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Extraction, characterization, and Multi-Target Biological Activity of *Cucurbita Maxima* from El Oued, Algeria; LC/MS profiling, *In Vitro*, *In Vivo* and *In Silico* Evaluation

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وبين السطور حكايةُ سعيٍ طويل، خُطّت بتعب الأيام، بدعاء لا ينقطع وبصبر لم يذهب سُدى، ها أنا اليوم أضع بين أيديكم ثمرة مسيرةٍ لم تكن سهلة، لكنها كانت مفعمة بالإيمان، بالعمل، وبالأمل...

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كرشو إسراء

إهداء

﴿لَئِن شَكَرْتُمْ لَأَزِيدَنَّكُمْ ﴾ المجادلة:[7]

لكل طريق بداية، ولكل بداية قلوب كانت أول النبض وأول النور

وها أنا أصل، بعد مشوارٍ لم يكن سهلاً، لكنه كان مليئًا بمن جعلوا عبوره ممكنًا، ومنحوني من عطائهم ما لا يُقاس بالكلمات

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وإلى كل من مرّ في هذه الرحلة ولو بخطوة أو بكلمة أو بدعاء صادق، شكرًا لأنكم كنتم جزءًا من هذا الإنجاز، حتى من خلف الستار

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حمادي آية

إهداء

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إلى من وهبني الله نعمة وجودهم في الحياة: أخواتي وزوجة أخي

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دركي إيناس

ABSTRACT

The objective of this study was to perform a comprehensive phytochemical and biological evaluation of *Cucurbita maxima* aqueous extracts using a multidisciplinary approach that included *in vitro*, *in vivo*, and *in silico* methods. The plant material was collected from El Oued, a Saharan region in southeastern Algeria. Aqueous extracts were prepared from the pulp, seeds, and leaves, and subjected to UPLC–ESI–MS/MS profiling, qualitative and quantitative phytochemical screening, and biological activity assessments.

Chemical profiling revealed the presence of key bioactive compounds, including chlorogenic acid, rosmarinic acid, rutin, quercetin, and kaempferol. Among the tested parts, the pulp extract demonstrated the highest antioxidant (IC_{50} = 210.48 µg/mL) and anti-inflammatory (IC_{50} = 0.309 mg/mL) activities, outperforming the reference drug diclofenac. *In vivo* wound healing studies showed that topical application of the leaves extract significantly enhanced wound closure and tissue regeneration, as confirmed by histological examination. Additionally, MEBO and Base treatment groups exhibited elevated lymphocyte counts ($p < 0.05$), while the *Cucurbita*-treated group maintained values close to normal, and platelet levels were significantly higher in the Base group ($p < 0.001$).

In the hypothyroidism model, BTU administration led to marked hormonal disruption, while supplementation with *Cucurbita* extracts (pulp, leaves, seeds) significantly restored T3 and T4 levels and reduced TSH concentrations, with the pulp and seed extracts showing the strongest effects. The pulp extract group recorded T3 at 5.2 pmol/L and T4 at 24.4 pmol/L. Furthermore, co-treatment with pulp extract significantly increased SOD levels ($p < 0.001$) and reduced MDA ($p < 0.05$), indicating strong antioxidative restoration.

In silico molecular docking supported the biological findings, confirming strong interactions between major compounds and key enzymes involved in inflammation and oxidative stress pathways.

Overall, the results demonstrate that *Cucurbita maxima* exhibit notable antioxidant, anti-inflammatory, and endocrine-modulating properties. Pulp and seed extracts, in particular, show strong potential as natural therapeutic agents against oxidative stress-related disorders such as inflammation and hypothyroidism, supporting their potential as safer alternatives to conventional treatments.

Key words: *Cucurbita maxima*, antioxidant, anti-inflammatory, phytochemicals, hypothyroidism, wound healing, El Oued, docking study.

الملخص

الهدف من هذه الدراسة هو إجراء دراسة شاملة للتركيب الكيميائي الحيوي والتقييم البيولوجي لمستخلصات نبات *Cucurbita maxima* المائية، وذلك باستخدام منهج متعدد يشمل اختبارات مخبرية(*in vitro*) ، وتجارب في الجسم الحي(*in vivo*) ، بالإضافة إلى محاكاة حاسوبية .(*in silico*) تم جمع العينات النباتية من ولاية الوادي، الواقعة في الجنوب الشرقي من الجزائر. تم تحضير مستخلصات مائية من اللب والبذور والأوراق، ثم إخضاعها لتوصيف بواسطةUPLC–ESI–MS/MS وتحاليل كيميائية نوعية وكمية، إلى جانب تقييم الفعالية البيولوجية.

أظهر التوصيف الكيميائي وجود مركبات نشطة بيولوجيًا مثل حمض الكلوروجينيك، وحمض الروزمارينيك، والروتين، والكيرسيتين، والكامبفيرول. أظهر مستخلص اللب أعلى نشاط مضاد للأكسدة (IC_{50} = 210.48 µg/mL) ومضاد للالتهاب(IC_{50} = 0.309 mg/mL) ، متفوقًا على الدواء المرجعي ديكلوفيناك. في نموذج التئام الجروح، ساهم تطبيق مستخلص الأوراق موضعيًا في تسريع إغلاق الجرح وتحسين تنظيم الأنسجة، كما أظهرت التحاليل الدموية ارتفاعًا في عدد اللمفاويات في مجموعات MEBO والأساس، في حين بقيت القيم في مجموعة اليقطين قريبة من السوية.

في نموذج قصور الغدة الدرقية، أدى إعطاء BTU إلى اختلالات هرمونية، في حين ساهمت مستخلصات اللب والبذور والأوراق في استعادة توازن الهرمونات بشكل ملحوظ، حيث ارتفعت مستويات T3 و T4وانخفضTSH ، مع تسجيل اللب والبذور لأفضل النتائج. كما ساهم المستخلص المائي للب في رفع SOD ($p < 0.001$) وخفض MDA ($p < 0.05$) ، مما يدل على استعادة التوازن المضاد للأكسدة.

أكدت نتائج المحاكاة الحاسوبية (Docking) قدرة المركبات الرئيسية على الارتباط بالأنزيمات المستهدفة للالتهاب والإجهاد التأكسدي. وبذلك، يثبت هذا العمل أن مستخلصات *Cucurbita maxima* تمتلك خصائص واعدة كمضادات للالتهاب، ومضادات أكسدة، ومنظمات لهرمونات الغدة الدرقية، مما يجعلها مرشحة طبيعية وآمنة للاستعمال العلاجي.

الكلمات المفتاحية: *Cucurbita Maxima*، مضاد للأكسدة، مضاد للالتهابات، مركبات فيتوكيميائية، قصور الغدة الدرقية، التئام الجروح، ولاية الوادي، دراسة الالتحام الجزيئي.

RESUME

L'objectif de cette étude est d'évaluer le potentiel biologique et la composition phytochimique des extraits aqueux de *Cucurbita maxima*, en combinant des approches *in vitro*, *in vivo* et *in silico*. Le matériel végétal a été collecté dans la région saharienne d'El Oued, au sud-est de l'Algérie. Des extraits aqueux de la pulpe, des graines et des feuilles ont été préparés, puis soumis à un profilage par UPLC–ESI–MS/MS, à des analyses phytochimiques qualitatives et quantitatives, et à des tests d'activités biologiques.

