و تقدير

بسم الله الرحمن الرحيم

الحمد الذي ما تم جهد ولا ختم سعي الا بفضله وتوفيقه ،فالحمد لله اقصى مبلغ الحمد والشكر لله الحمد لله من قبل ومن بعد.

نتقدم بخالص الشكر وعظيم الامتنان إلى الأستاذة الفاضلة وفاء بوضبية، مشرفتنا الكريمة، على ما قدمته لنا من توجيه علمي دقيق، وملاحظات بنّاءة، ومتابعة مستمرة طيلة فترة إعداد هذه المذكرة. لقد كان لتفانيها وإخلاصها في الإشراف بالغ الأثر في إنجاز هذا العمل.

كما نتوجه بجزيل الشكر والتقدير إلى الأستاذة عائشة تجاني على دعمها العلمي التي ساهمت في إثراء هذا البحث.

بكل فخر وامتنان، نتوجه نحن الممضيات أسفله بخالص عبارات الشكر وعظيم التقدير إلى لجنة المناقشة الموقّرة، السادة الأساتذة الأفاضل

لما أبدوه من اهتمام كريم وحرص صادق على تقييم هذا العمل العلمي، ولما أغنونا به من ملاحظات دقيقة وتوجيهات سديدة، كان لها بالغ الأثر في تقويم المذكرة والارتقاء بمستواها الأكاديمي.

إنّ تشريفكم لنا بالحضور ومرافقتكم العلمية الرصينة في هذه المحطة الختامية من مسارنا الجامعي، يعدّ وسامًا نعتز به، وحافزًا يدفعنا إلى مزيد من الجد والاجتهاد في قادم المحطات العلمية والمهنية.

فلكم منا أسمى آيات التقدير والعرفان، سائلين الله أن يجزيكم خير الجزاء، وأن يديم عليكم نعمة العلم والعطاء.

ولا يفوتنا أن نخص بالشكر والعرفان الأستاذ محمد العيد تليلي، رئيس القسم، لما قدمه من تسهيلات وتوجيهات ساعدتنا في تجاوز مختلف مراحل هذا المسار الأكاديمي

كما نعبر عن امتنانينا العميق لكل من ساهم في دعمينا وتشجيعينا، من أساتذة وزملاء وأفراد عائلاتنا الكريمة، الذين كان لهم الدور الكبير في مواصلتنا وتحقيقينا لهذا الإنجاز

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والله ولي التوفيق

Abstract

Chamaeleo chamaeleon has been traditionally used in folk medicine in the El Oued region of the Algerian Sahara for the treatment of various ailments, including tonsillitis, thyroid disorders, and persistent coughs. In light of its ethnomedicinal significance, This study aims to evaluate the immunological and biochemical effects of dried veiled chameleon (*Chamaeleo chamaeleon*) extracts, using two different types: a methanolic-aqueous extract and another enriched with multiple B-group vitamins (Multi B). The evaluation was carried out through both *in vitro* and *in vivo* tests using Wistar rats as the experimental model. Chemical analyses revealed that the methanolic-aqueous extract was particularly rich in vitamins B1, B3, and B9, while the Multi B extract contained significant levels of B2, B6, B7, and B12, indicating the potential nutritional and immunological value of these extracts. Regarding biological activity, the extracts showed strong antioxidant capacity in the DPPH assay, with inhibition exceeding 60% at the highest concentrations.

They also demonstrated considerable anti-inflammatory activity via the BSA protein denaturation test, in addition to promoting phagocytic activity and inhibiting the complement system. Animal trials confirmed these findings, showing enhanced immune performance characterized by increased spleen and thymus indices, improved phagocytic activity, and balanced biochemical (AST, ALT, CRP, Glucose) and hematological parameters. These results suggest that chameleon extracts possess promising immunological and biochemical properties, supporting their potential use as dietary supplements or as supportive therapy in cases of immune deficiency or oxidative stress.

Keywords: *traditional medicine, zootherapy, Chamaeleo chamaeleon, biochemical study, biological activities.*

ملخص

تم استخدام ***Chamaeleo chamaeleon*** تقليديًا في الطب الشعبي بمنطقة الوادي في الصحراء الجزائرية لعلاج مجموعة متنوعة من الأمراض مثل التهاب اللوزتين، واضطرابات الغدة الدرقية، والسعال المزمن. وبالنظر إلى أهميته في الطب الشعبي , تهدف هذه الدراسة إلى تقييم التأثيرات المناعية والبيوكيميائية لمستخلصات الحرباء المجففة *(Chamaeleo chamaeleon)* ، وذلك باستخدام نوعين مختلفين من المستخلصات: مستخلص مائي-ميثانولي، وآخر غني بفيتامينات متعددة من مجموعة B (Multi B) تم إجراء التقييم من خلال اختبارات *in vitro* و *in vivo* باستخدام نموذج حيواني يتمثل في جرذان من سلالة Wistar.

أظهرت التحاليل الكيميائية أن المستخلص المائي-الميثانولي يحتوي على نسب معتبرة من الفيتامينات B3،B1 ، وB9، في حين تميز مستخلص الـ Multi B بغناه بفيتامينات B2، B6، B7 ، وB12، مما يعكس القيمة الغذائية والمناعية المحتملة لهذه المستخلصات. من حيث النشاط الحيوي، بيّنت النتائج فعالية مضادة للأكسدة عبر اختبار DPPH بنسبة تجاوزت 60% عند أعلى التركيزات. كما كشفت عن قدرة ملحوظة في تثبيط الالتهاب من خلال اختبار نزع الطبيعة لبروتينBSA ، بالإضافة إلى تسجيل نشاط إيجابي في تحفيز البلعمة (Phagocytic activity) وتثبيط النظام المتمم

(Anti-complement activity) وقد أكدت التجارب الحيوانية هذه النتائج، حيث لوحظ تحسن في مؤشرات الأداء المناعي، شمل ارتفاع أوزان الطحال والغدة الزعترية، وزيادة النشاط البلعمي، إلى جانب الحفاظ على توازن في المؤشرات البيوكيميائية (AST، ALT، CRP، Glucose) والهيماتولوجية. وتدل هذه النتائج على أن مستخلصات الحرباء تمتلك خصائص مناعية وبيوكيميائية واعدة، مما يدعم إمكانية استخدامها كمكمل غذائي أو كعلاج مساعد في حالات ضعف المناعة أو الإجهاد التأكسدي

الكلمات المفتاحية : الطب التقليدي ،العلاج الحيواني ،الحرباء *Chamaeleo chamaeleon* ،دراسة بيوكيمائية ، الأنشطة البيولوجية.

Resumé

Le ***Chamaeleo chamaeleon*** a été traditionnellement utilisé dans la médecine populaire de la région d'El Oued, au Sahara algérien, pour traiter diverses affections telles que l'amygdalite, les troubles thyroïdiens et la toux persistante. Compte tenu de son importance ethnomédicinale, Cette étude vise à évaluer les effets immunologiques et biochimiques des extraits de caméléon voilé *(Chamaeleo chamaeleon*) séché, en utilisant deux types différents : un extrait aqueux-méthanolique et un autre enrichi en vitamines du groupe B (Multi B). L'évaluation a été réalisée à travers des tests *in vitro* et *in vivo* en utilisant des rats Wistar comme modèle expérimental. Les analyses chimiques ont révélé que l'extrait aqueux-méthanolique était particulièrement riche en vitamines B1, B3 et B9, tandis que l'extrait Multi B contenait des niveaux significatifs de B2, B6, B7 et B12, soulignant ainsi leur valeur nutritionnelle et immunologique potentielle. Sur le plan de l'activité biologique, les extraits ont démontré une capacité antioxydante marquée dans le test DPPH, avec un taux d'inhibition dépassant 60 % aux concentrations les plus élevées.

Ils ont également montré une activité anti-inflammatoire notable via le test de dénaturation de la protéine BSA, en plus de stimuler l'activité phagocytaire et d'inhiber le système du complément. Les essais sur animaux ont confirmé ces résultats, avec une amélioration des performances immunitaires, traduite par une augmentation des indices de la rate et du thymus, une meilleure activité phagocytaire, et un équilibre des paramètres biochimiques (AST, ALT, CRP, Glucose) et hématologiques. Ces résultats suggèrent que les extraits de caméléon possèdent des propriétés immunologiques et biochimiques prometteuses, soutenant leur potentiel en tant que compléments alimentaires ou thérapies d'appoint dans les cas de déficience immunitaire ou de stress oxydatif.

Mots clés : *médecine traditionnelle, zoothérapie, Chamaeleo chamaeleon, étude biochimique, activités biologiques.*

List of abbreviations

ACP: Acid Phosphatase
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline Phosphatase
ACD: Acid Citrate Dextrose
BSS: Balanced Salt Solution
BSA: Bovine Serum Albumin
COX-2: Cyclooxygenase-2
CRP: C-Reactive Protein
CD4: Cluster of Differentiation 4
CD8: Cluster of Differentiation 8
CVID: Common Variable Immunodeficiency
DPPH: 2,2'-Diphenyl-1-picrylhydrazyl
DNA: Deoxyribonucleic Acid
EDTA: Ethylenediaminetetraacetic acid
EGTA – Ethylene Glycol Tetraacetic Acid
FBS: Fasting blood sugar
GOT: Glutamate Oxaloacetate Transaminase
HCT: Hematocrit
HB: Hemoglobin
KCl – Potassium Chloride
LDH: Lactate Dehydrogenase
MeH2O: Methanol and water extract
NaCl – Sodium Chloride
NK: Natural Killer cells
PBS : Phosphate Buffered Saline
RBCs: Red Blood Cells
ROS: Reactive Oxygen Species
RNA: Ribonucleic Acid
SD: Standard Deviation
SLE: Systemic Lupus Erythematosus
TRAP: Tartrate-Resistant Acid Phosphatase

TLRs: Toll-Like Receptors
TNF: Tumor Necrosis Factor

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General Introduction

Humans are an integral part of nature and heavily rely on the Earth's land, oceans, atmosphere, and living organisms to obtain various resources such as air, water, soil, minerals, energy, plants, and animals. These natural resources are essential for producing food, fuel, and raw materials used in manufacturing different goods. All types of food consumed by humans originate from either plants or animals, and resources like coal, natural gas, and oil serve as key sources of heat, light, and energy **(Löbler, 2017).**

Since the dawn of time, humans have depended on nature for survival, utilizing plants, animals, and minerals to alleviate various illnesses. Fossil records indicate that the use of plants for medicinal purposes dates back at least 60,000 years (Yuan et al., 2016). Nevertheless, relying on natural remedies was not easy for early humans. It is likely that they often consumed poisonous plants during their search for food, resulting in symptoms such as nausea, vomiting, diarrhea, comas, or even death. However, these painful experiences played a crucial role in the learning process, helping early humans distinguish between safe food sources and medicinal plants **(Teschke et al., 2015).**In traditional medicine, humans have relied on plants, animals, and minerals to develop alternative remedies a practice that remains firmly rooted in many modern countries, particularly through phytotherapy and zootherapy **(Santos et al., 2019).**

Zootherapy refers to the treatment of human illnesses using medicines obtained from or derived from animals **(Costa-Neto, 2005)**. Since ancient times, various societies have utilized animals and their body parts as sources of therapeutic substances, and these practices continue to exist within certain traditional medical systems **(Lev, 2003)**. This form of therapy is notable for its wide geographical distribution and deep historical origins, making it a prominent therapeutic approach used worldwide and a relevant option in contemporary healthcare systems.

Multiple parts of both wild and domestic animals such as hooves, skins, bones, feathers, and tusks play a key role in producing medicines aimed at treatment, prevention, and healing. For instance, more than 1,500 animal species have been identified as having medicinal use in Traditional Chinese Medicine. In India, between 15% and 20% of Ayurvedic medicines are formulated using ingredients derived from animals **(Alves and Rosa, 2005)**. Similarly, over 180 medicinal animal species have been recorded in Bahia State, located in northeastern Brazil **(Costa-Neto, 2004).**

Reptiles are among the most commonly used animal groups in traditional medicine, holding significant value due to their therapeutic and preventive roles in various cultural and social contexts around the world **(Da Nóbrega Alves et al., 2008; Alves et al., 2012)**. These

animals are often available and in high demand in traditional medicine markets **(Williams et al., 2016)**.

In the Algerian desert, particularly in the El-Oued region, certain species of lizards are frequently used in the healing rituals practiced by indigenous communities. Among these, the chameleon is considered one of the most important reptiles in the preparation of traditional remedies, whether for disease prevention, immune system support, or the treatment of chest pain and coughs **(Williams and Whiting, 2016؛ Osunsina et al., 2012).**The dried chameleon (*Chamaeleo chamaeleon*) has proven effective in treating various common illnesses in the El-Oued region, which has led some local practitioners to recommend its use. It is employed in different ways to treat widespread conditions, most notably tonsillitis, in addition to being used for thyroid disorders and persistent coughs.

In light of the continuous increase in health concerns and the growing interest in finding effective methods to enhance the immune system, particularly amid the widespread prevalence of vitamin B deficiencies in humans, we have developed a dietary supplement derived from natural animal sources. This supplement is characterized by a composition rich in B vitamins, known for their essential role in supporting vital body functions and enhancing the efficiency of the immune system we conducted a study on the physicochemical properties of chameleon powder and evaluated the biological activity of the dried chameleon extracts (methanolic and aqueous).

This study examined, *in vitro* the extracts of dried *C. chamaeleon*'s Antioxidant activity,Anti-inflammatory activity, Phagocytic activity Anti-complement activity, In the animal experiments (*in vivo*), the efficacy of the *C. chamaeleon*'s , along with the assessment of phagocytic activity, biochemical analyses, and hematological analysis. This was done using B vitamin extract and methanol-water extract on Wistar rats.

First part

Bibliographic

Chapter I

Chamaeleo chamaeleon

I.1.Genelality :

Chameleons, derived from the Greek *khamaileôn*, meaning “lion that drags itself on the ground,” are vertebrate animals classified within the class *Reptilia* (from the Latin *reptilis*, meaning "crawling"). They belong to the order *Squamata* (from the Latin *squama*, meaning "scale"), the suborder *Sauria* (from the Greek *sauros*, meaning "lizard"), and the family *Chameleonidae*, also referred to as *Chameleontidae* or *Chamaeleonidae*. This family, primarily composed of arboreal lizards, was first defined by Constantine Samuel Rafinesque in 1815 (**Bourdain, 2006**).

Among vertebrates, the *Chameleonidae* family is one of the most distinctive taxa. *Chameleons* are among the most easily recognizable reptiles due to their unique characteristics and significant morphological diversity (**Bickel and Losos, 2002**). The family is divided into two subfamilies, *Chamaeleoninae* and *Brookesiinae*, and comprises over 200 recognized species and subspecies across eleven genera.

Chameleons inhabit a wide range of environments, from scorching desert dunes, where their body temperature (*Tb*) can exceed 39°C, to alpine regions above 3,500 meters, where temperatures drop below freezing (**Anderson, 2013**). They are diurnal reptiles, moving short distances between perching spots while scanning their surroundings for prey. They employ a foraging strategy that involves minimal movement while remaining vigilant against potential predators. The diet of European chameleons varies throughout the year, with a primary intake of bees, wasps, and grasshoppers, which they capture using their long, projectile tongues, especially during the spring, summer, and fall **(Jeroen et al., 2016**). Chameleons thrive in a variety of climatic conditions, including arid deserts, tropical rainforests, Mediterranean climates, and high-altitude ecosystems (**Tolley and Herrel, 2013**).

Morphologically, chameleons exhibit considerable diversity, with sizes ranging from 15 to 35 cm in length, depending on the species (**Bourdain, 2006**). Their bodies are laterally compressed, giving them a slender appearance, which helps them remain inconspicuous when perched on branches (**Tolley and Burger, 2007**). They possess pre;jnhbv hensile tails and specialized feet adapted for grasping. Their coloration changes in response to factors such as body temperature, social status (e.g., pregnancy or stress), and the need for camouflage **(Jeroen et al., 2016**).

One of their most distinctive adaptations is their highly specialized skull, which enables the ballistic projection of their tongue to capture prey (Diaz et al., 2018). Additionally, chameleons have independently mobile eyes, providing them with nearly 360-degree vision.

This allows them to observe their surroundings from multiple angles without needing to move their bodies (**Tolley et al., 2009**).

I.2. Definition

Chamaeleo chamaeleon, commonly known as the common chameleon, is a reptile species belonging to the family Chamaeleonidae. This chameleon is mainly arboreal and is distinguished by its ability to change color depending on its environment, physiological state, and ambient temperature **(Tolley & Herrel, 2014).** It has eyes capable of moving independently of each other, a long laterally compressed body, and an extensible sticky tongue that it uses to catch its prey, mainly insects **(Arnold & Ovenden, 2002).** This species is widely distributed around the Mediterranean basin, notably in North Africa, Southern Europe, and the Middle East. It prefers wooded habitats, scrublands, and coastal areas where it can find dense vegetation to camouflage itself and protect itself from predators **(IUCN, 2021).**

I.3. Taxonomy

The Chameleonidae family comprises 150 species, categorized into two subfamilies: Brookesiinae and Chamaeleoninae. The latter includes the genera *Chamaeleo, Furcifer, Bradypodion,* and *Calumma*. The Common Chameleon (*Chamaeleo chamaeleon*) is classified within the *Chamaeleo* genus **(Guillon, 2010).**

Table 01: Taxonomic Classification of *Chamaeleo chamaeleon (ITIS, 2023)*

Taxonomic Rank	Classification
Kingdom	Animalia
Subkingdom	Bilateria
Infrakingdom	Deuterostomia
Phylum	Chordata
Subphylum	Vertebrata
Infraphylum	Gnathostomata
Superclass	Tetrapoda
Class	Reptilia
Subclass	Lepidosauria
Order	Squamata
Suborder	Sauria
Infraorder	Iguania
Family	Chamaeleonidae
Subfamily	Chamaeleoninae

Genus	Chamaeleo
Species	*Chamaeleo chamaeleon* (Linnaeus, 1758)

I.4. Distribution

The common chameleon (*Chamaeleo chamaeleon*) has a broad distribution across the Mediterranean Basin, covering parts of Europe, North Africa, and the Middle East **(IUCN, 2021)**. In Europe, it is found in southern Spain, Portugal, Malta, Greece, Cyprus, and certain regions of Italy **(Salvador, 2014)**. Its presence in North Africa extends across Morocco, Algeria, Tunisia, Libya, and Egypt, while in the Middle East, it is recorded in Turkey, Lebanon, Syria, Palastin, and Jordan **(IUCN, 2021)**. This species thrives in warm, coastal, and forested environments with dense vegetation **(Tolley & Herrel, 2014)**, particularly in coastal woodlands, Mediterranean scrubland (maquis), agricultural zones with tree cover, as well as lowland and hilly landscapes that provide suitable shelter and food sources **(Arnold & Ovenden, 2002**).

