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Theme

Physicochemical composition and evaluation of biological activities of *Chamaeleo chamaeleon* L.

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My dear father's soul

To my dear Mather

To my brothers and sister

To all my friends

To all those who are dear to me

ABSTRACT

Dry *Chamaeleo chamaeleon* is used in the traditional medicine of the El Oued region in the Algerian Sahara to treat a variety of common ailments including tonsillitis, thyroid diseases, and coughs. Given these considerations, our study aims to characterize the physico-chemical properties of *C. chameleons*, as well as to evaluate the biological activities of its extracts.

Our research included an ethnopharmacological survey of the traditional use of *C.chamaeleon*, and then we examined the physicochemical properties of *C. chamaeleon* powder, by determinations of dry matter, moisture, ash, and minerals as well as estimation of carbohydrates, fats, proteins, and vitamins. After extraction using three solvents "hexane, methanol, and water" we examined *in vitro*, the antioxidant potency of extracts using the scavenger activity of the 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical, β-Carotene / linoleic acid technique, reducing power and technique of scavenging Superoxide radical ($O_2^{\bullet}$-). We also examined the extract's capacity to induce hemolysis, anti-inflammatory activity using the egg albumin denaturation method, antibacterial activity against six bacterial strains, and antiangiogenesis activity by the chick chorioallantoic membrane assay. *In vivo*, we tested the *C. chamaeleon* toxicity, as well as the antioxidant, anti-inflammatory and anti-hypothyroid activity of the *C. chamaeleon* powder and aqueous extract in Wistar rats exposed to carbimazole.

Based on the survey results, we determine that the use of *C. chamaeleon* is safe and effective in treating several diseases, including tonsillitis (66%), thyroid diseases (26%). The questionnaire also revealed that the sale/hunting of *C.chamaeleon* is not random, as they are hunted by experienced people while avoiding breeding times, which secures the risk of their extinction.

The obtained results demonstrate that the *C. chamaeleon* powder has a high quality in the drying procedure, with a pH of 5.43, dry matter of 90.56%, and moisture of 9.43%, which assures its prolonged shelf life. The mineral richness of the *C. chamaeleon* is demonstrated by the ash result of 22.44%, and the quantitative estimation revealed that it includes large levels of phosphorus (14.01%) as well as calcium (3.27%), magnesium (2.83%), sodium (1.51%), and potassium (0.49%). There are trace levels of iron, zinc, and copper. The results of estimating the chemical compounds of *C. chamaeleon* powder reveal a high percentage of proteins in contrast to carbohydrates and fats. And we found high values of the examined vitamins, E = 19.23 mg/100g, B1=21 mg/100g, B6 = 3.60 mg/100g, and B12= 5.61µg/100g.

According to the results of the antioxidant test, the *C. chamaeleon* extracts had an activity in scavenging DPPH radicals and a lot of activity in scavenging superoxide radicals. The findings of the hemolysis assay showed low percentages at high doses compared to sodium dodecyl sulfate, which showed very high rates of hemolysis in the presence of low concentrations, indicating that the extracts are non-toxic to red blood cells. The anti-inflammatory activity test yielded good results. Then, we also found that the extracts were anti-all the bacterial strains studied at a dose of 100 mg/ml. In terms of the anti-angiogenesis activity, we discovered that the extract had very high effectiveness since there are significant differences in the number of vessels, branching points, and inhibition zone compared to DMSO. There are no notable variations of extracts compared with bevacizumab.

According to the results of the *C. chamaeleon* toxicity test, it shows that *C. chamaeleon* is safe and non-toxic because it does not affect many factors, including alertness, insomnia, touch sensitivity, pain sensitivity, convulsions, constipation, urination, salivation, skin color, food intake, water intake, and mortality, and concerning the results *in vivo*, administration of carbimazole to rats caused a change in hematological parameters, leukocyte, thyroid function (T3, T4, TSH), hepatic enzyme markers, kidney function (urea, creatinine, protein) and testosterone. The antioxidant defenses of GSH, catalase, and SOD as well as MDA levels all altered in conjunction with these modifications. Histopathological studies also showed significant inflammation in the liver in the carbimazole and levothyroxine groups. However, treatment with *C. chameleons* improved most of the adverse effects of carbimazole.

These results lead us to conclude that *C. chamaeleon* is a nutritional supplement rich in minerals, proteins, and vitamins and has tremendous preventive and therapeutic potential against infectious diseases, inflammations, hypothyroidism, and tumor growth.

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

الملخص

تستخدم الحرباء الجافة *Chamaeleo chamaeleon* في الطب التقليدي لمنطقة الواد في الصحراء الجزائرية لعلاج مجموعة متنوعة من الأمراض الشائعة بما فيها التهاب اللوزتين وأمراض الغدة الدرقية والسعال. ولهذا فإن دراستنا تهدف الى تحديد الخصائص الفيزيائية والكيميائية للحرباء، وكذلك تقييم الأنشطة البيولوجية لمستخلصاتها.

تضمن بحثنا إستبيان حول الاستخدام التقليدي لـ *C. chamaeleon*، ثم قمنا بفحص الخصائص الفيزيائية والكيميائية لمسحوق *C.chamaeleon*، من خلال تحديد المادة الجافة والرطوبة والرماد والمعادن وكذلك تقدير الكربوهيدرات والدهون والبروتينات والفيتامينات. بعد الاستخلاص باستخدام ثلاثة مذيبات "الهكسان والميثانول والماء" فحصنا في المختبر الفاعلية المضادة للأكسدة للمستخلصات باستخدام النشاط الكاسح لـ(2,2'-Diphenyl-1-picrylhydrazyl (DPPH الجذري، تقنية β- كاروتين / حمض اللينوليك ، تقنية Reducing power وتقنية إزالة جذور الأكسيد الفائق ($O_2^{\bullet-}$). قمنا أيضًا بفحص قدرة المستخلص على إحداث انحلال الدم، والنشاط المضاد للالتهابات باستخدام طريقة تمسخ الزلال البيض، والنشاط المضاد للبكتيريا ضد ستة سلالات بكتيرية ، ونشاط مضاد لتكوُّن الأوعية عن طريق مقايسة الغشاء المشيمي للكتاكيت "CAM". في الجسم الحي، قمنا باختبار سمية *C.chamaeleon*، وكذلك النشاط المضاد للأكسدة، الالتهابات وضد قصور الغدة الدرقية لمسحوق *C.chamaeleon* والمستخلص المائي في جرذان ويستر المعرضة للكاربيما زول.

وبناءً على نتائج الإستبيان فقد وجدنا أن استخدام الحرباء آمن وفعال في علاج العديد من الأمراض مثل التهاب اللوزتين (66%) وأمراض الغدة الدرقية (26%). أظهر الاستبيان أيضًا أن بيع / صيد *C.chamaeleon* ليس عشوائيًا ، حيث يتم اصطيادهم من قبل أشخاص ذوي خبرة مع تجنب أوقات التكاثر لتفادي خطر انقراضهم.

توضح النتائج المتحصل عليها أن مسحوق *C.chamaeleon* له جودة تجفيف عالية، حيث يبلغ الرقم الهيدروجيني 5.43، والمواد الجافة بنسبة 90.56% ، والرطوبة بنسبة 9.43%، مما يضمن له مدة صلاحية طويلة. و يتضح الثراء المعدني للحرباء *C.chamaeleon* من نتيجة الرماد بنسبة 22.44%، وأظهر التقدير الكمي أنه يحتوي على مستويات عالية من الفوسفور(14.01%) وكذلك الكالسيوم (3.27%) والمغنيسيوم (2.83%) والصوديوم (1.51%) والبوتاسيوم (0.49%). هناك مستويات ضئيلة من الحديد والزنك و النحاس. أظهرت نتائج تقدير المركبات الكيميائية لمسحوق *C.chamaeleon* نسبة عالية من البروتينات على عكس الكربوهيدرات والدهون. ووجدنا قيم عالية من الفيتامينات التي تم فحصها، E = 19.23 mg/100g, B1=21 mg/100g, B6 = 3.60 mg/100g, B12= 5.61µg/100g.

وفقًا لنتائج اختبار مضادات الأكسدة، كان لمستخلصات *C.chamaeleon* قدرة لإزالة جذور DPPH وقدرة عالية لإزالة جذور الأكسيد الفائق. أظهرت نتائج فحص انحلال الدم نسب منخفضة عند الجرعات العالية مقارنة بكبريتات دوديسيل الصوديوم والتي أظهرت معدلات عالية جدا من انحلال الدم في وجود تراكيز منخفضة مما يشير إلى أن المستخلصات غير سامة لخلايا الدم الحمراء. أظهر اختبار النشاط المضاد للالتهابات عن نتائج جيدة. ووجدنا أيضًا أن المستخلصات كانت مضادة لجميع السلالات البكتيرية المدروسة بجرعة 100mg/ml. فيما يتعلق بالنشاط المضاد لتكوين الأوعية، اكتشفنا أن المستخلص كان له فعالية عالية جدًا نظرًا لوجود اختلافات كبيرة في أعداد الأوعية ونقاط التفرع ومنطقة التثبيط مقارنة بـ DMSO. ولا توجد اختلافات ملحوظة في المستخلصات مقارنة بـbevacizumab.

وفقًا لنتائج اختبار سمية *C. chamaeleon* ، فقد أظهر أن *C. chamaeleon* آمنة وغير سامة نظرا لعدم تأثيرها على العديد من العوامل ، منها اليقظة ، والأرق ، وحساسية اللمس ، وحساسية الألم، والتشنجات ، والإمساك، والتبول، وسيلان اللعاب، لون الجلد، وتناول الطعام، وكمية الماء، والوفيات، وفيما يتعلق بالنتائج في الجسم الحي ، تسبب إعطاء carbimazole للفئران في تغييرات في المعايير الدموية، الكريات البيض، وظيفة الغدة الدرقية (T3، T4، TSH)، علامات الإنزيمات الكبدية، وظائف الكلى (اليوريا ، الكرياتينين ، البروتين) وهرمون التستوستيرون. تم تغيير مضادات الأكسدة لـ GSH و catalase و SOD؛ وكذا مستويات MDA بالتزامن مع هذه التعديلات. كما أظهرت دراسات الأنسجة المرضية وجود التهاب كبير في الكبد في مجموعتي الكاربيمازول والليفوثيروكسين. ومع ذلك، فإن العلاج باستخدام الحرباء *C.chamaeleon* أدى إلى تحسين معظم الآثار الضارة لكاربيمازول.

تقودنا هذه النتائج إلى استنتاج أن *C.chamaeleon* هو مكمل غذائي غني بالمعادن والبروتينات والفيتامينات وله إمكانات وقائية وعلاجية هائلة ضد الأمراض المعدية والالتهابات وقصور الغدة الدرقية ونمو الأورام.

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

RESUME

Le *Chamaeleo chamaeleon* sec est utilisé dans la médecine traditionnelle de la région d'El Oued dans le Sahara Algérien pour traiter une variété de maladies courants, notamment l'amygdalite, les maladies de la thyroïde et la toux. Compte tenu de ces considérations, l'objectif de notre étude est de caractériser les propriétés physico-chimiques de *C. chamaeleon*, ainsi que d'évaluer les activités biologiques de ses extraits. Notre recherche comprenait une enquête ethnopharmacologique d'un usage traditionnel de *C. chamaeleon*, puis nous avons examiné les propriétés physico-chimiques de la poudre de *C. chamaeleon*, par des dosages de matière sèche, d'humidité, de cendres et de minéraux ainsi que estimation des glucides, lipides, protéines et vitamines. Après extraction avec trois solvants "hexane, méthanol et eau", nous avons examiné *in vitro* le pouvoir antioxydant des extraits en utilisant l'activité piégeuse du radical 2,2'-diphényl-1-picrylhydrazyle (DPPH), β-carotène / acide linoléique, pouvoir réducteur et technique de piégeage des radicaux Superoxyde ($O_2^{\bullet}$-). Nous avons également examiné la capacité de l'extrait à induire l'hémolyse, l'activité anti-inflammatoire par la méthode de dénaturation de l'albumine d'œuf, l'activité antibactérienne contre six souches bactériennes et l'activité antiangiogenèse par le test de la membrane chorioallantoïque du poulet. *In vivo*, nous avons testé la toxicité de *C. chamaeleon*, ainsi que l'activité antioxydante, anti-inflammatoire et antihypothyroïdienne de la poudre et de l'extrait aqueux de *C. chamaeleon* chez des rats Wistar exposés au carbimazole. Sur la base des résultats de l'enquête, nous déterminons que l'utilisation de *C. chamaeleon* est sûre et efficace dans le traitement de plusieurs maladies, amygdalite (66%), maladies thyroïdiennes (26%). Le questionnaire a également révélé que la vente/chasse de *C.chamaeleon* n'est pas aléatoire, car ils sont chassés par des personnes expérimentées tout en évitant les périodes de reproduction, ce qui sécurise le risque de leur extinction. Les résultats obtenus démontrent que la poudre de *C. chamaeleon* a une haute qualité dans la procédure de séchage, avec un pH de 5,43, une matière sèche de 90,56 % et une humidité de 9,43 %, ce qui assure sa durée de conservation prolongée. La richesse minérale du *C. chamaeleon* est démontrée par le résultat de cendres de 22,44%, et l'estimation quantitative a révélé qu'il comprend de grandes quantités de phosphore (14.01%) ainsi que du calcium (3.27%), du magnésium (2.83%), du sodium (1.51%) et du potassium (0.49%). Il existe des traces de fer, de zinc et de cuivre. Les résultats de l'estimation des composés chimiques de la poudre de *C. chamaeleon* révèlent un pourcentage élevé de protéines contrairement aux glucides et aux lipides. Et nous avons trouvé des valeurs élevées des vitamines examinées, E = 19,23 mg/100 g, B1 = 21 mg/100 g, B6 = 3,60 mg/100 g et B12 = 5,61 µg/100 g.

Selon les résultats du test antioxydant, les extraits de *C. chamaeleon* avaient une activité de piégeage des radicaux DPPH et une grande activité de piégeage du radical superoxyde. Les résultats du test d'hémolyse ont montré de faibles pourcentages à des doses élevées par rapport au dodécylsulfate de sodium, qui a montré des taux d'hémolyse très élevés en présence de faibles concentrations, indiquant que les extraits ne sont pas toxiques pour les globules rouges. Le test d'activité anti-inflammatoire a donné de bons résultats. Ensuite, nous avons également constaté que les extraits étaient anti-toutes les souches bactériennes étudiées à la dose de 100 mg/ml. En termes d'activité anti-angiogenèse, nous avons découvert que l'extrait avait une efficacité très élevée car il existe des différences significatives entre le nombre de vaisseaux, les points de ramification et la zone d'inhibition par rapport au DMSO. Il n'y a pas de variations notables d'extraits par rapport au bévacizumab. D'après les résultats du test de toxicité de *C. chamaeleon*, il montre que *C. chamaeleon* est sûr et non toxique parce qu'il n'affecte pas de nombreux facteurs, notamment la vigilance, l'insomnie, la sensibilité au toucher, la sensibilité à la douleur, les convulsions, la constipation, la miction, la salivation, la couleur de la peau, l'apport alimentaire, l'apport en eau et la mortalité, et concernant les résultats *in vivo*, l'administration de carbimazole à des rats a provoqué une modification des paramètres hématologiques, leucocyte, fonction thyroïdienne (T3,T4, TSH), marqueurs enzymatiques hépatiques, fonction rénale (urée, créatinine, protéine) et testostérone. Les défenses antioxydantes du GSH, de la catalase et de la SOD ainsi que les niveaux de MDA ont tous été alterés en conjonction avec ces modifications. Des études histopathologiques ont également montré une inflammation significative du foie dans les groupes carbimazole et lévothyroxine. Cependant, le traitement avec *C. chamaeleon* a amélioré la plupart des effets indésirables du carbimazole.

Ces résultats nous amènent à conclure que *C. chamaeleon* est un complément nutritionnel riche en minéraux, protéines et vitamines et possède un énorme potentiel préventif et thérapeutique contre les maladies infectieuses, les inflammations, l'hypothyroïdie et la croissance tumorale.

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

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LIST OF ABBREVIATIONS

ALb: Albuminemia

ALP: Alkaline phosphatase

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ATCC: American Type Culture Collection

BiL: Bilirubinemia

CAM: Chick chorioallantoic membrane

DMF: N,N-Dimethylformamide

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

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

DTNB: 5,5′-Dithiobis-(2-nitrobenzoic acid)

ECL: Electrochemiluminescence

ECs: Endothelial cells

EDTA: Ethylenediaminetetraacetic acid

EDTA: Ethylenediaminetetraacétique

FBS: Fasting blood sugar

$FeCl_3$: Ferric chloride test

GLDH: Glutamate dehydrogenase

GRA: Granulocytes

GSH: Glutathione

H_2O: Water

H_2O_2: Hydrogen peroxide

HCl: Hydrochloric acid

HGB: Hemoglobin

HOCl: Hypochlorous acid

LDH: Lactate dehydrogenase

LYM: Lymphocytes

MCV: Mean corpuscular volume

MCV: Mean corpuscular volume

MDA: Malondialdehyde

MDH: Malate dehydrogenase

NADPH: Nicotinamide adenine dinucleotide phosphate

NO•: Nitric oxide

$O_2^{\bullet}$–: Superoxide anion radical

OH•: Hydroxyl ion

PBS: Phosphate-buffered saline

pH: Potential of hydrogen

PLT: Platelets

POD: Peroxidase

RBC: Red blood cell

RO•: Alkoxyl

ROO•: Peroxyl

ROS: Reactive oxygen species

SOD: Superoxide dismutase

T.M.P: Average Preferential Temperature

T3: triiodothyronine

T4: thyroxine

TBS: Tris buffer saline solution

TCA: Trichloroacetic acid

Tes: testosterone

TP: Total protein

TSH: Thyroid Stimulating Hormone

UA: Uric Acid

VEGF: Vascular Endothelial Growth Factor

WBC: White blood cell

WHO: World Health Organization

SUMMARY

Second part : Experimental part

Chapter I : Meterial & Methods

Introduction

Introduction

Humans are part of nature, they depend on Earth's land, ocean, atmosphere, and biosphere for many different resources, including air, water, soil, minerals, metals, energy, plants, and animals. Natural resources are used to make food, fuel, and raw materials for the production of goods. All of the food that people eat comes from plants or animals. Natural resources such as coal, natural gas, and oil provide heat, light, and power **(Löbler, 2017)**. Since the dawn of time, man has resorted to nature as means of survival, and he has used plants, animals, and minerals to alleviate many of the illnesses that affect him. According to fossil records, humans have been using plants as medicine for at least 60,000 years **(Yuan *et al*., 2016)**. In fact, using natural remedies as medication has been quite difficult for early people. It is quite likely that early humans frequently ate poisonous plants while in search of food, which resulted in nausea, vomiting, diarrhea, comas, or other toxic reactions possibly even death. However, it was through this process that early people were able to learn about food sources and herbal remedies **(Teschke *et al*., 2015)**.

Traditional medicine sometimes referred to as ethnomedicine, folk medicine, native healing, or complementary and alternative medicine, is the first system of healthcare that has persisted through the generations. Humans have adapted and dealt with a variety of ailments that have threatened their life and survival by using this old form of treatment. Traditional medicine is hence extensive and varied **(Abdullahi, 2011)**. As a result, diverse communities have developed unique indigenous therapeutic techniques that fall within the general definition of traditional medicines, such as Chinese, Indian, and African traditional medicines **(Croizier, 1968)**. This explains why there isn't a single, widely recognized definition of the expression. Despite this, the World Health Organization (WHO) has provided one of the most accepted definitions of traditional medicines **(Lu *et al*., 2021)**. The World Health Organization defines traditional medicine as "the totality of knowledge, skills, and practices based on the theories, beliefs, and observations indigenous to diverse cultures used in the preservation of health, and also in the prevention, diagnosis, improvement, or treatment of mental and physical illnesses" **(Che *et al*., 2017)**. In traditional medicine, plants, animals, and minerals are used by humans to create alternative medicines, which are still well-established in modern countries, particularly phytotherapy and zootherapy **(Santos *et al*., 2019)**.

Zootherapy is the treatment of human illnesses utilizing therapies based on drugs taken from animals or derived from them **(Costa-Neto, 2005)**. Since ancient times, diverse societies have employed animals and products made from their various organs as a source of therapeutic compounds, these applications still exist in conventional medicine **(Lev, 2003)**. The phenomenon of zootherapy is distinguished by both a wide geographic range and extremely significant

historical roots. Zootherapy is one of several well-known therapies used across the globe and is a significant option in contemporary communities. Various parts of domestic and wild animals, including their hooves, skins, bones, feathers, and tusks, are crucial components in the creation of therapeutic, preventative, and curative medicines **(Alves *et al*., 2012)**. For example, more than 1500 animal species have been identified as having some therapeutic use in Traditional Chinese medicine. In India, between 15% and 20% of Ayurvedic medication is made from ingredients sourced from animals **(Alves and Rosa, 2005)**. Over 180 medicinal animals have been identified in Bahia State, located in northeastern Brazil **(Costa-Neto, 2004)**.