Le profil phytochimique a révélé la présence de composés bioactifs tels que l'acide chlorogénique, l'acide rosmarinique, la rutine, la quercétine et le kaempférol. Parmi les extraits testés, celui de la pulpe a montré la plus forte activité antioxydante (IC_{50} = 210.48 µg/mL) et anti-inflammatoire (IC_{50} = 0.309 mg/mL), dépassant le diclofénac standard. En modèle de cicatrisation, l'application topique de l'extrait foliaire a amélioré la fermeture des plaies, confirmée par l'examen histologique. Les groupes traités avec MEBO et la base ont montré une augmentation des lymphocytes ($p < 0.05$), tandis que les valeurs dans le groupe "citrouille" sont restées proches du témoin.

Dans le modèle d'hypothyroïdie, l'administration du BTU a perturbé les paramètres hormonaux, tandis que les extraits de citrouille (pulpe, feuilles, graines) ont permis une restauration significative des niveaux de T3 et T4, avec une diminution du TSH, la pulpe et les graines affichant les meilleurs résultats. L'extrait de pulpe a également augmenté significativement les niveaux de SOD ($p < 0.001$) et réduit ceux de MDA ($p < 0.05$), montrant un effet antioxydant marqué.

Les études *in silico* (étude de Docking moléculaire) ont confirmé les interactions fortes entre les principaux composés identifiés et les enzymes clés impliquées dans les voies inflammatoires et oxydatives.

Ainsi, cette recherche met en évidence le potentiel thérapeutique de *Cucurbita maxima* comme source naturelle d'agents antioxydants, anti-inflammatoires et régulateurs endocriniens.

Mots-clés: *Cucurbita maxima*, antioxydant, anti-inflammatoire, composés phytochimiques, hypothyroïdie, cicatrisation, El Oued, étude de Docking moléculaire.

Figures List

Tables List

Abbreviation List

ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity.

ALT: Alanine Aminotransferase.

ASLO: Anti-Streptolysin O.

AST: Aspartate Aminotransferase.

BHA: Butylated Hydroxyanisole.

BHT: Butylated Hydroxytoluene.

BTU: Benzylthiouracil.

C. maxima: *Cucurbita maxima*.

CAT: Catalase.

COX: Cyclooxygenase.

COX-1: Cyclooxygenase-1.

COX-2: Cyclooxygenase-2.

CRP: C-Reactive Protein.

CT: Creatinine.

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

Epik: Protonation state prediction tool.

ESI: Electrospray Ionization.

FBS: Fasting Blood Sugar.

GPx: Glutathione Peroxidase.

GRA: Granulocytes.

GSH: Glutathione.

HGB: Hemoglobin.

HPT axis: Hypothalamic-Pituitary-Thyroid Axis

IBU: Ibuprofen.

IC_{50}: Half Maximal Inhibitory Concentration.

IFD: Induced Fit Docking.

IL-1β: Interleukin-1 beta.

In silico: In the computer (computational modeling).

In vitro: Experiments outside living organisms.

In vivo: Experiments in living organisms.

iNOS: Inducible Nitric Oxide Synthase.

IPGRI: International Plant Genetic Resources Institute.

kcal/mol: Kilocalories per mole.

LAO: Linoleic Acid Oxidation.

LC-MS/MS: Liquid Chromatography–Tandem Mass Spectrometry.

LD_{50}: Lethal Dose for 50% of the population.

Levo: Levothyroxine.

LigPrep: Ligand Preparation**.**

LOX: Lipoxygenase.

LYM: Lymphocytes.

MAPK(s): Mitogen-Activated Protein Kinase(s)

MDA: Malondialdehyde.

MEBO: Moist Exposed Burn Ointment.

MS/MS: Tandem Mass Spectrometry.

NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells.

NO•: Nitric Oxide.

Nrf2: Nuclear factor erythroid 2–related factor 2.

NSAID: Non-Steroidal Anti-Inflammatory Drug.

OPLS4: Optimized Potentials for Liquid Simulations 4.

PDB ID: Protein Data Bank Identifier.

PF: Pumpkin Fruit (Pulp).

PL: Pumpkin Leaf.

PLT: Platelets.

PR: Protein.

PS: Pumpkin Seed.

R^2: Coefficient of Determination.

RBC: Red Blood Cells.

RNS: Reactive Nitrogen Species.

ROS: Reactive Oxygen Species.

RP: Reducing Power.

SOD: Superoxide Dismutase.

SP: Standard Precision.

Spp: Species plural.

T3: Triiodothyronine.

T4: Thyroxine.

TNF-α: Tumor Necrosis Factor-alpha.

TPO: Thyroid Peroxidase

TRH: Thyrotropin-Releasing Hormone.

TSH: Thyroid-Stimulating Hormone

UI: International Units.

UPLC: Ultra-Performance Liquid Chromatography.

UPLC–ESI–MS/MS: Ultra-Performance Liquid Chromatography–Electrospray Ionization–Tandem MS.

UPOV: Union for the Protection of New Varieties of Plants.

WBC: White Blood Cells.

α-tocopherol: Alpha-Tocopherol.

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INTROUDUCTION

Medicinal plants are considered a natural source of treatment due to their abundance of bioactive chemicals that can modify various physiological and biochemical pathways. This provides them advantageous for the management and prevention of complicated disorders [1]. Emphasis has been directed towards plants historically employed in traditional medicine due to their extensive empirical evidence and promising pharmacological properties substantiated by contemporary scientific research. Among these, *Cucurbita maxima* commonly known as winter squash or pumpkin has emerged as a multifunctional plant with both nutritional and medicinal significance[2]. A member of the Cucurbitaceae family, this species is widely cultivated and consumed in different parts of the world, including Algeria. It is characterized by its large fruit, which contains a rich matrix of secondary metabolites such as flavonoids, polyphenols, terpenoids, carotenoids, unsaturated fatty acids, and essential vitamins. This complex phytochemical profile endows *Cucurbita maxima* with potent antioxidant, anti-inflammatory, and nutraceutical properties [3], positioning it as a valuable candidate for functional food development and integrative medicine. A central mechanism in the pathogenesis of various chronic conditions is oxidative stress, which results from an imbalance between reactive oxygen species (ROS) and the antioxidant defense systems of the body[4].
This imbalance leads to molecular damage affecting lipids, proteins, and DNA, thereby accelerating aging and increasing susceptibility to diseases such as diabetes, cardiovascular disorders, neurodegenerative diseases, and cancer [5]. Furthermore, oxidative stress plays a critical role in the dysfunction of the thyroid gland, particularly hypothyroidism, by disrupting the synthesis of thyroid hormones (T3 and T4) and promoting inflammatory cascades that exacerbate the clinical symptoms of the disease. Recent findings have highlighted the interconnectedness between oxidative stress and inflammation, emphasizing the need for dual action compounds with both antioxidant and anti-inflammatory capacities. Natural plant-based compounds have shown promise in this area, especially those derived from *Cucurbita maxima*, which is traditionally used to manage digestive issues, cardiovascular symptoms, and metabolic disorders. However, the scientific validation of these effects, particularly in the context of chemically induced hypothyroidism, remains limited and requires further exploration through well-designed experimental models. Research Problem Despite the traditional use of *Cucurbita maxima* and preliminary studies supporting its biological activities, there remains a significant gap in understanding its precise mechanisms of action in relation to oxidative stress, inflammation, and thyroid dysfunction. Moreover, the specific phytochemical composition and therapeutic potential of the plant cultivated locally in El Oued (southern Algeria) have not been thoroughly investigated. This raises a critical scientific need to evaluate its bioactive constituents and test their pharmacological efficacy[6] .