Figure 01: Distribution of Mediterranean chameleon (Chamaeleo chamaeleon Linneus, 1758) Created with BioRender.com

I.5. Description

The common chameleon is a distinctive species across most of its range. Its skin color is influenced by the surrounding environment and temperature, allowing for rapid changes. At night, it typically appears light green. This *chameleon* spends most of its time climbing bushes and trees, using its prehensile tail and specialized feet, where each foot has two opposable groups of toes for a firm grip on branches **(Čerňanský, 2010).**

In terms of size, it measures 9 to 17 cm in body length, with an additional 12 to 14 cm for the tail. Males are generally slightly larger than females. During late summer, females lay 30-40 eggs, which hatch the following spring. The species has a lifespan of up to 20 years,

with a record of 22 years in captivity, though in the wild, it typically lives between 12 to 13 years **(Sidhom et al., 2020).**

I.5.1. Morphological particularities

❖ **Siz**

Chameleons also vary immensely in size by species. The smallest, Brookesia micra, is about 3 cm (1.18 inches) in length, and the largest, Furcifer oustaleti, can grow up to 68 cm (27 inches) in lengthThe size of a chameleon tends to be connected with its habitat; the big species inhabit open spaces, while the smaller species are more likely to inhabit confined areas **(Jackson, J. F.2008)** .

❖ **Head**

The chameleon's head is usually triangular or rounded, depending on the species, and may be moderately flattened or crested in shape. There are also some species that have large horns or bony crests on the head.One of the most striking features of the chameleon's head is its independently moving eyes. Each eye can move in opposite directions, allowing the chameleon almost 360-degree vision. *Chameleons a*lso have a specialized tongue that can extend rapidly to catch prey **(Bels.1990).**

❖ **Body**

Chameleons have a long, strong body formed for climbing and moving through thick vegetation.They possess a remarkable ability to alter color. This is attributed to chromatophores within the skin, which allow them to alter their color for camouflage, social interaction, and temperature control.The body is also covered with granular scales that render it invulnerable to predators and environmental dangers **(Cooper & Green 1992)**

❖ **Limbs (Members**

Chameleons have zygodactylous feet, with their toes in two opposing groups, which allows them to grasp branches tightly. This is another essential adaptation for their arboreal existence.Their legs are specially adapted for climbing and gripping, with sharp claws for extra grip**.(Stuart & Joubert 2019)** .

Figure 02: Front and hind limbs of Chamaeleo chamaeleon (Original photo).

❖ **Tail**

A chameleon's tail is prehensile, which means that it can grasp and wrap around things like a fifth limb. This assists them in balancing while climbing and moving about in trees. The tail is generally long and can coil when not in use **.(Perry, G.1999).**

Figure 03: Prehensile tail of the chameleon (Chamaeleo chamaeleon), (The Reptile Database)

I.5.2. Anatomical particularities

❖ **Cardio-Respiratory Apparatus**

- **Heart and Circulatory System**

Chameleons possess a unique three-chambered heart made up of two atria and a single ventricle. Interestingly, their ventricle is partially divided by a muscular septum, which helps

reduce the mixing of oxygenated and deoxygenated blood. This structural adaptation enhances their oxygen transport efficiency, vital for their slow, calculated movements and precise strikes when hunting**.(Wallach,& Broadley,2009)**

- **Circulatory Efficiency:**

Being ectothermic, chameleons experience changes in heart rate according to environmental temperature. Their cardiac rhythm speeds up in warm conditions and decelerates in cooler climates. This flexibility helps regulate their energy consumption and maintain appropriate metabolic functions throughout the day. Lillywhite . 2010).(

- **Vascular Adaptations:**

The circulatory system of chameleons features blood vessels adapted for efficient nutrient and oxygen delivery. This is especially important for arboreal species that must maintain stable circulation even while navigating vertical or unstable surfaces in their natural habitats**.(Feder, & Burggren, 1992).**

- **Respiratory System**

Chameleons have a relatively simple yet highly efficient respiratory system. They possess a single pair of lungs, internally divided into several chambers. The front portion, often referred to as the "saccular" lung, handles most of the gas exchange, while the rear section acts mainly as a reservoir for air storage**.(Schmidt-Nielsen, K.1997)**

- **Breathing Mechanism:**

Unlike mammals, chameleons do not have a diaphragm. Instead, they rely on the coordinated movement of their rib muscles to inflate and deflate the lungs. Their breathing rate is generally slow and carefully regulated, shifting based on physical activity and environmental conditions **(Perry, & Sander, 2004).**

- **Nasal Passages:**

The chameleon's nostrils, located near the tip of the snout, are adapted to serve as filters. These small nasal openings trap dust and other airborne particles, helping to maintain respiratory hygiene—especially important in the arid and dusty regions where many chameleons live**.(King, & McLelland, 1984).**

❖ **: Skin Apparatus**

Chameleon skin is composed of numerous layers that enable its astounding color-changing ability:

- **Epidermis:**

The outermost layer is the epidermis, which protects the body from outside harm and prevents excessive water loss. **(Clark, D. 2000).**

- **Chromatophores:**

Below the epidermis lie the pigment cells, or the chromatophores. They consist of different types of pigments such as erythrophores (red), xanthophores (yellow), and melanophores (dark brown to black), which fill or run out to change the color of the skin**.(Clark, D. 2000)**

- **Iridophores and Guanophores:**

Under the chromatophores are iridophores and guanophores that give rise to iridescent and reflective colors. The cells reflect and produce pale blue, green, and other tints and aid the chameleon in camouflage and communication through color change**.(Clark, D.2000)**

❖ **Color Change Mechanism :**

- **Camouflage:**

Chameleons turn colors in order to blend with the environment, escaping predators and hunting prey without detection . **(Schiøtz, A. 1999)**

- **Communication:**

Color change is also used in social signaling, especially in mating displays and territorial behaviors. The males are more colorful and bright in order to attract the females or signal other males **. (Schiøtz, A. 1999).**

- **Thermoregulation:**

The color change is also utilized in regulating body temperature. Darker colors capture more heat, and lighter colors reflect sunlight to keep the best temperature in the chameleon. **(Schiøtz, A.1999).**

❖ **Skin Functions :**

- **Protection:**

The chameleon's skin provides significant protection against physical harm, shielding the animal from injuries caused by sharp objects, predators, and extreme temperature conditions. The tough and layered structure of the skin is key to its durability and defensive role. **(Rösler, & Schmitz,2006).**

- **Hydration :**

Chameleon skin plays an essential role in moisture retention, which is particularly crucial for species inhabiting arid or semi-arid environments. Some chameleon species have the ability to absorb water directly through their skin, helping them maintain hydration in dry conditions **. (Senter, P. 2007).**

- **Defense and Camouflage :**

The skin provides a protective mechanism with its ability to allow the chameleon to blend in the surroundings. They are difficult to detect by predators due to their skin texture and color. Some other species of chameleons have spiny or scaly skin that acts as a predator defense since they are undesirable or difficult to capture**.(Schiøtz, A. 1999**)

❖ **-Other Anatomical Features**

- **Jaw Structure and Feeding:**

Chameleons possess a unique jaw structure that aids in their specialized feeding technique. Their jaws are capable of rapid extension, allowing them to capture prey with precision. Rather than chewing, chameleons use their long, sticky tongue to seize and ingest their food, with their teeth firmly anchored to the jaw**.(Herrel, A., et al. (2001).**

- **Tongue and Feeding:**

Chameleons are renowned for their highly efficient projectile tongues, which can extend up to twice the length of their body. This remarkable ability allows them to catch prey from a distance, minimizing their exposure to potential predators and enhancing their predatory success**.(Irschick, et al. 1996)**

- **Nostrils and Sensory Adaptations:**

Chameleons' nostrils at the end of their snouts are not just the site of air entry but also help in dust and particle removal from the air. Additionally, their keen eye sight and vibration sensitivity through their skin make them very sensitive to their environment, which allows them to sense both predators and prey**.(Pienkowski, M.2007).**

I.5.2.1. Digestive apparatus

The digestive system of the chameleon is not highly complex. The stomach is tubular in shape and secretes enzymes and hydrochloric acid. The intestines are short and end at the cloaca **(Bourdain, 2006).** Chameleons possess small, conical teeth that are all similar and rudimentary, inserted directly into the upper edge of the jawbone. These teeth are primarily used to hold prey inside the mouth. The oral cavity is often colored, and some chameleon species display it when threatened to scare off opponents. The mouth contains glands that secrete mucus, and others that produce non-sticky saliva of varying viscosity **(Tolley and Herrel, 2013).**

The chameleon’s tongue consists of a circular accelerator muscle connected to a cartilaginous structure known as the hyoid horn, which is linked to the hyoid bone by hyoid muscles. The hyoid bone is itself attached to the sternum by powerful muscles and is shaped like a "U" with high mobility **(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 tongue is covered with numerous epithelial glands and papillae that adhere to the irregular surfaces of prey like sticky hooks **(Schwenk, 1983).**

The contraction of the accelerator muscle, combined with the backward motion of the hyoid horn, generates high pressure that ejects the tongue from the mouth in a catapult-like manner. When extended, the tongue can reach the length of the chameleon's body. When not in use, it simply rests at the back of the throat, with its tip folded inward (**Fouda et al., 2015).**

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

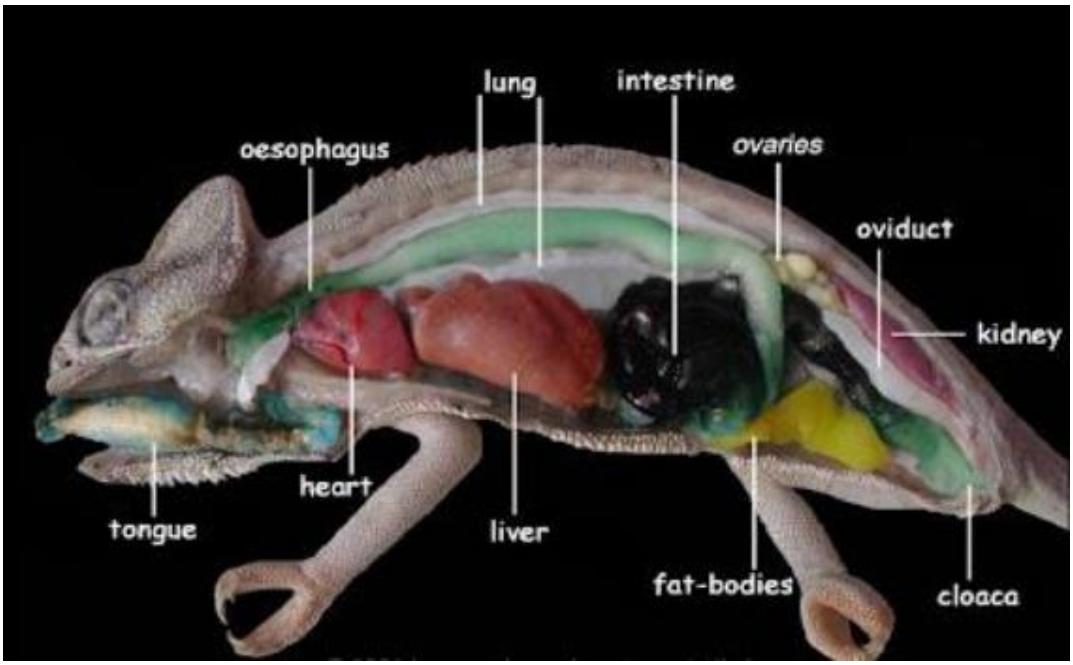


Figure 04: Gross anatomy of female chameleon (Kusuma, 2008)

Chapter II

Immuno-modulator activity

I.1. Immunity

The function of immunity was realized from time immemorial when individuals who had survived certain illnesses were discovered to be immune to secondary infection. Nonetheless, the scientific basis of immunology was developed during the late 18th century when Edward Jenner invented the smallpox vaccine in 1796, which was a landmark in the prevention of diseases **(Riedel, 2005)**. Louis Pasteur generalized principles of vaccination during the 19th century, and Élie Metchnikoff's discovery of phagocytosis made great contributions to understanding innate immunity **(Silverstein, 2009**).

The 20th century saw revolutionary discoveries in immunology, such as the discovery of antibodies, new developments in organ transplantation, and the use of genetic engineering. These have created vaccines and immunotherapies that are today used to combat infectious diseases, autoimmune diseases, and cancers. **(Abbas, Lichtman, & Pillai. 2022).**

II.2. Definition :

Immunity is the body's defense mechanism against infection by microorganisms such as bacteria, viruses, and parasites. In addition, it participates in the detection and elimination of abnormal cells, including cancer cells. The immune system, which is a complex network of cells, tissues, and organs, acts to defend the body against invasion. **(Abbas et al., 2022)**

II.3. Significance of Immunity

The immune system is essential for maintaining health and physiological balance, as :

- Protects against infections: It acts as a barrier to prevent pathogens from entering the body and efficiently kills those that find their way in**(Janeway et al., 2012).**
- Screens and kills abnormal cells: It detects and kills cancer cells or damaged cells before they multiply **(Murphy & Weaver, 2017)** .
- Creates immunological memory: When a pathogen is encountered, the immune system stores a memory of the invader, enabling it to respond more quickly and effectively on the next encounter **(Goldsby et al., 2003)** .
- Provides immune tolerance: It ensures self-non-self discrimination, keeping the immune system from attacking the body's own tissues. When this fails, autoimmune disorders might be triggered **(Parham, 2014)** .

II.4. Mechanisms of Immune Protection

The immune system functions through two primary defense mechanisms :

Innate immunity (non-specific): The body's first line of defense that encompasses physical and chemical barriers such as the skin and mucous membranes, and immune cells that react to invading pathogens at once **(Murphy & Weaver, 2017)** .

Adaptive immunity (specific): This mechanism is formed by past experience with infections or vaccinations. It relies on the specific immune cells, T cells, and B cells, which release targeted immune responses and assist in long-term immunity by releasing antibodies **(Janeway et al., 2012)**

II.4.1.Innate Immunity :

Innate immunity, also known as natural immunity, is the first line of defense of the body against microbes. It is present from birth and does not require any prior exposure to microbes. It is a nonspecific immune response because it acts on all foreign substances in a general manner without specificity for the pathogen type

(Abbas, Lichtman, & Pillai. 2022).

Components of Innate Immunity

1-Physical and Chemical Barriers

- **Skin:** Acts as a barrier, excluding microorganism**.(Alberts, et al.2014).**
- **Mucous Membranes:** Cover the respiratory, digestive, and reproductive tracts and produce mucus, which traps pathogens**.(Alberts, Johnson, Lewis, Raff, Roberts, & Walter.2014).**
- **Chemical Secretions:** Sweat, tears, and saliva are secretions that are antimicrobial because they contain enzymes like lysozyme that break down bacterial cell walls.(Alberts, Johnson, Lewis, Raff, Roberts, & Walter.2014)..
- **Acidic pH:** Stomach secretes hydrochloric acid that kills most microbes that enter it.(Alberts, Johnson, Lewis, Raff, Roberts, & Walter.2014).