In traditional folk medicine, reptiles are among the most often used animal species. They are important in treating and/or preventing disease in a variety of social and cultural contexts around the world **(Da Nóbrega Alves *et al*., 2008; Alves *et al*., 2012)**. Reptiles are regularly found and in high demand in African traditional medicine marketplaces, particularly crocodiles and pythons **(Williams *et al*., 2016)**. Some species of lizards are frequently consumed in native peoples' rituals in Algeria's desert, particularly in the El-Oued region. Chameleons are an essential lizard in the creation of therapeutic, preventative, and curative drugs for disease immunity, defense against witchcraft and ill luck, and treatment of chest pain and cough **(Williams and Whiting. 2016; Osunsina *et al*., 2012)**. It is known that the dried *Chamaeleo chamaeleon* can be used to cure a variety of diseases that are common in the El-Oued region of the Algerian desert. Even local doctors advise their patients to take it because of its proven efficacy, which has been used in a variety of ways to treat many diseases, most notably for the treatment of tonsillitis. It was also used to treat various conditions, such as thyroid illness and cough.

Animal extracts, in contrast to plant extracts, lack the scientific proof to support their biological utility; as a result, our study aims to conduct an ethnopharmacological survey of the traditional use, hunter/seller of the *Chamaeleo chamaeleon*, and then to examine the physicochemical properties of *C. chamaeleon* powder as well as to assess the biological activity of the extracts (hexane, methanolic and aqueous) of dry *C. chamaeleon*. This study examined, *in vitro* the extracts of dried *C. chamaeleon*'s antioxidant, hemolysis assay, anti-inflammatory, and antibacterial activity, *in ovo* antiangiogenic properties. *In vivo*, we tested *C. chamaeleon* toxicity, and also antioxidant, anti-inflammatory, and anti-hypothyroidism activity of the *C. chamaeleon* powder and aqueous extract in Wistar rats exposed to carbimazole.

First part

Bibliographic

Chapter I

Chamaeleo chamaeleon

I.1. Genelality

Chameleons, from the Greek khamaileôn “lion that drags itself on the ground”, are vertebrate animals belonging to the class of Reptiles (from the Latin reptilis “crawling”). They are part of the order Squamates (from the Latin squama “scale”), the suborder Saurians (from the Greek sauros, “lizard”) and the family Chameleonidae, sometimes called Chameleontidae or Chamaeleonidae (family of mainly arboreal saurians, defined by Constantine Samuel Rafinesque in 1815) **(Bourdain, 2006)**. The lizard family Chameleonidae is one of the most unique taxa of all vertebrates. Chameleons are certainly among the most recognizable vertebrates in the world, the Chameleonidae display significant morphological variety in addition to being distinct in these characteristics **(Bickel and Losos, 2002)**.This family includes two subfamilies: Chamaeleoninae and Brookesiinae, and over 200 species and subspecies currently recognized in eleven genera. Chameleons live in habitats ranging from desert sand dunes where body temperature (Tb) exceeds 39 °C to alpine regions above 3500 metres, with ambient temperatures below freezing **(Anderson, 2013)**. Chameleons are diurnal and move between their living stations over short distances to scan their surroundings for prey using a "foraging" approach that involves a little flight (while being wary of potential predators). The diet of European species changes throughout the year, but the majority of their food are bees, wasps, and grasshoppers, which they catch with a long, protruding tongue throughout the spring, summer, and fall **(Jeroen *et al.*, 2016)**. There are many different thermal zones and environmental circumstances where chameleons can be found, including as hot, dry desert habitats, tropical rainforests, Mediterranean climates, and high-altitude settings **(Tolley and Herrel, 2013)**.

Chameleons have a large number of different morphological shapes, as the size of chameleons varies depending on the species, ranging from fifteen to thirty-five centimeters in length **(Bourdain, 2006)**. Their bodies are compressed laterally, as if their sides were pushed inward, giving them their slender appearance. This allows them to walk along a stick or branch that is relatively invisible from above or below **(Tolley and Burger, 2007)**. Prehensile tails and feet that are converted into pincers. Colors may vary depending on the individual, body temperature, social status (pregnancy, stress), and camouflage requirements **(Jeroen *et al.*, 2016)**. A highly modified skull with a ballistically projected tongue used to capture prey **(Diaz *et al.*, 2018)**, characterized by the independent mobility of their eyes **(Jeroen *et al.*, 2016)**. This gives them an almost 360 view of their surroundings, meaning they can see in almost all directions (except right behind the head) without moving or turning their bodies **(Tolley *et al.*, 2009)**.

I.2. Definition

Chamaeleo chamaeleon, the Common Chameleon, is a species of saurian in the Chamaeleonidae family that is widely known for its ability to change skin color and pattern. In the Mediterranean region, plantations and natural woods are home to the arboreal common chameleon (*C. chamaeleon*) **(Hodar *et al.*, 2000)**. Chameleons are sluggish diurnal lizards that have evolved to live in trees **(Bickel and Losos, 2002)**. *C. chamaeleon* is a medium-sized reptile (28-30 cm total head-tail length). Body compressed at the sides, head provided with a well-developed crest, with bulging eyes covered with a conical eyelid, prehensile tail and fingers opposed in groups of two or three, forming claws. The eyes can move in all directions and independently of each other. It is able to change color very quickly thanks to a complex system of pigment structures in skin cells. This allows it to blend into the landscape changing color to match its surroundings depending on mood, health, or temperature **(Cuadrado *et al.*, 2001)**.

I.3. Taxonomy

There are 150 species in the Chameleonidae family, which is subdivided into the subfamilies Brookesiinae and Chamaeleoninae (genera Chamaeleo, Furcifer, Bradypodion, and Calumma). The Common Chameleon, *C. chamaeleon*, belongs to the Chamaeleo genus **(Guillon, 2010)**.

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

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

I.4. Distribution

The Mediterranean chameleon (*Chamaeleo chamaeleon* Linneus, 1758) is present in the southern Mediterranean of Europe (Spain, Portugal, Italy and Greece), in North Africa (coastal areas Western Sahara, Morocco, Algeria, Tunisia, Libya and Egypt) and South-West Asia (Turkey of the south, Syria, Lebanon, Palestine, Jordan, Saudi Arabia and Yemen) (fig01) **(Dimaki *et al*., 2015)**.

In Europe, it is present in Malta, in some Greek islands (Crete, Samos and Chios), and in the southern part of the Iberian Peninsula. In Italy, some sporadic reports have been received in the past from Sicily but there are accidental introductions of specimens from North Africa: a recent review puts *C. chamaleon* among species whose presence in Sicily does not has not been confirmed. More recently, acclimatatate populations have been reported in Apulia and Calabria; both populations are the result of relatively recent introduction (Tunisia and Palestine respectively) **(Miraldo *et al*., 2005)**.

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

I.5. Description

The common chameleon is unique in most of its range. The color of its skin is determined by the surrounding environment and temperature and is capable of sudden changes. It is usually light green at night. It spends most of its time climbing bushes, clinging to branches with its tail and with modified legs (toes of each foot are formed by two prehensile claws) **(Čerňanský, 2010)**. Measures 9 to 17 cm, plus 12 to 14 tails, the male it is a bit larger female part. The latter lays late summer 30-40 eggs, which will open the next spring. Lives up to 20 years, the record in captivity is 22 years, but usually lives 12-13 years **(Sidhom *et al*., 2020)**.

I.5.1. Morphological particularities

I.5.1.1. Size

The size of chameleons varies depending on the species from fifteen to thirty-five centimeters in length. The common chameleon (scientific name: *Chamaeleo chamaeleon*) is a medium-sized chameleon (up to 30 cm long, at most), with hatchlings only measuring 15 mm **(Cuadrado and Loman, 1999)**.

I.5.1.2. Head

Chameleon heads have a pyramidal form, trong bone keels support the head, and the occipital lobe extends to the top of the helmet (The helmet is the area of the head behind the eyes) **(Bourdain, 2006)**, The parietal crest is curved and taller than the lateral crests, which s'interrupt immediately behind the temporal region **(Lustig *et al*., 2012; Lev-Ari, 2016)**.

I.5.1.3. Body

The body of *C.chamaeleon* is compressed laterally which gives these animals an elliptical shape. Using their lungs and muscles, chameleons can change the shape of their body, causing it to swell or flatten dorso-ventrally. By modifying their conformation, they can regulate their internal temperature, camouflage themselves or communicate with other individuals: they flatten out as much as possible in the morning to increase their body surface in order to capture as much solar energy as possible, they flatten out behind a branch and oscillate to imitate the movement of the leaves of the trees, they inflate to threaten their adversaries (**Morsy *et al*., 2012; Bourdain, 2006)**.

I.5.1.4. Members

Chameleons have distinctive limbs that are customized to a life in the trees (fig02); their legs are long and strong, the extremely mobile hip and shoulder joints make chameleons very flexible **(Bourdain, 2006)**. The fingers are partially welded together, thus forming claws to firmly grip the branches. Two external and three internal fingers are welded on the front legs. Three external fingers and two internal ones are welded on the hind legs. All fingers have a curved claw. The claw is usually white to yellowish, sometimes transparent. The scales of the palmar and plantar surfaces of the legs, as well as those of the ventral part of the end of the tail have a modified structure which forms adhesive cushions facilitating movement on smooth surfaces **(Schleich, 1985)**.

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

I.5.1.5. Tail

The tail is solid, cylinder-shaped, and prehensile. In other words, it serves as a fifth limb by grabbing the things around it. The tail is either shorter than the body or the same length. 23.8 cm make up the entire length. It typically rolls up under the cloaca when the animal is at rest and unfolds when it needs to grasp. The tail is kept back or raised in an oblique "S" spiral when moving onshore **(Cuadrado *et al*., 2003)**.

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

I.5.2. Anatomical particularities

I.5.2.1. Cardio-respiratory apparatus

Heart

The chameleon's heart is divided into three chambers with two atria topped by a single ventricle which is partly divided by an incomplete interventricular septum **(Fouda *et al.*, 2015)**. Therefore, venous and arterial blood mixes. The heart rate is involved in the thermoregulation of the chameleon and the heart rate depends on many factors: the body temperature of the animal where optimal cardiac muscle performance is at the preferential temperature for the chameleon, the size of the individual (frequency is inversely proportional to obesity), the level of metabolism and the rate of respiration (slow Cardiac occurs during apnea) and sensory stimulation **(Bourdain, 2006)**.

Blood

The volume of blood in chameleons is about 3 percent of body weight, all reptile blood cells have a nucleus, erythrocytes have the peculiarity of the ability to divide and platelets themselves can turn into red blood cells **(Divers and Mader, (2005)**. Granulocytes are heterogeneous (homogeneous to neutrophils in mammals) and basophils can account for up to 40 percent of the reptile blood population **(Schilliger, 2004)**. Lymphocytes are the main white blood cells in the leukocyte formula. Their number varies according to the seasons, as it decreases with low temperatures in winter and rises in summer, according to the sex of the animal, as there are more females, according to the nutritional status of the chameleon (the number of lymphocytes decreases during malnutrition) and according to its state of health (lymphocytosis is observed during parasitic or viral diseases) **(Divers and Mader, 2005)**.

Lungs

Chameleons can breathe through their noses and mouths. The palate is perforated by a pair of sinuses that allow air to pass from the nostrils into the lungs. Air inhaled through the mouth passes through the larynx and glottis under the tongue into the trachea **(Bourdain, 2006)**. When the ambient temperature raises, the volume of inhaled air increases, as does the frequency of respiratory beats, Chameleons have a pair of lungs; the inner part of the lungs is divided into several chambers divided by many septa. Chameleons do not have a membrane. It is the contractions of the striated skeletal muscles (intercostal muscles and muscles of the limbs) that allow breathing movements. When feeling threatened, chameleons can also inflate their lungs to appear larger **(Perry and Duncker, 1978)**.

I.5.2.2. Skin apparatus

Scales

Chameleons, like all reptiles, have keratinous thickenings that form scales all over the body surface. Scales are produced by the basal layer of the epidermis. They have various shapes depending on their position on the body. Thus we distinguish the standard forms, conical, tubercular, lenticular, the forms of plate, crescent (in particular around the mouth). Standard scales cover most of the animal's body and are round **(Tolley and Herrel, 2013)**.

Skin

The skin has the two classic layers, namely a superficial layer, the epidermis, and a deep layer, the dermis. The upper dermis is much vascularized, it has a nourishing role and it participates in heat exchanges with the outside. The deep dermis is mainly made up of collagen; it contains vessels, nerves, fibroblasts and chromatophore cells. With the exception of the femoral glands which serve to mark territory, there are no sebaceous glands in reptiles. The skin of chameleons is thus always dry. The thickness of the skin varies depending on where you live. In desert regions, chameleons are protected from desiccation by thick, dry skin. And in mountainous regions have thinner, more moisture-permeable skin **(Fouda and El Mansi, 2017)**.

Shed

The superficial layer of the skin is composed of highly keratinized and therefore dead cells. It no longer grows and is renewed during shedding. The old skin dries out, stiffens and then falls off. The animal can rub itself to promote their fall and the skin exfoliates in shreds. shedding is more frequent during growth than in adulthood: an adult moults on average every four months or so and a young after a few weeks. They last depending on the environment, from several hours to several days. Unlike snakes, chameleons continue to feed normally before the shedding period **(Uversky, 2003)**.

I.5.2.3. Digestive apparatus

The digestive system of chameleons is not highly specialized. The stomach is tubular and secretes enzymes and hydrochloric acid. The intestines are short and lead to the cloaca **(Bourdain, 2006)**. Chameleons have small conical teeth, all identical and rudimentary, inserted directly on the upper edge of the jaw bone. The teeth are mainly used to hold prey in the mouth. The inside of the oral cavity is often colored. When threatened, some chameleons

show the inside of their mouths to scare away their opponents. The mouth contains glands that secrete mucus and glands that produce non-sticky saliva of varying viscosity **(Tolley and Herrel, 2013)**. The tongue of the chameleon is made up of an annular accelerator muscle which is inserted into a cartilaginous process called the hyoid horn. The hyoid horn is itself connected by the hyoid muscles to the hyoid bone which is attached to the sternum by powerful muscles. The hyoid bone has a "U" shape and is extremely mobile **(Wainwright *et al.*, 1991)**. The retractor muscles (or hyoglossus muscles) sheath the accelerator muscle and insert on the underside of the tip of the tongue **(Chen *et al.*, 2020)**. The tip of the chameleon's tongue is covered with numerous epithelial glands as well as numerous papillae which adhere to the irregularities of the surface of the prey like sticky hooks **(Schwenk, 1983)**. The contraction of the accelerator muscle associated with the displacement of the hyoid horn backwards creates a strong pressure which ejects the tongue out of the mouth like a catapult. When extended, the tongue is as long as the body. When not in use, the tongue simply rests at the back of the throat, its tip invaginated **(Fouda *et al.*, 2015)**. Chameleons have a greenish-blue colored liver consisting of two lobes. The left lobe is the largest. The gallbladder is attached to it. The pancreas is yellowish and is located behind the stomach near the spleen, which is small and purple **(Bourdain, 2006)**.

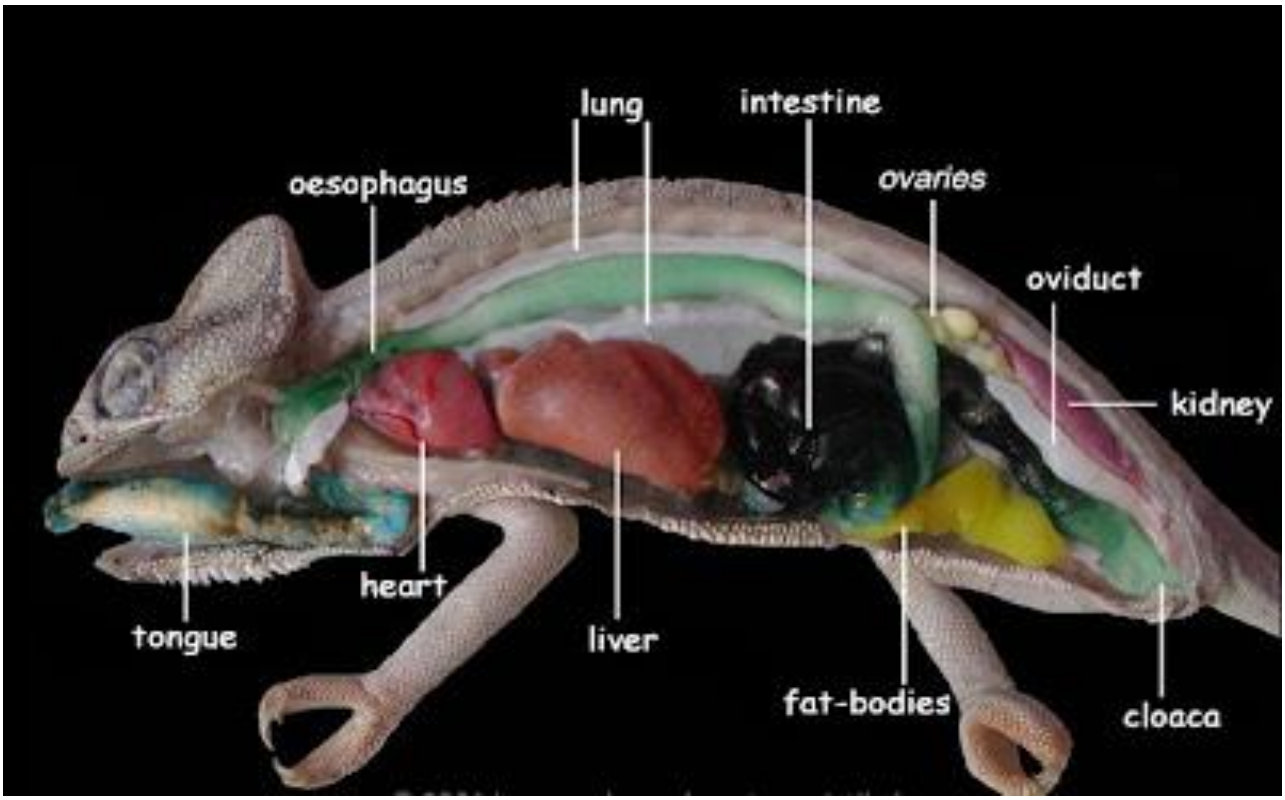

Figure 04: Gross anatomy of female chameleon **(Kusuma, 2008)**.

I.5.2.4. Musculoskeletal apparatus

The musculoskeletal apparatus of the chameleon is adapted to arboreal life **(Higham and Jayne, 2004)**. Its cartilaginous ribs limit pain during falls; its clip ends ensure a firm hold of the support even in high winds; his spine is endowed with great flexibility; the highly specialized muscles of its extensible tongue allow the capture of insects from a distance and the highly developed musculature of its independent eyes makes it possible to detect predators or prey in all directions. Research has also revealed a high content in his muscles of so-called "tonic" muscle fibers as opposed to the so-called "kinetic" muscle fibers which allow movement. These tonic fibers maintain a static, energy-saving state in a passive way, which allows the chameleon to maintain a posture effortlessly **(Tolley and Herrel, 2013)**.

I.5.2.5. Sensory apparatus

Nervous system

The chameleon is like all reptiles, smooth brain. The whole brain-cerebellum does not represent more than 1 percent of the body mass of the lizard **(Mader, 1996)**. Its cerebral cortex is nevertheless developed with two well differentiated hemispheres. It has twelve pairs of cranial nerves including the highly developed optic nerve. The spinal cord extends to the end of the tail, unlike that of mammals.

Visual organs

The eye is the most developed sensory organ in the chameleon. The eyelids, made up of small scales, cover most of the eye except for a small circular opening next to the pupil. They provide excellent protection against desiccation and trauma but limit the field of vision. The chameleon widens its field of vision thanks to large eyeballs located on each side of the head and multidirectional movements of each of its eyes. Thus he is able to see an object located at a vertical angle of 90° and a horizontal angle of 180° **(Necas, 2004)**. Both eyes move independently which provides the brain with two images at once. In addition, the chameleon has very good visual acuity. Its performance is comparable to that of a telephoto lens with a focal length of 100 to 150 millimeters **(Le Berre, 1995)**. It is able to perceive colors that range from red to violet, i.e. from 375 nanometers to 610 nanometers, but sees neither infrared nor ultraviolet **(Bowmaker *et al*., 2005)**.