Our research, based on a deep dependence on nature, was motivated by an important instance of Qur'anic miraculousness the story of the Prophet Yunus (peace be upon him), where after his release from the whale, God caused a pumpkin plant to flourish above him, giving food, healing, and mercy. The Qur'an states: "And We caused to grow over him a gourd plant" [As-Saffat: 146]. This theological foundation directed our scientific investigation of pumpkin (*Cucurbita maxima*), not alone as a dietary resource, but as a plant of significant therapeutic value. We aimed to investigate if contemporary research corroborates the therapeutic properties traditionally ascribed to this plant. The study illustrates a vision that synthesizes faith and science, linking historical knowledge with current problems in searching for natural and effective solutions to modern health issues. Therefore, the aim of this study is to extract and characterize the phytochemical components of *Cucurbita maxima* grown in El Oued, and to assess their antioxidant and anti-inflammatory properties, with a special focus on their potential protective and therapeutic effects against chemically induced hypothyroidism in a controlled experimental model. This research intends to contribute to the development of plant-based strategies for managing thyroid dysfunctions and related oxidative conditions, aligned with the growing interest in alternative and complementary medicine.

First part

Bibliographic synthesis

I. *Cucurbita Maxima*

1. An Overview

The Cucurbitaceae family, commonly known as the gourd or squash family, is a diverse and economically significant group of flowering plants comprising over "825 species" across approximately "118 genera". It is predominantly tropical and subtropical in distribution, with a strong presence in Africa, Asia, and the Americas [7]. Major cultivated species include:

- ✓ ***Cucurbita spp***. (pumpkins and squashes)
- ✓ ***Cucumis spp***. (cucumber and melons)
- ✓ ***Lagenaria*** (bottle gourd)
- ✓ ***Luffa*** (sponge gourd), alongside numerous wild relatives with ecological and medicinal value.

The most economically significant species of Cucurbita in the world are *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*. The former is extensively cultivated, while the latter two are of socio-economic value as the best pumpkin species, coming second in production rate and being considered functional foods [8].

Morphological characterization studies of local landraces across Algerian regions particularly in Chlef, Mitidja, and Mascara have revealed substantial phenotypic diversity. Key traits assessed include fruit size and shape, rind texture and color, seed morphology, and plant growth habit. These evaluations follow standardized agro-morphological descriptors established by the International Union for the Protection of New Varieties of Plants (UPOV) and the International Plant Genetic Resources Institute (IPGRI), enabling regional and international comparative analyses [9].

Agriculturally, *C. maxima* grow best in warm climates with optimal temperatures ranging from 20°C to 27°C. The plant is sensitive to frost and cold, making it suitable for cultivation in Algeria's agroecological zones such as Chlef, Mitidja, Mascara, and the southeastern province of El Oued. In these regions, traditional oasis agriculture and irrigation systems support its growth. Harvest typically occurs between August and October, depending on sowing dates and local conditions. When properly stored in well-ventilated facilities, pumpkins can remain available throughout the year, contributing to local market supply and food security [10].

2. Phenotypic profiling

Cucurbita maxima is an annual, herbaceous plant with distinct male and female flowers on the same individual (monoecious). Its root system can extend to a depth of about 1.8 meters, though the majority is concentrated in the upper 60 cm of soil. The stems are rough, often angular, and capable of producing adventitious roots at the nodes. The plant exhibits two main growth habits: creeping vines that can reach 10 to 30 meters in length, and semi-erect forms with shorter internodes. The leaves are large, nearly circular in shape, and often display shallow lobes (Figure 01).

Figure 1. Flower, Leaves and fruit of *Cucurbita Maxima* [7].

Usually, flowers are single and yellow. Male flowers have long stalks and three free filamented, linear, partially fused anthers, one of which is unilocular. Short-stalked female flowers have a lower-placed ovary with several ovules and a stigma split into three to five lobes. The stigmas stay receptive for up to 12 hours and pollination is entomophilous (insect-mediated). A big, indehiscent berry with various forms and hues, the fruit is A central cavity holds fibrous tissue and many seeds inside.

Ranging in size from 50 to 250 mg, the seeds are flat (Figure 02), oval-shaped. They have no useful endosperm; rather, the embryo occupies the whole seed, mostly in the cotyledons storing energy reserves of lipids and proteins. While temperatures below 15°C slow the process, ideal germination takes place between 25°C and 30°C. Germination takes place between 25°C and 30°C; temperatures below 15°C hinder the process [7].

Figure 2.*Cucurbita maxima* seeds [7].

3. Botanical Classification

A thorough comprehension of the botanical characteristics of *Cucurbita maxima* (Table 01) is essential for identifying its unique features and enhancing techniques for the extraction and processing of its many byproducts. An accurate botanical characterization promotes taxonomic resolution, informs agronomic techniques, and supports effective application in nutritional and industrial. [11]

Table 1. Taxonomy of *Cucurbita maxima* [11].

Division	Spermatophyta
Subdivision	Angiospermae
Class	Dicotyledonae
Subclass	Poly patellae
Series	Caliciflorae
Order	Passiflorales
Family	Cucurbitaceae
Genus	Cucurbita
Species	Maximus

4. Phytochemical Composition

Phytochemicals are naturally occurring, non-nutritive chemical substances found in plants or generated from plant sources. The genus Cucurbita, specifically, constitutes a substantial and varied source of these bioactive chemicals [12]. *Cucurbita* species are extensively cultivated for their delicious fruits and have achieved global recognition for their nutritional (Table 02) and medicinal benefits. *Cucurbita maxima* have garnered heightened interest in recent years as a commercially significant crop with a unique phytochemical composition. Numerous categories of bioactive chemicals, such as sesquiterpenoids, phenolic acids, flavonoids, secondary metabolites, and phytoecdysones, have been recognized in *C. maxima* (Table 03). The clarification of these phytochemicals is fundamental for future pharmacological research, since comprehending their composition is vital for delineating the mechanisms that govern the species' biological activity [13].