2-Innate Immune Cells:

- **Phagocytes (Macrophages and Neutrophils):** Engulf and devour pathogens through the process of phagocytosis. These cells are specialized to do this **(Murphy, & Weaver. 2016).**
- **Natural Killer (NK) Cells:** Kill virus-infected and cancer cells by causing apoptosis**(Murphy, & Weaver. 2016).**

3-Immune Proteins:

- **Interferons:** Signaling proteins released by virus-infected cells to induce antiviral responses in neighboring cells **(Abbas, Lichtman, & Pillai.2022).**
- **Complement System:** A cascade of proteins that enhance pathogen removal and enhance phagocytosis **(Abbas, Lichtman, & Pillai.2022).**

4-Inflammatory Response:

An inflammatory response is created during infection, which is characterized by:

- **Redness:** Due to increased blood supply to the site of infection **(Murphy, & Weaver.2016).**
- **Heat:** Which inhibits the growth of microorganisms**(Murphy, & Weaver.2016).**
- **Swelling and Pain:** Resulting from the accumulation of immune cells and fluids at the infection site **(Murphy, & Weaver.2016).**

5- Characteristics of Innate Immunity :

- **Rapid Response:** Acts instantaneously against pathogen invasion
- **Nonspecific:** Provides a general defense against all foreign particles
- **Lack of Immunological Memory:** Unlike adaptive immunity, it does not "remember" previous infections**(Abbas,Lichtman, & Pillai. 2022).**

II.4.2. Acquired Immunity:

Acquired immunity is an important component of the immune system that develops due to exposure to antigens through natural infection or vaccination. It exists in two large categories**(Abbas, & Lichtman. 2017).**

1- Humoral Immunity

Supported by antibodies produced by B cells, Antibodies recognize and kill antigens, fighting infections, Very effective against extracellular bacteria and viruses**(Murphy, & Weaver. 2016)**: **2-Cell-Mediated Immunity:**

Is founded on T cells rather than antibodies, Targets virus-infected cells, cancer cells, and foreign tissue, Involves cytotoxic T cells ($CD8^+$), which kill infected cells directly, and helper T cells ($CD4^+$), which regulate the immune response**(Janeway, Travers, Walport, & Shlomchik.2001)**

2- The Role of Antibodies

Antibodies or immunoglobulins are specialized proteins produced by plasma cells (activated B cells). They have roles in immunity through different mechanism

- Neutralization – Preventing infection by binding with antigens
- Complement Activation – Triggering the complement system to enhance microbial killing
- Opsonization – Facilitating phagocytosis by labelling pathogens for recognition by macrophages**(Abbas, & Lichtman. 2017)**

The five major classes of antibodies are:

IgG – Most abundant; can cross placenta to provide fetal immunity , IgA – Present in body secretions, e.g., saliva and milk, IgM – First antibody produced during primary immune response, IgE – Involved in allergic reactions and protection against parasites, IgD – Primarily present on immature B cells, role not yet discovered **(Murphy,& Weaver.2016)**

❖ **T Cells and B Cells**

- B Cells: Humoral immunity's key players; they differentiate into plasma cells to produce antibodies**. (Janeway, Travers, Walport, & Shlomchik. 2001**) .
- **T Cells:** Cell-mediated immunity's backbone and categorized as, Helper T cells ($CD4^+$)
- Activate and regulate immune responses

Cytotoxic T cells ($CD8^+$) – Destroy infected or cancerous cells Regulatory T cells – Downregulate excessive immune responses**.(Janeway, Travers, Walport, & Shlomchik. 2001)**

II.5. Components of the Immune System :

The immune system is an orderly system of organs, cells, and signaling molecules that work together to protect the body from infection and disease. It is composed of primary and secondary lymphoid organs, immune cells, and cytokines, which are all involved in basic immune defense and regulation **(Murphy & Weaver, 2017).**

II.5.1. Lymphoid Organs

A. Primary Lymphoid Organs

❖ **Bone Marrow**

The primary site of hematopoiesis, where all the blood cells, including immune cells such as B and T lymphocytes, neutrophils, and macrophages, are producedB cells mature in the bone marrow before they migrate to secondary lymphoid organs, where they participate in adaptive immunity **(Murphy & Weaver, 2017)**

❖ **Thymus**

An essential organ for T cell maturation, as long as developing T cells are able to recognize foreign antigens and tolerate self-antigensT cells that fail to differentiate properly are eliminated by apoptosis to prevent autoimmune diseases **(Goldsby et al., 2003)**

B. Secondary Lymphoid Organs

❖ **Lymph Nodes**

Serve as centers of filtration where antigens get trapped and presented to lymphocytes.Macrophages and dendritic cells of lymph nodes play a pivotal function in antigen presentation, activating B and T cells **(Janeway et al., 2012)**

❖ **Spleen**

Primarily engaged in the filtration of blood, removing old red blood cells, and in the detection of blood-borne pathogens Central role in B cell activation and antibody production **(Abbas et al., 2022)**

Mucosa-Associated Lymphoid Tissue (MALT3-)

Includes organs such as the tonsils and Peyer's patches that confer immune protection against infection entering via mucosal surfaces of the respiratory and gastrointestinal tracts **(Parham, 2014)**

II.5.2. Immune Cells and Their Functions :

A. Innate Immune Cells

Innate immune cells provide an immediate, non-specific response to infections

❖ **Phagocytes**

Macrophages: Engulf pathogens, process, and present antigens to T cells, and produce inflammatory mediators that regulate immune responses **(Murphy & Weaver, 2017(**

Neutrophils: The most populous granular white blood cell, used to combat bacterial and fungal infection through phagocytosis **(Goldsby et al., 2003)**

❖ **Natural Killer (NK) Cells**

Lympocytes specialized to recognize and kill virus-infected and cancer cells without prior antigen sensitization **(Abbas et al., 2022)**

❖ **Dendritic Cells**

Serve as mature antigen-presenting cells that connect the innate and adaptive immune systems by processing and presenting antigens to T cells **(Janeway et al., 2012).**

B. Adaptive Immune Cells

❖ **B Cells**

Responsible for producing antibodies that neutralize invading pathogens. Some B cells differentiate into memory B cells, facilitating rapid immune responses upon reinfction **(Murphy, & Weaver.2017)**

❖ **T Cells**

Helper T Cells ($CD4^+$): Regulate immune responses by releasing cytokines that stimulate B cells, macrophages, and other immune cells **(Parham, 2014)**

Cytotoxic T Cells ($CD8^+$): Destroy virus-infected and cancer cells directly by triggering apoptosis **(Janeway et al., 2012)**

Regulatory T Cells: Suppress excessive immune activation to prevent autoimmune disorders **(Abbas et al., 2022)**

❖ **Cytokines and Immune Signaling**

A. Overview of Cytokines

Cytokines are small signaling molecules that allow intercellular communication and coordinate immune responses **(Murphy & Weaver, 2017)**

1- Interleukins (ILs)

Stimulate immune cell differentiation, activation, and proliferation

Example: IL-2 promotes T cell proliferation, enhancing adaptive immunity

(Goldsby,et al,2003).

2 -Interferons (IFNs)

Play a central role in antiviral immunity by inhibiting viral replication .Example: IFN-γ enhances macrophage activation and antigen presentation**(Janeway, et al,2012)**

3 -Tumor Necrosis Factor (TNF)

Involved in inflammation and programmed cell death (apoptosis) of infected or cancerous cells**(Parham.2014).**

4-Chemokines

Direct immune cells to sites of infection or injury, facilitating coordinated immune responses **(Murphy, & Weaver,2017**)

II.6.Mechanism of Immune Response:

The immune system serves as the body's primary defense mechanism, protecting against infectious agents through a highly coordinated response. This process involves recognizing antigens, mounting an initial immune response upon first exposure, and generating a more efficient secondary response upon subsequent encounters. The ability to retain immunological memory ensures long-term immunity and effective protection against recurrent infections**(Murphy, & Weaver.2017)**

1-Antigen Recognition :

A. Definition of Antigen

An antigen is any foreign substance, typically a protein or polysaccharide, that elicits an immune response. Antigens are commonly derived from pathogens, such as viruses, bacteria, and fungi, and include surface proteins, toxins, and other molecular structures**(Janeway, 2012).**

B. Mechanisms of Antigen Recognition

- **Innate**

The innate immune system identifies antigens through pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), which detect pathogen-associated molecular patterns (PAMPs). Macrophages and dendritic cells play a critical role in recognizing these molecular patterns and initiating an immediate immune response(**Murphy, & Weaver,2017).**

- **Adaptive Immunity:**

Adaptive immunity relies on B and T lymphocytes, which possess highly specific receptors—B cell receptors (BCRs) and T cell receptors (TCRs)—for antigen recognition. Unlike innate immunity, T lymphocytes require antigen presentation via major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) such as dendritic cells and macrophages to activate an immune response**(Abbas,et al, 2022).**

2 -Primary and Secondary Immune Responses

A. Primary Immune Response

The primary immune response occurs upon first exposure to an antigen and typically requires several days to fully develop. B cells are activated and initially produce immunoglobulin M (IgM), which is later replaced by immunoglobulin G (IgG) for a more sustained immune defense. Helper T cells ($CD4^+$) regulate the immune response by activating B cells and cytotoxic T cells ($CD8^+$), which eliminate infected cells(**Murphy,& Weaver,2017)**

B. Secondary Immune Response

A secondary immune response is triggered upon re-exposure to the same antigen and is significantly faster and more robust than the primary response. Memory B cells rapidly differentiate into antibody-producing plasma cells, leading to a heightened and prolonged production of IgG antibodies. Memory T cells enable a swift immune reaction, effectively neutralizing the pathogen before it can cause significant harm **(Abbas,et al, 2022**).

3 -Immunological Memory

A. Definition of Immunological Memory

Immunological memory is a hallmark of adaptive immunity, characterized by the persistence of memory B and T cells that enable a more rapid and efficient immune response upon subsequent antigen exposure. This feature is essential for long-term protection against infectious diseases **(Parham.2014)**

B. Types of Memory Cells

- **Memory B Cells**

These cells reside in the lymphoid tissues and bloodstream, facilitating a rapid antibody-mediated response upon re-exposure to an antigen **(Abbas,et al, 2022)**

- **Memory T Cells**

$CD4^+$ Memory T Cells: Support immune responses by activating B cells and coordinating cellular immunity ,$CD8^+$ Memory T Cells: Rapidly recognize and destroy infected cells, enhancing immune efficiency upon reinfection**(Janeway,et al ,2012)**

C. Role of Immunological Memory in Vaccination

Vaccination harnesses immunological memory by stimulating a controlled primary immune response without causing disease. This process leads to the development of memory cells, which provide rapid and effective immunity upon future pathogen exposure. Vaccines for diseases such as measles and rubella rely on this mechanism to confer long-lasting protection **(Murphy, & Weaver , 2017).**

II.7. Immune System Disorders

The immune system plays a crucial role in protecting the body from harmful pathogens. However, when it malfunctions, it can lead to immune system disorders, which can be broadly classified into three main categories: immunodeficiency disorders, autoimmune diseases, and hypersensitivity reactions **(Abbas,et al , 2022)**.

1 -Immunodeficiency Disorders:

Immunodeficiency occurs when the immune system is weakened, making the body more susceptible to infections. These disorders can be:

Primary (Congenital): Genetic disorders present from birth

Secondary (Acquired): Develop later due to infections (e.g., HIV), medical treatments (e.g., chemotherapy), or malnutrition

Examples of Immunodeficiency Disorders:

Severe Combined Immunodeficiency (SCID): A rare genetic disorder that significantly weakens the immune system. **(Mayo Clinic).**

Common Variable Immunodeficiency (CVID): A condition characterized by low antibody levels and frequent infections **(Mayo Clinic).**

HIV/AIDS: A viral infection that progressively destroys immune system function **(Mayo Clinic).**

2- Autoimmune Diseases

Autoimmune diseases occur when the immune system mistakenly attacks the body's healthy cells, mistaking them for foreign invaders. The exact cause is often unknown, but genetic and environmental factors are believed to contribute

Examples of Autoimmune Diseases

Rheumatoid Arthritis: The immune system targets the joints, causing inflammation and pain Systemic Lupus Erythematosus (SLE): A condition in which the immune system attacks multiple organs and tissues

Type 1 Diabetes: The immune system destroys insulin-producing cells in the pancreas

(National Institute)

3- Hypersensitivity Reactions (Allergic Disorders)

Hypersensitivity disorders occur when the immune system overreacts to harmless substances (allergens) such as pollen, pet dander, or certain foods. These reactions can range from mild symptoms (e.g., sneezing, skin rashes) to severe life-threatening conditions (e.g., anaphylaxis)

Examples of Hypersensitivity Disorders

- **Asthma:** Inflammation and narrowing of the airways triggered by allergens or irritants
- **Anaphylaxis:** A severe allergic reaction requiring immediate medical attention
- **Hay Fever (Allergic Rhinitis):** Allergic response to airborne allergens like pollen or dust mites

II.8. Immunomodulation :

Immunomodulation refers to the modulation of the immune response to upregulate or downregulate immune responses. It has attracted significant attention over the years on the basis of its potential therapeutic uses against a number of diseases, including autoimmune diseases, infections, and organ transplantation. The following sections present significant information regarding immunomodulation, from its mechanisms, therapeutic use, and limitations**(Chauhan,et al,2024).**

1-Mechanisms of Immunomodulation

- **Immunostimulation vs. Immunosuppression:** Immunomodulation has the ability to enhance (immunostimulation) or repress (immunosuppression) immune function, based on the clinical application**(Chauhan et al., 2024)**
- **Specific vs. Non-Specific Modulation:** Specific immunomodulation is antigen-specific, but non-specific modulation encompasses wider immune response, allowing for multiple therapeutic applications**(Chauhan et al., 2024)**

2-Therapeutic Applications

- **Autoimmune Disorders:** Immunomodulatory drugs are increasingly being used to treat autoimmune diseases by specifically targeting single immune pathways ("Immunomodulatory pharmaceuticals for the treatment of immune(**dysfunction", 2022)**
- **Infectious Diseases:** Repurposing existing immunomodulatory drugs in drug-resistant tuberculosis has proved effective against the disease**(Khan et al., 2022)**
- **Transplantation:** Recent efforts aim to engineer donor tissue to reduce rates of rejection, restricting the need for long-term immunosuppression**(Faustman, 2014)**

3-Challenges and Future Directions

- **Safety and Efficacy:** Designing safer and more effective immunomodulatory therapy remains challenging, and there is a constant need for research and clinical trials**(Strzelec et al., 2023)**
- **Precision Therapy:** Tailoring immunomodulatory therapy based on individual patient needs and disease pathomechanisms is a new focus in clinical practice("Immunomodulatory pharmaceuticals for the treatment of immune(**dysfunction", 2022).**

Second part

Experimental part

Chapter I

Material &Method

I.1. Materials

I.1.1.Equipment and Small Tools

A number of specific equipment and small laboratory tools were used in our study, including:

- Refrigerated centrifuge• Optical microscope • Two incubators, one set at 37°C and the other at 56°C • Water bath / Magnetic stirrer • Electronic balance • Precision balance • pH meter / Thermometer • Rotator / Soxhlet extractor • Heating plate • Vortex / Oven • Spectrophotometer • Hot plate with magnetic strirrer • Micropipettes, graduated pipettes, suction bulb, beakers, Erlenmeyer flasks, volumetric flasks, Eppendorf tubes, Petri dishes, test tubes, funnels, spatula, slides, cover slips, Pasteur pipettes, filter paper

I.1.2. Chemicals, Reagents, and Biological Materials

- Buffers: PBS (Phosphate Buffered Saline) / EGTA (Ethyleneglycol Tetraacetic Acid) with a neutral pH •ACP buffer / EGTA / PBS with $MgCl_2$ at a neutral pH • HBSS buffer

  • Agarose gel • Chicken blood (used as a source of erythrocytes, treated with 20% (v/v) ACD anticoagulant and stored at 4°C) •Sample of normal human plasma (NHP) collected from a healthy adult donor • Ascorbic acid, acetonitrile, acetic acid, methanol • NaCl, KCl, Na_2HPO_4, KH_2PO_4 .

I.2. Méthodes

I.2.1. MeH_2O Extraction

We measured 10 grams of the sample (*C. chamaeleon* powder) in a cartridge, then added 200ml of methanol (70%) and 100ml of water (30%) to a round-bottom flask, and then put the two components in a Soxhlet apparatus. Afte 6 cycles of in the apparatus continuously we got a solution of methanol and water which contained polar compounds, after taking the solution, we put it in a Rotary evaporator device where the methanol is removed from the mixture by pressure evaporation and after that we placed the solution in an oven at 45° to 50°C for 24 hours, After the drying process, the extract was weighed to obtain it in powdered form, and its yield was then calculated.

I.2.2. Vitamins B Extraction

5g of *C.Chamaeleon* powder was extracted using 6 mL of cold an aqueous buffer composed of 50% acetonitrile, 1% acetic acid, 0.1% ascorbic acid, and 30ml distilled water . The mixture was homogenized using a vortex and between each stage, samples were frozen. Then C.Chamaeleon homogenate were incubated in a water bath for 15 minutes at 50 °C, and swiftly cooling on ice, and then centrifuged for 15 minutes at 2000 g. Next, 20 mL of cold acetonitrile was added to start the second round of deproteinization **(Xu et al., 2020)**.

I.2.3. Dosage :

The dosage of B vitamins present in both the "Multi B" extract and the methanolic-water extract was determined. Subsequently, absorbance was measured at specific wavelengths, (**Jin et al., 2012; Chen et al., 2011; Guggisberg et al., 2012**). as shown in the accompanying table. Vitamin Absorption Wavelengths

Table 02: Absorption Wavelengths of B Vitamins in Chamaeleo chamaeleon Extracts

Vitamin	Absorption Wavelength (nm)
B1	250
B2	370
B3	260
B6	290
B7	220
B9	280
B12	361

During three different measurements, the standard curves were produced using drug les neuf B (vitamin B1, B2,B3,B5, B6, B7, B8, B9, and B12), and their values were expressed in mg/100g.

I.2.4. In vitro assays of *Chamaeleo chamaeleon* extracts

I.2.4.1. Antioxidant activity

The antioxidant activity of the extracts of C. chamaeleon was evaluated using several complementary techniques; including the scavenger activity of the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical.

Scavenging activities on DPPH radical

❖ **Principe**

The chemical compound 2,2-diphenyl-1-picrylhydrazyl (a, a-diphenyl-ß picrylhydrazyl) is one of the first free radicals used to study the structure-antioxidant activity relationship of compounds. The effectiveness of an antioxidant is measured by measuring the decrease in violet coloration, due to a reduction of the DPPH radicals towards the corresponding pale. yellow of hydrazine, measurable spectrophotometric at 515-518nm **(Xie and Schaich, 2014).**

❖ **Procedure**

The 1,1-diphenyl-2-picrylhydrazyl solution is prepared by dissolving 4 mg of DPPH in 100 ml of methanol. 1 ml of each chameleon extract (14, 15, 16 ,17 ,18 ,19 ,20 µg /ml) or ascorbic acid as standard is added to 1 ml of the solution of DPPH prepared previously. The reaction mixture is immediately shaken, then kept in the dark for 30 min at room temperature for the reaction to be completed. The absorbance of the reaction medium is measured at 517 nm against the control **(Talbi et al., 2015).**

The percentage of anti-radical activity is estimated using the equation below :

(%1) DPPH radical scavenging = [(A0-A1)/A0] × 100

With:

- 1%:percentage of inhibition, A0: absorbance of the control, Al: absorbance of the sample- The IC50 is the concentration of the sample tested necessary to reduce 50% of the DPPH radical, they are calculated graphically by percentages of inhibition according to different concentrations of the extracts tested. The results are expressed in mg/ml. The antioxidant capacity of a compound is all the more high that its IC50 is small and compared with that of ascorbic acid **(Morabbi Najafabad and Jamei, 2014).**

I.2.4.2. Anti-inflammatory activity

We selected the BSA denaturation method described by **Chandra et al., (2012)** with some modifications to test the anti-inflammatory. where we combined 1 ml of C.Chamaeleon extracts at various doses (10, 11, 12 ,13, 14 ,15 ,16 ,17, 18 ,19, 20 µg/ml) with 0.2 ml of BSA 1.4 ml of PBS (phosphate-buffered saline, pH6.4), And we used distilled water with the same volume as a control. The mixture was then incubated for 15 minutes at 37 °C before being immediately placed in a water bath for 5 minutes at 70 °C. After cooling, it is centrifuged for 10 minutes at 3000 rmp. Following that, measure the absorbance at 660 nm. Acid acetyl salicylic was used as an anti-inflammatory reference medicament.