Hearing organs

The chameleon has neither outer ear nor tympanic membrane. It has a simplified inner ear with only a developed and fluid-filled cochlea. It is the soft tissues and bones of the skull (including a developed pterygoid process) located on each side of the head behind the eyes that transmit sound vibrations and allow the chameleon to detect low frequency sounds essentially between 200 and 600 Hertz **(Bourdain, 2006)**.

Olfactory and taste organs

Smells and flavors are captured in the chameleon by Jacobson's organ located in the oral cavity in front of the palate. Present in mammals and highly developed in reptiles, Jacobson's organ or vomeronasal organ is composed of two cavities lined with sensory cells covered with a thin layer of mucus and is innervated by the first pair of cranial nerves. Taste buds have also been found on the chameleon's tongue **(Schwenk, 1985)** which occasionally allows it to control the environment by licking surfaces **(Ogilvie, 1966)**.

I.5.3. Physiological particularities

I.5.3.1. Thermoregulation

The chameleon is, like all reptiles, ectothermic. This means that its internal temperature is fluctuating and entirely dependent on environmental factors. For each species there is an optimal temperature threshold, also called T.M.P (Average Preferential Temperature) that each animal tries to reach. If an individual's body temperature drops too far below their T.M.P., they cannot digest or defend themselves against disease, their kidneys do not function properly and uric acid deposits form in the body **(Bourdain, 2006)**. To warm up, the chameleon exposes itself to the sun by positioning itself as perpendicular as possible to the rays, flattens itself and adopts a dark coloring of the skin which allows it to effectively absorb the infrared rays, the most energetic. The superficial capillaries dilate; the heart rate accelerates which increases the absorption of heat. On the other hand, if the internal temperature rises too much, the animal withdraws into the shade, clears up, hyperventilates with its mouth wide open, its tongue sticking out and even looks for underground shelters **(Stuart-Fox and Moussalli. 2009)**.

The T.M.P. of an individual varies according to his biotope, his age and his physiological state. The T.M.P of the *Chamaeleo chamaeleon* in southwestern Spain averaged 28 1C in October and 30 1C in June. Slopes of regressions of T.M.P on Ta (ambient temperature at

perch height) indicated that individuals were able to maintain a preferred body temperature of about 30 °C in June but not in October **(Andrews, 2008)**.

In general, the chameleon is more resistant to hypothermia than to hyperthermia, being able to even withstand night frosts in their country of origin. When the surrounding climatic conditions are unfavorable, the chameleon can go into hibernation or aestivation. Hibernation concerns chameleons in high mountain regions or subtropical regions where temperatures drop in winter. Aestivation is used when the temperature rises too high or during prolonged periods of drought. In both cases, the animals take shelter under a layer of moss, leaves or branches as long as the humidity and temperature are not favorable and they cease all activity **(Bennett, 2004)**.

I.5.3.2. Color change

Chameleon skin coloring involves several types of specialized cells located in the dermis. The epidermis is made up of transparent cells. The most superficial are the iridocytes. Their cytoplasm contains purine particles in suspension. Depending on their spatial conformation, they absorb or not the light rays by Tyndall effect, masking more or less the colors of the underlying cells. Pigmented cells, called chromatophores, are of several types **(Goda, 2017)**. The most superficial contain carotenoid pigments in their cytoplasm: these are xanthophores (for yellow) and erythrophores (for red). Deeper down are the guanophores, which, thanks to the transparent crystals of guanine in their cytoplasm, passively reflect or not the blue of the light, depending on the incidence of the rays. Thus if the layer of cells located above the guanophores is made up of xanthophores, the reflected light will appear green by association of blue and yellow. Finally, there are the melanophores, cells with long dendritic processes containing melanin responsible for the dark color of the chameleon's skin **(Dong *et al.*, 2019)**.

Chromatophores are contractile cells that can cause their pigments to move within their cytoplasm under the action of both the central nervous system and the endocrine system **(Ma *et al.*, 2002)**. The alpha-M.S.H. (Alpha Melanophore Stimulating Hormone) and catecholamines, in particular adrenaline, are among the substances with hormonal action highlighted in the chameleon **(Teyssier *et al.*, 2015)**. M.S.H is produced by the pituitary and darkens the skin. Adrenaline is synthesized by the adrenals and makes skin colors more vivid. In the chameleon at rest, the pigments are evenly distributed within the chromatophores, the light is diffused by the iridocytes to the guanophores which reflect it towards the epidermis: the skin then sports brilliant colours. Under the action of a stress such as the sight of an adversary, the light is diffused to the dermis and the xanthophores contract rapidly: the animal

then displays bright colors and dark. When the chameleon is active but not threatened, the melanophores contract and the colors are bright and brilliant. All color variations are therefore possible depending on the position of the iridocytes in the dermis, the incidence of light rays on the guanophores and the state of contractility of the chromatophores. However, it is wrong to think that a given individual is capable of expressing all colors. Indeed, each species has its own palette with a flexible but limited body scheme. The most frequently used colors are brown, black, yellow and green **(Zhao *et al*., 2019)**.

I.6. Reproduction

The entry into the sexual season is initiated by weather changes. The chameleon lives in isolation all year round, except for the breeding period, which extends from mid-July to the end of September. The receptive female displays bright or colored patterns and remains calm when seeing the male. This approaches the female and climbs onto her back **(Andrews and Donoghue, 2004)**. One of the two male hemipenes is external when the two are in contact. Copulation lasts about ten minutes. Females are somewhat passive and end up pushing their partner away. Females indicate their pregnancy by a specific color, often orange, and are more aggressive towards their counterparts **(Keren-Rotem *et al*., 2016)**. They feed and drink more at the beginning of pregnancy, while they almost stop in the days leading up to childbirth. Females lay eggs after a gestation period of about two months. The female descends to the ground, digs a hole about one and a half times as long at the base of a shrub in relatively loose soil, lays 10 to 40 eggs measuring 17/11 mm, covers them and returns to its arboreal territory. Incubation varies with temperature and soil moisture, but lasts about 200 to 290 days, with embryonic development halting during the winter. In May, hatch over 1-2 weeks and the young are 35 mm long from snout to vent **(Andrews *et al*., 2008)**.

I.7. Use in traditional medicine

Since ancient times, humans have used animals to produce drugs, treat diseases, and heal wounds. A number of researchers showed that there is considerable evidence of the use of fauna for therapeutic purposes in various human communities **(Santos *et al*., 2019)**. One of the animal species that is most frequently used in traditional medicine is the reptile. According to **Alves *et al*., (2007)**, a total of 165 species of reptiles are utilized in popular medicine throughout the world. **Ogoanah and Omijie, (2017)** results showed that the use of chameleons dominated (12%) in animal species in Benin City, Nigeria. Where **Osunsina *et al*., (2012)** study in support zone villages around some Nigeria national parks indicated that chameleon meat is used in the treatment of chest pain and cough.

Chapter II

Biological activities

II.1. Antioxidant activity

In order to combat the negative effects of free radicals and other oxidants, the human body possesses a complicated system of naturally produced enzymatic and non-enzymatic antioxidant defenses **(Gulcin, 2020)**. Numerous illnesses, such as cancer, cardiovascular disease, neurological disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol-induced liver disease, ulcerative colitis, aging, and atherosclerosis are brought on by free radicals **(Alam *et al*., 2013)**. Consuming enough dietary antioxidants can improve protection against free radicals. According to a large body of research, antioxidant-rich meals in general and antioxidant nutrients in particular may play a crucial role in disease prevention. Scientists are coming to the conclusion that antioxidant combinations, as opposed to individual substances, may be more potent in the long run. By delaying the onset of degenerative diseases or preventing them altogether, antioxidants may be extremely helpful in improving life quality. Additionally, they have the potential to significantly reduce the price of providing healthcare **(Wang *et al*., 2016)**.

II.1.1.Oxidative stress and reactive oxygen species

Oxidative stress is a phenomena brought on by an imbalance between a biological system's capacity to detoxify these reactive byproducts and the creation and buildup of oxygen reactive species (ROS) in tissues and cells **(Migdal and Serres, 2011)**. ROS have long been thought of being undesirable byproducts of the mitochondria's regular aerobic metabolism process and have been linked to a wide range of illnesses. However, there is mounting evidence that controlled ROS generation also has physiological roles, particularly in controlling cell signaling and redox homeostasis **(Pizzino *et al*., 2017)**.

The oxygen is nonmetal highly reactive oxidizing agent that easily forms oxides with the majority of elements as well as with other substances. It is a stable triplet biradical ($^3O_2$) that is present in the environment in its ground state and goes through a stepwise reduction process **(Taslimi and Gulçin, 2018; Rezai *et al*., 2018)**. Two unpaired electrons with parallel spins are present in each of the two distinct anti-bonding orbitals of molecular oxygen in its ground state. This spin limitation allows it to accept two electrons from an electron source. Redox, on the other hand, is a crucial metabolic process where electrons are moved from one species to another in a living system. Where these are the major chemical processes in living systems that allow an organism to utilize oxygen from the environment for oxidation and to produce ATP **(Gulcin, 2012)**. In living organisms, oxidation-reduction and enzymatic processes are highly intertwined into the production of oxygen. Additionally, as oxygen is the

final electron acceptor in the electron flow system that generates energy in the form of ATP, it serves as a crucial component of aerobic life and our metabolism **(Gülçin *et al.*, 2005, Elmastas *et al.*, 2018)**. However, issues could develop if the electron flow separates (transfer of unpaired single electrons), contributing to the production of free radicals. Free radicals are highly unstable atoms, molecules, or ions with unpaired electrons that are eager to interact chemically with other molecules. Three elements—oxygen, nitrogen, and sulfur—are their sources. Reactive oxygen species (ROS), which include superoxide ($O_2^{\cdot}-$), hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), and nitric oxide ($NO^{\cdot}$), are examples of oxygen-centered free radicals. The damage induced by the hydroxyl (half-life of 10^{-9}S) and alkoxyl (half-life of seconds) free radicals is likely irreparable and is handled by repair processes because they are very reactive and quickly assault the molecules in neighboring cells. And $O_2^{\cdot}-$, $NO^{\cdot}$, and lipid hydroperoxides are less reactive **(Han *et al.*, 2018; Bulut *et al.*, 2018)**. Other nonradical ROS, such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl), exist in living things in addition to these ROS radicals **(Pietta, 2000)**.

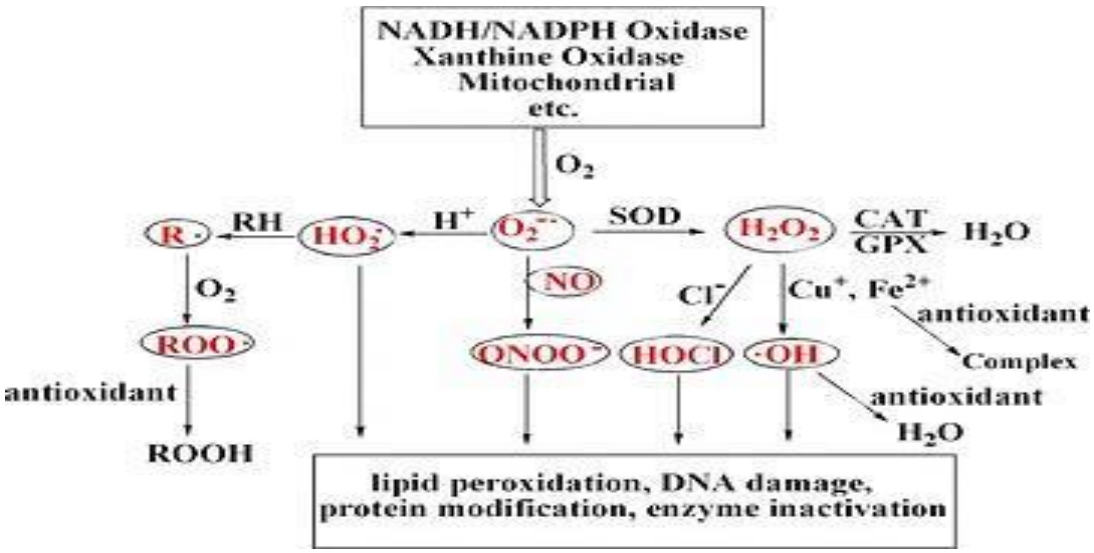

Figure 05: Summary of ROS types and sources, and action point of antioxidants **(Lü *et al.*, 2009)**.

II.1.2. Antioxidant compounds

An antioxidant is a chemical that can prevent other molecules from oxidizing. Antioxidants are essential for reducing oxidative processes and the negative consequences of ROS in both food systems and the human body **(Gulcin, 2020)**. An antioxidant was defined as "any substance that delays, prevents, or removes oxidative damage to a target molecule" but was later changed to "any substance that significantly delays or inhibits the oxidation of that substrate when present in low concentrations compared to that of an oxidizable substrate".

Another definition of an antioxidant states that it "is a compound that directly scavenges ROS or indirectly up-regulates antioxidant defenses or inhibits ROS generation." The process of lipid peroxidation, which is one of the main causes of the degradation of food and pharmaceutical items during processing and storage, can be delayed by antioxidant chemicals, increasing shelf life by scavenging free radicals. Additionally, antioxidants help defend the body against the negative effects of ROS and free radicals. Numerous chronic illnesses are slowed down by them **(Francenia *et al*., 2019)**. Antioxidants are small molecules such as vitamin C, vitamin E, uric acid, and glutathione that play important roles as cellular antioxidants. Synthetic antioxidants such as tert-butylhydroxyl-toluene, tert-butylhydroxyanisole and tert-butylhydroquinone are widely used in the food industry to delay fat oxidation. And plant extracts are considered as antioxidant food supplements. Most natural antioxidants come from fruits, vegetables, spices, grains, and herbs. They contain a variety of antioxidant compounds **(Lü *et al*., 2009)**.

II.1.3. Antioxidant mechanism

The mechanisms that antioxidant defense uses are as follows:

- Preventing the generation of free radicals
- Scavenging of free radicals
- The process of changing damaging free radicals into less toxicants
- Preventing the synthesis of more damaging metabolites
- Blocking the secondary oxidants' ability to spread in a chain
- Repairing the injured molecules
- Activation and improvement of the body's natural antioxidant defense mechanism.

To protect the organism from oxidative stress, each of these defense mechanisms works in concert with the others. The body's antioxidant systems are made up of potent enzymatic and non-enzymatic antioxidants, as well as a system for repair molecules **(Hunyadi, 2019)**.

II.1.4. Antioxidant methods

There are many methods for estimating antioxidant activity that differ according to their purpose and principle of action, including *in vitro* and *in vivo*.

II.1.4.1. *In vitro* methods

Actually, numerous *in vitro* test protocols are really used to assess the antioxidant activity of the target samples. Antioxidant test models differ in a number of ways. There are the several

analytical techniques for assessing antioxidant capacity can be divided into three groups: spectrometry (Colorimetry, Fluorescence, Chemiluminescence), electrochemical (Cyclic voltammetry, Amperometry, Biamperometry), and chromatography (Gas chromatography, High performance liquid chromatography) **(Azat *et al*., 2019)**.

II.1.4.1. *In vivo* methods

The samples that are to be evaluated for all *in vivo* procedures are typically given to the testing animals (mice, rats, etc.) at a certain dose regimen as defined by the relevant method. The animals are typically slaughtered after a predetermined amount of time and their blood or tissues are used for the test **(Alam *et al*., 2013)**.

Table 02: Some methods used in the evaluation of antioxidant activity **(Alam *et al*., 2013)**.

***In vitro* methods**	***In vivo* methods**
DPPH scavenging activity	Ferric reducing ability of plasma
Hydrogen peroxide scavenging (H_2O_2) assay	Reduced glutathione (GSH) estimation
Nitric oxide scavenging activity	Glutathione peroxidase (GSHPx) estimation
Peroxynitrite radical scavenging activity	Glutathione-S-transferase (GSt)
Trolox equivalent antioxidant capacity (TEAC) method	Superoxide dismutase (SOD) method
	Catalase (CAT)
ABTS radical cation decolorization assay	γ-Glutamyl transpeptidase activity (GGT) assay
Total radical-trapping antioxidant parameter (TRAP)	Glutathione reductase (GR) assay
method	Lipid peroxidation (LPO) assay
Ferric reducing-antioxidant power (FRAP) assay	LDL assay
Superoxide radical scavenging activity (SOD)	
Hydroxyl radical scavenging activity	
Hydroxyl radical averting capacity (HORAC) method	
Oxygen radical absorbance capacity (ORAC) Method	
Reducing power method (RP)	
Phosphomolybdenum method	
Ferric thiocyanate (FTC) method	
Thiobarbituric acid (TBA) method	
DMPD (N,N-dimethyl-p-phenylene diamine dihydrochloride) method	
ß-carotene linoleic acid method/conjugated diene assay	
Xanthine oxidase method	
Cupric ion reducing antioxidant capacity (CUPRAC) method	
Metal chelating activity	

II.2. Hemolysis activity

II.2.1. Hemolysis

Hemolysis is defined as the rupture or disintegration of red blood cells (erythrocytes) and then the release of their contents (cytoplasm, including hemoglobin) at the level of the surrounding fluid whatever, and in most cases blood plasma. Hemolysis may occur *in vivo* or *in vitro* **(Madigan, 2010)**. One of the causes of hemolysis is the action of hemolysins, some bacterial or fungal toxins, heat and it can happen from intense physical exercise **(Witek *et al.*, 2017)**. Hemolysins affect the cytoplasmic membrane of red blood cells, resulting in lysis and death of the cells. Because hemoglobin is released into the blood plasma during hemolysis, hemoglobinemia can result. Hemoglobin plays a crucial role in the pathophysiology of sepsis and can increase the risk of infection because it suppresses the innate immune system **(Effenberger-Neidnicht and Hartmann, 2018)**.

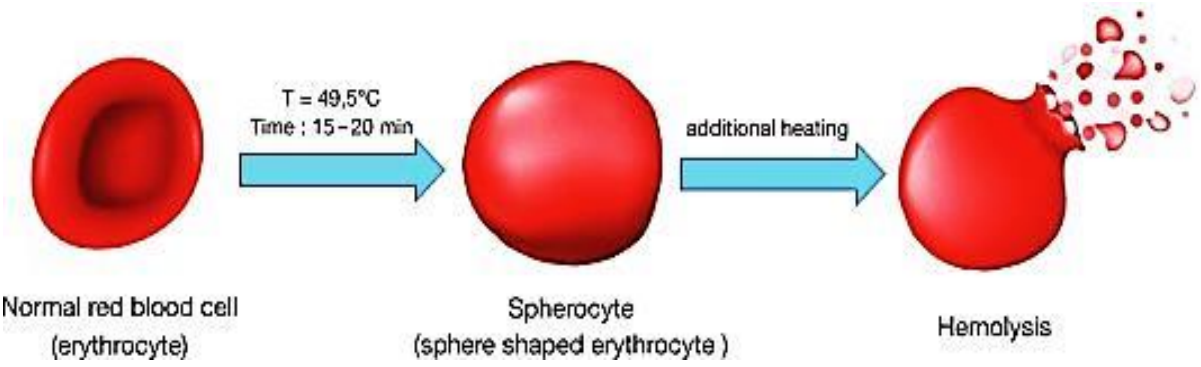

Figure 06: Heat-damaged of Red Blood Cells **(Sočan, 2022)**.

II.2.2. Hemolytic activity

The hemolytic activity of any molecule is its ability to destroy red blood cells, which leads to the release of hemoglobin. Many natural extracts contain chemicals with side effects that may have a hemolytic or anti-hemolytic effect on human red blood cells. Numerous reports indicate that human erythrocyte membranes are blood types that have variable stability as determined by mean body fragility. Natural extracts can positively affect the red cell membrane and many have serious adverse effects **(Vinjamuri *et al.*, 2015)**.

II.2.3. Hemolysis assay

Hemolysis testing represents the most common initial toxicity evaluation, and the use of human erythrocytes (RBC) is the most common option for initial in vitro testing for hemolytic activity. This is why the RBC model has been widely used because it provides a direct

indication of the toxicity of directly injectable formulations in the human body as well as a general indicator of membrane toxicity **(Greco *et al.*, 2020)**. Another advantage of the erythrocyte model is that blood is readily available and cells are easy to isolate from blood; Moreover, its membrane has similarities with that of other cells. Hemolysis results from the destruction of red blood cells resulting from the breakdown of the lipid bilayer. The hemolysis of any extract relates first to the presence of compounds with toxic effects on red blood cells and may be affected by the concentration and effectiveness of the extract **(Vinjamuri *et al.*, 2015)**.