Table 2: Nutrient values per 100 grams of *C.maxima* seeds [13]**.**

Nutrient	Value per 100 g
Protein	30.23 g
Energy	559 kcal
Total lipid	49.05 g
Fiber	6 g
Carbohydrates	10.7 g
Micronutrients (Vitamins)	
Vitamin C	1.9 µg
Niacin	4.98 mg
Thiamine	0.27 mg
Riboflavin	0.15 mg
Pantothenic Acid	0.75 mg
Vitamin A	16 IU
Vitamin E	35.1 mg
Folate	58 µg
Mineral disposites	
Iron	8.82 mg
Phosphorus	1223 mg
Magnesium	592 mg
Potassium	809 mg
Zinc	7.81 mg
Manganese	4.54 mg
Copper	1.3 mg
Selenium	9.4 µg
Sodium	7 mg
Phytochemicals	
β-Carotene	9 µg
β-Cryptoxanthin	1 µg
Leutin-Zeaxanthin	74 µg

Table 3. Comparison of amino acid % composition of pumpkin seeds Cucurbita spp and *Cucurbita maxima* [7].

Amino acid	*Cucurbita spp*	*Cucurbita maxima*
Alanine	3.7	5.12
Arginine	16.4	15.80
Aspartic acid	9.0	9.56
Cysteine	1.1	-
Glutamic acid	17.9	23.23
Glycine	4.2	6.01
Histidine	2.4	2.66
Isoleucine	3.9	3.59
Leucine	6.9	7.25
Lysine	3.8	3.71
Methionine	2.1	1.83
Phenylalanine	5.5	5.29
Proline	3.4	-
Serine	5.2	5.85
Threonine	3.1	3.04
Tryptophan	2.7	1.10
Tyrosine	3.9	3.26
Valine	4.7	4.45

5. Nutritional benefits of *Cucurbita maxima*

C. maxima are recognized as a highly nutritious food crop with extensive health benefits. It is a rich source of vitamins (A, C, E), minerals (potassium, magnesium, iron), carotenoids (such as β-carotene and lutein), polyphenols, and dietary fiber, making it both a functional food and a nutraceutical. Research supports its use in promoting heart health, glycemic control, immune modulation, antioxidative defense, and even in neuroprotective and anti-inflammatory roles. Pumpkin seeds also offer high-quality fats, protein, and phytosterols, enhancing their utility in combating chronic diseases[14]. *Cucurbita* Saponins have the property of precipitating and coagulating red blood and are used to stop bleeding and in treating wound. Tannins have astringent properties; hasten the healing of wounds and inflamed mucous membrane[12].

Oil seeds are the main source of fat-soluble vitamins and are involved in the formation of steroid hormones because they contain squalene. In addition, they have anti-inflammatory, antitumor, antithrombotic, and antimicrobial properties, and they can show biological functions such as immunomodulator and satiety [15]. *Cucurbita spp* has been used as an anthelmintic, taeniacide, and diuretic in the traditional medicine of many cultures[16]. In addition to these, it has been used in traditional Chinese medicine to treat gallbladder disorders and prostate problems [15].

5.1 Anti-Inflammatory Activity

Cucurbita maxima, rich in such compounds, has long been used in traditional medicine to treat gout, arthritis, and topical inflammation. Scientific studies have confirmed its folkloric use, attributing its anti-inflammatory, antioxidant, and analgesic effects to a synergistic action of bioactive constituents like Cucurbitacins, flavonoids, and carotenoids. Both in vitro and in vivo results demonstrate that *C. maxima* possess significant anti-inflammatory and antiarthritic activity, suggesting its potential in managing osteoarthritis (OA) and rheumatoid arthritis (RA). Moreover, extracts from its seeds and stem have shown promising results in reducing inflammation, comparable to standard medications like ibuprofen [17].

5.2 Antioxidant Activity

Cucurbita maxima demonstrate significant antioxidant activity due to its high content of phenolic compounds, flavonoids, tannins, and other bioactive phytochemicals. Extracts from seeds, pulp, peel, and leaves have shown potent free radical scavenging ability through assays such as DPPH, ABTS, reducing power (RP), and inhibition of linoleic acid oxidation (LAO), with activity levels often surpassing synthetic antioxidants like BHT [18]. The antioxidant effects are concentration-dependent and associated with the presence of polyphenols such as caffeic acid, chlorogenic acid, and isoferulic acid, which also contribute to DNA protection and superoxide radical scavenging [19]. *C. maxima* seed oil, rich in unsaturated fatty acids, tocopherols, and phytosterols, exhibits the highest total phenolic content and strong antioxidant and anti-inflammatory effects through mechanisms involving hydrogen donation, reductive capacity, xanthine oxidase inhibition, and suppression of cyclooxygenase (COX) activity [20][21].

5.3 Antibacterial Activity

Cucurbita maxima extracts have demonstrated antibacterial effects against common wound-infecting bacteria such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Studies showed inhibition zones up to 15 mm and MIC values between 2.5–10 mg/mL. These effects are attributed to phenolic compounds and fatty acids that disrupt bacterial cell membranes. Thus, *C. maxima* show potential as a natural agent for managing wound infections [22].

6. Toxicological profile of *Cucurbita maxima*

The safety and potential toxicity of *Cucurbita maxima* have been evaluated in several experimental studies, which collectively support its low toxicological risk under normal usage conditions. In a foundational investigation by De Queiroz-Neto [23], both acute and sub-acute oral administration of *C. maxima* seed extracts to rats and swine produced no significant alterations in clinical biochemical parameters, hematological values, or histopathological outcomes. These findings were corroborated by Wahid [17], who reported no behavioral or physiological abnormalities in rodents even at doses up to 1,000 mg/kg of ethanolic seed extract. Similarly, Cruz [24] observed no signs of toxicity in mice administered hydroalcoholic extracts, with an LD_{50} value exceeding 5,000 mg/kg, indicating a high safety margin.

In summary, *C. maxima* exhibit a strong toxicological safety profile across various experimental models, particularly in its commonly consumed seed and pulp forms. However, caution is advised in cases where bitterness indicates abnormal cucurbitacin content.

II. Oxidative stress

1. Definition

Oxidative stress is defined as an imbalance between the production of reactive species oxygen/nitrogen (ROS/RNS) and the antioxidant systems (Figure 3), in favour of the former .Reactive oxygen species or free radicals can be produced by normal cellular metabolism and react with biomolecules like protein, lipid, and DNA to cause cellular damage and responsible for degenerative changes and to play a central role in the pathophysiology of many different disorders [25] .This situation may result dysfunction of the mitochondrial chain (ischemia–reperfusion, aging), activation of enzymatic systems (xanthine oxidase, NADPH oxidase, glucose oxidase, monoamine oxidase), a release of free iron from chelating proteins (ferritin) or a oxidation of certain molecules (glucose, hemoglobin, catecholamines, etc.)[26]