The following equation was used to calculate the percentage of protein denaturation inhibition:

Inhibition = ((Ac-As)/ Ac) × 100 %

With: Ac: the absorbance of control, As: the absorbance of sample.

I.2.4.3. Phagocytic activity

One of the key potential effects of immunostimulants is their ability to enhance or restore the immune defenses of humans and animals against bacterial, viral, fungal, or parasitic infections. In general, most effective immunostimulants have demonstrated the capacity to improve nonspecific anti-infectious resistance (**Lagrange and Hurtrel, 1986).**

- **principe**

The phagocytosis assay relies on the activation of phagocytic cells using vitamin B extracts derived from *chamealeo chamealeon* powder. This experiment is used to evaluate the extent to which the phagocytic capacity of these cells increases following opsonization**(Harun. et al.,2015).**

- **Blood sampling**

A 5 mL blood sample was obtained from a healthy donor using EDTA-containing tubes to prevent coagulation. The sample was centrifuged at 2000 rpm for 10 minutes, after which the cellular pellet was discarded and the resulting serum was carefully collected.**(Vinjamuri et al., 2015).**

- **Préparation Saccharomyces cerevisiae**

A quantity of Saccharomyces cerevisiae was added to sugar and a specific amount of PBS solution. After that, the components was mixed well and incubated at 37°C. Finally, the absorbance was mesured using a spectrophotometer at a wavelength of 260 nm so that the absorbance value equals one (El-Kirat-CHatel,2010).

❖ **Preparation of phosphate-buffered saline (PBS)**

Table 03: Composition of PBS according (Burnat et al (2023)

Components	Quantity (g.l-)
NaCl	0,8g
KCl	0 ,2g
Na2HPO4	1,44g
KH2PO4	0,24 g
Distilled water	Up to 1 litter

- **Preparation Method**

The solid components (NaCl, Na_2HPO_4, KH_2PO_4, and KCl) were dissolved in approximately800mLofdistilledwater.The volume was adjusted to 1 liter by adding more distilledwater.The pH was measured using a pH meter and was adjusted to 7.4 using an acid orbasesolutionifnecessary.The solution was stored in a tightly sealed container and was used as needed.**(Atlas,2010).**

❖ **Experimental Procedure**

- **Preparation of Solutions and Experiment Procedure:**

✓ **Preparation of Stock Solutions:**

– **Solution 1**: Dissolve 2.5 mg of extract of "Multi B" in 5 mL of Hbss.

– **Solution 2:** Dissolve 2.5 mg of extract of "Methanol and water " in 5 mL of Hbss.

Preparation of Test Tubes with Different Concentrations.

Varyious amounts of the stock solutions was placed in test tubes to obtain the following concentrations: 0.5, 0.375, 0.25, 0.125, 0.0625, Hbss was added to each tube to bring the total volume to 1 mL.

✓ **Preparation of Control Tubes:**

– **Positive Control Tube:** Contains "Zenc" at a concentration of 0,5mg/mL .

– Negative Control Tube: Contains only Hbss.

✓ **Experimental Procedure:**

40 µL of each concentrations was transferred into new test tubes, then 200 µL of the previously prepared serum was added to each tube, the tubes were incubated in a water bath at 37°C for 30 minutes, 40 µL of Saccharomyces cerevisiae was included to each tube, and

return them to the water bath at 37°C for 10 minutes, Then 1 mL of PBS solution was introduced to each tube, Centrifuge the tubes were centrifugated at 2000 rpm for 5 minutes, the pellet were collected from each tube and placed on microscope slides, then a few drops of "Blood Methylene" were added to examine the slides under an electron microscope (El-Kirat-Chatel, 2010) .

I.2.4.4. Anti-complement test

A. Preparation of the First Extract:

5 mg of Multi-B extract are dissolved in 5 mL of PBS (phosphate-buffered saline)

B. Preparation of the Second Extract:

5 mg of a methanol and water mixture are dissolved in 5 mL of PBS, After preparation, different volumes of these solutions are taken at various concentrations, as shown in the following table.

Let me know if you want the table:

Table 04: Serial Concentration Preparation of ACP Solution for Use in the Complement Inhibition Assay

Concentration (mL)	0.2	0.4	0.6	0.8	1.0
Stoock Solution(Extract1, Extract2)	200	400	600	800	1000
ACP	800	600	400	200	0

C. Preparation Method:

- **Preparation of Red Blood Cells:**

Blood was collected from a chicken and centrifuged at 2500 rpm for 10 minutes. The supernatant was removed, and the pellet containing the red blood cells was retained. The pellet was washed twice with PBS (Phosphate Buffered Saline), then once with ACP solution.

- **Preparation of Human Serum:**

Blood was collected from a healthy person and centrifuged under the same conditions (2500 rpm for 10 minutes).The serum was retained after removing the red blood cells.

- **Preparation of Diluted Red Blood Cell Solution:**

0.5 mL of the prepared red blood cells was mixed with 4.5 mL of PBS to obtain a diluted solution.

❖ **Preparation of agarose gel Solution:**

0.52 g of agarose gel was weighed and dissolved in 26 mL of ACP solution. The mixture was placed in a water bath and stirred until the temperature reached 85°C to ensure complete dissolution.

After dissolution, 19.6 mL of ACP solution and 0.4 mL of the previously diluted red blood cell solution were added (Diallo et al. 2001; Wang et al. 2016; Lee et al. 2018).

D. Control and Treatment:

- Heparin at a concentration of 1 mg/mL is used as the positive control.
- ACP (1000 µL) is used as the negative control. (Vinjamuri et al., (2015)

a) Preparation of Agar Plates:

1. The agarose gel mixture was prepared and poured into sterile Petri dishes.
2. The plates were left to cool until the agar solidified.
3. Using a 5 mm diameter punch, evenly spaced wells were created on the agar surface.

b) Sample Application:

- 25 µL of each concentration of the first and second extracts was added into the wells .
- 25 µL of serum was added into its designated wells.
- One well was designated for the positive control (Heparin) and another for the negative control (ACP).

c) Incubation:

- The plates were incubated at 37°C for 72 hours. (Gao et al. 2013)

ACP Solution Preparation

Table 05: Composition of ACP Solution

Composition	Quantity
PBS	20 mL (pH = 7.4)
EGTA	2 mL
$MgCl_2$	2 mL

I.2.5. *In Vivo* Immunomodulatrice

I.2.5.1. Animals Care

The ***in vivo*** study was conducted using 25 adult female Albino Wistar rats**,** with an average body weight of 223.8 ± 25.5 g**,** obtained from the Pasteur Institute in Algiers. The animals underwent an acclimatization period of 15 days under standard laboratory conditions, including a temperature of 25°C**,** relative humidity of 62%**,** and a 12-hour light/12-hour dark photoperiod**.**

The rats were housed in polypropylene cages measuring 50 × 35 × 20 cm**,** with **5** animals per cage**,** and were given free access to food and water throughout the experiment. The cages were lined with sawdust and cleaned daily to ensure proper hygiene. The animals were fed a standard diet, as described by **(Southon et al.1984),** and tap water was provided as their drinking source.

All animal handling procedures were carried out in accordance with the ethical guidelines outlined in the Guide for the Care and Use of Laboratory Animals **(Albus, 2012).**

I.2.5.2. Experimental process

After an adaptation period lasting 15 days, the rats were divided into five experimental groups, with each group consisting of five rats. Each group was treated separately, as will be explained in the following sections.

- **Group 01(Control):** As the control group, the rats in this group were given regular drinking water and were fed only standard food throughout the experimental period.
- **Group 02 (Ink**):): All animals in this group were fed standard food only and provided with regular drinking water throughout the experimental period. They were also injected with ink 15 minutes prior to sacrifice**.**
- **Group 03 (Ink+MeH_2O):** All animals in this group were fed standard food and provided with regular drinking water throughout the experimental period. Subsequently, they received aqueous and methanolic extract treatments via gavage at a concentration of 50mg /kg for three days and were injected with ink 15 minutes prior to sacrifice."
- **Group 04 (Ink + multi B):** All animals in this group were fed standard food and provided with regular drinking water throughout the experimental period. Subsequently, they received multi B extract treatments via gavage at a concentration of 50mg /kg for three days and were injected with ink 15 minutes prior to sacrifice
- **Group 05 (Ink + neuf B):):** All animals in this group were fed standard food and provided with regular drinking water throughout the experimental period. Subsequently,

they received drug les neuf B extract treatments via gavage at a concentration of 50mg /kg for three days and were injected with ink 15 minutes prior to sacrifice.

I.2.5.3. Sacrifice of rats and collection of blood and organs

After 16 hours of fasting, the rats are anesthetized using 94% chloroform and then sacrificed. Blood samples are collected at the time of sacrifice: into EDTA tubes for hematological analysis and into dry tubes for biochemical analysis. Following dissection, Spleen , Thymus and Bone marrow carefully removed rinsed first with distilled water and then with 0.9% NaCl solution, and subsequently weighed,The organs are preserved in formalin solution for histological examination.

I.2.5.4. Organ Weight Index :

We have the following organs: the spleen, the bone marrow, and the thymus gland. We weighed them according to this protocol.

Organ Weight Index (%)=(Body Weight (g)/Organ Weight (g))×100

I.2.5.5. Phagocytic activity

Phagocytic activity is expressed by the phagocytic index (K), which measures the function of the entire reticuloendothelial system in contact with circulating blood. The clearance rate is expressed as the half-life of carbon in the blood ($t_1/_2$, in minutes). These are calculated using the following equations:

K= Ln DO1-Ln DO2/ t2-t1 , 1/2 = 0.693/K

Where:

- OD1 and OD2 are the optical densities at times t1 and t2, respectively."

I.2.5.6. Biochemical analysis :

❖ **Blood glucose**

Beta-D-glucose present in plasma is oxidized by the enzyme glucose oxidase to form D-glucono-1,5-lactone, accompanied by the production of hydrogen peroxide ($H_2\ O_2$). The lactone is then gradually hydrolyzed to D-gluconic acid. The hydrogen peroxide generated is subsequently broken down by the enzyme peroxidase into water and oxygen. The liberated oxygen reacts with a chromogenic oxygen acceptor, resulting in the formation of a colored compound. The intensity of this color is measured colorimetrically to determine the glucose concentration (Trinder, 1969).

- Glucose + glucose oxidase + $O_2 \rightarrow$ Gluconic Acid $+ \mathbf{H_2O_2}$
- $\mathbf{H_2O_2}$ + Peroxidase + Oxygen acceptor (Colourless) $\rightarrow$ H.O + Oxidised acceptor (**Coloured**)

❖ **C- reactive protein**

The concentration of C-reactive protein (CRP) in serum was measured using a turbidimetric method on the COBAS INTEGRA 400 analyzer. This method relies on a highly specific, antibody-based equilibrium turbidimetric immunoassay to detect CRP in blood. Polyethylene glycol-6000 is used to accelerate and enhance the immunoprecipitation reaction, while Tween-20 serves as a surfactant to minimize interference and stabilize the blank values of the samples (Otsuji et al., 1982).

❖ **Aspartate Transaminase (AST):**

The activity of AST was measured using a spectrophotometer based on an enzymatic kinetic method. Aspartate aminotransferase, also known as glutamate oxaloacetate transaminase (GOT), catalyzes the transfer of an amino group from aspartate to α-ketoglutarate, resulting in the formation of glutamate and oxaloacetate. Subsequently, oxaloacetate is converted to malate by malate dehydrogenase (MDH) using NADH. The rate of decrease in NADH concentration is directly proportional to the AST activity in the sample and is measured spectrophotometrically at a wavelength of 340 nm (Schumann et al., 2010).

- Aspartate + 2-Oxoglutarate $\rightarrow$ Oxalacetate + Glutamate
- Oxalacetate + NADH+H+ $\rightarrow$ Lactate + NAD^+

❖ **Alanine Transaminase (ALT):**

ALT activity was determined using a spectrophotometer following an enzymatic kinetic method. Alanine aminotransferase, also referred to as glutamate pyruvate transaminase (GPT), catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, resulting in the production of L-glutamate and pyruvate. Pyruvate is then converted to lactate by lactate dehydrogenase (LDH) in the presence of NADH. The ALT activity in the sample is proportional to the concentration of NADH, and absorbance is measured at a wavelength of 340 nm (Schumann et al., 2010).

- Alanine + 2-Oxoglutarate $\rightarrow$ Pyruvate + Glutamate
- Pyruvate + NADH+H+ $\rightarrow$ Lactate + NAD+

I.2.5.7. Hematologic analysis

❖ **Blood Smear:**

1. Prepare the Slides: The microscope slides should be clean and completely free of any dirt or grease, and must be new and unused.

2. Collect the Blood Sample: Disinfect the skin using an alcohol swab. Use a needle to extract a small amount of blood (approximately 1–2 drops), and place the drop near one end of the microscope slide, about 1 cm from the edge.

3. Make the Smear: Hold a second microscope slide at an angle of 30 to 45 degrees against the first slide. Gently touch the edge of the angled slide to the blood drop, then quickly and smoothly push it forward to spread the blood across the surface. Move the slide steadily along the length of the first slide to ensure an even distribution. The smear should gradually thin out, forming a "feathered edge" at the opposite end.

4.Air Dry the Slide: Allow the blood smear to air dry naturally and completely. Avoid blowing on the slide, as this may lead to uneven drying of the cells.

5. Stain the Smear: Wright-Giemsa stain is a blend of basic dyes like methylene blue, which gives a blue color, and acidic dyes like eosin, which produce a red stain. As a result, acidic cell components (such as the nucleus, cytoplasmic RNA, and basophilic granules) appear blue or purple, while basic components (like hemoglobin and eosinophilic granules) are stained red or orange.

To apply the stain, immerse the dried blood smear in the staining solution for approximately 1–2 minutes.

After staining, gently rinse the slide with buffered water or saline to remove any excess stain—avoid flooding the slide.

Allow the slide to air dry naturally again without blotting.

6. Microscopic Examination: higher magnification (40x or 100x) for detailed examination.

7. Analyze the Cells: Blood consists of three types of cells: white blood cells (WBCs), red blood cells (RBCs), and platelets. White blood cells are classified into five types: neutrophils, eosinophils, basophils, lymphocytes, and monocytes.

I.2.5.8. Histopathological Study

For the histopathological analysis, tissue fragments from the organs of all batches are collected. They must be removed promptly to prevent autolysis, which begins shortly after the animal's death. The samples are first rinsed with distilled water, followed by 0.9% NaCl solution, and then immediately immersed in a fixative solution containing 10% formaldehyde **(Leclerc-Mercier et al., 2016).**

The procedure involves several steps: tissue samples are placed in special cassettes with perforated walls to allow fluid passage. The samples are then dehydrated using an automated device that enables gradual transfer through ethanol baths of increasing concentrations (70%, 95%, and 100%). Subsequently, the tissues are immersed in baths of liquid paraffin.

After being maintained and soaked in paraffin, the embedding stage follows, during which the paraffin-impregnated tissue is placed into a paraffin block. As the paraffin

solidifies, it facilitates the sectioning process. This step uses “embedding devices,” which include a reservoir of molten paraffin kept in a liquid state by a heating system, a small tap, and a refrigerated metal plate to enable rapid solidification of the paraffin block containing the tissue. Thin sections of just a few mi The preparation of tissue sections (with an average thickness of a few micrometers) is made possible by using specialized instruments known as microtomes. These sections are placed onto microscope slides, smoothed, and fixed using heated gelatinous water. For staining, the Hematoxylin-Eosin technique (also referred to as Hematein-Eosin) is employed **(Elmalti et al., 2007).** This method requires acid alcohol (a mixture of 100 ml of 70% ethyl alcohol and 50 ml of HCl acid), ammoniacal water (100 ml of distilled water mixed with 2 ml of ammonia), and eosin solution (prepared from 100 ml of 3% eosin aqueous solution, 125 ml of 95% ethyl alcohol, 375 ml of distilled water, and 2 drops of acetic acid).

The staining procedure includes the following steps: first, slides are deparaffinized and rehydrated using tap water, followed by rinsing with distilled water. The samples are then immersed in Harris Hematoxylin for 15 minutes, which stains basophilic structures (nuclei) a purplish-blue color. Afterwards, the slides are briefly dipped (1 to 2 times) in acid alcohol to remove excess stain, followed by a tap water rinse. The differentiation process is verified under a microscope. Next, the slides are immersed in ammoniacal water, rinsed again, and then placed in an eosin bath (for 15 seconds to 2 minutes), which stains acidophilic structures (cytoplasm) pink. All steps are interspersed with tap water rinses.

Finally, the prepared slides are dried and examined under an optical microscope, with images captured using a camera **(Bremond-Gignac et al., 2004).**crons are then prepared for examination.

Chapter II

Results & Discussion

II.1. Results

II.1.1 Extraction yield

The image shows a bar chart comparing the extraction yield (%) between two solvents or solvent mixtures:

- MultB, with a yield of 7.4%
- MeH_2O, with a yield of 19.1%

It is clear from the chart that MeH_2O achieved a significantly higher extraction yield compared to MultB.