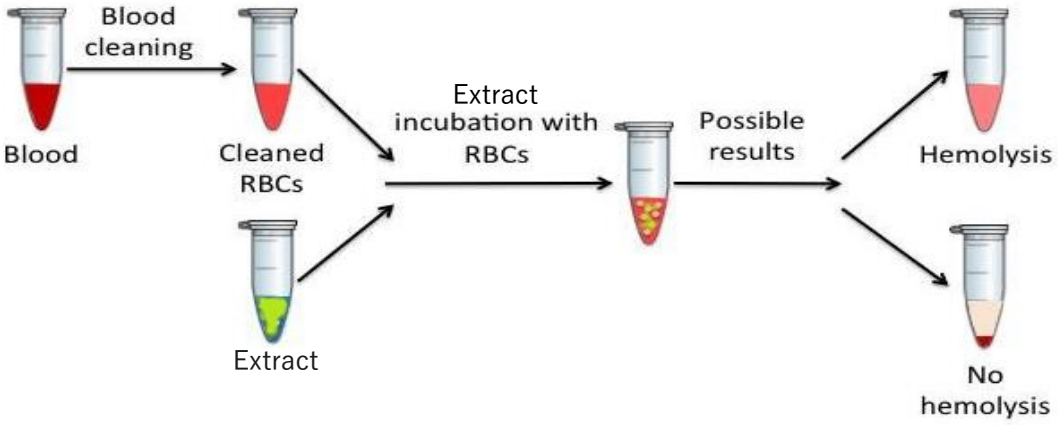

Figure 07: Schematic representation illustrating the hemolysis assay. RBCs: red blood cells (**Fornaguera and Solans, 2017)**.

II.3. Anti-inflammatory activity

II.3.1. Inflammatory

Inflammation is a complex but well-regulated biological response of vascular tissue against aggressive agents, and it occurs when white blood cells fight to protect us from infection agents such as bacteria and viruses. Inflammation may also occur as a result of injury, as in cases of muscle tearing or stretching during exercise, which often leads to pain, swelling and inflammation. Infections can be classified as acute or chronic, and involve a series of biochemical events involving the local vascular system, the immune system, and the different types of cells present in the affected tissues **(Sá *et al.*, 2013)**. Acute inflammation represents the initial response and is characterized by increased movement of plasma and immune system cells from the blood into infected tissues. In chronic inflammation, a gradual change in the type of cells present at the site of the inflammatory reaction is characterized by the simultaneous destruction and healing of the affected tissues **(Azab *et al.*, 2016)**.

The mechanisms involved in the inflammatory process are universal, regardless of the triggering factor, and the typical symptoms of inflammation include increased blood flow, increased cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids, and cellular influx. Cells' reactions to inflammation can result in pathological symptoms as redness, heat, swelling, and discomfort as well as compromised physiological processes **(Esho *et al*., 2021)**.

II.3.2. Inflammation and protein denaturation

Inflammatory response formation is a complicated but tightly controlled process. Polyunsaturated fatty acid arachidonic acid is released from phospholipids in cell membranes by the hydrolysis of phospholipase A2 enzymes. The metabolism of arachidonic acid then occurs via two different enzyme routes, cyclooxygenase into prostaglandins and lipoxygenase into leukotrienes **(Esho *et al*., 2021)**. Members of the eicosanoid family, prostaglandins are the primary mediator of inflammation in the majority of inflammatory illnesses. They are produced by practically all human body cells. The plasma proteins or cells, such as mast cells, platelets, neutrophils, and monocytes/macrophages that are triggered by bacterial products or host proteins are the source of the inflammatory mediators **(Khan *et al*., 2015)**. They bind to certain receptors and cause oxidative damage, vascular permeability, neutrophil chemotaxis, smooth muscle contraction, and discomfort. The majority of the mediators have brief but negative impacts. Denatured proteins are one of the inflammatory mediators, so medications that prevent the precipitation of denatured protein aggregates and protein condensation are beneficial for treating conditions like rheumatic disorders, cataracts, and Alzheimer's disease **(Osman *et al*., 2016)**.

II.3.3. Albumin denaturation inhibitory activity

Protein denaturation is closely associated with the occurrence of the inflammatory response and leads to various inflammatory diseases including arthritis. Protein denaturation is defined as a process caused by external agents such as heat, a strong acid, or a strong base; the organic solvent or concentrated inorganic salt denatures the protein which means that the protein's tertiary structure and secondary structure are confused **(Dharmadeva *et al*., 2018)**. Natural extracts are an essential source of novel chemicals with therapeutic anti-inflammatory effects that prevent protein denaturation. Albumins (bovine serum albumin (BSA), egg albumin or human serum albumin (HSA)) are usually used to test the anti-denaturation activity of the protein. The egg albumin method provides an inexpensive alternative method for testing the anti-inflammatory activity of drugs from natural sources using a denaturation technique

(Osman *et al*., 2016). Where the albumin is exposed to heat or strong acid in the presence or absence of the natural extract, then the absorbance of the denatured albumin is measured and the inhibition percentage is calculated. The extract has a strong effectiveness anti-inflammatory when the percentage is greater than 50% **(Romana *et al*., 2017)**.

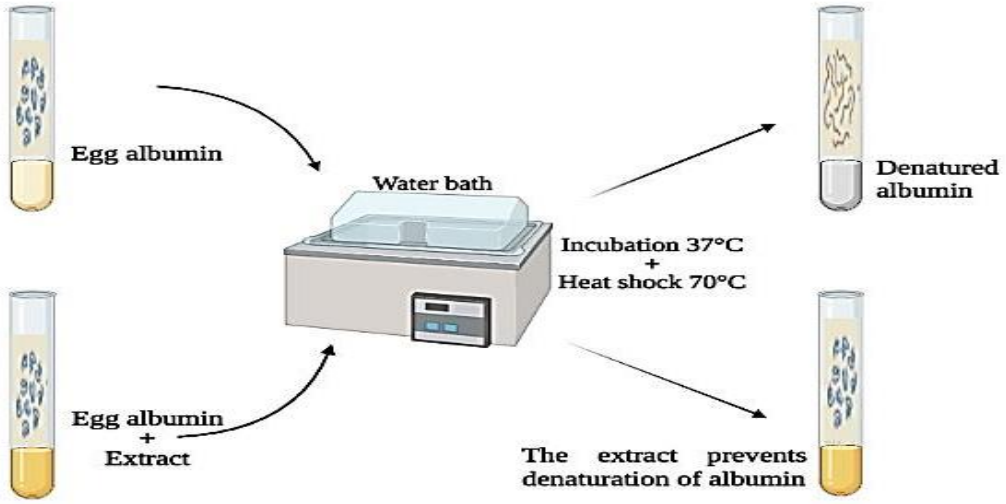

Figure 08: Schematic representation illustrating the albumin denaturation inhibitory activity (Created with **BioRender.com)**.

II.4. Antibacterial activity

II.4.1. Bacteria

Bacteria are a ubiquitous, unicellular and nucleusless micro-organism (prokaryote) whose genome consists of DNA. This consists of a single chromosome, and the presence of plasmids (small piece of circular DNA) may be noted. All bacteria form the kingdom of eubacteria (Eubacteria). They are among the earliest known life forms on Earth. There are thousands of different types, and they live in every possible environment, all over the world. They live in the land, in the sea and in the depths of the earth's crust **(Cohan, 2002)**. Some bacteria have even been reported to live in radioactive waste. Many bacteria live on and in the body of humans and animals (on the skin and in the respiratory tract, mouth, digestive tract, genito-urinary tract) without causing harm. Such bacteria are called resident flora, or microbiome. There are at least as many bacteria in the resident flora as there are cells in the body. Most resident flora bacteria are actually useful to humans, for example, by aiding in the digestion of food or preventing the growth of other more dangerous bacteria **(Pallen and Wren, 2007)**. Only a small number of bacteria cause disease. They are called pathogens. Sometimes, under certain conditions, resident bacterial flora can act as a pathogen and cause disease. Bacteria

can cause disease by making harmful substances (toxins), by invading tissues, or both. Certain bacteria can trigger inflammation that can affect the heart, lungs, nervous system, kidneys or digestive tract. Certain bacteria (such as Helicobacter pylori) increase the risk of cancer **(Proft and Fraser, 2003)**.

II.4.2. Infectious diseases and antibiotics

Worldwide, infectious diseases are the second leading cause of death, with developed countries indicating that the third leading cause of death is infectious diseases. In the United States, infection with bacteria is the most common source of death. Antibiotics are no longer effective in most cases, causing scarcity of treatment options for some bacteria **(Kiehlbauch *et al*., 2000)**. In hospitals, bacterial resistance increases because antibiotics are frequently used. The emergence of new antibiotics from industrial sources always leads to resistance to bacteria and pathogens, which ultimately limits the effectiveness and lifespan of each antibiotic. Besides, the evolutionary process of antibiotic therapy has led to hereditary bacterial resistance **(Milo *et al*., 2005)**.

II.4.3. Antibacterial activity

Antibacterial activity is the most important characteristic of medical textiles, to provide adequate protection against microorganisms, biological fluids and aerosols, as well as disease transmission. Antibacterial activity is a very complex process involving living organisms that every step of their life such as food source, metabolism, respiration and ability to reproduce can be affected by the presence of toxic substances **(Qaddoori, 2016)**. The search for natural antibacterial sources is another potential pathway for bacterial resistance as compounds are sought that act either. They prevent bacteria from growing (bacteriostatic agents) or by killing them completely (bactericidal agents). Natural products have been a rich source of compounds in the discovery of antibiotics as most antibiotic drugs are derived from a natural product. The use of animal resources constitutes an important therapeutic alternative for many populations, and they have been cited for their use against diseases apparently caused by pathogenic microorganisms **(Oliveira *et al*., 2014)**. The antimicrobial activity of the natural extracts is evaluated using laboratory reference strains (Gram-positive and/or Gram-negative bacteria) and the strains are selected according to the traditional use of natural products **(The *et al*., 2017)**. There are many ways to test the antibacterial activity, the most famous of which is the method of dissemination of the disk in the medium of agar, where the determination of the antibacterial activity is estimated by measuring the diameter (mm) of the zone of inhibition caused by different concentrations around the disks **(Bhargav *et al*., 2016)**.

II.5. Anti-angiogenesis activity

II.5.1. Angiogenesis

The development of new blood vessels from the existing vasculature is known as "angiogenesis". It starts in utero and lasts all the way through old age, happening in both health and disease. A blood capillary, which is created by the angiogenesis process, is never more than a few hundred micrometers away from any metabolically active tissue in the body. All tissues require capillaries to facilitate the diffusion exchange of nutrients and metabolites. Angiogenesis increases proportionally with variations in metabolic activity, which causes proportional changes in capillarity. A key factor in this regulation is oxygen. The longevity of vascular networks and structural modifications of vessel walls depend on hemodynamic variables **(Adair and Montani, 2010)**.

II.5.2. Angiogenesis process

The dynamic process of angiogenesis is how new blood vessels are created from pre-existing ones (fig09). There are three different types of angiogenesis: splitting angiogenesis, intussusception, and sprouting angiogenesis, which is the most frequent **(Teleanu *et al.*, 2020)**, and typically consists of the following actions: Enzymatic breakdown of the vascular basement membrane, vascular endothelial growth factor (VEGF)-stimulating endothelial cells (ECs), vascular endothelial cell proliferation, migration, and germination, branching, and tube creation. The process of angiogenesis is controlled by the net balance between pro- and anti-angiogenic factors in physiologically normal individuals, and blood vessels remain static and hardly ever generate new branches **(Yang *et al.*, 2022)**.

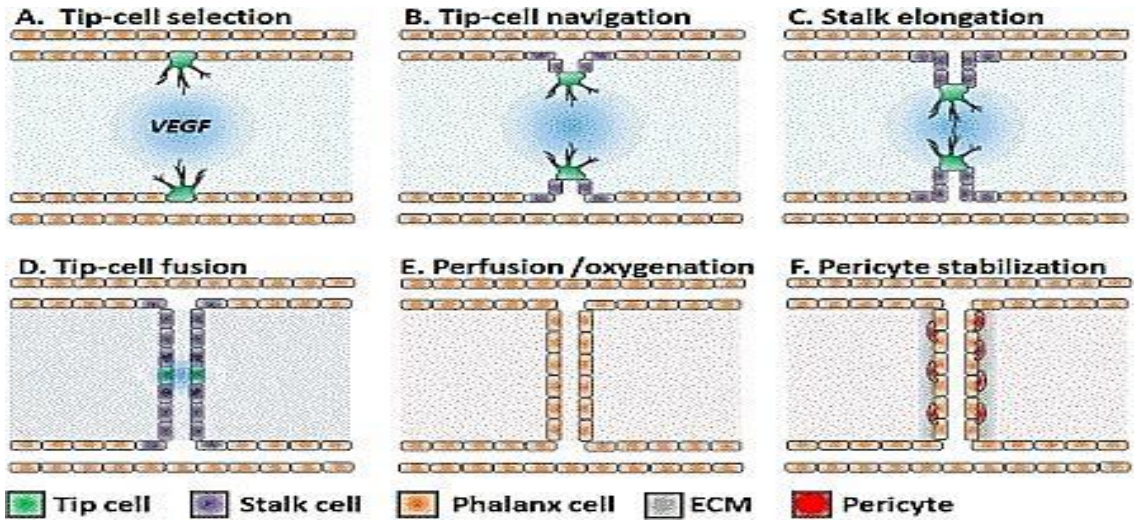

Figure 09: The stages of sprouting angiogenesis, VEGF: Vascular Endothelial Growth Factor, ECM; ExtraCellular Matrix **(Carmeliet *et al.*, 2009)**.

Angiogenesis, the process by which new blood vessels are created from pre-existing ones, happens routinely in the body under physiologically normal circumstances **(Ribeiro *et al.* 2018)**. An imbalance between stimulatory and inhibitory factors that results in a pro-angiogenic state is thought to be the cause of the switch to angiogenes in cancer. Solid tumors require a blood supply in order to develop larger than a few millimeters; hence Angiogenes is essential to the process of tumor genesis. The majority of tumors may go into dormancy at a small diameter, perhaps 2-3 mm, in the absence of neovascularization **(Saman *et al.*, 2020)**.

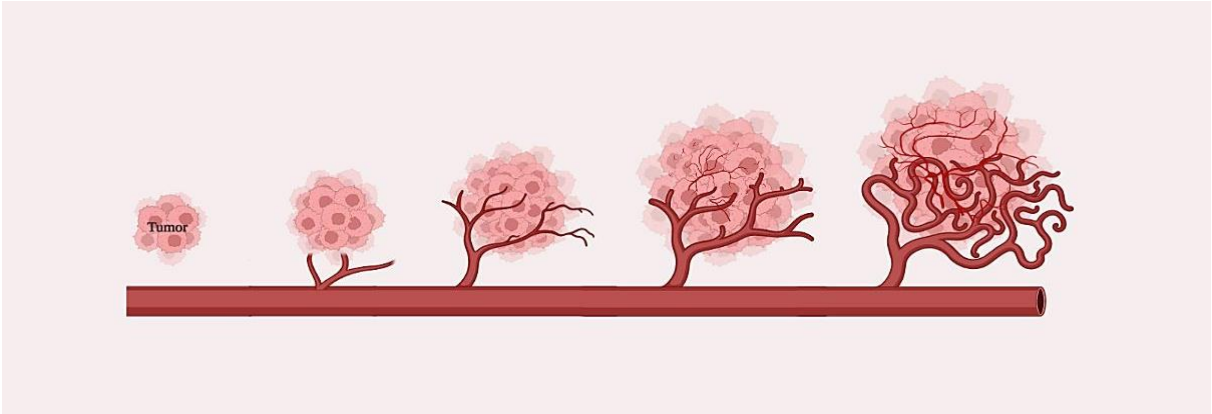

Figure 10: Creation of new blood vessels from existing vessels to feed the tumor (Created with **BioRender.com)**.

II.5.3. Anti-angiogenesis

The process of angiogenesis is controlled by the net balance between pro- and anti-angiogenic factors in physiologically normal individuals, and blood vessels remain static and hardly ever generate new branches **(Yang *et al.*, 2022)**. Over the past 40 years, there has been a lot of interest sparked by the realization that controlling angiogenesis may have therapeutic benefits. In the treatment of peripheral artery disease, ischemic heart disease, and wound healing, angiogenesis can be stimulated. Rheumatoid arthritis, cancer, and other illnesses can all benefit from reducing or inhibiting angiogenesis **(Adair and Montani, 2010)**.

Controlling tumor-associated angiogenesis is a promising strategy for slowing the spread of cancer since angiogenesis is necessary for tumor growth and metastasis **(Weis and Cheresh, 2011)**. Targeting VEGF has emerged as the most potential therapeutic approach for angiogenesis inhibition and cancer treatment due to its critical involvement in the stimulation of tumor angiogenesis. However, anti-angiogenic tactics were created, and antibodies like bevacizumab are now accessible for the treatment of cancer **(Vasudev and Reynolds, 2014)**. It was the initial anti-angiogenic medication authorized for use in people **(Lopes-Coelho *et al.*, 2021)**.

II.6. Anti-hypothyroidism activity

II.6.1. Thyroid gland

The thyroid gland is a small butterfly-shaped gland. It is located at the base of the neck and produces two hormones: triiodothyronine (T3) and thyroxine (T4) **(Porazzi *et al.*, 2009)**. Production of hormones by the thyroid is regulated by a stimulating hormone, TSH (Thyroid Stimulating Hormone). TSH is secreted by the pituitary, a small gland located at the base of the brain. This regulation is based on the principle of the thermostat: a drop in the blood levels of T3/T4, as is the case with hypothyroidism, leads to an increase in the blood level of TSH in order to stimulate the thyroid to produce more T3/T4. Conversely, an excessive blood level of T3/T4 (hyperthyroidism) causes a drop in the blood level of TSH to slow down the activity of the thyroid **(Malik and Hodgson, 2002)**. Thyroid hormones act on the body's basic metabolism by accelerating it. They participate in the regulation of body temperature, body weight, muscle strength, appetite, respiration, growth, the reproductive system as well as cardiac, cerebral and renal functions. A dysfunction of the thyroid gland can therefore give rise to a significant hormonal imbalance affecting the whole body **(Gy *et al.*, 2002)**.

II.6.2. Hypothyroidism

Hypothyroidism is the most common thyroid disorder **(Vanderpump and Tunbridge, 2002)**. This trouble occurs when the thyroid gland becomes less active and no longer releases enough hormones. The metabolic rate goes down and the normal functions of the body slow down. In hypothyroidism, the thyroid gland does not produce enough triiodothyronine (T3) and thyroxine (T4) **(Chiovato *et al.*, 2019)**. The production of thyroid-stimulating hormone (TSH) increases in response to the drop in T3/T4 in order to stimulate their production. This type of thyroid disorder is more common in women, especially around the age of menopause, but hypothyroidism can occur at any age **(Hollowell, 2002)**. Hypothyroidism can be the consequence of a disease of the thyroid gland itself (so-called "peripheral" hypothyroidism) or of insufficient secretion of TSH by the pituitary gland (so-called "central" hypothyroidism) **(Canaris *et al.*, 2000)**.

II.6.3. Causes of hypothyroidism

Central hypothyroidism occurs when the pituitary gland fails to secrete sufficient amounts of thyroid stimulating hormone (TSH), which is necessary for normal stimulation of the thyroid. Secondary hypothyroidism is much rarer than primary hypothyroidism **(Lania *et al.*, 2008)**.

Peripheral hypothyroidism is due to a disorder of the thyroid itself, most common causes are: Hashimoto's thyroiditis: Hypothyroidism develops as the thyroid is gradually destroyed; inflammation of the thyroid; chronic dietary iodine deficiency; Radiation to the head and neck; and rarer causes of hypothyroidism include certain inherited disorders **(Grasberger and Refetoff, 2011)**.

Treatment of hyperthyroidism or thyroid cancer can cause hypothyroidism because radioactive iodine or the drugs used in treatment interfere with the body's ability to make thyroid hormones. Surgical removal of the thyroid leads to a deficit in the production of thyroid hormones **(Almandoz and Gharib, 2012)**.

II.6.4. Carbimazole causes hypothyroidism

Carbimazole is a drug indicated against Graves' disease. Carbimazole is a thyrostatic, that is to say a drug capable of reducing the secretion of thyroid hormones. Carbimazole is a prodrug that is transformed in the blood into its active form, thiamazole (also called methimazole). Thiamazole inhibits the formation of thyroid enzymes (T3 and T4) **(Diav-Citrin and Ornoy, 2002)**. Adverse effects of carbimazole usually appear within the first 8 weeks of treatment. The most frequent reactions are nausea, headache, arthralgia, gastric disturbances, skin rash, pruritus and enlargement of the goitre, which may suggest hypothyroidism by overdose **(Hashem *et al.*, 2016)**. Carbimazole prevents the incorporation of iodine into thyroglobulin residues and prevents conjugation of iodotyrosyl residues to form Iodothyronine inhibits the synthesis of thyroid hormones (T3, T4). Then the pituitary gland responds in the opposite way and raises the level of TSH hormone in an attempt to increase the activity of the thyroid gland. When healthy rats are given significant doses of carbimazole, it causes a decrease in T3/T4 secretion and, consequently, a retrograde increase in TSH **(Sarwar and Parveen, 2005)**.