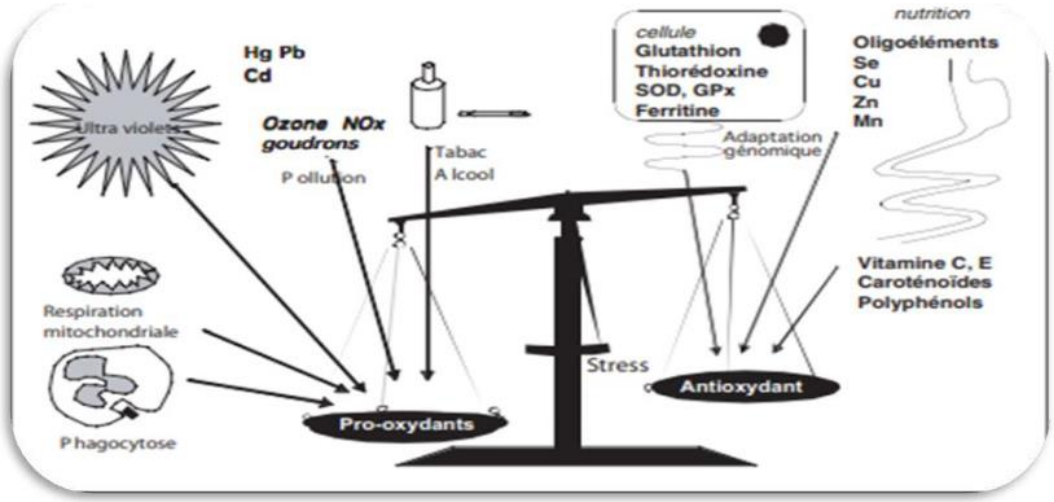


Figure 3. The balance between the pro and antioxidant systems[26].

2. Free radicals

Free radicals are atoms or molecules that contain one or more unpaired electrons in their outer orbitals, making them highly reactive and unstable. Due to their unpaired electrons, free radicals seek to stabilize themselves by capturing electrons from nearby molecules, often leading to a chain reaction of molecular damage. In biological systems, the most common free radicals include reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2), as well as reactive nitrogen species (RNS) like nitric oxide (NO•). These reactive species can damage lipids, proteins, and nucleic acids, contributing to cellular dysfunction and a variety of diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions[6].

2.1. Sources

2.1.1. Endogenous sources

They are produced, in the majority, at the level of the respiratory-mitochondrial chains of the cells of aerobic organisms. The plasma membrane and the endoplasmic reticulum are the main sites of free radical release. Secondary contributors to endogenous oxidant generation are enzymes, such as xanthine oxidase, membrane oxidases, nitric oxide synthases, which physiologically produce oxidants [26].

2.1.2. Exogenous sources

Exogenous sources can be represented by radiation (X-rays and UV light), drugs, xenobiotics, toxins, air pollutants (N, NO2), organic solvents, pesticides, tobacco smoke, etc. (Figure 4) [26]

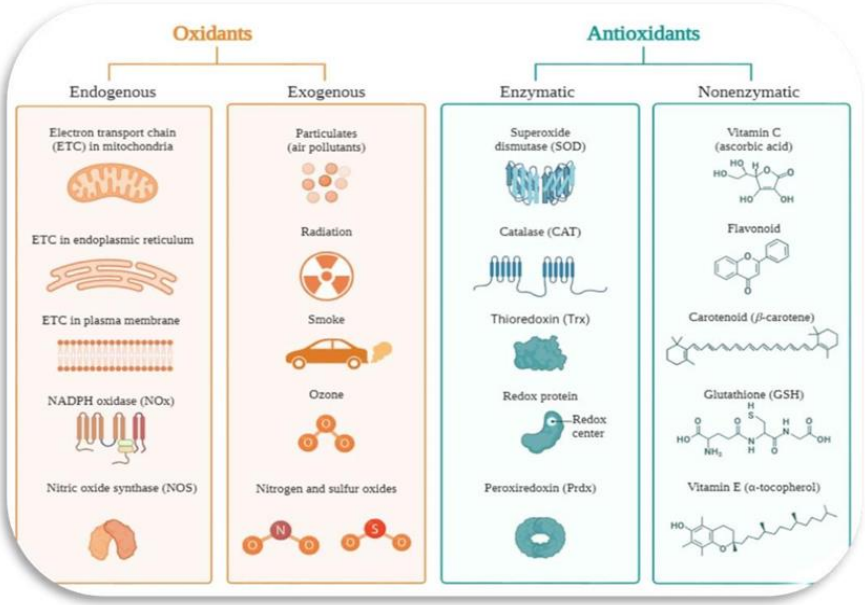


Figure 4. Sources of Oxidants and Antioxidants[26].

3. The induced development of hypothyroidism by oxidative stress

Excessive accumulation of reactive oxygen species (ROS) adversely affects thyroid follicular cells (thyrocytes), impairing their capacity to synthesize and secrete thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4), hence disrupting thyroid function. In addition to cytotoxicity, oxidative stress is the primary factor inhibiting the deiodinase enzyme that converts T4 into its active form, T3, hence reducing effective hormone production even when T4 levels appear normal. Notwithstanding ostensibly normal endocrine signals, this enzymatic failure results in a biochemical profile characterized by diminished peripheral thyroid hormone activity. Furthermore, oxidative stress can disrupt the hypothalamic-pituitary-thyroid (HPT) axis by influencing the production and secretion of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH), hence diminishing thyroid function downstream. Numerous studies, including those by Guo [27] and Yamamoto[28] have demonstrated that

mitochondrial damage and impaired redox signaling in thyroid tissue elevate ROS generation, thereby establishing a detrimental cycle of oxidative injury and endocrine disruption. In autoimmune thyroid disorders such as Hashimoto's thyroiditis, reactive oxygen species (ROS) contribute to cellular damage and the activation of immunological pathways. Chronic oxidative stress exacerbates tissue damage and hormone deficiency by enhancing lymphocyte infiltration, activating nuclear factor kappa B (NF-κB) signaling, and facilitating the release of pro-inflammatory cytokines [29][30]. Oxidative stress has been associated with epigenetic alterations and protein misfolding, resulting in defective protein aggregates that lead to increased cellular damage and apoptosis. These findings highlight the several factors contributing to oxidative stress in thyroid dysfunction, including hormone production, peripheral activation, immunological surveillance, and intracellular signaling. This expanding knowledge substantiates the potential for targeting oxidative stress through antioxidant-based therapeutic strategies. The modulation of transcriptional regulators like NF-κB and Nrf2 has demonstrated protective benefits against oxidative damage and inflammation, presenting interesting options for adjunct therapy in the management of hypothyroidism [31][30]. Further research should focus on the therapeutic application of antioxidant compounds and the development of redox-modulating pharmaceuticals to rectify thyroid dysfunction and reestablish endocrine equilibrium.

4. The impact of *Cucurbita Maxima* as an antioxidant on oxidative stress pathway

Cucurbita maxima have been increasingly recognized for its potent antioxidant properties, which play a vital role in mitigating oxidative stress. The plant is rich in bioactive compounds such as phenolics, flavonoids, carotenoids (notably beta-carotene), vitamins C and E, and essential trace elements like zinc and selenium especially in the seeds [32][33]. These constituents contribute to the scavenging of reactive oxygen species (ROS), thus protecting cells from oxidative damage to lipids, proteins, and DNA[21]. Several in vitro studies have demonstrated strong antioxidant capacity of C. maxima extracts through DPPH and ABTS assays, while in vivo experiments in animal models indicate significant reductions in malondialdehyde (MDA) levels and improvements in enzymatic antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [34]. Moreover, pumpkin extracts have shown protective effects against oxidative stress in pathological conditions like diabetes, hepatotoxicity, and neurodegeneration [20]. These findings underscore the potential application of *Cucurbita maxima* as a natural antioxidant source in functional foods and therapeutic formulations.