Extraction yield

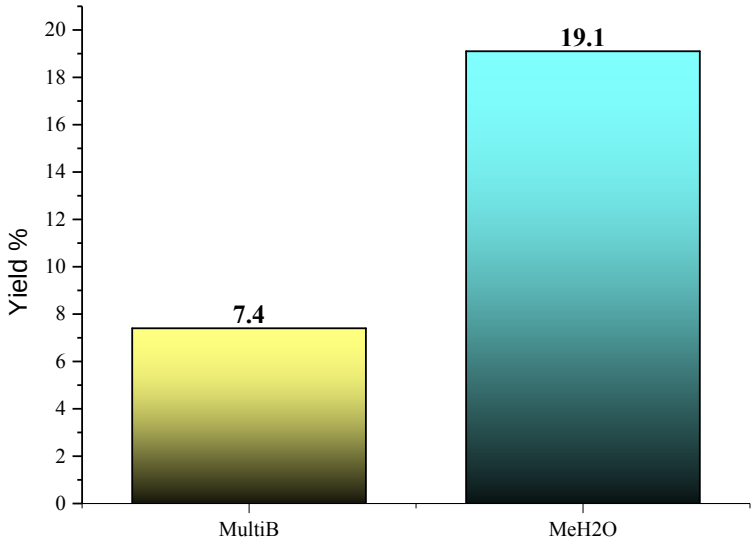


Figure 05: A diagram illustrating the extraction yield of both the methanolic extract and the Multi B extract.

II.1.2. Vitamins B content

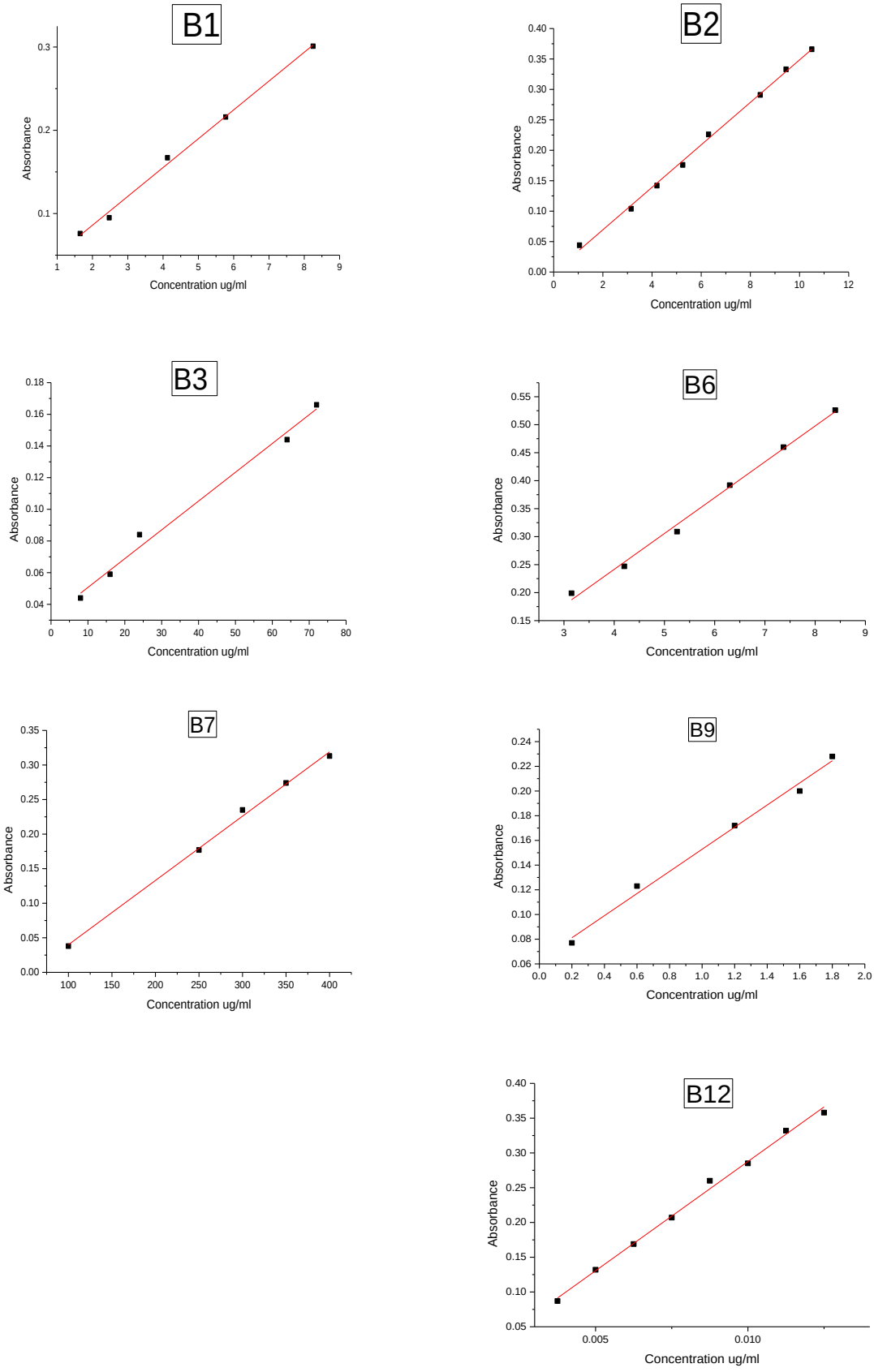


Figure 06: Linear regression of the absorbance by concentration of vitamins B

❖ **Vitamin B1 (Thiamine)**

The curve shows a clear linear relationship between vitamin B1 concentration and absorbance, indicating a good analytical response of the measurement system (e.g., spectrophotometry). Thiamine is involved in carbohydrate metabolism and is essential for proper nerve function.

❖ **Vitamin B2 (Riboflavin)**

The curve demonstrates a consistent linear response, reflecting the reliability of the measurement. Riboflavin plays a key role in metabolic processes, especially as a coenzyme in oxidation-reduction reactions.

❖ **Vitamin B3 (Niacin)**

The graph displays a positive linear correlation between absorbance and concentration, suggesting this method can be used to accurately quantify niacin. Niacin is crucial for energy production through its role in forming NAD^+ and $NADP^+$.

❖ **Vitamin B6 (Pyridoxine)**

The B6 curve shows a straight-line relationship, indicating precise quantitative response. Pyridoxine is necessary for amino acid metabolism and supports proper nervous system function.

❖ **Vitamin B7 (Biotin)**

Although the concentration range is wider, the relationship remains linear. Biotin is known for its role in fat and carbohydrate metabolism and in maintaining healthy skin and hair.

❖ **Vitamin B9 (Folic Acid) :**

The graph shows a clear linear relationship between the concentration of vitamin B9 (x-axis, µg/ml) and its absorbance (y-axis). It is evident that as the concentration increases, the absorbance increases proportionally in a linear manner, indicating good compliance with the Beer-Lambert Law. This suggests that absorbance can be used as a reliable indicator for determining the concentration of B9 in samples, supporting the use of this method for the quantitative analysis of folic acid in solutions.

❖ **Vitamin B12 (Cyanocobalamin) :**

This plot also demonstrates a linear relationship between the concentration of vitamin B12 and its absorbance. The concentration range is relatively narrow (approximately 0.05 to 0.10 µg/ml), yet the linear trend is clearly observed, indicating the precision and sensitivity of the analytical method used. The linear regression indicates that absorbance is directly

proportional to B12 concentration within this range, making this method suitable for the quantitative analysis of this vitamin in biological or nutritional samples.

The quantitative analysis of B vitamins in the methanol-water extract (MeH_2O) and a Multi B extract, both derived from Chamaeleo chamaeleon, revealed notable variations in their vitamin B profiles. The MeH_2O extract exhibited higher concentrations of vitamin B1 (thiamine, 27.14 µg/mg), vitamin B3 (niacin, 148.5 µg/mg), and vitamin B9 (folic acid, 2.59 µg/mg) compared to the Multi B extract, which contained 25.82 µg/mg, 138.01 µg/mg, and 2.44 µg/mg of the respective vitamins. These findings suggest that the methanol-water extract may serve as a richer natural source for specific B vitamins, particularly thiamine, niacin, and folic acid. Conversely, the Multi B extract showed elevated levels of vitamin B2 (riboflavin, 22.26 µg/mg), vitamin B6 (pyridoxine, 23.14 µg/mg), vitamin B7 (biotin, 1172.9 µg/mg), and vitamin B12 (cobalamin, 31.37 ηg/mg), surpassing those found in the MeH_2O extract (16.53 µg/mg, 18.19 µg/mg, 865.1 µg/mg, and 21.75 ηg/mg, respectively). These results indicate that while both extraction methods yield a broad spectrum of B vitamins, the Multi B extract may offer a more potent and diverse B-complex profile. Overall, Chamaeleo chamaeleon appears to be a promising novel source of natural B vitamins.

Table 06: Quantitative Vitamin B Content in Methanol-Water (MeH_2O) and Multi B Extracts of Chamaeleo *chamaeleon*

	Vitamins B content	
	MeH_2O	MultiB
B1 (µg/mg)	27.14	25.82
B2 (µg/mg)	16.53	22.26
B3 (µg/mg)	148.5	138.01
B6 (µg/mg)	18.19	23.14
B7 (µg/mg)	865.1	1172.9
B9 (µg/mg)	2.59	2.44
B12 (ηg/mg)	21.75	31.37

II.1.3. *In vitro* assays of *Chamaeleo chamaeleon* extracts

II.1.3.1. Antioxidant activity

The antioxidant potential of Chamaeleon-derived extracts (MeH_2O and Multi B) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with ascorbic acid (Vitamin C) as the positive control. Both extracts demonstrated dose-dependent free radical scavenging activity across the tested concentrations (14–20 µg/mL). At 14 µg/mL, MeH_2O and Multi B achieved DPPH inhibition rates of 19.6% and 18.2%,

respectively, while Vitamin C showed a markedly higher inhibition of 32.1%. The scavenging activity of the extracts increased with concentration, reaching maximum values of 56.6% (MeH_2O) and 62.0% (Multi B) at 20 µg/mL. These values, although lower than the 83.1% inhibition observed for Vitamin C, indicate substantial antioxidant capacity. The IC_{50} values were 19.45 µg/mL for MeH_2O and 18.80 µg/mL for Multi B, compared to 16.19 µg/mL for Vitamin C, reflecting relatively strong radical scavenging efficiency, particularly for the Multi B extract. These findings suggest that both Chamaeleon-derived extracts possess considerable antioxidant activity, which may contribute to their potential therapeutic applications in oxidative stress-related conditions.

Table 07: Antioxidant activity of *Chamaeleo chamaeleon* extracts.

Concentration (µg/mL)	DPPH %		
	MeH_2O	MultiB	Vit C
14	19.6	18.2	32.1
15	26.3	23.0	41.6
16	32.1	28.3	49.2
17	35.5	34.4	55.1
18	39.2	41.0	63.2
19	45.3	52	71.2
20	56.6	62	83.1
IC_{50} Values	19.45 µg/mL	18.80 µg/mL	16.19µg/Ml

II.1.3.2. Anti-inflammatory activity

The anti-inflammatory potential of MeH_2O and Multi B was assessed using the bovine serum albumin (BSA) denaturation inhibitory assay, with diclofenac sodium serving as the standard reference drug. Both extracts exhibited a concentration-dependent inhibition of BSA denaturation, indicating promising anti-inflammatory activity. At the lowest concentration tested (10 µg/mL), MeH_2O and Multi B inhibited BSA denaturation by 7.14% and 7.45%, respectively, compared to 4.33% inhibition by diclofenac. As concentrations increased, both extracts demonstrated progressively stronger inhibitory effects, reaching 72.64% (MeH_2O) and 69.30% (Multi B) at 20 µg/mL, closely approaching the inhibition shown by diclofenac (73.11%) at the same concentration. The IC_{50} values were calculated to be 17.41 µg/mL for MeH_2O and 18.21 µg/mL for Multi B, which are comparable to that of diclofenac (17.54 µg/mL), further supporting the extracts' efficacy. Overall, the results suggest that

Chamaeleon-derived extracts, particularly MeH_2O, possess significant anti-inflammatory properties.

Table 08: In vitro Anti-inflammatory activity of Chamaeleon-Derived Extracts (MeH_2O and Multi B) by BSA inhibitory assay

Concentration (µg/mL)	Percentage of BSA Inhibition %		
	MeH_2O	MultiB	Diclofenac
10	7.142	7.446	4.331
11	14.285	10.182	9.543
12	20.516	16.413	12.456
13	27.051	22.492	17.334
14	31.458	25.227	22.122
15	37.993	32.674	31.678
16	41.337	37.993	38.234
17	47.264	46.808	44.123
18	52.431	54.863	52.226
19	63.525	61.550	61.225
20	72.644	69.300	73.112
IC_{50} Values	17.41 µg/mL	18.21 µg/mL	17.54µg/mL

II.1.3.3. Phagocytic activity

The graph illustrates the phagocytic index (%) of two extracts (MeH_2O and Multi B), both derived from *Chamaeleo chamaeleon*, across four concentrations (0.062, 0.125, 0.25, and 0.37 mg/mL). The activity was compared to two controls: HBSS as a negative control (49.66%) and Zinc 0.5% as a positive control (75%).

MeH_2O extract exhibited a concentration-dependent increase in phagocytic activity, peaking at 0.25 mg/mL (71.42%), and then slightly declining at 0.37 mg/mL (61.85%). This pattern suggests a stimulatory effect that reaches an optimal concentration before potential saturation.

Multi B extract showed a different profile, with a drop at 0.125 mg/mL (32.38%) but a gradual increase thereafter, reaching 66.04% at 0.37 mg/mL. This delayed response might reflect a different mechanism of action or slower cellular uptake compared to MeH_2O.

Compared to the negative control (HBSS, 49.66%), both extracts demonstrated enhanced phagocytic activity at higher concentrations, indicating immune-stimulating

potential. However, only MeH_2O at 0.25 mg/mL approached the activity level of the positive control (Zinc 0.5mg/ml, 75%), suggesting its promising immunomodulatory effect.

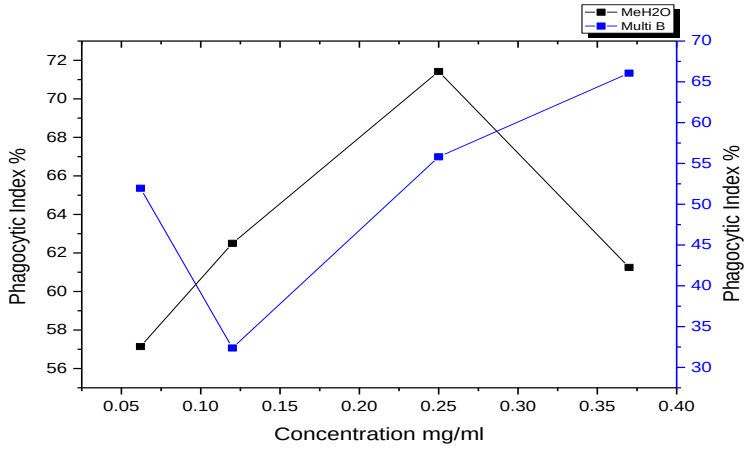


Figure 07: Phagocytic index of MeH_2O and Multi B at different concentrations.

II.1.3.4. Anti-complement activity

The anticomplement potential of *Chamaeleo chamaeleon* extracts was assessed using the hemolytic inhibition assay, in which inhibition zone diameters were measured and compared across various concentrations (0.2–1.0 mg/mL). Results are presented as mean inhibition zone diameters (mm ± SD) and benchmarked against both negative and positive controls.

At 0.2 mg/mL, the negative control exhibited no inhibition of complement activity (0.60 ± 0.00 mm), confirming the absence of any active compounds. The positive control at the same concentration produced a measurable inhibition zone (0.83 ± 0.10 mm), validating the sensitivity of the assay and serving as a reference for comparing the test samples.

The Multi B extract showed superior anticomplement activity across most tested concentrations. At 0.2 mg/mL, it produced a larger inhibition zone (0.93 ± 0.15 mm) than the positive control, indicating effective complement system interference even at low doses. Notably, the maximum inhibitory effect was observed at 0.4 mg/mL (1.20 ± 0.12 mm), exceeding all other groups and concentrations. While the activity slightly declined at higher concentrations—0.6 mg/mL (1.00 ± 0.00 mm), 0.8 mg/mL (0.80 ± 0.00 mm), and 1.0 mg/mL (0.73 ± 0.10 mm)—the extract maintained consistent inhibition.

In contrast, the methanolic extract (MeH_2O) exhibited moderate and more stable anticomplement activity. At 0.2 mg/mL, it was comparable to the positive control (0.90 ± 0.17 mm), and showed minor variations across concentrations: 0.4 mg/mL (0.83 ± 0.06 mm), 0.6 and 0.8 mg/mL (both 0.73 ± 0.10 mm), and 1.0 mg/mL (0.80 ± 0.00 mm). The relatively

uniform inhibition zones suggest a plateau effect and indicate the presence of bioactive compounds capable of interfering with complement activation, though likely at lower potency compared to the Multi B formulation.

Overall, the results highlight the strong anticomplement activity of Multi B, particularly at mid-range concentrations, and support the therapeutic potential of *Chamaeleo chamaeleon* derivatives in modulating innate immune responses.

Table 09: Anticomplement activity of *Chamaeleo chamaeleon* extracts and controls

Expressed as inhibition zone (mm ± SD, n = 3)

Groups	Concentration (mg/mL)	Inhibition Zone (mm ± SD)
ACP Control	0.2	0.60 ± 0.00
Heparin Control	0.2	0.83 ± 0.10
Multi B	0.2	0.93 ± 0.15
	0.4	1.2 ± 0.12
	0.6	1.00 ± 0.00
	0.8	0.8 ± 0.00
	1.0	0.73 ± 0.1
MeH_2O	0.2	0.90 ± 0.17
	0.4	0.83 ± 0.06
	0.6	0.73 ± 0.1
	0.8	0.73 ± 0.1
	1.0	0.80 ± 0.00

II.1.4. In vivo immunomodulatory activity

II.1.4.1. Phagocytic Index

To evaluate the immunomodulatory potential of *Chamaeleo chamaeleon* extracts, phagocytic activity was assessed in rats using the carbon clearance assay. Rats were grouped and treated with a reference immunostimulant (Neuf B), methanolic-water extract (MeH_2O), or a multi-vitamins B formulation (Multi B), and compared to control and ink-only groups. The phagocytic index was calculated and expressed as mean ± standard deviation (SD). The ink group exhibited an increased phagocytic index compared to the control (0.967 ± 0.429 vs. 0.796 ± 0.181, $p < 0.05$). Notably, treatment with MeH_2O and Multi B extracts significantly enhanced the phagocytic index (1.126 ± 0.245 and 1.603 ± 0.455, respectively; $p < 0.001$ vs. control), indicating pronounced immunostimulant properties. Conversely, the reference drug Neuf B did not elicit a significant change compared to the control group ($p > 0.05$). These results suggest that both the methanolic-water and multi-vitamins B of *Chamaeleo*

chamaeleon may enhance innate immune responses more effectively than the standard reference drug under the tested conditions.