II.6.5. Treatment

Standard treatment is daily use of the synthetic thyroid hormone levothyroxine (Levo-T, Synthroid, others). This oral medication restores sufficient levels of the hormone while restoring its functions **(Colucci *et al.*, 2010)**. There may be side effects or complications for medications to treat thyroid hormone deficiency. This is why some natural products may help improve the condition of the thyroid gland. Therefore, herbal thyroid treatment aims to treat the cause of the lethargy problem “Hypothyroidism”. Changing the diet and taking certain herbs can help treat an underactive thyroid gland or help the patient respond better to thyroid medications **(Norakant *et al.*, 2017)**.

Second part

Experimental part

Chapter I

Material &Method

I.1. Characterization by survey

I.1.1. Aim of the survey

The aim of our survey is to deepen knowledge about the use, hunting and marketing (sale) of chameleon in the El-Oued region; and to collect as much data as possible on the use of this reptile in traditional medicine.

I.1.2. Description and delimitation of the study area

The study area is located in the Wilaya of El Oued, One of the main oases of the Algerian northern Sahara. The study area covers 18 municipalities (El Oued, Bayadha, Robbah, Kouinine, Guemar, Taghzout, Hassani Abdelkrim, Debila, Sidi Aoun, Magrane, Hassi Khelifa, Reguiba, Mihouensa, Oued Alenda, Ogla, Nakhla, Ourmes et Trifaoui) **(Abdelmonem and Bouallem, 2012)**. The administrative boundaries of the Wilaya of El Oued are: North Tébessa and Khenchla, East Tunisia, South Ouargla and West Beskra and Ouargla. An area of approximately 11200 Km^2, limited by the following geographical coordinates: Longitude: 06 ° 20 'and 07 ° 50' East and latitudes; 32 ° 50 'and 34 ° 30' North.

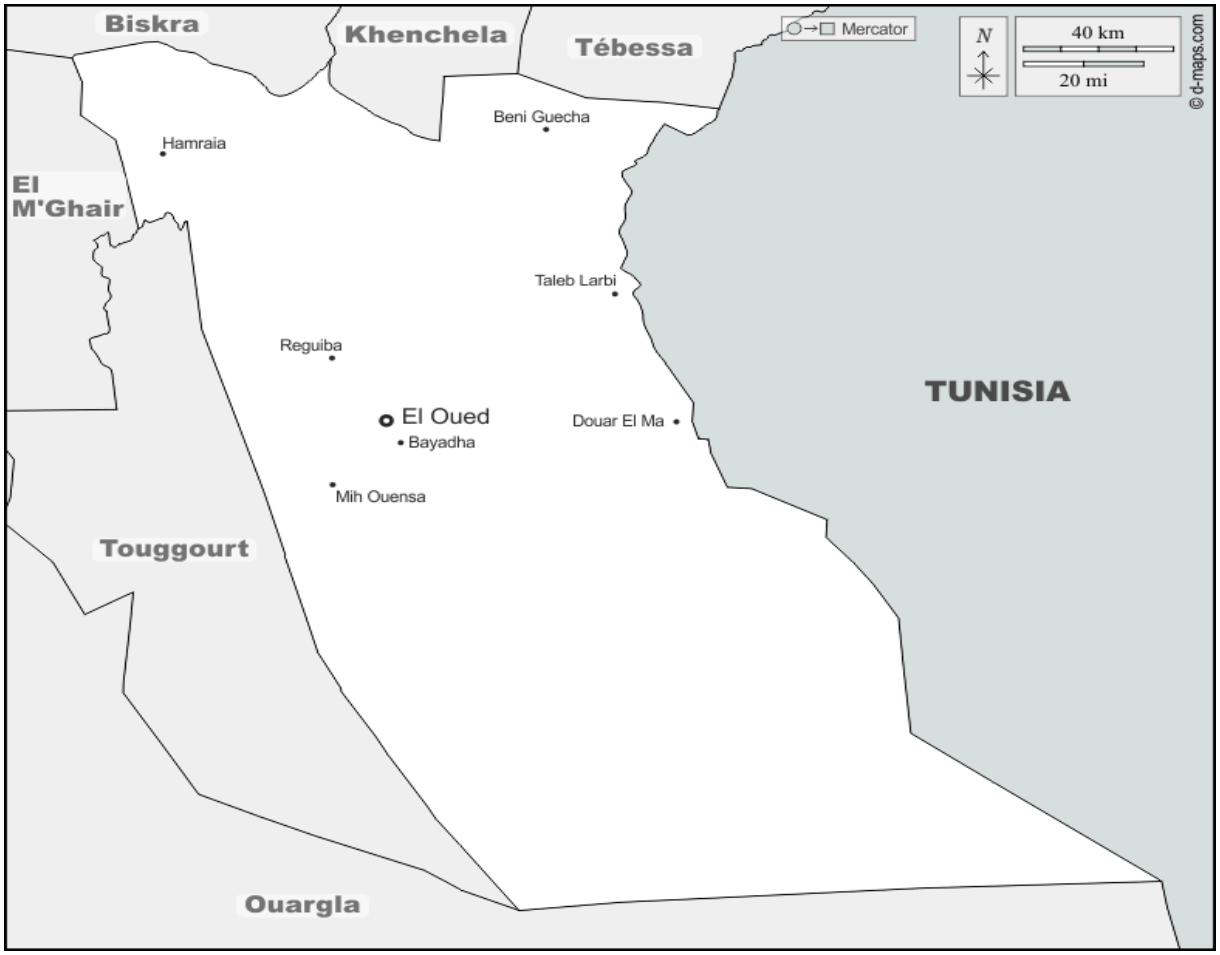

Figure 11: Geographical map of the Wilaya of El-Oued.

The region of El Oued is characterized by an arid climate of the Sahara desert type, in winter the temperature drops below 0 °C while in summer it reaches 50 °C; the average rainfall varies between 80 and 100 mm / year (period from October to February). The climate in El-Oued resembles that of the Sahara with the particularity that the nights are cooler than elsewhere given the difference in temperature felt across the large expanses of sand **(Abdelmonem and Bouallem, 2012)**.

The soil of the Souf region is typical of the Saharan regions. It is a soil poor in organic matter, with a sandy texture and a structure characterized by very high water. The soil of Souf takes two aspects. The most dominant is the dune set. These are large sandy accumulations. The region of Oued Souf is characterized by light soils, predominantly sandy, with a particular structure. These soils are known for their low organic matter content, high salinity, alkaline pH and good aeration **(Kadri and Chaouche, 2018)**.

I.1.3. Study population

The survey was carried out at the level of 18 municipalities. The target population is the collection of users, hunters and sellers who display chameleons. We identified questions for the target population on the use of this reptile in traditional medicine, their hunting and marketing. The questionnaire was paper and electronic. At the end of the survey, we were able to interview 1000 users and 100 hunters and vendors. The distribution of the subjects surveyed according to the municipalities of the Wilaya will be detailed in the results and discussion section.

I.1.4. Survey tools

Our questionnaire (Annex 1) has two parts: one part for users and the other for hunters / sellers, each part includes a series of questions of different types, namely:

- Closed questions;

- Semi open questions;

- Open questions

The questions asked are simple and understandable in order to shorten the duration of the interrogation. The filling of the questionnaire is done by our-selves if the questionnaires are on paper. But if the electronic questionnaire was completed by the respondents themselves, to facilitate communication with the respondents, we used the questionnaire in Arabic language.

a. Users section

The user section includes a series of 18 questions divided into two groups containing personal information on the user and information on use, source of supply, diseases treated, methods of preparation and how used, the interview thus conceived may last 10 to 15 m.

b. Hunter / sellers section

In this section, we chose 14 questions which were divided into two groups, from which we were able to collect information concerning: the place and the hunting period, number of chameleons hunted / sold daily, variations in availability and price and form of sale. Questions can be answered from 10 to 15 m.

I.1.5. Survey process

Before the start of the survey, the finalized questionnaire was tested on around twenty people in order to:

- See if the questions were understandable and unambiguous;
- Confirm, support and enrich the questionnaire and especially to clarify it;
- Eliminate some unnecessary questions;
- Add or remove some propositions in the case of multiple choice questions;
- Rephrase and modify incomprehensible questions.

Our field study took place from March to December 2020. The online questionnaire (electronic questionnaire) was distributed through social media, while the paper questionnaire was directly questioned of the target person.

I.2. Experimental characterization of *Chamaeleo chamaeleon* dry

I.2.1. Aim

The aim of the experimental characterization is to determine nutritional and therapeutic value of the dry *C.Chamaeleon* in the El-Oued region through physicochemical and biochemical parameters, namely: Dry matter; ash; Minerals content; Protein content; Lipid content; Sugar content and vitamins.

I.2.2. Collect samples

I.2.3. Preparation of samples

Chamaeleo Chamaeleon samples were obtained by hunting them in the Algerian desert, specifically in the El Oued region, then the chameleons are slaughtered at the laboratory level. After evisceration and washing: Whole *C. chamaeleon* was dried using the traditional method, whereby we filled the body cavity of the specimens with table salt (coarse salt). Then we arranged it in a cardboard to dry it. The drying conditions required that the specimens be protected from direct sunlight as we placed them in the shade in a well-ventilated area, at a temperature of 35 to 40 °C for a week. After drying the samples, we washed them well with distilled water and placed them in the oven at a temperature of 45 ° C for 24 hours. Using a manual mill, we grinded the samples (Fig.12). The obtained ground material (powder) is kept for subsequent analysis.

Figure 12: Sample preparation steps.

I.2.4. Physicochemical characterization of samples

Determinations of dry matter, moisture and ash, as well as estimation of carbohydrates, fats and vitamins were carried out in the Laboratory of Biology, Environment and Health and pedagogical laboratories at the level of the Faculty of Natural Sciences and Life at the University of Echahid Hamma Lakhdar in El-Oued, Algeria, the determination of proteins and minerals in Fatilab laboratory.

I.2.4.1. Determination of pH value

For pH measurements, 5 g of samples were homogenized with 45 mL distilled water in a blender for 1 min and left to stand for 30 min before measurement with a digital pH meter **(Petit *et al.*, 2014)**, the pH value will be the average of three tests.

I.2.4.2. Determination of moisture and dry matter content

The dry matter is the substance or part remaining from the sample after the moisture has been completely expelled from it in the drying oven, and it includes all the components of the sample. The dry matter content of the samples is determined by drying the sample at 105 ° C in a ventilated oven until a constant weight is obtained. The difference in weight corresponds to the loss of moisture and the residue characterizes the dry matter content of the sample. To avoid any absorption of humidity, it is advisable to operate in tare vessels, placed in a desiccator. The measurements are carried out in triplicate **(Ferreira *et al.*, 2013)**.

Calculations of moisture and dry matter ratio

$$\%\mathbf{M} = \frac{\mathbf{W0} - \mathbf{W1}}{\mathbf{Ws}} \times \mathbf{100} \qquad \%\mathbf{DM} = \mathbf{100} - \%\mathbf{M}$$

With:

M: weight of moisture (weight of the crucible and the sample before drying − weight of the crucible and the sample after drying)

W0: weight of the crucible and the sample before drying

W1: weight of the crucible and the sample after drying

Ws: weight of sample (weight of the crucible and the sample before drying − weight of the empty crucible)

DM: weight of dry matter (weight of sample − weight of moistur)

I.2.4.3. Determination of total ash

Total ash is the residue of mineral compounds that remains after incineration of a sample containing organic substances of animal, plant or synthetic origin. It consists of incineration in a muffle furnace, in porcelain crucibles, at a temperature of 550 ° C for 6 hours until the residue turns white after cooling **(Ferreira *et al.*, 2013)**.

Calculations of ash ratio

$$\mathbf{Ash}\% = (\mathbf{W2} - \mathbf{W3} / \mathbf{W2} - \mathbf{W1}) \times \mathbf{100}$$

Whose:

W1: empty crucible weight.

W2: crucible weight + sample before incineration**.**

W3: crucible weight + sample after incineration

I.2.4.4. Metal content determinations

In order to estimate the minerals we burn the organic material in the sample with a muffle furnace to get ash, then we heat the ash in a hotplate, to which was added nitric acid. The whole is stirred until it becomes homogeneous. Finally, the samples are filtered using filter paper, then diluted to 20ml with bi-distilled water. Contents of iron, zinc, sodium, potassium, calcium, magnesium and copper in the ash were determined by an atomic absorption spectrometer, type of JENWAY (PFP7 Flame Photometer) **(Gençcelep *et al.*, 2009)**. The molybdovanadate technique was used to determine the phosphorus content of the samples **(AOAC, 1990)**. In the molybdovanadate procedure, an acid medium is used to combine orthophosphate and molybdate, resulting in a mixed phosphate/molybdate complex. When vanadium is present Yellow molybdovanadophosphoric acid is created, the intensity of the yellow color is proportional to the phosphate concentration. The spectrophotometer's measuring wavelength is 430 nm.

I.2.4.5. Determination of protein content

The Kjeldahl method (1883) is the reference method for determining total nitrogen and proteins **(Bennani *et al.*, 1995)**. It consists of three stages: digestion, distillation and titration. Where the organic matter can be broken down using concentrated H_2SO_4, heat, K_2SO_4 (to raise the boiling point), and a catalyst (like selenium) to speed up the reaction. Through this procedure, any nitrogen in the sample is converted to ammonium sulfate, the digestate then

neutralized by adding NaOH, which transforms the ammonium sulfate into ammonia. Ammonia is then distilled out and collected in a receiving flask of surplus boric acid to create ammonium borate. To determine the sample's total nitrogen concentration, the leftover boric acid is titrated with a standard acid using a suitable end-point indicator. The measured nitrogen content must be converted to the crude protein content using a specified conversion factor after the total nitrogen has been determined **(Goulding *et al.*, 2020)**. Nitrogen to meat protein conversion factor is 6.25 **(Toumi-Nesr, 2018)**.

I.2.4.6. Determination of total sugars

Total sugars are determined according to the method of **DuBois *et al.*, (1956)** whose principle is based on the following reaction: concentrated sulfuric acid causes, when hot, the departure of several molecules of water from the monosaccharides. This dehydration is accompanied by the formation of a hydroxy-methylfurfural (HMF) in the case of hexose and of a furfural in the case of a pentose. These compounds condense with phenol to give colored complexes (yellow-orange). The intensity of the coloration is proportional to the concentration of the oses. The optical density is measured at 490 nm using a spectrophotometer.

I.2.4.7. Determination of total lipid content

Lipids are cold extracted according to the method of **Folch *et al.*, (1957)** in which 4 g of sample are placed in 45 mL of a mixture of organic solvents (chloroform/methanol 1/2, v/v) and then separated using a mill rotating at 12,000 rpm.

Grinding is done in a sequence of 3 cycles of 1 minute each, 1 minute apart to avoid any heating. The homogenate is vacuum filtered through a conical flask topped with a funnel fitted with sintered glass.

The filter paste is recovered and the process is repeated twice according to the same grinding cycle. The three filters (#150 mL) are combined in a separation funnel and added with 37 mL of distilled water solution containing 0.73% NaCl to allow separation of the chloroform phase (containing lipids) from the methanol/water phase (containing water-soluble impurities in mostly).

The chloroform phase is withdrawn. The chloroform was evaporated under vacuum and the dry extract was weighed at a constant weight. The total fat content is expressed in g/100g of fresh tissue.

I.2.4.8. Vitamins content

- **Analysis of vitamin Group-B content (B1, B6, B12)**

For determination group-B vitamins content (B1, B6, and B12) we followed the next steps: 1g 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 0.1% 2-mercaptoethanol. 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, 2 mL of cold acetonitrile was added to start the second round of deproteinization **(Xu *et al.*, 2020)**. A spectrophotometer was used to measure the clear supernatant at wavelengths of B1, B6, and B12 at 280, 260, and 361, respectively (**Jin *et al.*, 2012; Chen *et al.*, 2011; Guggisberg *et al.*, 2012**). During three different measurements, the standard curves were produced using drug tri B (vitamin B1, B6, and B12), and their values were expressed in mg/100g.

- **Analysis of vitamin E (α-tocopherol)**

In order to measure the amount of α-tocopherol (Vitamin E), we mix 1 gram of the sample with 5 ml of hexane, homogenize it with a vortex, and then centrifuge it for 10 minutes at 5312 rpm. Next, we take 2 ml of the suspension that produces, then at 40 °C, wait for it to evaporate, at that time added 2 ml of 2-propanol into it. The estimation was performed in a spectrophotometer at 290 nm and tested three times. A standard curve for the estimation was made using α-Tocopherol standard. And their measurements were presented in mg/100g **(Tchègnon *et al.*, 2017)**.

I.3. Exploration of the biological activity

I.3.1. Extraction

I.3.1.1. Hexane extract

Twenty grams of the sample (*C. chamaeleon* powder) were placed inside a cartridge for the hexane extraction process, which was carried out using a device Soxhlet. Next, 300 ml of the hexane solution were added to the round-bottom flask of the same apparatus, and after six cycles, a solution containing the sample was obtained (Lipids, vitamin E and non-polar compounds). The solution was then placed in the Rotary evaporator apparatus, which uses pressure evaporation to separate the extract from the hexane. To guarantee that the hexane is completely evaporated, we place the solution in the beaker and place it in the oven at a temperature of 45–50°C for 24 hours.

I.3.1.2. Methanol extract

We weighed 20 grams of the sample (*C. chamaeleon* powder), placed it in a cartridge, added 300 ml of methanol to a round-bottom flask, and then put the two components in a Soxhlet apparatus. After 10 cycles in the apparatus in a continuous process, we obtained a solution of methanol that contained polar compounds. Then, we transport this solution to a Rotary evaporator device where methanol is extracted from the mixture using pressure evaporation. And to eventually obtain the necessary extract, the solution then placed it in an oven set at 45 to 50°C for 24 hours.

I.3.1.3. Aqueous extract

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

I.3.1.4. Extraction yield

The extraction yield (%) of the three extracts was calculated using the following formula: Extraction yield (%) = (W1/W2) 100. Where W1: the weight of the extract after the solvent has evaporated and W2: the dry weight of the sample **(Truong *et al*., 2019)**.

I.3.2. *In vitro* assays of *Chamaeleo chamaeleon* extracts

I.3.2.1. 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, β-Carotene / linoleic acid technique, Reducing power technique and technique of scavenging Superoxide radical ($O_2^{\bullet-}$).

a) Scavenging activities on DPPH • radical

- Principle

The chemical compound 2,2-diphenyl-1-picrylhydrazyl (α, α-diphenyl-β 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 (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg/ml) or ascorbic acid as standard (0.01, 0.02, 0.03, 0.04, 0.04, 0.06, 0.07 mg/ml) 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:

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

With:

- I%: percentage of inhibition,

A0: absorbance of the control,

A1: 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)**.

b) β-Carotene/Linoleic Acid Assay

- Principle

The oxidation of linoleic acid generates peroxide radicals, these free radicals will subsequently oxidize β-carotene causing the disappearance of its red color, which is followed by spectrometry at 470 nm. However, the presence of an antioxidant could neutralize free radicals derived from linoleic acid and therefore prevent the oxidation and bleaching of β-carotene. In this test the antioxidant capacity is determined by measuring the inhibition of the oxidative degradation of β-carotene (discoloration) by the oxidation products of linoleic acid **(Kumaran and Joel Karunakaran, 2006)**.

- Procedure

Tow mg of β-carotene were dissolved in 10 ml of chloroform. 1 ml of this solution is taken which is added to a flask containing 45 µL of linoleic acid and 400 mg of Tween 40 beforehand. After mixing the two phases, the chloroform was subsequently evaporated using a vacuum evaporator. Subsequently, 100 ml of distilled water saturated with oxygen was added to the above mixture with rigorous stirring. 4.5 ml of the resulting emulsion were added to a series of tubes containing 0.5 ml of the extracts to be tested or of Gallic acid (control antioxidant). A spectrophotometer was used to measure the zero time absorbance at 470 nm as instantly as the sample was added to each tube. Next, the tubes were placed in the dark in a 50 ° C water bath for 120 min. Then, the absorbance of each extract is measured at 470 nm at t = 120 min. As a blank, emulsion without β-carotene was applied in measurements. All tests are repeated three times **(Nickavar and Esbati, 2012)**. With the following equation, the inhibition percentage of bleaching (I bleaching %) was determined:

$$\textbf{I bleaching (\%)} = (A1/A0) \times 100$$

Where: **A0**: Initial absorbance, **A1**: Absorbance after 2 h of assay

c) Reducing Power assay

- Principle

The reducing power test method was based on the idea that compounds with reduction potential combine with potassium ferricyanide (Fe^{3+}) to generate potassium ferrocyanide (Fe^{2+}), which subsequently reacts with ferric chloride to form ferric-ferrous complex, which has an absorption maximum at 700 nm **(Irshad *et al*., 2012)**.