Second part

Experimental part

Materials & Methods

I. Introduction

The Materials and Methods provides a comprehensive account of the experimental procedures and methodologies employed in this study, ensuring transparency and reproducibility of the research findings. This investigation was conducted collaboratively between two specialized laboratories: the Valorization and Technology of Sahara Resources (VTRS) Laboratory at the University of El Oued and the Scientific and Technical Research Centre in Physico-Chemical Analysis (CRAPC). The VTRS Laboratory facilitated the extraction of plant materials, conducted *in vivo* and *in vitro* assays, and performed *in silico* analyses. Meanwhile, CRAPC was responsible for the precise liquid chromatography-mass spectrometry (LC-MS) analysis of the plant extracts. This collaborative effort ensured thorough and accurate experimentation, combining expertise and resources from both institutions.The subsequent sections detail the specific methodologies employed, including the collection and identification of plant materials, phytochemical analyses and *in vivo* studies with appropriate ethical considerations, *in vitro* bioassays, and *in silico* analyses. Each subsection is meticulously structured to provide clarity and facilitate reproducibility, adhering to the highest standards of scientific inquiry.

II. Plant Material

1. Collection and Identification of Plant Species

The collection and identification of plant specimens were conducted with meticulous attention to ensure the authenticity and integrity of the botanical materials used in this study. The geographical locations and environmental conditions of the collection sites are detailed below.

❖ ***Cucurbita Maxima***

Cucurbita Maxima, a member of the Cucurbitaceae family, was collected during October and November 2024 from the Trifaoui region in El Oued State, situated in southeastern Algeria. This area is characterized by a desert climate with minimal annual rainfall and high temperatures. The specific attributes of the collection site are as follow:

- **Geographic Coordinates**: Latitude ″20°33″ North, Longitude ″ 53°6″ East.
- **Elevation:** Approximately 60 Centimeter of soil above.
- **Distance from Coastline**: Approximately 600 kilometers inland.
- **Bioclimatic Characterization:** Arid desert environment.

2. Preparation of Plant Extracts

The extraction of bioactive compounds from *Cucurbita Maxima* was performed using a maceration method with using water as solvent [35]. This approach aimed to evaluate the efficiency of the solvent in extracting phytochemicals from the plant materials [36]. The following standardized procedures were meticulously carried out to ensure the integrity and reproducibility of the extracts.

2.1. Sample Collection and Preparation

- ✓ **Collection and Cleaning:** Different parts of *Cucurbita maxima*, including the leaves, seeds, and fruit, were harvested from its respective natural habitats in El Oued state which located in southeastern of Algeria. We collected 100 grams of fresh material from plant. The samples were thoroughly washed under running water to eliminate visible dirt and surface impurities, followed by a second wash using distilled water to remove any remaining soluble impurities and to minimize microbial contamination, ensuring that the phytochemical content would not be altered by surface contaminants [37].
- ✓ **Sorting and Cutting:** A wet sorting process was performed to select the healthiest, undamaged, and most representative samples from the collected plant materials. Following selection, the samples were cut into smaller, uniform segments using sterile tools to facilitate consistent and efficient drying, as uniformity in sample size ensures even moisture loss and preserves the integrity of bioactive compounds during processing [38].

2.2. Drying Process

The collected parts of *Cucurbita maxima* (leaves, seeds, and fruit) were subjected to drying under different conditions based on their physical characteristics and sensitivity to heat, in order to preserve their phytochemical integrity.

- ✓ **Leaves:** Dried at room temperature (25 ± 2°C) in a shaded, well-ventilated environment to minimize the degradation of heat-sensitive bioactive compounds.
- ✓ **Seeds:** Air-dried in the shade at ambient temperature to prevent oxidation of unsaturated fatty acids and sensitive oils.
- ✓ **Fruit (Pulp):** Sliced into thin pieces and dried in the shade at room temperature to preserve carotenoids, vitamins, and water-soluble compounds that are susceptible to heat and light degradation.

2.3. Grinding

After complete drying, each plant part of *Cucurbita maxima* (leaves, stems, seeds, and fruit) was individually ground into a fine, homogeneous powder using a clean, dry mechanical grinder. This grinding process increases the surface area of the plant material, thereby enhancing the efficiency of solvent penetration and the yield of bioactive compound extraction in subsequent steps[39].

2.4. Extraction Process

The powdered plant materials underwent maceration using distilled water as the sole solvent. Each extraction was performed separately to compare the efficacy of the solvent. The procedure was as follows:

- ✓ **Maceration:** For each extraction, 100 grams of the powdered plant material were mixed with 1000 mL of the distilled water, maintaining a standard plant-to-solvent ratio of 1:10 (w/v). The mixtures were placed in clean, sealed glass containers and stored at room temperature in a dark environment for 24 hours to prevent the degradation of light-sensitive. Periodic agitation was performed throughout the maceration process to enhance solvent penetration and facilitate the diffusion of soluble phytochemicals into the aqueous phase. To ensure maximum extraction efficiency, the maceration was repeated three consecutive times for each plant part using fresh solvent [40].
- ✓ **Filtration**: After each cycle, the mixtures were filtered using Whatman No. 2 filter paper to separate the liquid extract from the plant residues.
- ✓ **Combination of Filtrates**: The aqueous filtrates obtained from the three successive maceration cycles were pooled together for each plant part in order to obtain a concentrated extract containing the maximum yield of water-soluble bioactive compounds. This combination step ensures improved extraction efficiency and minimizes compound loss across multiple maceration rounds.

2.5. Solvent Removal and Concentration

- ✓ **Concentration:** The combined filtrates were concentrated using a Büchi Rotavapor R-200 rotary evaporator equipped with a B-490 heating bath and vacuum pump V-3300 [41]. The evaporation process was conducted at 45°C and 5mbar ± (2mbar) vacuum to remove the solvents, yielding the crude extracts.
- ✓ **Lyophilization:** The water-based extracts were subjected to lyophilization (freeze-drying) to remove remain water content without exposing the extracts to elevated temperatures, thereby preserving thermolabile compounds [42]. Lyophilization was performed using the SED-XDG Series Laboratory Freeze Dryer, a pilot-scale laboratory freeze dryer suitable for research and experimental purposes [43]. Lyophilization involves freezing the aqueous extract followed by sublimation under reduced pressure, resulting in a dry, stable powder. This method is advantageous as it maintains the structural and chemical integrity of the bioactive compounds [44].

2.6. Extraction Yield

The extraction yield for each solvent was calculated using the formula [45]:

$$\text{Yield (\%)} = \left(\frac{Mass\ of\ Dried\ Extract\,(g)}{Initial\ Mass\ of\ Plant\ Material\,(g)} \right) \times 100 \qquad \textbf{(1)}$$

3.Phytochemical Analysis

Phytochemical analysis identifies and quantifies bioactive compounds in plant extracts. This study examines *Cucurbita Maxima*. using qualitative screenings, quantitative assessments, and Liquid Chromatography-Mass Spectrometry (LC-MS) for detailed chemical characterization.