Table 10: Effect of *Chamaeleo chamaeleon* extracts and reference drug Neuf B on phagocytic activity in rats assessed by carbon clearance test.

Treated groups	Phagocytic index	Mean phagocytic index K
Control	0.955, 0.833, 0.600	0.796 ± 0.181
Ink	0.471, 1.208, 1.222	0.967 ± 0.429 a
Ink+MeH2O	1.353, 1.158, 0.867	1.126 ± 0.245 c ***
Ink + Multi B	1.875, 1.077, 1.857	1.603 ± 0.455 c ***
Ink + Neuf B	0.750, 0.706, 0.786	0.747± 0.040 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: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

II.1.4.2. Organ Weight Index

The organ weight index, calculated as the ratio of organ weight to body weight, is a useful biomarker for assessing systemic effects of toxic agents and immunomodulatory interventions. In this study, changes in spleen and thymus indices were evaluated across experimental groups following exposure to ink and treatments including a methanol–water extract of Chamaeleon (MeH_2O), a vitamin B-rich extract from Chamaeleon (Multi B), and a reference drug, Neuf B.

In the **spleen**, the control group showed a weight index of $0.23 \pm 0.005\%$. Ink exposure led to a non-significant increase to $0.27 \pm 0.006\%$, suggesting a mild splenic response, possibly related to immune activation. Further increases were observed in the Ink + MeH_2O ($0.30 \pm 0.017\%$), Ink + Multi B ($0.29 \pm 0.02\%$), and Ink + Neuf B ($0.28 \pm 0.05\%$) groups, although none reached statistical significance. These upward trends suggest that Chamaeleon extracts and the reference drug may contribute to enhanced splenic activity, potentially reflecting an ongoing immune response, expansion of lymphoid tissue, or increased blood cell turnover.

For the **thymus**, the control index was $0.14 \pm 0.008\%$. Ink exposure significantly increased the thymus index to 0.21 ± 0.01 ($p < 0.05$), indicating possible thymic hyperplasia or increased T-cell development in response to systemic stress. The thymus index remained elevated in all treatment groups: $0.21 \pm 0.021\%$ in the Ink + MeH_2O group, $0.23 \pm 0.01\%$ in the Ink + Multi B group, and $0.22 \pm 0.008\%$ in the Ink + Neuf B group. However, these differences were not statistically significant when compared to the control. This persistent

elevation implies that the treatments did not reverse the ink-induced thymic changes, but may have supported or maintained the heightened thymic activity.

The data demonstrate that ink exposure alone increases the thymus index significantly, with a trend toward increased spleen index. The Chamaeleon-derived treatments and the reference drug Neuf B appeared to maintain or slightly enhance organ indices, suggesting mild immune activation or preservation of lymphoid organ function. The absence of statistically significant reductions in organ size across treatments implies a lack of overt toxicity and supports the relative safety of these interventions under the tested conditions.

Table 11: Organ weight Index of different experimental groups

	Organ Weight Index %	
	Spleen	Thymus
Control	0.23±0.005	0.14±0.008
Ink	0.27±0.006 ^{NS}	0.21±0.01 ^{a}
Ink+MeH$_2$O	0.30±0.017 ^{NS}	0.21±0.021 $^{a\ NS}$
Ink + multi B	0.29±0.02 ^{NS}	0.23±0.01 ^{NS}
Ink + neuf B	0.28±0.05 ^{NS}	0.22±0.008 ^{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: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

II.1.4.3. Hematological analysis

The present study examined the effects of ink exposure and various treatments including a methanol-water extract of Chamaeleon (MeH$_2$O), a vitamin B complex (Multi B), and a vitamin B-based medicament (Neuf B) on leukocyte subpopulations. The hematological parameters evaluated included the percentages of polynuclear neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Comparisons were made against a control group, with statistical significance assessed using standard notations.

Polynuclear neutrophil levels increased modestly in the ink group ($20.33 \pm 0.88\%$) compared to the control ($13.0 \pm 1.73\%$), though the change was not statistically significant. However, a significant and robust elevation was observed in the Ink + MeH$_2$O group ($66.67 \pm 2.33\%$; $p < 0.01$ vs. control, *** vs. ink), indicating a strong neutrophilic response likely related to immune stimulation. Multi B and Neuf B treatments also increased neutrophil levels ($30.0 \pm 0.57\%$ and $25.0 \pm 4.5\%$, respectively), with the former showing a statistically significant increase. These findings suggest that both Chamaeleon extract and vitamin B complexes can enhance neutrophil recruitment.

Polynuclear eosinophils, which are often involved in allergic reactions or parasitic responses, were significantly elevated following ink exposure ($8.67 \pm 1.2\%$; $p < 0.01$). This trend continued with MeH_2O, Multi B, and Neuf B treatments ($13.0 \pm 1.5\%$, $15.0 \pm 1.15\%$, and $11.0 \pm 0.57\%$, respectively), with the latter two achieving statistical significance. These findings imply that both the ink and the administered extracts may stimulate eosinophilic responses, potentially through immune activation.

Polynuclear basophil percentages remained largely unchanged across treatment groups, with only the Ink + MeH_2O group showing a statistically significant increase ($5.0 \pm 0.57\%$; $p < 0.05$ vs. control). Basophils play a role in allergic responses and inflammation; therefore, this result may reflect a minor allergic or histamine-mediated component in response to the Chamaeleon extract, while other treatments had no meaningful impact on this cell population.

Lymphocyte levels were significantly elevated across all ink-treated groups, with the most notable increases seen in the Ink + MeH_2O ($62.0 \pm 1.15\%$) and Ink + Multi B ($64.67 \pm 2.6\%$) groups, compared to control values ($25.0 \pm 1.73\%$). These changes ($p < 0.01$ vs. control) indicate a marked lymphoproliferative response, suggestive of adaptive immune system activation. The Neuf B treatment also significantly increased lymphocyte levels ($46.0 \pm 3.78\%$; $p < 0.05$), though to a lesser extent, reflecting a potential stimulatory effect on lymphocyte maturation or activation.

Monocyte percentages were also markedly affected. Ink alone increased monocytes significantly ($24.3 \pm 1.45\%$; $p < 0.01$ vs. control), suggesting a pro-inflammatory response. This effect was further intensified in the Multi B and Neuf B groups ($41.67 \pm 1.2\%$ and $39.0 \pm 1.0\%$, respectively; $p < 0.001$ and $p < 0.01$), indicating that these treatments may promote monocytosis, possibly to support phagocytic activity and tissue repair. In contrast, the Ink + MeH_2O group showed a reduction in monocyte percentage ($12.0 \pm 1.15\%$; $p < 0.05$), suggesting an anti-inflammatory or modulatory effect of the Chamaeleon extract.

The data indicate that exposure to ink induces an inflammatory response, as evidenced by increases in neutrophils, lymphocytes, and monocytes. The Chamaeleon methanol-water extract (MeH_2O) enhanced neutrophil and lymphocyte counts while suppressing monocyte elevation, implying complex immunomodulatory effects. In contrast, vitamin B formulations (Multi B and Neuf B) consistently amplified immune cell responses, particularly lymphocytes and monocytes, pointing to their potential use as immune enhancers.

Table 12: Effects of Ink, Chamaeleon-Derived Extracts (MeH_2O and Multi B), and a Reference Drug (Neuf B) on Differential Leukocyte Counts in Peripheral Blood

	Polynuclear neutrophils %	Polynuclear eosinophils %	Polynuclear basophils %	Lymphocytes %	Monocytes %
Control	13.0±1.73	5.0±1.0	2.0±0.57	25.0±1.73	6.67±0.66
Ink	20.33±0.88 NS	8.67±1.2 b	3.67±0.33 NS	32.67±1.45 NS	24.3±1.45 b
Ink+MeH_2O	66.67±2.33 b***	13.0±1.5 NS	5.0±0.57 a	62.0±1.15 b	12.0±1.15 a*
Ink + multi B	30.0±0.57 b*	15.0±1.15 a	4.67±0.66 NS	64.67±2.6 b	41.67±1.2 b***
Ink + neuf B	25.0±4.5 NS	11.0±0.57 a	2.33±0.33 NS	46.0±3.78 a	39.0±1.0 c**

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: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

II.1.4.4. Biochemical parameters

This study evaluated the impact of ink exposure and subsequent treatments—including Chamaeleon methanol–water extract (MeH_2O), a vitamin B-rich extract (Multi B), and a reference drug (Neuf B)—on key biochemical markers: fasting blood sugar (FBS), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and C-reactive protein (CRP). These parameters are essential for assessing metabolic status, liver function, and systemic inflammation.

The **fasting blood sugar (FBS)** level in the control group was 0.86 ± 0.01 g/L. Ink exposure elevated this to 1.56 ± 0.48 g/L, although the difference was not statistically significant. Notably, the Ink + Neuf B group showed a further increase to 1.67 ± 0.09 g/L, reaching statistical significance ($p < 0.05$ vs. control). Conversely, treatment with Multi B significantly reduced FBS levels to 0.74 ± 0.02 g/L ($p < 0.01$ vs. control), suggesting a hypoglycemic or glucose-stabilizing effect of this Chamaeleon-derived vitamin B extract. MeH_2O treatment also reduced FBS (1.32 ± 0.05 g/L) relative to the Ink group, though not significantly.

Liver function enzymes, AST and ALT, remained mostly unchanged across groups. AST levels were consistent, ranging from 69.0 to 83.67 U/L, with no significant differences observed. ALT levels also remained close to control values, with one exception: the Ink + Multi B group exhibited a significant decrease in ALT (70.67 ± 2.1 U/L; $p < 0.05$ vs. control,

* vs. Ink), indicating a potential hepatoprotective effect of this extract. These results suggest that neither ink nor the tested treatments caused significant liver enzyme elevation, and that Multi B may help reduce hepatic stress or enzyme leakage.

In terms of systemic inflammation, measured by **CRP (C-reactive protein)**, the control group had a baseline value of 1.86 ± 0.28 mg/L. Ink exposure markedly increased CRP to 4.03 ± 0.061 mg/L ($p < 0.01$), indicating an acute inflammatory response. Treatment with MeH_2O (2.26 ± 0.08 mg/L), Multi B (2.2 ± 0.11 mg/L), and Neuf B (2.3 ± 0.11 mg/L) significantly reduced CRP levels compared to the ink group, with strong statistical significance observed ($p < 0.01$ to $p < 0.001$). These results confirm that all three treatments attenuated the ink-induced inflammatory response, with Multi B showing the most pronounced CRP-lowering effect.

Table 13: Glycemia, CRP, liver function parameters of different experimental groups

	FBS (g/L)	AST (U/L)	ALT (U/L)	CRP (mg/L)
Control	0.86±0.01	81.33±3.33	86.0±2.3	1.86±0.28
Ink	1.56±0.48 ^{NS}	81.67±6.17 ^{NS}	79.67±3.1 ^{NS}	4.03±0.061 ^{b}
Ink+MeH_2O	1.32±0.05 ^{NS}	83.67±4.66 ^{NS}	84.3±1.4 ^{NS}	2.26±0.08 ^{NS**}
Ink + multi B	0.74±0.02 $^{b\ NS}$	69.0±0. 57 ^{NS}	70.67±2.1 $^{a\ *}$	2.2±0.11 ^{NS***}
Ink + neuf B	1.67±0.09 $^{a\ NS}$	80.0±2.88 ^{NS}	84.0±4.5 ^{NS}	2.3±0.11 $^{NS\ **}$

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: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

II.1.4.5. Histological study

A. Bone marrow

1. Control (C):

The bone marrow tissue displays a normal histoarchitecture with a balanced density of white blood cells, including precursor myeloid cells, lymphocytes, and immature neutrophils. No signs of abnormal activation or inflammation are noted, indicating a physiological state of bone marrow immune activity .

2. Ink :

A moderate increase in leukocyte density is observed, particularly neutrophils and monocytes. These cells exhibit features of activation, such as multilobed nuclei and granular cytoplasm, suggesting a localized inflammatory response to the ink stimulus. The presence of particulate matter likely triggers innate immune activation, including phagocytosis by neutrophils and macrophage precursors .

3. Neuf B (Vitamin B Complex):

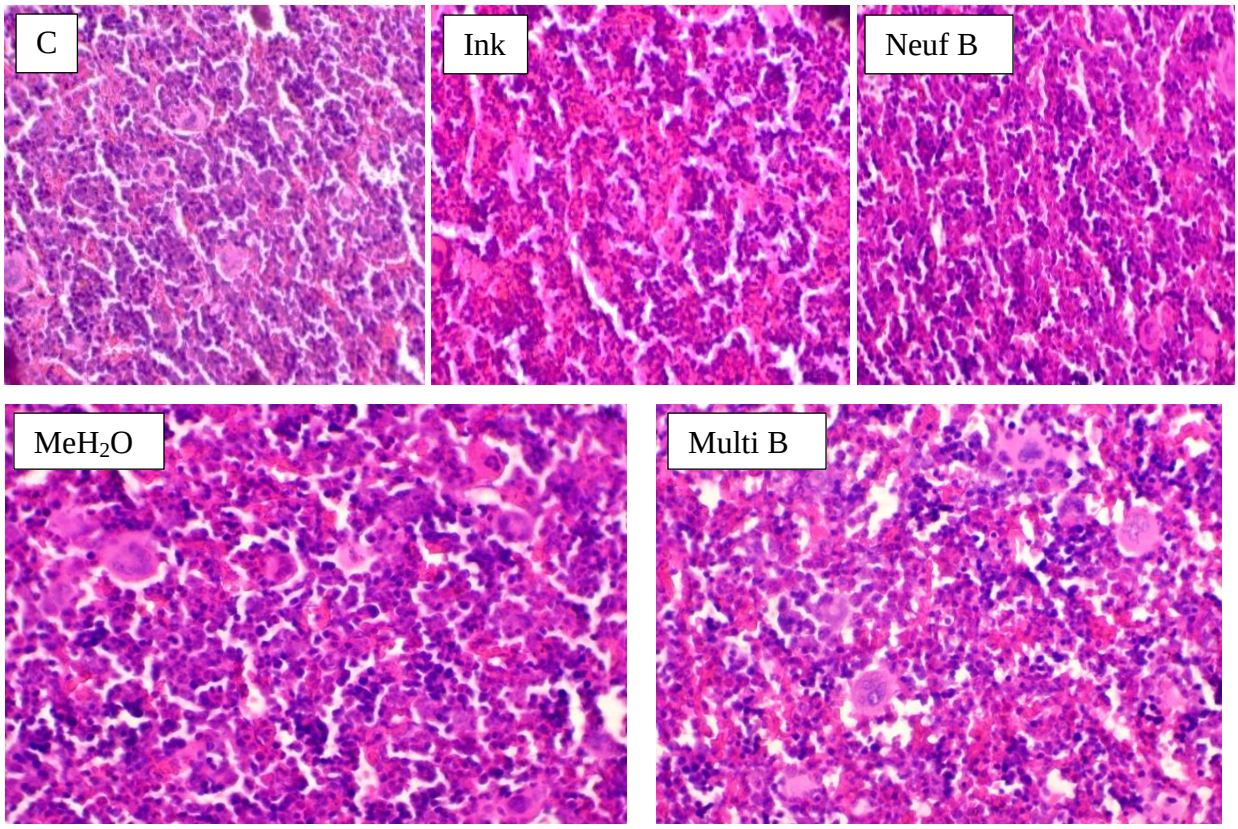
The leukocytes appear balanced, with a slight increase in lymphocytes. This may be due to the known immunomodulatory effects of certain B vitamins (e.g., B6 and B12) on hematopoiesis and lymphocyte development. The bone marrow reflects mild immunological activation without evidence of cellular stress or inflammation .

4. MeH_2O (Methanol + Water Extract):

A noticeable rise in white blood cell count is evident, particularly neutrophils and monocytes. These cells exhibit larger nuclei and dense cytoplasm, consistent with immune activation.

5. Multi B:

This group demonstrates the highest leukocyte activity and density. Numerous activated neutrophils and macrophages are observed, with enlarged nuclei and expanded cytoplasm, indicative of enhanced phagocytic and immune activity. These findings suggest that the Multi B formulation exerts a potent stimulatory effect on the innate immune system, likely due to a synergistic action of its bioactive components .



B. Spleen

A histological analysis was conducted on spleen sections treated with various substances, using Hematoxylin and Eosin (H&E) staining, in order to evaluate structural and cellular changes—particularly regarding the distribution and density of lymphocytes within the white pulp regions.

- **Control Group (C):**

The spleen sections in this group exhibited a normal histological architecture, with well-organized lymphoid follicles in the white pulp densely populated with lymphocytes. This indicates a healthy immune activity and the absence of pathological or inflammatory effects.

- **Ink:**

A slight reduction in lymphocyte density within the follicles was observed, along with minor alterations in the structural organization of the white pulp. These changes likely reflect a non-specific immune response to the injected substance, suggesting a mild inflammatory reaction.