- Procedure

Practically, 250 µL of different concentrations of extracts (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg/ml) are mixed with 625 µL of phosphate buffer (pH 6.6, 0.2M) and 625 µL of 1% potassium ferricyanide solution $K_3[Fe(CN)_6]$. After incubation in a water bath at 50 °C for 20 minutes, 625 µL of Trichloroacetic acid (TCA 10%) was added to the mixture, after which the mixture was centrifuged at 3000 rpm for ten minutes. To 625 µL of the supernatant, add 625 µL of distilled water and 125 µL of ferric chloride. The absorbance was measured at a wavelength of 700 nm. The results were compared with the use of vitamin C as a positive control. The increase in the absorbance of the reaction mixture indicates an increase in the reductive capacity **(Pan *et al*., 2008)**.

d) Superoxide radical ($O_2^{\bullet -}$) trapping test

The inhibitory activity of the superoxide radical anion is determined by cyclic voltammetry. The superoxide radical anion is generated by commercial molecular oxygen dissolved in N,N-Dimethylformamide (DMF) which contains 0.02M of Bu4NBF4 at room temperature (28 ± 1 ° VS). The slew rate is maintained at 100mV / s. The applied potential range was from -1.6V to 0.0V. The extracts studied is added to the superoxide radical dissolved in DMF and the voltammograms were recorded **(Benabdesselam, 2017)**.

The following equation determines the extract's capacity to scavenge superoxide radicals (O2•-) **(Lanez, 2019)**:

$$\%\mathbf{O2^{\cdot -}}\ \boldsymbol{radical}\ \mathbf{scavenging\ activity} = \frac{ipa0 - ipa}{ipa0} \times 100$$

Where: *ip0* and *ipa* are the intensity of the anodic oxygen peak current, respectively without and with test compound.

I.3.2.2. Hemolysis assay

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

$$\%\ hemolysis\ inhibition\ = 100 - \left(\frac{\text{Sample}}{\text{Control}}\right) \times 100$$

I.3.2.3. Anti-inflammatory activity

We selected the egg albumen 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 (0.4, 0.6, and 0.8 mg/ml) with 0.2 ml of fresh hen's egg albumin, 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:

$$\%\boldsymbol{Inhibition}\ = \left(\frac{Ac - As}{Ac}\right) \times 100$$

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

I.3.2.4. Antibacterial activity

The methods used to study the interaction between *C. chamaeleon* extracts and microbial species are based on the diffusion of this extract in culture media to inhibit the growth of a pathogenic microorganism. The diffusion method on agar medium or aromatogram was used to test the extracts of *C. chamaeleon*'s antibacterial activity. The antibiogram was the method's inspiration for the aromatogram, which measures the diameter of inhibition around an extracts impregnated disc to quantify the extract's inhibitory activity **(Anthoney Swamy *et al.*, 2014)**. Each extract was examined at four different concentrations (100, 75, 50, and 25 mg/ml) diluted in DMSO (dimethyl sulfoxide) with 20µl in disc. The six strains of bacteria that were used as a basis for the selection are: *Bacillus subtilis, Listeria innocua, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella typhimurium*.

Table 03: The six strains of the studied bacteria.

Strains		Code	Gram
S1	*Bacillus subtilis*	ATCC 6633	+
S2	*Listeria innocua*	CLIP 74915	+
S3	*Escherichia coli*	ATCC 25922	-
S4	*Pseudomonas aeruginosa*	ATCC 9027	-
S5	*Staphylococcus aureus*	ATCC 6538	+
S6	*Salmonella typhimurium*	ATCC 14028	-

The extracts is placed to a disc 6 mm in diameter made of sterile Whatman paper, which is then placed in a Petri dish 90 mm in diameter that is filled with a "Mueller Hinton" agar medium that is 4 mm thick, previously inoculated by swab with the inoculum. The latter is produced at a 0.5 Marc Ferland concentration, with 106–108 CFU.mL-1 for each strain. The control boxes are applied with discs impregnated with DMSO and antibiotics (Azithromycin, Gentamicin), for each test, there are three repetitions. The Petri dishes are incubated at 37°C for 24 hours. Measurement of the diameter of the zone of inhibition is used to obtain the results, where creates a translucent halo around each disc; the presence or absence of a halo would indicate whether the germs were sensitive to the extracts under test or not **(Boughendjioua, 2017; Selam *et al*., 2022)**.

I.3.3. *In ovo* assays of *Chamaeleo chamaeleon* extracts

The chick chorioallantoic membrane (CAM) assay has been used for a long time to investigate how compounds affect angiogenesis in vivo, giving researchers' access to a high throughput, and easily accessible, self-sustaining screen without the requirement for facilities for keeping animals. It is acknowledged as a valid in vivo method for evaluating the early tissue response to biomaterials. Here, we explain the usefulness of the CAM assay for studying *C. chamaeleon* extracts' effectiveness for preventing angiogenesis **(Ribatti, 2017)**.

During the initial days of development, eggs are incubated in a humidified incubator at a constant relative humidity of 60-80% and a temperature of 37 °C. In order to prevent the fetus from adhering to the membranes of the egg shell, the fertilized chicken eggs are placed horizontally and rotated repeatedly in the incubator **(Abdulsahib *et al*., 2016; Kennedy *et al*., 2022)**. After three days of incubation, ethanol 70% was employed to thoroughly wash the egg's surface. Sterilized forceps were used to make a tiny hole in the shell, and then approximately 2 ml of egg albumin were taken out using a hypodermic needle to assist the CAM and yolk sac to separate from the shell membrane **(Mousseau *et al*., 2014; Kim *et al*., 2000)**. Then the egg was saved for one more day of incubation at 37 °C. On day four, the egg's sac was pierced, creating a small opening (3–4 cm) in the shell. To guarantee sterility and maintain humidity, the created window was covered with a sterile laboratory wrap or plastic cover, and then the eggs were placed back into the incubator until day seven in the development of the chick embryo.

On the seventh day, we divided the chicken embryos into five groups (6 in each group), Group 01: DMSO, Group 02: Bevacizumab, Group 03: hexane extract, Group 04: methanol extract, Group 05: aqueous extract. The extracts were prepared at a concentration 50 mg/ml), and 20 µl of extracts were placed on a round disc of Whatman paper, which was then allowed to desiccate before being transported to the CAM, eggs are sealed and then kept in the incubator again for a 72 hours (up to day 11) **(Abdulsahib *et al*., 2016)**.

On the eleventh day, the numbers of blood vessels was evaluated; the vessel branching locations in the square region were examined for each treatment group. The angiogenesis score is computed using the mean and standard error of the mean (SEM) of the new branching points in each set of data. Depending on the quantity of branching points, each egg received an angiogenesis score ranging from 0 to 4. If there are ≥35 branching points, the angiogenesis score is 4. If the branches measure between 25 and 34, the score is 3, and between 15 and 24 it is 2. If there are <15 points, the score is 1 **(Kamili *et al*., 2019)**. And all cases were

photographed using a cell phone (Poco X3), and the necessary pictures were obtained. The factors include the quantity of blood vessels and their branches, the level of angiogenesis, and the area of inhibition assessed in the samples **(Mousseau *et al.*, 2014)**.

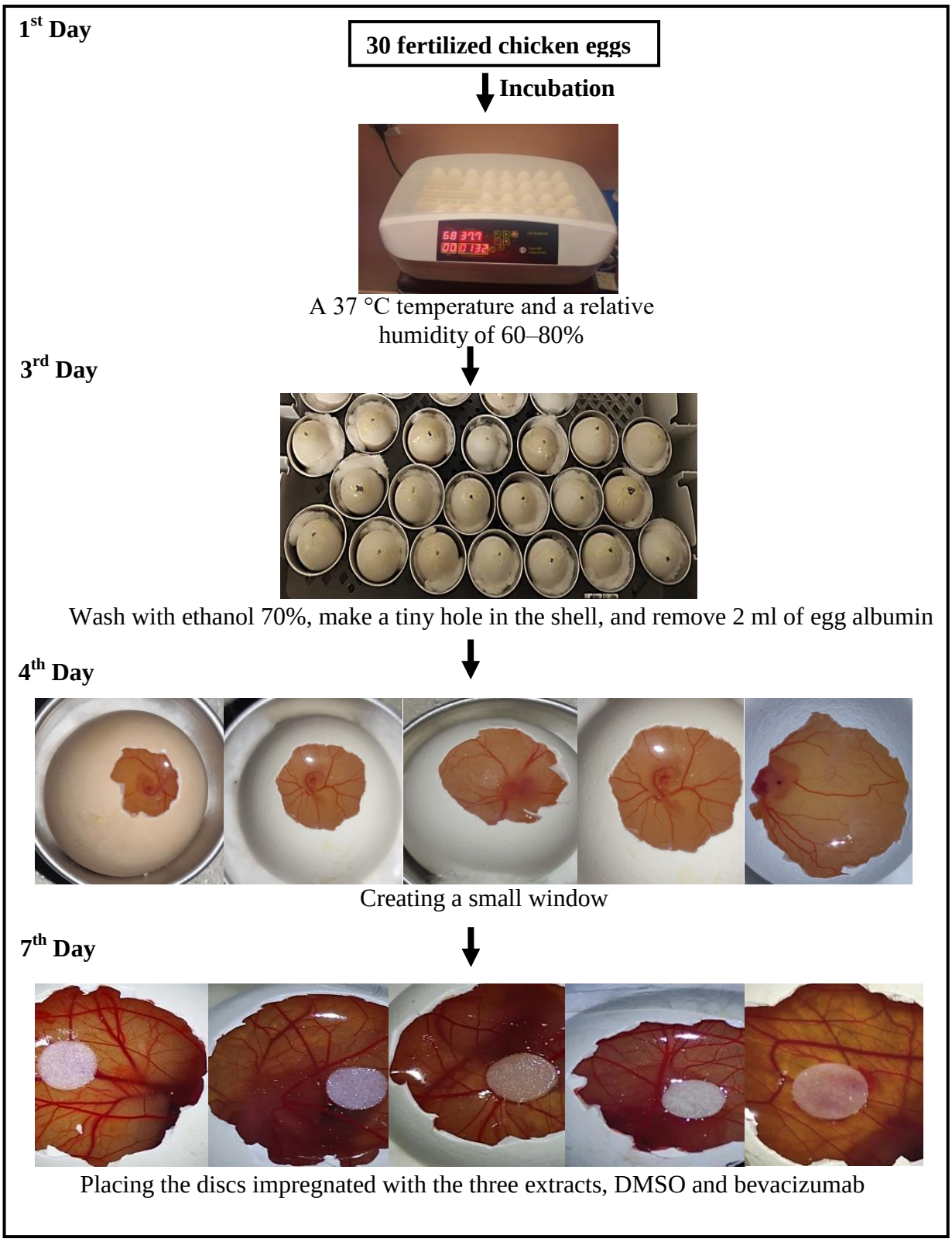

Figure 13: Steps to perform the chick chorionic membrane (CAM) test.

I.3.4. *In vivo* assays of *Chamaeleo chamaeleon* extract

I.3.4.1. Acute toxicity

The aqueous extract was tested for toxicity by Intraperitoneal injection, and the *C. chamaeleon* powder was tested for toxicity by being consumed in various diet compositions. Healthy adult albino Wister rats weighing between 140 and 215 g were used in the tests. The animals were separated into nine groups at random, with six rats in each group housed in polypropylene cages of 50×35×20 cm with sawdust, and maintained at a temperature of 30±2 °C. The lighting is organized to provide 12 hours of light and 12 hours of darkness for every 24 hours. Animals were weighed before receiving peritoneal injections of the extract at doses of 50, 100, 500, 1000, and 2000 mg/kg. After a 12-hour period of fasting, the rats were given chameleon powder mixed with chow in the following ratios: 10%, 25%, 50%, and 75%.

For the first four hours, then 24 hours, then 72 hours, then seven days, then fifteen days, clinical symptoms, les changes in the skin, fur, eyes, and mucous membranes, were noticed. Gross behaviors, body postures, locomotion, rearing, tremors, and gait, as well as the impact of *C. chamaeleon* extract on grip strength, pain response, and righting reflex, were studied for a total of 15 days. The consumption of food and water, as well as mortality, was also observed **(Subha and Geetha, 2017)**.

I.3.4.2. Evaluation of *C. chamaeleon* effectiveness in Wistar rats exposed to carbimazole

1. **Animal treatment**

The study was in vivo using a total of 30 adult male Albino Wister rats, with an average weight of 223.8±25.5, obtained from the Pasteur Institute in Algiers. These rats were subjected to a conditioning period for a period of 15 days to animal house conditions at a temperature of 25 °C, 62% humidity, and a photoperiod of 12 hours of light and 12 hours of darkness. They were treated according to the guidelines stipulated in the Guide for the Care and Use of Experimental Animals **(Albus, 2012)**. Polypropylene cages with a size of "50 x 35 x 20" divided into six rats in each cage with free access to water and food. The cages are lined with sawdust and cleaned daily until the end of the experiment and they were fed standard food according to **Southon *et al.*, (1984)** they were given tap water to drink throughout the experiment.

2. Experimental process

After adaptation period, which lasted 15 days, the rats were divided into five experimental groups, each group containing six rats, each group was treated separately, as we discuss in the sections below:

⇒ **Group 01(Cont):** As a group as a control, they were given normal drinking water and fed only standard food during the experimental period.

⇒ **Group 02 (Carb):** All members of this group were fed only standard food and given drinking water cloudy with carbimazole at a rate of 0.005% for 30 days, then they were injected intraperitoneally with physiological water for seven days.

⇒ **Group 03 (Carb + Levo):** All individuals in this group were fed standard food only, and given drinking water clouded with carbimazole at a rate of 0.005% for 30 days, Then give them drinking water cloudy with levothyroxine sodium 0.02% for 7 days.

⇒ **Group 04 (Carb + Ext):** All individuals in this group were fed standard food only, given drinking water infused with carbimazole at a rate of 0.005% for 30 days. Then they were given the aqueous extract of dry *C.Chamaeleon* for seven days by intraperitoneal injection at a concentration of 50 mg/kg.

⇒ **Group 05 (Carb + Cha):** All members in this group were fed standard food only, where they were given drinking water containing 0.005 carbimazole for 30 days, Then give them the dry C.Chamaeleon powder mixed with their food at a rate of 10% for 7 days.

3. Sacrifice of rats and collection of blood and organs

The rats are anesthetized with chloroform (94%) after 16 hours of fasting and are sacrificed. The blood sample is taken at the time of sacrifice in EDTA tubes for hematological analyzes dry tubes for biochemical analyzes.

After the dissection, the livers, kidneys, and testis are carefully removed, rinsed with distilled water then with 0.9% NaCl and then weighed. Some of the organs are used for the preparation of the homogenate and the other is kept for histological sections. The homogenates of the organs are used for the determination of the parameters of oxidative stress (malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)).

4. Methods of blood analysis

4.1. Hematological parameters assay

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

4.2. Biochemical parameters assay

Blood glucose

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

Glucose + **glucose oxidase** + $O_2 \rightarrow$ Gluconic Acid + $\mathbf{H_2O_2}$

$\mathbf{H_2O_2}$ + Peroxidase + Oxygen acceptor (Colourless) $\rightarrow$ H.O + Oxidised acceptor (**Coloured**)

Calcium

The calcium measurement is based on the formation of a colored complex between the calcium in the sample and 2,7-(bis[2-arsonophenylazo])-1,8-dihydroxynaphthalene 3,6-disulphonic acid (Arsenazo III) , in an alkaline medium, and the intensity of the color formed is directly proportional to the concentration of calcium present in the sample tested **(Michaylova and Ilkova, 1971)**.

Calcium (Ca) + Arsenazo III $\rightarrow$ **[Ca-(Arsenazo III)]**

Phosphor

The photometric analysis of molybdenum blue, which is produced by the reduction of phosphor molybdenum, is used to measure the amount of phosphorus in the blood. While organic phosphate does not react with molybdic acid, inorganic phosphate must be created using H_2SO_4 or $HClO_3$ **(Leiboff. 1928)**.

$PO_4^{=}$ + $(NH_4)_6$ Mo_7 $O_{27}.4H_2O$ + H_2SO_4 or $HClO_3 \rightarrow$ Mo / PO_4 (**yellow complex**)

Mo / PO_4 + ascorbic acid $\rightarrow$ **Hetro polymeric blue molybedenum blue**

Testosterone

Serum testosterone assay is performed by cobas e411 analyzer is a fully automated immunology analyzer, which uses the electrochemiluminescence (ECL) technique. And this is done by carefully choosing the electrochemically active "substrates" (such as tris-propylamine, TPA, and ruthenium chelates). The substrate will first come into contact with the cathode where it will absorb too many electrons and become negatively charged. The analyte will then reach the anode, where it will release an electron and become positively charged. Third, the substrate and the analyte come into contact. The extra electron from the substrate is transferred to the analyte with low electron density. The idea of electron valency change serves as the foundation for electron transmission. Finally, the transmission of an electron results in the transmission of a photon, which may be measured and used to determine the analyte's quantity **(Wiwanitkit, 2007)**.

Thyroid hormones

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

C- reactive protein

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

Aspartate transaminase

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

$$\text{Aspartate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + \text{Glutamate}$$

$$\text{Oxalacetate} + \text{NADH+H+} \xrightarrow{\text{MDH}} \text{Lactate} + \textbf{NAD+}$$

Alanine transaminase

The measurement of ALT activity was carried out by the spectrophotometer following an enzymatic kinetic method. Alanine aminotransferase (ALT), also known to as glutamate-pyruvate-transaminase, is a transaminase (GPT). L-glutamate and pyruvate are produced as a result of the amino group transfer from L-alanine to -ketoglutarate, which is catalyzed by ALT. Lactate dehydrogenase (LDH) and NADH convert pyruvate to lactate. The alanine transferase activity in the sample is correlated with the NADH concentration. At a wavelength of =340 nm, spectrophotometry is used to take the measurement **(Schumann *et al*. 2010)**.

$$\text{Alanine} + \text{2-Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH+H+} \xrightarrow{\text{LDH}} \text{Lactate} + \textbf{NAD+}$$

Albumin

The indicator's change in color from yellow-green to green-blue whenever albumin with bromcresol green are present at a slightly acidic pH. The relationship between the amount of albumin in the sample and the color intensity that results is proportional **(Webster, 1974)**.

Bilirubin

Diazotized sulfanilic acid transforms bilirubin into colored azobilirubin, which is then photometrically evaluated. There are two fractions present in serum: bilirubinglucuromide and free bilirubin loosely linked to albumin (bilirubin indirect). Only the former responds immediately in aqueous solution (bilirubin direct), while free bilirubin requires solubilization with dimethylsulfoxide (DMSO) to react (bilirubin indirect).The findings of determining both the direct and indirect bilirubins correspond to total bilirubin. The sample's bilirubin concentration has a direct relationship with the color's intensity **(Malloy and Evelyn, 1937)**.

Alkaline phosphatase

Alkaline phosphatase (ALP) catalyzes the transfer of the phosphate group from 2-amino-methyl-1-propanol to p-nitrophenylphosphate (pNPP), releasing p-nitrophenol and phosphate. The amount of catalytic alkaline phosphatase present in the sample directly correlates with the rate of p-nitrophenol synthesis as measured photometrically. By using spectrophotometry, reading is done at wavelength 405 nm **(Rosalki *et al*., 1993)**.

$$\text{p-Nitrophenylphosphate} + \text{AMP} \xrightarrow{\text{ALP}} \textbf{p-Nitrophenol} + \text{Phosphate}$$

Total protein

In an alkaline medium, proteins produce an intense violet-blue complex with copper salts. Antioxidant iodide is incorporated. The amount of total protein present in the sample directly relates to the color's intensity **(Gornall *et al*., 1949)**.

Urea

Urease catalyzes the hemolysis of urea, present in the sample, into ammonia (NH4+) and carbon dioxide (CO2). The ammonia formed is incorporated into α-ketoglutarate by the action of glutamate dehydrogenase (GLDH) with parallel oxidation of NADH to NAD. The decrease in NADH concentration in the method is proportional to the concentration of urea in the sample tested **(Kaplan, 1984)**.