3.1. Qualitative Phytochemical Screening

This preliminary analysis detects the presence of various phytochemical groups, including polyphenols, alkaloids, flavonoids, tannins, saponins, terpenoids, reducing sugars and glycosides. Standard protocols were followed for each assay:

3.1.1. Polyphenols Detection

- ✓ **Method:** The Folin-Ciocalteu method involves mixing 1 mL of the plant extract with the Folin-Ciocalteu reagent and sodium carbonate [46].
- ✓ **Positive Observation:** Development of a blue coloration indicates the presence of polyphenols.

3.1.2. Flavonoids Detection

- ✓ **Method:** Add a few drops of 10% aluminum chloride ($AlCl_3$) solution to 1 mL of the extract[47].
- ✓ **Positive Observation:** A yellow coloration signifies the presence of flavonoids.

3.1.3. Alkaloids Detection

- ✓ **Method:** To 1 mL of the plant extract, add a few drops of Mayer's reagent [48].
- ✓ **Positive Observation:** The formation of a white or creamy precipitate indicates the presence of alkaloids.

3.1.4. Terpenoids Detection

- ✓ **Method:** Combine 2 mL of the extract with 2 mL of acetic anhydride ($(CH_3CO)_2O$), followed by 2-3 drops of concentrated sulfuric acid (H_2SO_4) [49].
- ✓ **Positive Observation:** A deep red coloration indicates terpenoids.

3.1.5. Tannins Detection

- ✓ **Method:** Mix 2 mL of the extract with 2 mL of water, then add 2-3 drops of 5% ferric chloride ($FeCl_3$) solution [50].
- ✓ **Positive Observation:** Formation of a green precipitate suggests the presence of tannins.

3.1.6. Saponins Detection

- ✓ **Method:** Shake 5 mL of the extract with 5 mL of water vigorously [51].
- ✓ **Positive Observation:** Persistent froth formation indicates saponins.

3.1.7. Reducing Sugars Detection

- ✓ **Method:** To 2 mL of the extract, add 10 mL of water, 2 drops of 20% ethanolic α-naphthol, and 2 mL of concentrated sulfuric acid (H_2SO_4) [52].
- ✓ **Positive Observation:** A red precipitate signifies reducing sugars.

3.1.8. Glycosides Detection

- ✓ **Method:** Treat 2 mL of the extract with 2 mL of chloroform ($CHCl_3$) and 2 mL of acetic acid (CH_3COOH) [53].
- ✓ **Positive Observation:** A color change from violet to blue to green indicates glycosides.

Following the detailed descriptions of each phytochemical assay, we conducted these tests on *Cucurbita Maxima* are summarized in Table 4:

Table 4. Phytochemical screening of different parts of Cucurbita Maxima extract (Leaves, Seeds, pulp).

Phytochemicals	Name of the test	Leaves	Pulp	Seeds
Polyphenols	Ferric cyanide	+	-	+
Flavonoids	Lead acetate	+	+	+
Alkaloids	Wagner' s	+	-	+
Tannins	Braymer' s	+	-	+
Saponin	Foam	+	-	+
Terpenoids	Salkowski reaction	+	+	+
Steroids	Salkowski reaction	+	+	+
Glycosides		+	-	+
Protein		+	+	-

(+): Positive test, **(–):** Negative test

3.2. Quantitative Phytochemical Analysis

In the present study, we conducted a comprehensive quantitative phytochemical analysis to determine the concentrations of key bioactive compounds [Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Condensed Tannin Content (CTC)] in *Cucurbita Maxima*.

3.2.1. Estimation of Total Polyphenols Content (TPC)

Polyphenolic content was quantified using a modified Folin-Ciocalteu spectrophotometric method, as per [54]. In this procedure, 0.2 mL of varying concentrations of the dry crude extracts of *Cucurbita Maxima*, obtained using water, were combined with 1 mL of 10% Folin–Ciocalteu reagent in test tubes. After a 5-minute incubation, 0.8 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added. The reaction mixtures were then incubated in the dark at room temperature for 30 minutes. Subsequently, absorbance readings were taken at 765 nm using a UV-Vis spectrophotometer. A standard calibration curve was constructed using gallic acid concentrations ranging from 20 to 160 µg/mL. The polyphenolic content of the extracts was expressed as micrograms of gallic acid equivalents per milligram of extract (mg GAE/g).

3.2.2. Estimation of Total Flavonoids Content (TFC)

The flavonoid content in *Cucurbita Maxima*. extracts was quantified using a colorimetric assay involving aluminum chloride ($AlCl_3$), following the methodology outlined by [55]. Specifically, 0.5 mL of each extract was combined with 0.5 mL of 2% $AlCl_3$ solution. The mixtures were incubated in the dark at room temperature for 1 hour to facilitate complex formation. Subsequent absorbance measurements were taken at 420 nm using a UV-Vis spectrophotometer. A standard calibration curve was generated utilizing quercetin concentrations ranging from 0 to 50 µg/mL. The flavonoid content was then expressed as micrograms of quercetin equivalents per milligram of extract (µg QE/mg E).

3.2.3. Estimation of Condensed Tannin Content (CTC)

The quantification of condensed tannins in *Cucurbita Maxima*. extracts was conducted following the vanillin-hydrochloric acid spectrophotometric method, as outlined by [56]. This technique is based on the reaction between vanillin's aldehyde group and carbon 6 of the catechin A-ring, forming a red-colored complex with characteristic absorption at 500 nm. In this procedure, 500 µL of each extract, was mixed with an equal volume of concentrated hydrochloric acid. After 15 minutes of incubation at room temperature in the dark, absorbance was recorded at 500 nm using a UV-Vis spectrophotometer. The condensed tannin content was quantified using a standard catechin calibration curve (0–1.5µg/mL) and expressed as micrograms of catechin equivalent per milligram of extract (mg CE/g E).

3.2.4. Ultra-performance liquid chromatography-mass spectrometry (UPLC/MS–MS) analysis

- ✓ **UPLC-ESI-MS/MS System:** Analyses were conducted using a Shimadzu LCMS-8040 system, renowned for its ultra-fast multiple reaction monitoring capabilities and high sensitivity.
- ✓ **Chromatographic Separation:**
  - **Column:** Restek Ultra C18 column (150 mm × 4.6 mm, 3 µm particle size), selected for its high-purity, type-B silica, ensuring reliable high-performance liquid chromatography (HPLC) applications.
  - **Mobile Phase:** Solvent A consisted of water with 0.1% formic acid, while Solvent B was methanol.
  - **Gradient Program:**
    - 0.0–0.2 min: 98% A
    - 0.2–2.5 min: linear gradient to 25% A
    - 2.5–4.0 min: linear gradient to 0% A
    - 4.0–7.0 min: maintained at 0% A
    - 7.0–7.1 min: return to 98% A
    - 7.1–12.0 min: re-equilibration at 98% A
  - **Flow Rate:** 0.2 mL/min.
  - **Injection Volume:** 5 µL.