- **Neuf B Group (single-compound drug):**

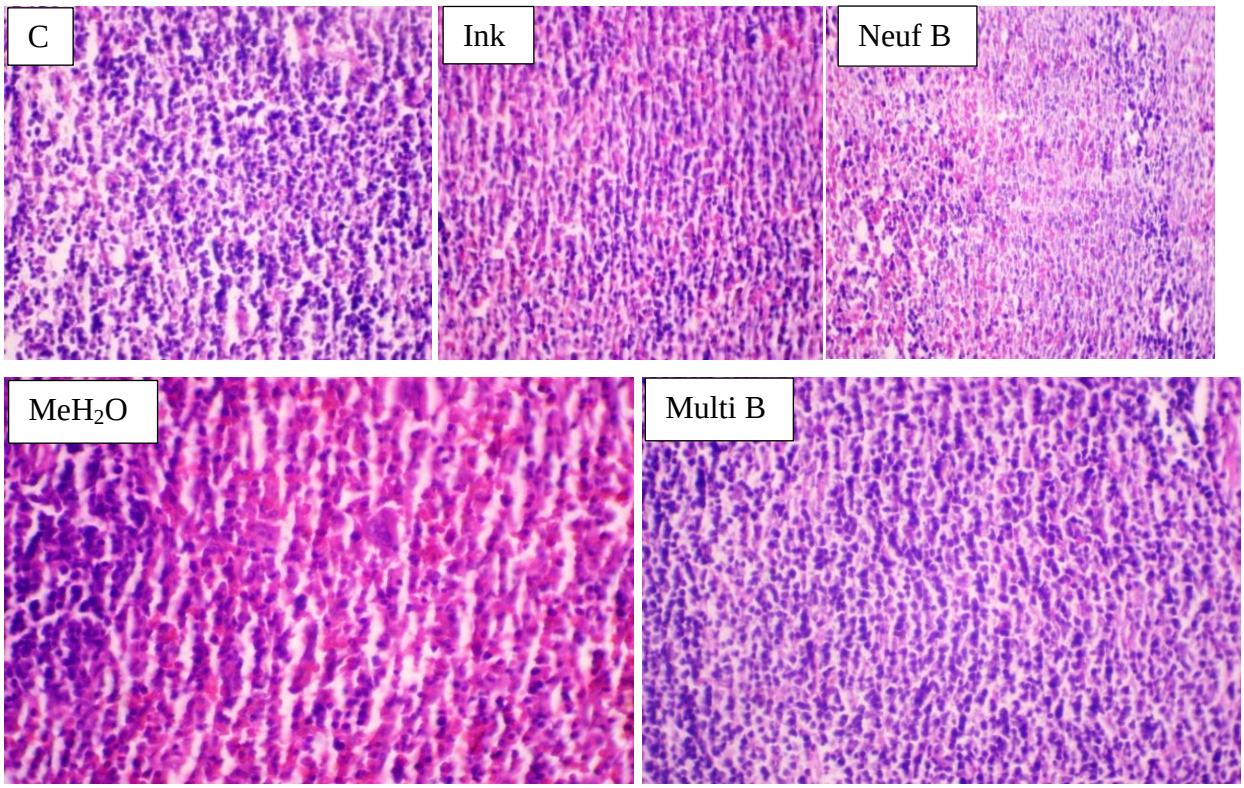
The tissue sections showed a marked decrease in lymphocyte numbers and disorganization of the white pulp follicles, with relatively empty interstitial spaces. These findings suggest a possible immunosuppressive or cytotoxic effect of this compound on lymphoid tissue.

- **MeH_2O Group (methanol/water solvent):**

The tissue displayed a generally preserved splenic architecture, with moderate cellular density and relatively organized lymphoid follicles. No significant indicators of toxicity or structural disruption were observed, suggesting the solvent itself does not adversely affect lymphocytes.

- **Multi B Group (multi-compound drug):**

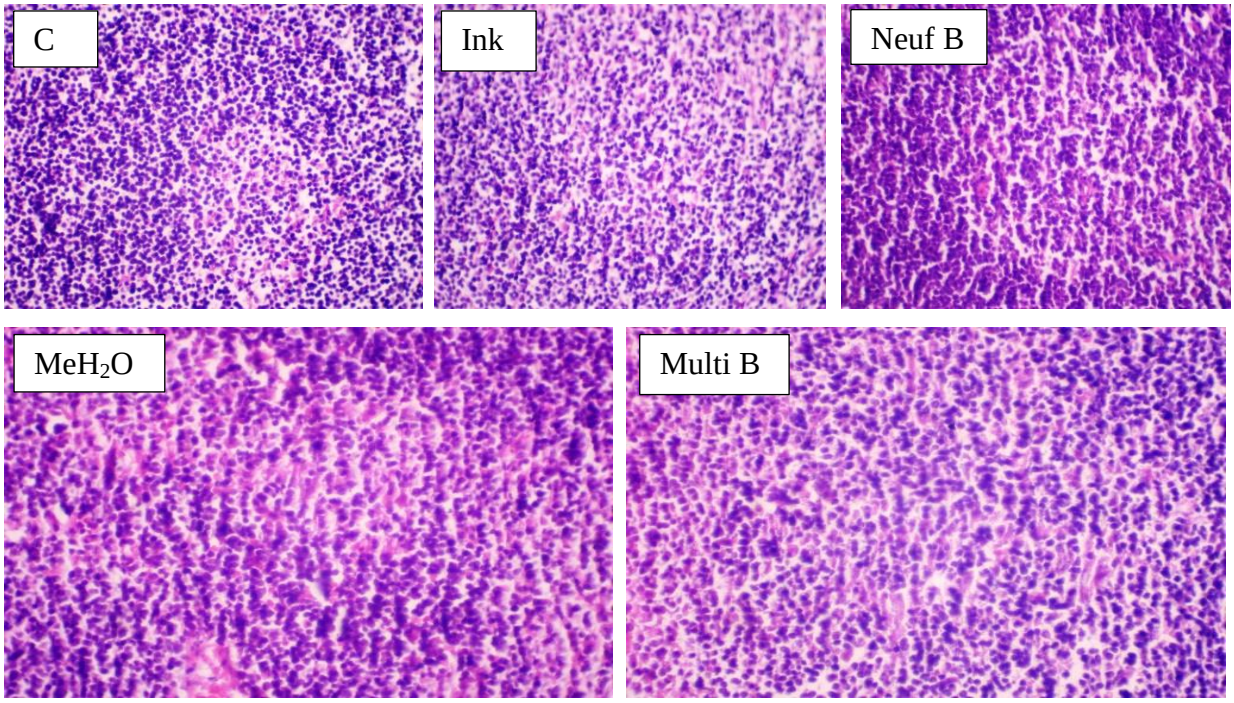
The sections revealed noticeable improvement in white pulp architecture, with more distinct and densely populated lymphoid follicles compared to the Neuf B group. This suggests that the multi-compound formulation may exert a protective or supportive effect on immune cells, enhancing splenic immune competence and mitigating potential toxic effects.



C. Thymus

- **C (Control):** Dense tissue composed of small, closely packed lymphocytes with dark, round nuclei and uniform distribution, with a clear reduction in intercellular spaces.
- **Ink:** Lymphocytes remain dense but show slight cellular gaps and less homogeneous distribution compared to the control, with slight separation between cells.
- **Neuf B:** Lymphocytes are scattered, with clearly visible intercellular spaces and variability in nuclear distribution, along with a marked decrease in cell density.
- **MeH_2O:** A noticeable decrease in cell density is observed, with lymphocytes showing irregular distribution and a large number of white spaces between cells.
- **Multi B:** Lymphocytes are moderately dense, spread with a nearly uniform distribution, showing some intercellular spaces, and the nuclei remain clear and well-defined.

❖


C
Ink
Neuf B
MeH2O
Multi B

II.2. Discussion

The results indicate that the methanol/water mixture (MeH_2O) provides a significantly higher extraction yield (19.1%) compared to MultB (7.4%). This difference can be explained by the variation in solvent polarity. Methanol mixed with water forms a medium-to-high polarity solvent, making it more effective in dissolving bioactive molecules commonly found in *chamaeleo chamaeleon* extracts (**Dai & Mumper, 2010**).Moreover, the presence of water in the methanol mixture enhances cell wall permeability and improves diffusion and mass transfer, facilitating the release of target compounds into the solvent system **(Azwanida, 2015)**. In contrast, MultB—whose chemical composition is not specified here—may be less polar or may contain components that hinder efficient extraction. Previous studies have shown that methanol/water mixtures are among the most effective solvents for extracting antioxidants and bioactive compounds, outperforming pure organic solvents or those with lower polarity **(Do et al., 2014)**. Methanol is also widely used in experimental extraction due to its high efficiency and relatively low cost.

The current findings revealed significant differences in the quantitative composition of B vitamins between the two different extracts of *Chamaeleo chamaeleon* (Methanol-Water extract [MeH_2O] and Multi B extract). The MeH_2O extract exhibited higher concentrations of vitamin B1 (thiamine), B3 (niacin), and B9 (folic acid) compared to the Multi B extract. This superiority suggests that the methanol-water solvent mixture may be more efficient in extracting these specific vitamins, possibly due to differences in polarity or the bio-distribution of these vitamins in the tissue source.

Previous studies have shown that vitamin extraction efficiency greatly depends on the type of solvent used. Similar results have indicated that polar solvents like methanol or methanol-water mixtures are particularly effective in extracting thiamine and niacin from animal sources **(Zhang et al., 2021; Santos et al., 2020)**.Conversely, the Multi B extract was characterized by higher concentrations of vitamins B2 (riboflavin), B6 (pyridoxine), B7 (biotin), and B12 (cobalamin). This suggests that the Multi B extract may have been formulated or processed in a way that enhances the recovery of these vitamins, possibly through the inclusion of stabilizing compounds or optimized extraction conditions. This finding aligns with previous studies showing that vitamin B12(cobalamin) in particular is highly sensitive to extraction conditions and requires specific media for stability **(Allen, 2008; Knez et al., 2017)**.

The current study demonstrated that the MeH_2O and Multi B extracts derived from *Chamaeleo chamaeleon* exhibit significant radical scavenging activity as measured by the

DPPH assay, a widely used and reliable method for assessing antioxidant potential in natural compounds. The results revealed a dose-dependent behavior, indicating that the antioxidant activity increased proportionally with the concentration. Notably, the Multi B extract showed slightly higher radical scavenging efficiency compared to MeH_2O at most concentrations, which may suggest a higher content of bioactive (**Brand-Williams et al., 1995**).

At the lowest tested concentration (14 µg/mL), DPPH inhibition by MeH_2O and Multi B reached 19.6% and 18.2%, respectively, which were lower than that of Vitamin C (32.1%). As the concentration increased to 20 µg/mL, the inhibition rates rose to 56.6% for MeH_2O and 62.0% for Multi B, whereas Vitamin C reached 83.1%, reflecting the strong standard antioxidant activity of ascorbic acid. These findings indicate that, although less potent than the standard, the extracts possess substantial radical scavenging capabilities **(Rice-Evans et al., 1997**).The IC_{50} values, representing the concentration required to inhibit 50% of DPPH radicals, further support the antioxidant potential of the extracts. The IC_{50} was approximately 19.45 µg/mL for MeH_2O and 18.80 µg/mL for Multi B, which are relatively close to that of Vitamin C (16.19 µg/mL). These results underscore the promising antioxidant efficiency of the extracts, especially Multi B, which showed activity approaching that of the standard. Such data highlight the potential application of these natural extracts as antioxidant agents in pharmaceutical or food industries, particularly in combating oxidative stress-related disorders **(Halliwell & Gutteridge, 2015).**

Protein denaturation is a well-established mechanism in inflammation, as it can lead to the production of autoantigens that trigger inflammatory responses. Therefore, inhibiting protein denaturation is considered a relevant marker for anti-inflammatory potential **(Grant et al., 1970**).The current findings demonstrate that both MeH_2O and Multi B extracts possess strong anti-inflammatory activity, as evidenced by their ability to inhibit BSA denaturation in a dose-dependent manner. Notably, MeH_2O extract exhibited slightly superior performance, with a lower IC_{50} value (17.41 µg/mL) than Multi B (18.21 µg/mL), closely matching the reference drug diclofenac (17.54 µg/mL). These values highlight the potential therapeutic value of these natural extracts.The observed anti-inflammatory activity may be attributed to the presence of vitamin B B9 (folic acid) B7 (biotin,)compounds in the extracts, which are known for their ability to stabilize proteins and inhibit inflammatory mediators **(Owoyele et al., 2005; Kumar et al., 2011)**. Methanol/water mixtures (as in MeH_2O) are particularly effective at extracting such bioactive compounds, likely explaining the stronger performance of this extract compared to Multi B **(Do et al., 2014).**These results align with other studies reporting BSA denaturation inhibition as a reliable in vitro model for screening anti-

inflammatory agents, especially those derived from *chamaeleo chamaeleon* **(Sakat et al., 2010)**.

The phagocytic index results illustrate distinct immunomodulatory behaviors of the MeH_2O and Multi B extracts of *Chamaeleo chamaeleon*. Phagocytosis is a central process in innate immunity, involving the uptake and destruction of pathogens by immune cells such as macrophages and neutrophils. Compounds that enhance phagocytic activity are considered potential immunostimulants and are of growing interest in natural product-based therapeutic development **(Delves et al., 2017).**The MeH_2O extract demonstrated a clear concentration-dependent stimulatory effect on phagocytic activity. The response peaked at 0.25 mg/mL with a phagocytic index of 71.42%, approaching the activity level of the positive control (Zinc 0.5%, 75%). This indicates that the MeH_2O extract contains bioactive constituents that activate phagocytic cells efficiently. The slight decline observed at 0.37 mg/mL (61.85%) may suggest the onset of a saturation point or feedback regulation in macrophage activation **(Chandrasekaran et al., 2011)**.In contrast, the Multi B extract exhibited a biphasic response. It showed an initial decline in activity at 0.125 mg/mL (32.38%), followed by a gradual increase reaching 66.04% at 0.37 mg/mL. This delayed stimulatory effect suggests that the bioactive compounds in Multi B may require metabolic activation, have slower uptake, or act through indirect immune pathways, such as cytokine induction **(Mahima et al., 2012)**. The lower initial activity could also reflect cytotoxic effects at lower concentrations that are overcome at higher doses.When compared to the negative control (HBSS, 49.66%), both extracts significantly enhanced phagocytic activity at higher concentrations, confirming their potential as immunostimulants. However, MeH_2O appeared more potent and consistent, particularly at 0.25 mg/mL. This aligns with previous findings that *chamaeleo chamaeleon*-derived can modulate immune functions by enhancing phagocytosis and reactive oxygen species (ROS) generation (**Kim et al., 2010).**

The results obtained from the hemolytic inhibition assay demonstrate that *Chamaeleo chamaeleon* extracts possess notable anti-complement activity, indicating their potential role in modulating innate immune responses. The complement system is a key component of the innate immune defense, playing a pivotal role in inflammation and microbial clearance. However, its overactivation is associated with several inflammatory and autoimmune conditions, making it a critical target for therapeutic intervention (**Ricklin et al., 2010**).Among the tested extracts, the Multi B formulation exhibited superior complement inhibitory effects. At a concentration of 0.4 mg/mL, it showed the highest inhibition zone (1.20 ± 0.12 mm), outperforming both the positive control and other extract types. This

suggests the presence of potent bioactive compounds capable of interfering with complement activation at relatively low to moderate concentrations. Compounds such as vitamin B9(folic acid) B12(cobalamin) and known for their immunomodulatory properties, may contribute to this activity **(Zhang et al., 2009).**In contrast, the methanolic extract (MeH_2O) displayed moderate and more consistent anti-complement effects across concentrations. While its inhibition zones were comparable to the positive control at low concentrations (e.g., 0.90 ± 0.17 mm at 0.2 mg/mL), they remained relatively stable across higher concentrations. This plateau effect might indicate receptor saturation, limited solubility of active compounds, or weaker inhibitory kinetics **(Piozzi & Bruno, 2011)**.The clear distinction in activity between the two extracts underscores the importance of extraction methods in determining the efficacy of vitamin B These findings align with growing interest in natural sources for complement-targeted therapies, particularly those with lower toxicity and higher specificity than synthetic alternatives (**Markiewski & Lambris, 2007)**.

The results of the carbon clearance assay demonstrate the immunostimulatory potential of *Chamaeleo chamaeleon* extracts in enhancing innate immune responses in rats by increasing phagocytic activity. An increase in the phagocytic index is considered a key indicator of macrophage activation, which forms one of the primary lines of innate immune defense (**Tizard, 2018)**. The ink group alone exhibited a moderate increase in phagocytic activity compared to the control group, confirming the effect of carbon ink as a stimulant of the immune response.

More importantly, treatment with the methanolic-water extract (MeH_2O) and the multi-vitamin B formulation (Multi B) led to a statistically significant increase in the phagocytic index compared to the control group ($p < 0.001$). This indicates that these formulations possess clear immunostimulant properties that surpass those of the reference drug Neuf B, which did not induce a statistically significant change ($p > 0.05$). This may reflect differences in the mechanism of action or chemical composition between natural extracts and conventional pharmaceutical agents **(Delves et al., 2020).**

These findings support the hypothesis that *Chamaeleo chamaeleon* extracts contain biologically active compounds capable of stimulating the immune system, potentially by activating surface receptors on macrophages or enhancing the production of cytokines associated with inflammatory responses. Bioactive compounds such as vitamin B1(thiamine) B2(riboflavin), may be contributing to this observed effect, as reported in previous studies on natural immunomodulators (**Patwardhan et al., 2005).**

The organ weight index, particularly of the spleen and thymus, is an essential preliminary tool for evaluating systemic effects of toxic agents or immunomodulatory therapies. Changes in organ weights may reflect conditions such as inflammation, hyperplasia, or immune suppression, making them significant indicators in preclinical toxicity studies **(Michael et al., 2007).**

Ink exposure showed a slight but non-significant increase in spleen weight, which may suggest limited immune stimulation. This increase could be related to lymphoid tissue expansion or an increase in immune cell populations in response to an external stimulus **(Ramos et al., 2006)**.