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2 \qquad \text{GLDH}$$

$$NH_4^+ + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

Creatinine

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

Uric acid

By using the enzyme uricase, uric acid is converted to allantoin and hydrogen peroxide ($2H_2O_2$), which when mixed with peroxidase (POD), 4-aminophenazone (4-AF), and 2-4 Diclorophenolsulphonate (DCPS) results in the formation of red quinonaimine. The amount of red quinonaimine that forms is proportional to the amount of uric acid that is present in the sample tested **(Schultz, 1984)**.

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{DCPS} \xrightarrow{\text{POD}} \textbf{Quinoneimine} + 4H_2O$$

5. Determination of oxidative stress parameters

Preparation of homogenates

One gram of tissue (liver, kidney, and testis) from each rat in the different groups studied was used. After grinding and homogenizing the tissues in TBS (50 mM Tris, 150 mM NaCl, pH 7.4), the cell suspension is centrifuged at 3000 rpm for 15 minutes. Then the supernatant obtained is kept on ice for the assays of the oxidative stress parameters.

Determination of protein level

The Bradford method was used to spectrophotometrically measure the protein content of the supernatants using bovine serum albumin as a reference **(Bradford, 1976)**. 100 mg of Coomassie Brilliant Blue were dissolved in 50 ml of ethanol to make the Bradford Reagent, which was then agitated vigorously for two hours with an agitator. Next, incorporate 850 ml of water and 100 ml of phosphoric acid (to obtain 1L of solution). Whatman filter paper was used to filter the Reagent. Where, 40 µl of the homogenate and the standard were added to 2

ml of Bradford Reagent in tubes in the dark. After five minutes of incubation, the absorbance at 595 nm was measured. Protein concentrations are assessed by comparison to a standard range of bovine serum albumin carried out under identical conditions.

Determination of Malondialdehyde level

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

$$MDA\ (nmol/mg\ prot) = \frac{OD\ sample\ \ x\ 10^6}{1 \times 1.56 \times 10^5 \times DF \times mg\ prot}$$

Where: OD: Optical density at 532 nm, **1.56 x10^5 M^{-1} cm^{-1}**: Molar extinction coefficient of the MDA, **DF**: Dilution factor, **1**: Optical path length.

Determination of reduced glutathione level

The GSH concentration determination Based on the development of a yellow color when DTNB (5,5′-Dithiobis-(2-nitrobenzoic acid)) is added to compounds containing sulfhydryl groups. The mixture of 0.8 ml tissue homogenate and 0.2 ml 0.25% sulphosalicylic acid was agitated, and then chilled at 4 °C for 15 minutes. The mixes were then centrifuged for 5 minutes at 1000 rpm. A final mixture of 0.5 ml of supernatant, 1 ml of TBS (pH 7.4), and 0.025 ml of 0.01 M DTNB was created. The absorbance at 412 nm was measured after 5 minutes. The amount of total GSH was calculated as nmol GSH/mg protein **(Weckbecker and Cory, 1988)**.

$$GSH\ \left(\frac{nmol}{mgprot}\right) = \frac{OD\ \times 10^6\ \times 1.525}{13100 \times 0.8 \times 0.5 \times DF \times mg\ prot}$$

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

Determination of Superoxide dismutase activity

The method of assaying SOD activity using NBT by the superoxide anion ($O_2^{\cdot}$), is used as a basis for detecting the presence of SOD **(Beauchamp and Fridovich, 1971).** Of which, mix 500µL of EDTA-Met, 900µL of Phosphate Buffer (pH = 7.8), 25µL of Sample, and 50µL of

NBT. Then put the mixture in a water bath for 5min at 25°C and then add 25 µL of Riboflavin to the mixture then put the mixture in the light for 20 min, the same steps for the control with 0µL of the Sample replaced by the addition of 25µL of Buffer phosphate. the absorbance is read at λ=560 nm.

$$I\% = \frac{OD_C - OD_S}{OD_C} \times 100$$

With: **I%**: % inhibition of NBT reduction by SOD, **OD_C**: Optical density of the control, **OD_S**: Optical density of the sample.

And: The 50% inhibition is equal to 1 unit of enzyme. 50% inhibition = 1 unit of SOD, so the antioxidant activity of the enzyme equals SOD units / mg of pro.

Determination of Catalase activity

Catalase activity was determined using **Aebi, (1984)** methodology. The reaction was initiated by mixing 20 ml of supernatant with 780 ml of phosphate buffer (KH_2PO_4, 0.1 M; pH 7.5) and 200 µl of H_2O_2 (0.030 M). The drop in absorbance at 240 nm was observed every 30 seconds for 2 minutes in order to track the breakdown of H_2O_2. International units per minute and per gram of protein (IU/min/g of protein) were used to express the enzymatic activity.

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

Where*:* **A1**: Absorbance at the first minute, **A2**: Absorbance at the second minute, **T:** Time interval in minutes.

6. Histopathological study

For the histological study, the fragments of the organs of all batches are taken. They must be removed quickly to avoid autolysis, which takes place after a few moments of the death of the animal. After rinsing the samples with distilled water then with 0.9% NaCl, they are immediately immersed in a fixative (10% formaldehyde) **(Leclerc-Mercier *et al.*, 2016)**. The technique used includes the following steps: placing these tissue samples in special cassettes with turned walls to allow the passage of liquids. Then dehydrating the samples at using an automatic device which allows the automatic and progressive passage of the samples in ethanol baths of increasing concentration (70%, 95% and 100%). and after that the parts are then immersed in baths of liquid paraffin. The tissues being maintained and soaked in paraffin, then comes the embedding stage which consists of including the impregnated tissue in a block of paraffin which, by solidifying, will allow its cutting. This operation uses “so-called inclusion” devices closing a reservoir of paraffin maintained in the liquid state by a heating system, a small tap and a refrigerated metal plate to obtain the rapid solidification of the paraffin block containing the tissue. The production of thin sections of a few microns (5

µm on average) is possible thanks to special devices called "Microtomes". These sections are spread on microscope slides, smoothed and fixed on the slide by the use of heated gelatinous water. and for staining, the technique used is that with Hematoxylin-Eosin or (Hematein-Eosin) **(Elmalti *et al*., 2007)**; which requires the presence of acid alcohol (100 ml of 70% ethyl alcohol + 50 ml of HCl acid), ammoniacal water (100 ml of distilled water + 2 ml Ammonia) and Eosin solution (100ml Eosin 3% aqueous solution, 125 ml 95% ethyl alcohol, 375 ml distilled water and 2 drops of acetic acid). The staining follows the following steps: deparaffinization and hydration of the slides with tap water then rinsing with distilled water. Then are placed in a Harris Hematoxylin bath (15 minutes) which colors the basophilic structures (nuclei) purplish blue. The blades are immersed in acid alcohol (1 to 2 dives); then in a tap water bath with verification of the differentiation under the microscope. Bet in an ammoniacal water bath, then in an Eosin bath (15 seconds to 2 minutes) which colors the acidophilic structures (cytoplasm) pink. All of these baths are separated by tap water washes. Finally, Observation under the microscope: the preparations of which were then dried and then observed under an optical microscope and photographed using a camera **(Bremond-Gignac *et al*., 2004)**.

I.3.5. Statistical analysis

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

Chapter II

Results & Discussion

Conclusion

Conclusion

One of the most important natural biological resources is the wildlife in the prairies, forests, and deserts, whether plant or animal. The desert is a biological environment rich in natural resources with important therapeutic effects. Compared to plants, studies on the evaluation of natural products of animal origin are crucial because they are currently unexplored, which could lead to major medical developments.

The *Chamaeleo chamaeleon* is well known to be used in the El-Oued region to treat several diseases, and from this our research aims to prove their biological effectiveness. Where our study includes a questionnaire directed to users and another directed to hunters/sellers, and we also studied the physiochemical characteristics of dried *C. chamaeleon* powder, and then evaluated the biological activities of *C. chamaeleon* extracts.

Based on the survey, we determine that the use of *C. chamaeleon* is safe and effective in treating several diseases, namely tonsillitis, thyroid diseases, cough, skin diseases (vitiligo, melasma, and leprosy…), scorpion sting, urinary tract infection, and leukemia. The questionnaire also revealed that the sale/hunting of *C. chamaeleon* is not random, as they are hunted by experienced people while avoiding breeding times, which secures the risk of their extinction.

In our current study, we discovered that dry *C. chamaeleon* powder has a high nutritional and therapeutic value since it contains a significant amount of proteins and numerous beneficial minerals like phosphorus, calcium, magnesium, sodium, and potassium. Additionally, there are vitamins such as vitamin E and group B vitamins B1, B6, and B12. As a result, the extracts of *C. chamaeleon* powder had significant antioxidant activity, also giving it substantial medical significance in treating infectious and inflammatory diseases; this is what contributes primarily to the treatment of tonsillitis. All extracts had high activity against angiogenesis, making them a strong candidate for the prevention of tumor growth and the treatment of cancer through its capacity to prevent the cancer cells in the tumor from receiving nutrition and oxygen, leading to their death and the tumor's disappearance.

According to the results of the *C. chamaeleon* toxicity test in Wistar Albino rats, it shows that *C. chamaeleon* is safe and non-toxic, and *in vivo* study proves that the powder and aqueous extract of chameleon have antioxidant activity and efficacy in the treatment of inflammation. In addition, our findings show that *C. chamaeleon* has anti-hypothyroidism activity that strongly recommends them for the treatment of hypothyroidism.

In view of the novelty of this topic, its scientific value, and its biological importance, it would be interesting to pursue further research regarding a further determination of the physiochemical properties in the characterization of the amino acids constituent of therapeutic *C. chamaeleon* peptides, as well as the fatty acids constituent of chameleon lipids, and the identification of additional types of vitamins present in *C. chamaeleon*;

- It is interesting to continue searching for more results using micro-devices with other types of solvents to detect the active compounds in *C. chamaeleon* powder;
- An *ex vivo* study on tumor cell culture complements our anticancer findings;
- Experimenting *in vivo*, the effectiveness of *C. chamaeleon* in the treatment of skin diseases, as well as the treatment of scorpion stings;
- Application of clinical studies of the topic.

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Annexes

Annex 01: Questionnaire sheets

Survey 01

The use of *Chamaeleo chamaeleon* (El-Bouyeh) in alternative medicine

Place: …….

Age: Less than 18 years □ [18-30] □ [30-45] □ [45-60] □ Over 60 years' old □

Sxe: Women □ Men □

Study level: primary □ middle □ secondary □ university □

Scientific name: *Chamaeleo chamaeleon* Family: Chamaeleonidae

1. **Treated Diseases:**

Tonsillitis □ - Cough □ - Thyroid diseases □ - Others.....

2. **Preparation:**

……
…….....

3. **Methods of use :**

.....
.....

4. **Used parts :**

- head □ - whole body □ - others.....
_ Why ? ....

5. **How is the dose taken?**

- Eat □ - Drink □ - Anointing □ - Put a piece under the tongue □ - Other.....

6. **Dose time:**

- In the morning □ - In the evening □ - With food □ - Before bed □ - Other.....

7. **Number of doses per day:**

-Once □ - Twice □ - Three times □ - Others……

8. **Duration of treatment**

-Day □ -Two days □ -Week □ -Stop at healing result □

9. **Dose value**

……...

10. **Are there side effects? - Yes □ - No □ - Maybe □**
11. **Are you satisfied with the chameleon therapy? - Yes □ - No □**
12. **Are there plants to add with it? What is it?**

……
……...

13. **Source to get it -Herbalists □ -From the desert □**
14. **Non-medical uses**

……...

Survey 02

About *Chamaeleo chamaeleon* hunting and selling (El-Bouyeh)

Place: …….

Age: Less than 18 years □ [18-30] □ [30-45] □ [45-60] □ Over 60 years' old □

Sxe: Women □ Men □

Study level: primary □ middle □ secondary □ university □

1. **Are you a hunter or seller of *C.chamaeleon*?**

- Hunter □ - Seller □ - together □

If you are a hunter:

2. **Hunting Place**

……

3. **Hunting method**

……

4. **When (hunting time)?**

……

5. **How many chameleons are hunted per day?**

……

6. **Why (the purpose of hunting them)?**

- For self-use □ - Sell it to herbalist □ - Sell it to user □ - Others.....

If you are a seller

7. **Number of *C.Chamaeleon* sold per day?**

……...

8. **Who is the *C.Chamaeleon* sold to?**

- Elderly □ - middle age □ - Children □ – Others……..

9. **Which season most requested?**

……...

10. **Form to sell it? -Live □ - Dry □**

Annex 02: Calibration curves of vitamins determined

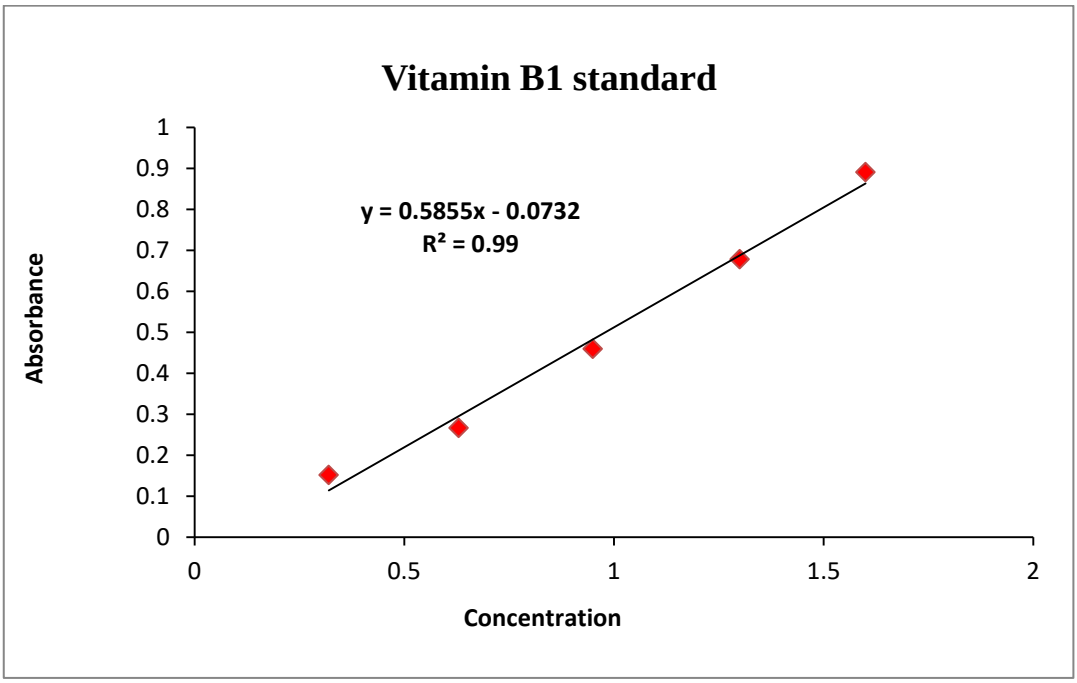

Figure 01: Calibration curve of thiamine (Vit B1) for determination of vit B1 concentration in *Chamaeleo chamaeleon*.

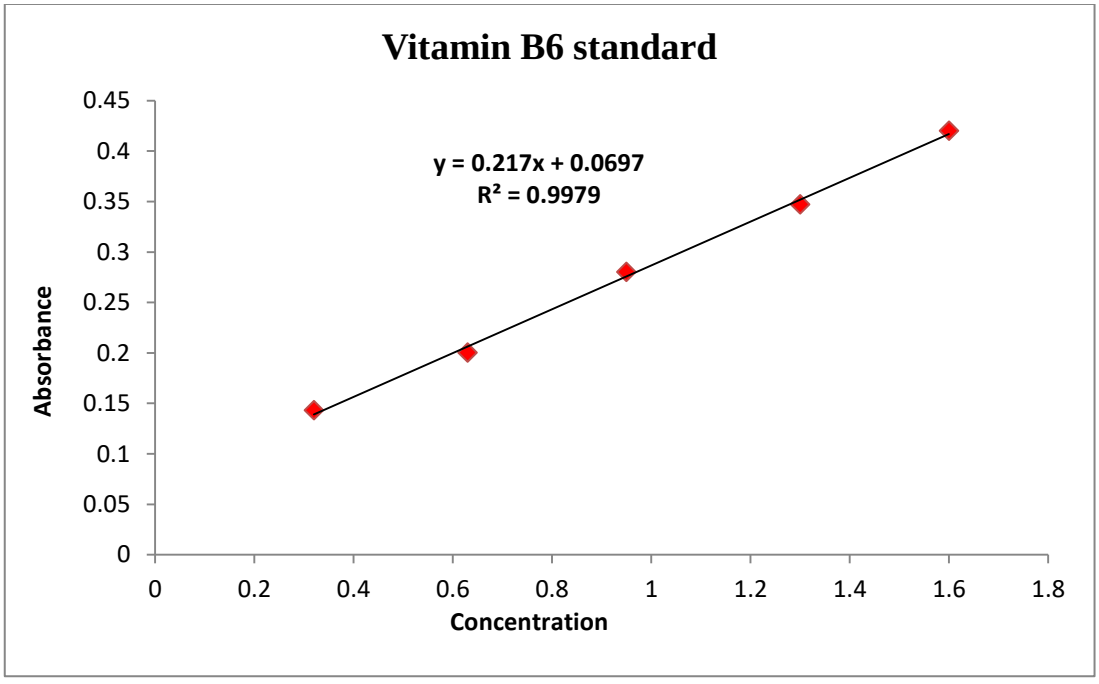

Figure 02: Calibration curve of pyridoxine (Vit B6) for determination of vit B6 concentration in *Chamaeleo chamaeleon*.

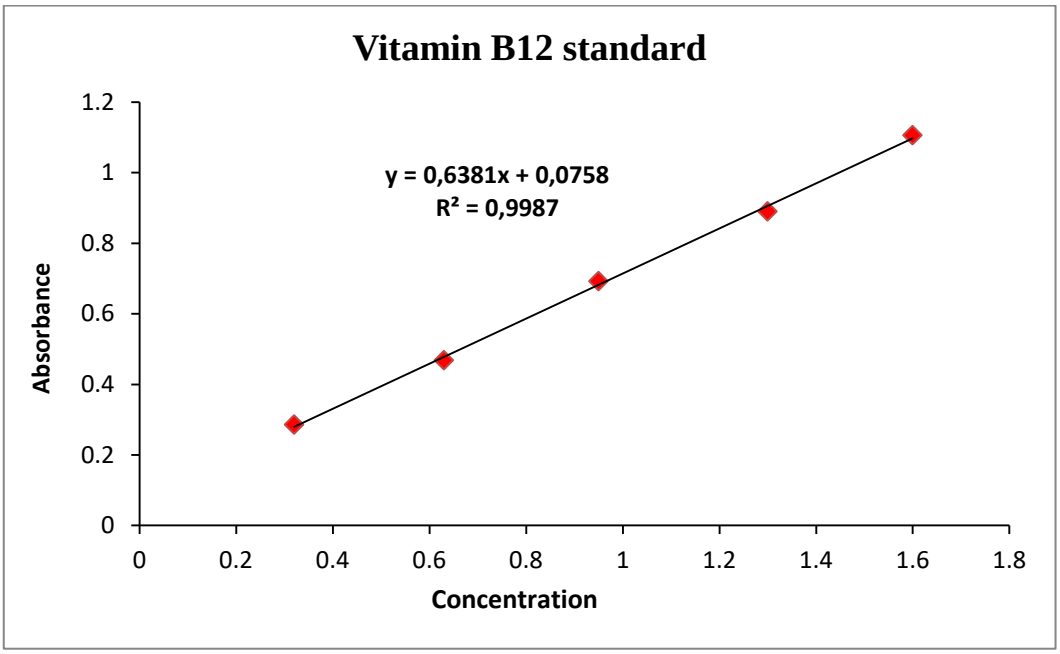

Figure 03: Calibration curve of cobalamin (Vit B12) for determination of vit B12 concentration in *Chamaeleo chamaeleon*.