Chamaeleo chamaeleon extracts showed slight increases in spleen index compared to the ink group, though without statistical significance. This suggests that Chamaeleon extracts may contribute to mild immune activation or support spleen function without exhibiting overt toxicity **(Wagner & Ulrich-Merzenich, 2009)**.

A significant increase in thymus weight following ink exposure indicates a clear immune response. This may reflect increased T-cell production or hyperplasia due to immune stress or inflammation **(Roth & Tulis, 1981)**.

The persistence of elevated thymus indices in treatment groups suggests that these substances did not reverse the ink-induced effect, but they may have helped maintain immune activity or protected thymic structure **(Kemper et al., 2001)**.

The absence of reductions in organ weights across the treatment groups indicates a lack of observable toxicity for the *chamaeleo chamaeleon* extracts and the reference drug, supporting their potential safe use as immunomodulatory agents **(WHO, 2004; EMA, 2006).**

The results reveal significant variations in leukocyte subpopulations in response to ink exposure and subsequent treatments, reflecting distinct immunomodulatory profiles.

Neutrophils represent the first line of defense in the innate immune response, participating in phagocytosis and inflammation. A modest increase was observed in the ink group, suggesting a mild inflammatory reaction. However, the substantial and statistically significant elevation in the Ink + MeH_2O group (66.67%) indicates a strong innate immune stimulation

Studies suggest that animal extracts such as Chamaeleon may activate innate immunity by enhancing cytokine production and neutrophil activation **(Zhou et al., 2020).**

Additionally, both Multi B and Neuf B increased neutrophil levels, highlighting the potential of B vitamins to enhance innate immune functions.

Research has shown that B vitamins, particularly B6(pyridoxine) and B12(cyanocobalamin), influence white blood cell production and maturation **(Kennedy, 2016).**

The significant increase in eosinophils after ink exposure (8.67%) suggests an immune response possibly linked to allergenic or toxic components. The increase continued with all treatments, particularly Multi B (15.0%), indicating activation of eosinophils, which are involved in allergic and antiparasitic responses.

This is supported by evidence that certain animal or vitamin supplements can enhance eosinophil production via Th2 pathways and IL-5 cytokine signaling **(Rothenberg, 2004)**.

Basophil percentages remained largely stable, except for a statistically significant increase in the Ink + MeH_2O group. These cells play roles in allergic reactions and histamine release. The change may reflect a mild histamine-mediated effect from the pt extract, while other treatments had no major impact.

Lymphocyte levels significantly increased in all ink-treated groups, especially Ink + MeH_2O and Ink + Multi B, suggesting strong adaptive immune activation.

Lymphocytes, especially T and B cells, proliferate upon antigen exposure. Vitamin B6(pyridoxine) is essential for T cell differentiation and proliferation (**Mocchegiani et al., 2014**).

Ink exposure significantly elevated monocyte levels (24.3%), indicating an inflammatory and phagocytic response. Interestingly, Ink + MeH_2O reduced monocyte percentages (12.0%), suggesting an anti-inflammatory or immunoregulatory effect.

This may be due to the presence of vitamin in Chamaeleon extracts, known for their anti-inflammatory and antioxidant properties (**Zhang et al., 2016).**

In contrast, Multi B and Neuf B markedly increased monocyte levels, potentially enhancing phagocytic and tissue-repair function

This study aimed to evaluate the biochemical effects of ink exposure and potential treatments derived from Chamaeleon (MeH_2O and Multi B), in comparison with the reference drug Neuf B, by analyzing key markers:

fasting blood sugar (FBS), liver enzymes (AST and ALT), and C-reactive protein (CRP) a key indicator of systemic inflammation.

Ink exposure led to an apparent increase in fasting blood glucose compared to the control group, indicating a possible disruption in glucose homeostasis, potentially linked to oxidative stress or inflammation induced by ink exposure **(Yin et al., 2014**). Notably, treatment with the Multi B extract significantly reduced blood glucose (0.74±0.02 g/L; $p <$

0.01), suggesting a hypoglycemic or glucose-stabilizing effect. This may be attributed to the extract's content of B vitamins such as B1(thiamine) and B6 (pyridoxine), which play a regulatory role in carbohydrate metabolism **(Volkert et al., 2010)**. MeH_2O also reduced glucose levels relative to the Ink group, although without statistical significance.

Liver enzyme levels remained largely stable across groups, suggesting that ink exposure did not induce notable hepatocellular damage. However, the Ink + Multi B group showed a significant reduction in ALT levels (70.67±2.1 U/L; $p < 0.05$), indicating a potential hepatoprotective effect. This may be due to antioxidant and anti-inflammatory components in the extract that enhance membrane stability and reduce cellular leakage of hepatic enzymes (**Ahsan et al., 2009).**

CRP levels in the ink-exposed group rose significantly (4.03±0.061 mg/L; $p < 0.01$), indicating an acute inflammatory response, consistent with literature linking chemical pollutants to systemic inflammation **(Libby, 2007).** Treatment with MeH_2O, Multi B, and Neuf B all significantly reduced CRP levels, confirming their anti-inflammatory potential, with Multi B showing the most pronounced effect. This may result from the ability of active chemicals to inhibit inflammatory pathways such as NF-κB and COX-2 **(Wang et al., 2012).**

In the control group, the marrow retained a physiological balance of myeloid and lymphoid elements, consistent with normal hematopoietic function. This is in line with established descriptions of steady-state bone marrow homeostasis **(Kumar et al., 2022).**

The Ink group exhibited signs of innate immune activation, with increased neutrophilic and monocytic presence. The introduction of India ink likely acted as a foreign particulate stimulant, eliciting a phagocytic response typical of macrophage and neutrophil recruitment. This mirrors findings from studies showing that particulate matter can trigger localized immune activation via pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) on phagocytes (**Tizard, 2023; Akira et al., 2006**).Administration of Vitamin B complex (Neuf B) resulted in a subtle increase in lymphocytes without overt inflammatory changes, suggesting a role in immunomodulation rather than direct activation. B vitamins are critical cofactors in nucleic acid metabolism and lymphocyte proliferation, and their supplementation has been shown to enhance adaptive immune responses without triggering innate cell overactivation **(Wintergerst et al., 2007; Tanaka et al., 2019).**The MeH_2O extract, rich in chemicals from *Chamaeleo chamaeleon*, induced a marked increase in neutrophils and monocytes with morphological signs of activation. These effects can be attributed to bioactive compounds such as vitamins , known to enhance hematopoiesis and

stimulate cytokine release. This supports prior literature on the immunostimulatory potential of methanol-based *chamaeleo chamaelon* extracts **(Kang et al., 2020; Lin et al., 2018).**

Notably, the Multi B group displayed the most pronounced activation of the immune compartment in the marrow, with intense phagocytic morphology and high leukocyte density. Such a response suggests synergistic interactions among the bioactive constituents within the Multi B formulation, potentially enhancing both myeloid lineage proliferation and functional activation of phagocytic cells. This aligns with findings from **Delgado et al. (2021**), who reported enhanced immune responses.

In the control group (C), the spleen tissue displayed normal architecture, with well-organized lymphoid follicles in the white blood cells and a high density of lymphocytes, reflecting a healthy immune status and intact tissue structure. This is consistent with the known histological features of a normal spleen **(Steiniger & Barth, 2000).**In the Ink (Ink), a slight reduction in lymphocyte density and minor alterations in white pulp organization were observed. These findings likely represent a nonspecific immune reaction to the foreign particulate material, as injection of such substances can trigger a macrophage-mediated response and T-cell activation **(Liu et al., 2019).** In contrast, the Neuf B group exhibited clear disruptions in the white blood cells structure and a marked decrease in lymphocyte density, suggesting potential immunosuppressive or cytotoxic effects of the single-compound drug. This may reflect a direct negative impact on lymphocyte maturation or survival, as similarly reported for certain immunotoxic pharmaceutical agents **(Ullah et al., 2020).**The MeH_2O group showed relatively preserved splenic architecture, with moderate cellular density and organized lymphoid follicles. The absence of pathological features suggests that the solvent itself does not exert significant adverse effects on immune tissue when used at non-toxic concentrations.

Notably, the Multi B group demonstrated a partial restoration of white pulp structure and an increase in lymphocyte density compared to the Neuf B group. This suggests that the multi-compound formulation may exert synergistic immunoprotective effects or enhance lymphoid activity. Such effects may stem from antioxidant or immunostimulatory properties of some of the components involved **(Khan et al., 2021),** warranting further investigation into their mechanisms of action.

The histological findings reveal clear differences in the distribution and cellular density of lymphocytes between the control group and the groups treated with various agents. These changes reflect the effects of the treatments on lymphoid tissue or the general inflammatory state of the tissues. Control Group (C):This group represents normal tissue, where

lymphocytes appear densely packed and closely arranged, with dark, round nuclei and uniform distribution. This pattern reflects healthy tissue with precise structural organization of lymphocytes, as typically observed in normal lymph nodes or spleen. It aligns with classical descriptions of organized lymphoid tissue in histological studies **(Kumar et al., 2020).** Ink Group:Although lymphocyte density remains high, the appearance of slight intercellular gaps and less homogeneous distribution may suggest the onset of mild physiological or inflammatory changes caused by the ink or compound used. These changes might be related to mild oxidative stress or localized immune activation **(Abbas et al., 2021).** Neuf B Group:This group shows a marked reduction in cellular density and variation in nuclear distribution, possibly indicating immune suppression or partial lymphocyte destruction. Such a pattern may suggest cytotoxic or immunosuppressive effects of the compound or the activation of programmed cell death (apoptosis), which is common in chronic inflammation or oxidative stress conditions **(Elmore, 2007).** MeH_2O Group:This group exhibits the highest degree of cell loss, with clearly dispersed lymphocytes and prominent white spaces, reflecting evident tissue damage. This may be due to a direct cytotoxic effect of the treatment, possibly linked to increased membrane permeability or degradation of the tissue environment **(Majno & Joris, 1995).**

Multi B Group:This group shows a relatively moderate response, with medium lymphocyte density and nearly uniform distribution. The nuclei remain clearly defined, indicating partial preservation of tissue structure. This pattern suggests a balanced immune response, potentially reflecting a state of immune homeostasis without significant tissue damage. This condition is described in the literature as an effective immunoregulatory state that balances activation and surveillance without triggering acute inflammation **(Murphy & Weaver, 2016).**

Conclusion

❖ Conclusion

Wildlife in natural habitats such as prairies, forests, and deserts represents one of the most important biological resources, whether plant-based or animal-based. The desert, in particular, is a biologically rich environment containing natural resources with significant therapeutic potential. While plant-derived products have been widely studied, natural products of animal origin remain largely unexplored, offering promising opportunities for major medical advancements.

Chamaeleo chamaeleon is traditionally used in the El-Oued region for the treatment of various diseases. Building on this traditional knowledge, our research aims to scientifically validate its biological effectiveness.

This study concluded that *Chamaeleo chamaeleon* extracts, whether methanol-water or vitamin B complex-based, possess promising biological and immunological properties, qualifying them as potential natural immune-boosting dietary supplements. Chemical analysis revealed significant concentrations of B-group vitamins, supporting the extracts' role in enhancing vital physiological and immune functions. Biologically, the extracts demonstrated notable antioxidant activity through their ability to scavenge free radicals and exhibited significant anti-inflammatory effects by inhibiting BSA protein denaturation.

Furthermore, both *in vitro* phagocytic tests confirmed an increase in immune cell activity against pathogens, along with partial inhibition of the complement system. *In vivo* experiments also showed improved immune performance indicators, such as increased phagocytic index and favorable liver and hematological markers, without signs of toxicity. These findings suggest that Chamaeleon extracts have strong potential in natural immunotherapy or preventive health strategies and support the scientific validation of their use in traditional medicine. The study recommends further research to isolate and characterize the active compounds and assess their efficacy in disease-specific or clinical settings.

❖ Research Perspectives

1. **Studying the Effects of Chameleon Extracts on Specific Immune Diseases:** Future research can be directed toward evaluating the impact of chameleon extracts on models of chronic immune disorders (such as lupus or multiple sclerosis). This includes examining whether the extracts possess regulatory or suppressive effects on immune system hyperactivity.
2. **Analysis of Bioactive Compounds:** Isolating and identifying the bioactive chemical compounds responsible for the immunological and antioxidant effects observed in the

extracts. Advanced techniques such as HPLC and GC-MS can be used to obtain precise chemical profiling.

3. **Clinical Studies on Humans:** Transitioning from in vitro and animal studies to potential clinical trials on human volunteers to determine the safety and efficacy of these extracts in humans. This also includes evaluating potential side effects or toxicity levels.
4. **Development of Natural Dietary Supplements:** Formulating dietary supplements in the form of capsules or powders enriched with B vitamins and antioxidants derived from chameleon extracts. These products would require rigorous safety and quality control tests according to international standards.
5. **Investigation of Antimicrobial and Antiviral Properties:** Expanding the research to include the antimicrobial potential of the extracts, particularly their effectiveness against antibiotic-resistant bacterial strains and certain viruses.
6. **Application in Veterinary Medicine:** Exploring the use of chameleon-derived extracts to stimulate immunity in domestic animals or livestock, especially in regions where traditional medicine is still widely practiced.
7. **Ecological and Ethical Considerations:** Studying the environmental impact of chameleon harvesting on biodiversity and ecosystem balance. It is essential to propose strategies for the sustainable use of animals in traditional medicine while respecting environmental laws and animal welfare ethics.

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Annex

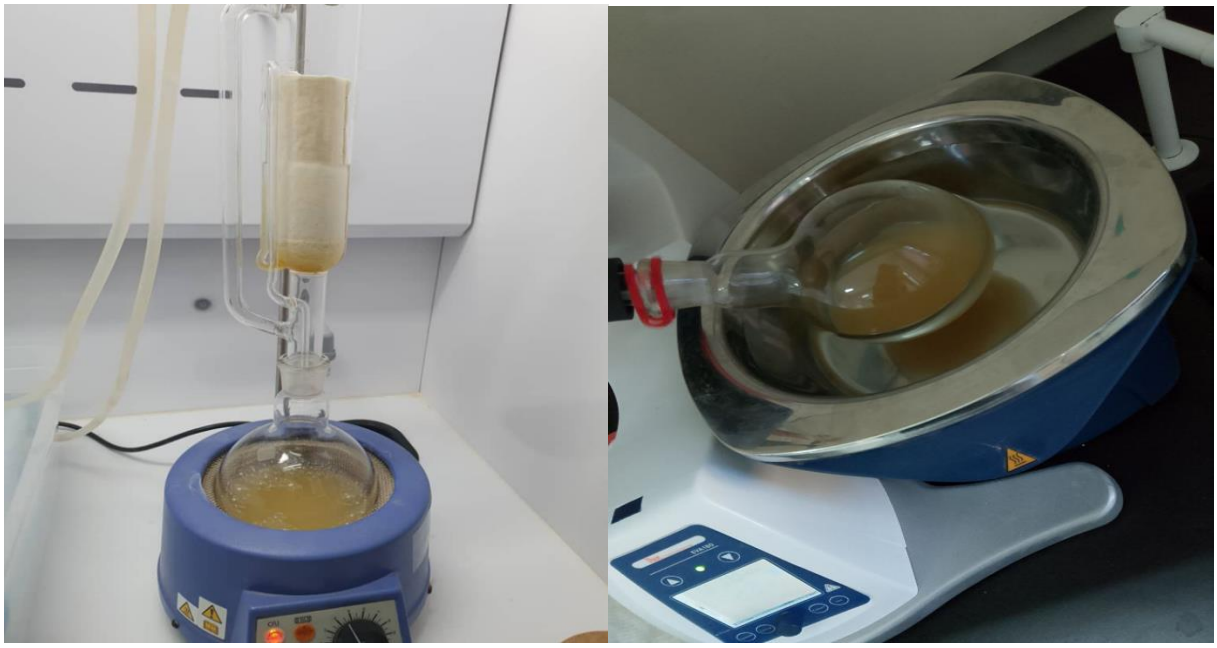

Figure 01: Extraction method of methanol and water mixture.

Figure 02: Preparation of multi b extract.

Figure 03: Chamealeo chamealeon powder

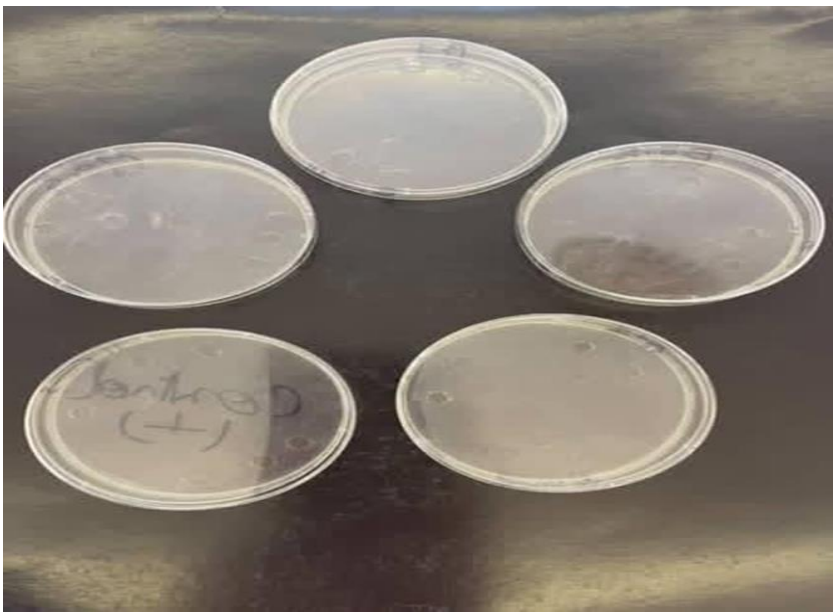

Figure 04: Anti_complement activity pietures.

Figure 05: Female Albino Wistar rats.

Figure 08: acrifice of rats and collection of blood and organs.

Figure 09: Preparation of ink.

Figure 10: the Hematoxylin-Eosin technique.