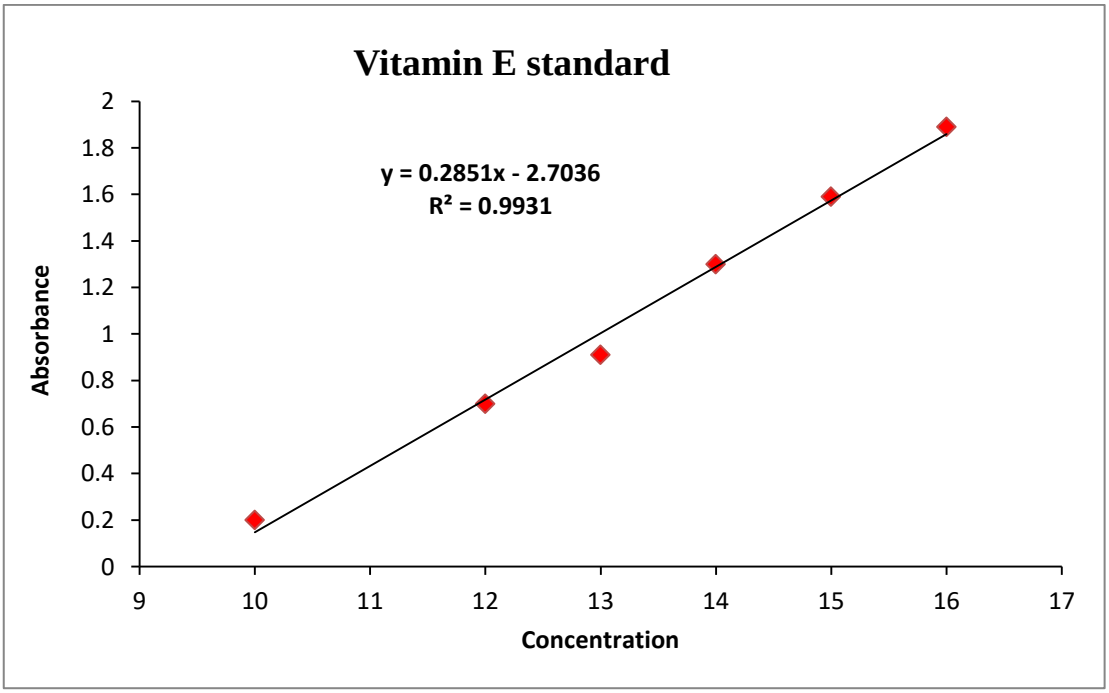

Figure 04: Calibration curve of a-tocopherol (Vit E) for determination of vit E concentration in *Chamaeleo chamaeleon*.

Annex 03: the Cyclic voltammograms of ($O_2^{\bullet-}$) radical in the absence and presence of increasing concentrations of the extracts

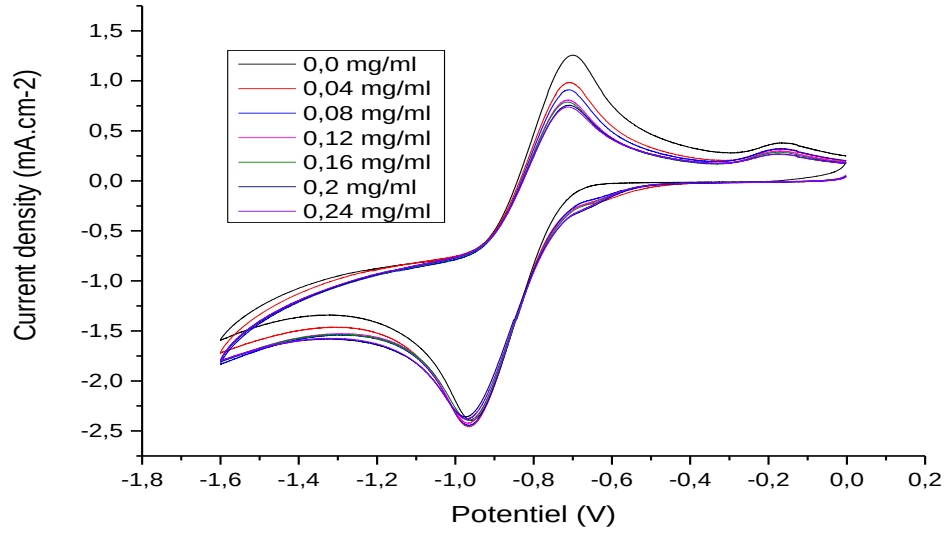

Figure 05: Cyclic voltammograms of ($O_2^{\bullet-}$) radical in the absence and presence of increasing concentrations of the aqueous extract were recorded on a glassy carbon electrode at a potential sweep rate of 100 mV/s.

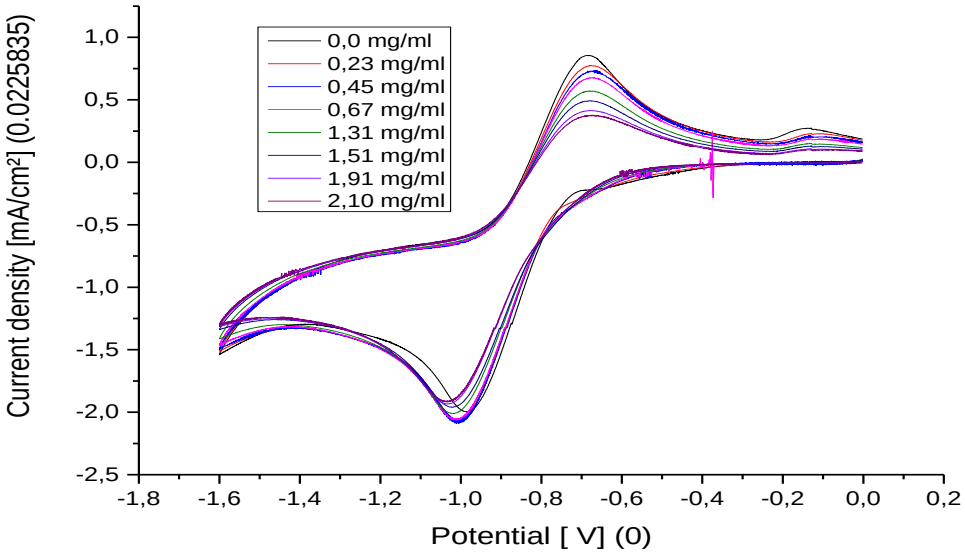

Figure 06: Cyclic voltammograms of ($O_2^{\bullet-}$) radical in the absence and presence of increasing concentrations of the methanolic extract were recorded on a glassy carbon electrode at a potential sweep rate of 100 mV/s.

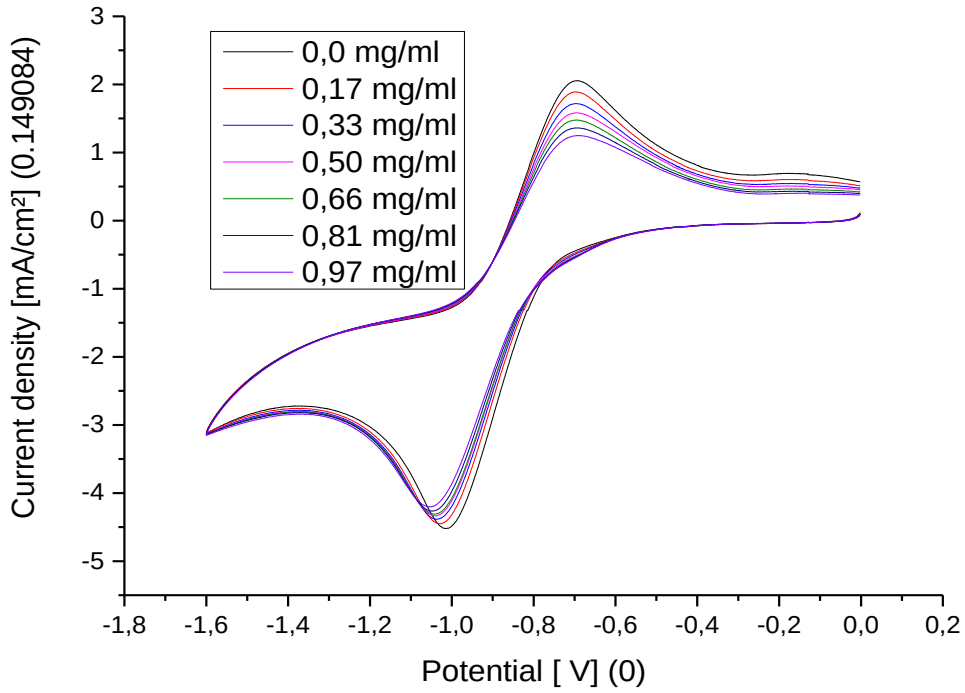

Figure 07: Cyclic voltammograms of ($O_2^{\bullet-}$) radical in the absence and presence of increasing concentrations of the hexane extract were recorded on a glassy carbon electrode at a potential sweep rate of 100 mV/s.

Annex 04: Hemolysis percentage of sodium dodecyl sulfate (SDS) and percentage inhibition of albumin denaturation by Acid acetylsalicylic

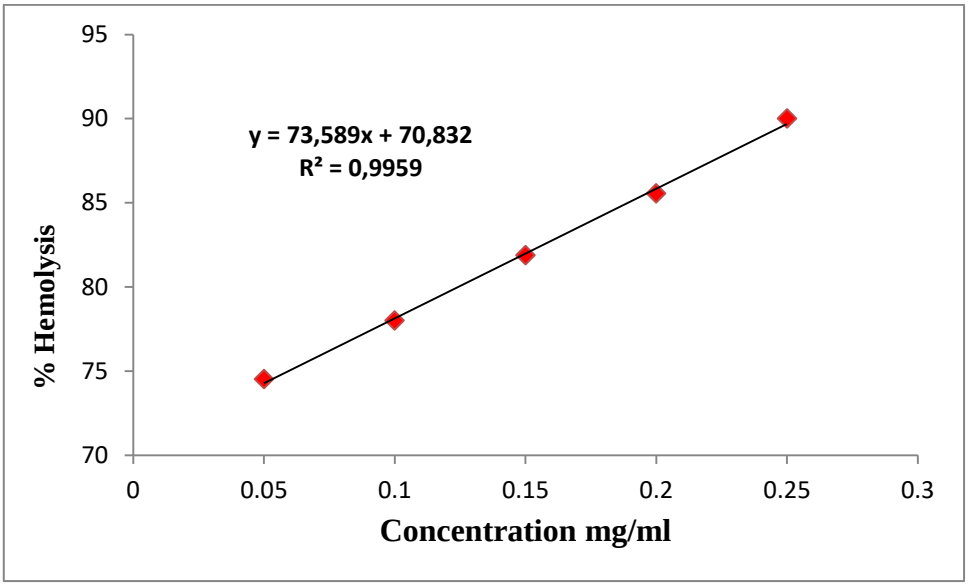

Figure 08: Hemolysis percentage of sodium dodecyl sulfate (SDS)

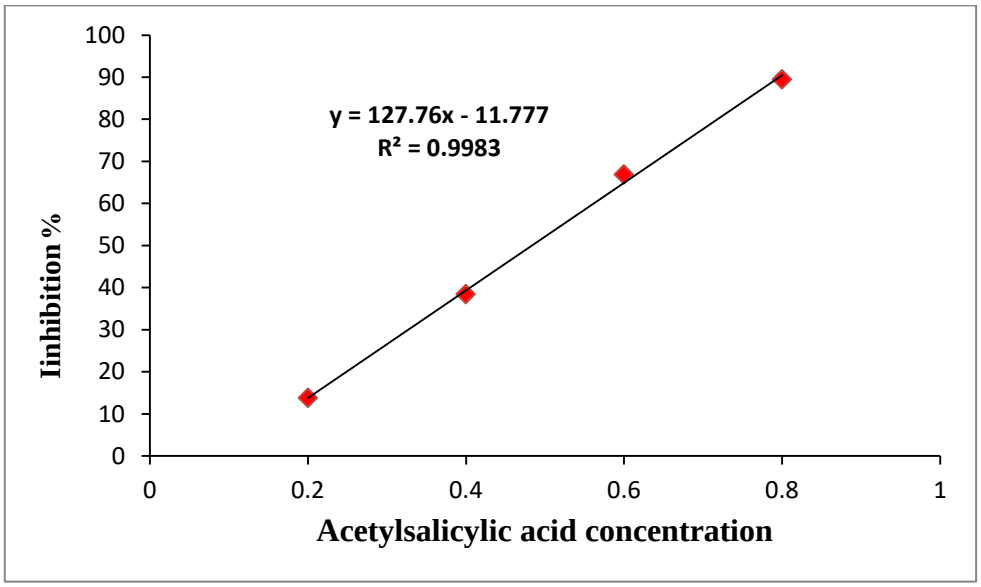

Figure 09: Percentage inhibition of albumin denaturation by Acid acetylsalicylic

Annex 05: Antibacterial activity pictures

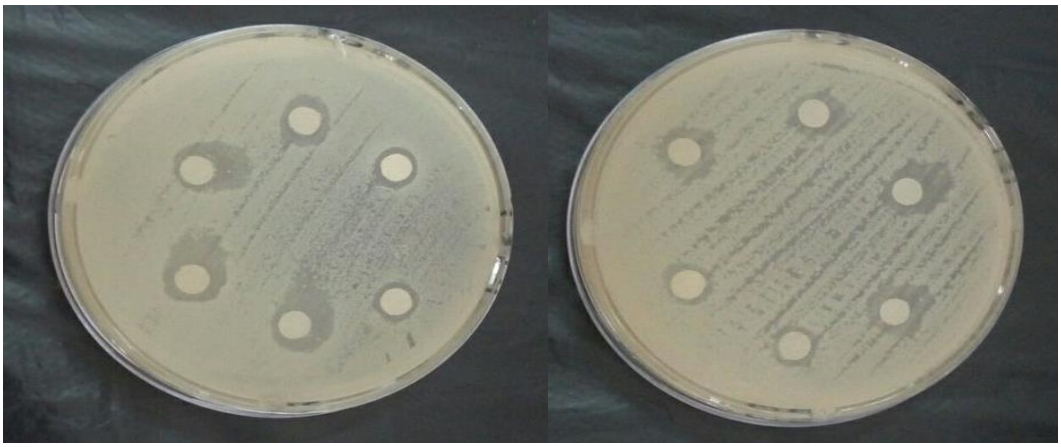

Figure 10: Pictures of the antibacterial activity of the methanolic extract

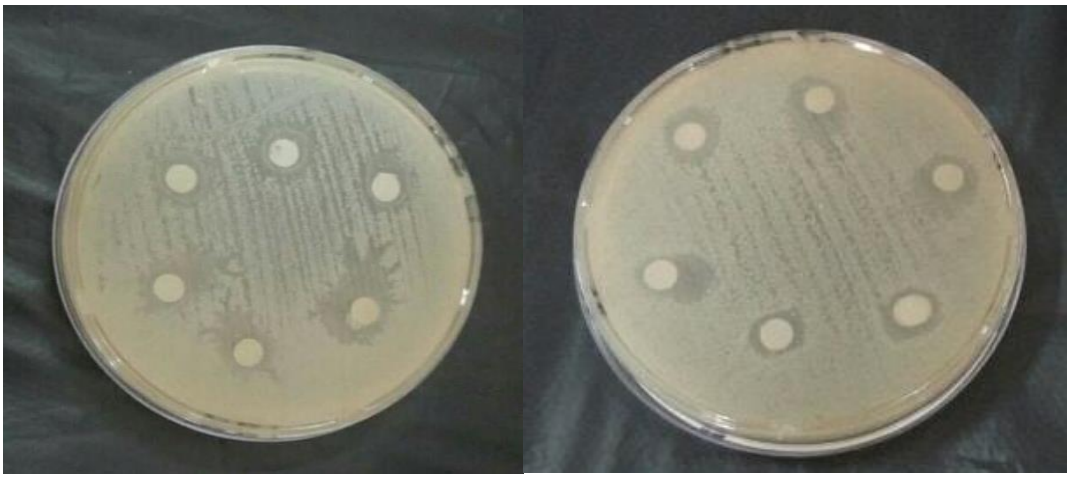

Figure 11: Pictures of the antibacterial activity of the hexane extract

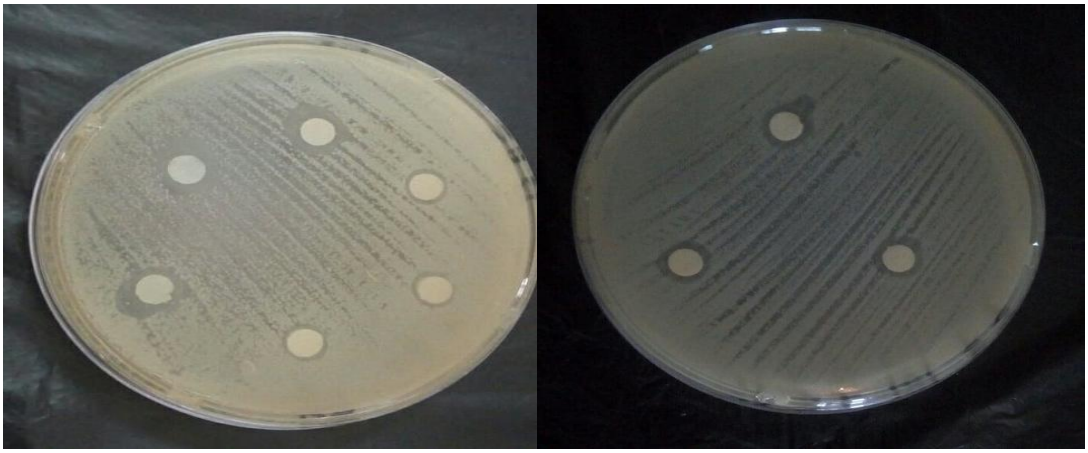

Figure 12: Pictures of the antibacterial activity of the aqueous extract

Annex 06: Picture of the egg incubator and Bevacizumab used in anti-angiogenesis process

Figure 13: The egg incubator used during the experiment of anti-angiogenesis

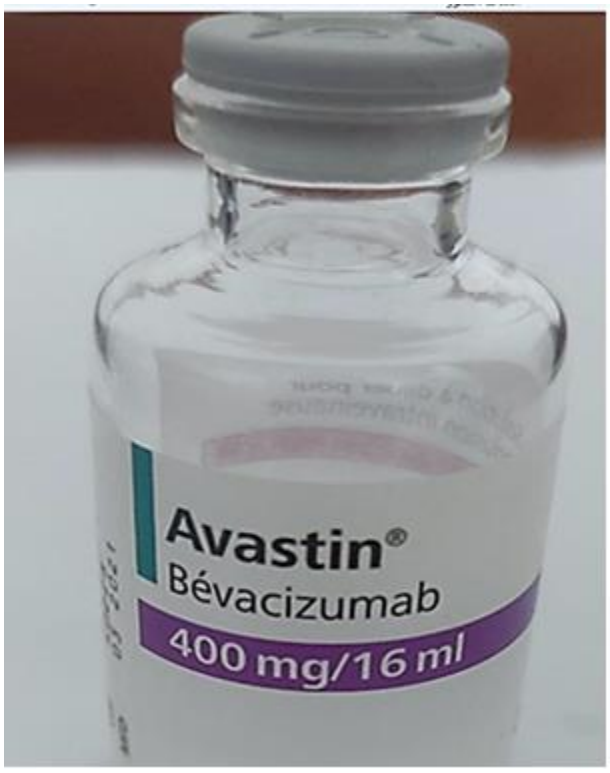

Figure 14: The drug used anti-angiogenesis "Bevacizumab"

Annex 07: Pictures from the *in vivo* study

Figure 15: Preparation of carbimazole in rat drinking water

Figure 16: Intraperitoneal injection

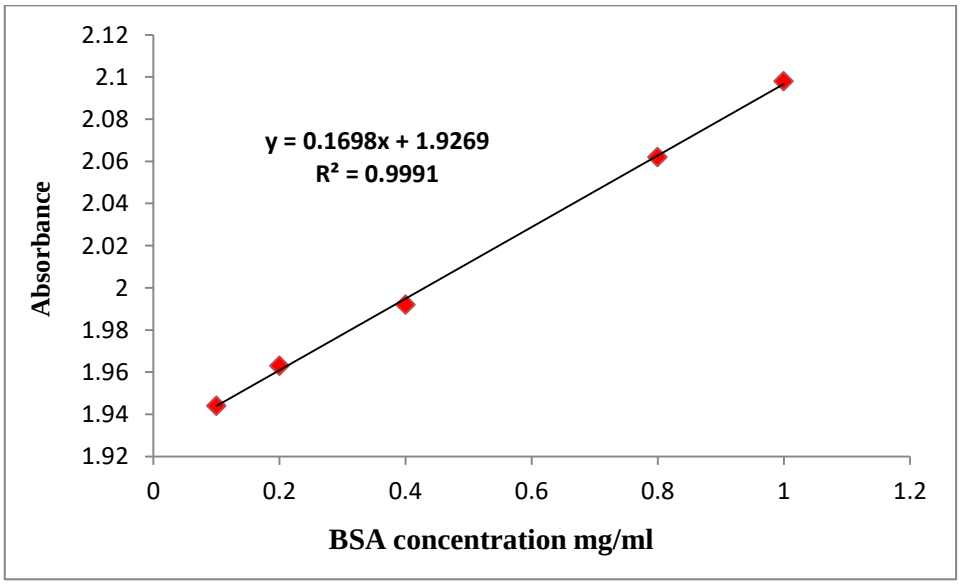

Figure 17: Calibration curve of BSA for determination of protein level