✓ **Mass Spectrometric Conditions:**

- **Ionization Source:** Electrospray ionization (ESI) in both positive and negative modes.
- **Interface Parameters:**
  - Desolvation Line (DL) Temperature: 250°C
  - Heat Block Temperature: 400°C
  - Nebulizing Gas Flow: 3.0 L/min
  - Drying Gas Flow: 10 L/min
- **Collision-Induced Dissociation (CID) Gas Pressure:** 230 kPa.
- **Conversion Dynode Voltage:** −6.00 kV.

III. *In Vitro* Bioassays

1. Equipment and Apparatus

- A Shimadzu UV-1800 spectrophotometer equipped with a 96-well microplate adapter was used for absorbance measurements.
- Micropipettes (1–1000 µL) – for accurate liquid handling
- Analytical balance – for precise weighing of chemicals and extracts
- Incubator (25°C, dark conditions) – for controlled reactions
- Water bath (70°C) – used in the BSA denaturation assay

2. Anti-inflammatory Activity: Bovine Serum Albumin (BSA) Denaturation Assay

2.1. Overview

Protein denaturation is a key process in inflammatory disorders [57], making BSA denaturation inhibition an effective model for assessing anti-inflammatory potential [58]. This assay evaluates the ability of *Cucurbita Maxima* extracts to prevent protein denaturation, which may indicate their therapeutic potential for inflammation-related diseases.

2.2. Chemicals and Reagents

✓ Bovine serum albumin (BSA, 5% w/v) from Sigma-Aldrich

✓ Plant extracts (0.1–1 mg/mL)

✓ Diclofenac sodium (positive control, anti-inflammatory drug)

2.3. Procedure

In the present study, the assay was conducted following established methodologies [59] with slight modifications to optimize experimental conditions. The reaction mixture was prepared by combining 500µL of a 5% BSA solution with 250 µL of the test sample at varying concentrations (100 – 1000 µg/mL). Diclofenac sodium was used as a positive control, while the negative control consisted of BSA solution mixed with distilled water under identical conditions. The prepared mixtures were incubated at 37°C for 20

minutes to allow interaction between BSA and the test compounds. Following this, the solutions were subjected to heat-induced denaturation by maintaining them at 70°C for 20 minutes. After the heating phase, the samples were cooled to room temperature and diluted with 500µL with deionized water before measuring their absorbance at 660 nm using a UV-Vis spectrophotometer. Blank was prepared with mixing: 1mL water + 250 µL DMSO. The percentage inhibition of protein denaturation was calculated using the Equation 4:

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad \textbf{(2)}$$

Where $A_{control}$ corresponds to the absorbance of the negative control, and A_{sample} represents the absorbance of the test sample or positive control. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

3. Antioxidant Activity: DPPH Radical Scavenging Assay

3.1. Overview

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating antioxidant potential by measuring the ability of plant extracts to neutralize free radicals [60]. The reduction of DPPH radicals results in a color change from purple to yellow, which is quantified spectrophotometrically [61]. In the present study, the antioxidant potential of the plant extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay using the following established methodologies [62] with slight modifications.

3.2. Chemicals and Reagents

- ✓ DPPH solution (0.1 mM in DMSO) from Sigma-Aldrich
- ✓ Plant extracts (100 - 1000 µg/mL, dissolved in DMSO)
- ✓ Alpha-tocopherol (positive control, reference antioxidant)

3.3. Procedure

A freshly prepared 0.0634 mM DPPH solution in DMSO was used as the oxidant. In a typical reaction, 1 mL of the DPPH solution was combined with 250µl of the test sample at different concentrations (100 – 1000 µg/mL). The mixture was allowed to incubate in the dark at ambient temperature for 30 minutes to ensure sufficient interaction between the antioxidants and the free radicals. The absorbance of the reaction system was subsequently recorded at 517 nm using a UV-Vis spectrophotometer, DMSO was used as a blank. A control sample, containing only the DPPH solution without any test compounds, was prepared under identical conditions. Alpha-tocopherol served as the reference antioxidant. The ability of the test

samples to scavenge DPPH radicals was expressed as a percentage inhibition, calculated using the Equation 5:

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad \textbf{(3)}$$

Where $A_{control}$ represents the absorbance of the DPPH solution alone, and A_{sample} corresponds to the absorbance of the sample-containing reaction mixture. Each experiment was conducted in triplicate, and the results were presented as mean ± standard deviation (SD).

4.Preparation of Cosmetic Cream

4.1. Procedure

A natural cosmetic cream was formulated with a balanced composition aimed at moisturizing the skin and providing anti-inflammatory and antioxidant effects. The active ingredient used is *Cucurbita maxima* leaf extract.

The aqueous base of the cream consists of distilled water (64.6%), which hydrates the skin and serves as a carrier for other ingredients, along with glycerin (3%), a natural humectant that retains skin moisture and enhances smoothness. Shea butter (1%) was added for its emollient and cell-regenerating properties.

In the oil phase, olive oil (10.5%) was used in place of the generic vegetable oil mix. Olive oil is rich in vitamin E and monounsaturated fatty acids, providing deep nourishment and reinforcing the skin barrier. Beeswax (1%) acts as a natural emulsifier and antimicrobial agent, while stearic acid (2%) contributes to cream consistency and emulsion stability. To achieve a uniform and stable emulsion, emulsifiers including Lanette O (4%), Lanette 16 (2%), and Eumulgin B2 (2%) were incorporated. Tween 80 (1%) was used as an additional emulsifying agent to support homogeneity.

The active plant extract (8%), derived from leaves, was added in the oil phase for its healing action. An essential oil (0.5%) of choice was included for fragrance and soothing aromatherapeutic effects. Additionally, Vitamin E (0.2%) was introduced as a natural antioxidant to protect the formulation, and a naturel preservative (0.2%) was used to ensure microbiological safety[63].

The sterilization of instruments and maintenance of a hygienic workspace are crucial during the entire procedure.

4.2. Physical analysis of the formulated cream

4.2.1.PH Test

The pH of the cream is determined by diluting a sample with water and then filtering it through Whatman N° 04 filter paper. The pH analysis is conducted at a temperature of 20 °C use a pH meter [64].

4.2.2. Stability Test

The test was conducted on the sample following centrifugation at 3000 rpm for 30 minutes at the room temperature. The degree of separation between the two phases is indicated by the total stability percentage, where 100 represents stable and 0 signifies unstable. The cream showed a shelf life exceeding 12 months, signifying its stability over an extended duration. This observation was validated through initial stability assessments, where the cream preserved its consistency, pH, and efficacy without any indications of phase separation or ingredient degradation throughout this duration[64].

IV. *In Vivo* Experiments

1. Experimental Animals

1.1. Animal Selection and Procurement

A total of:

- ✓ 20 adult Female Albino Wistar rats (150 ± 25.5 – 200 ± 25.5 g) for the Local inflammation experiment (Wound Healing activity).
- ✓ 35 adult Male Albino Wister rats (150 ± 25.5 – 200 ± 25.5 g) for the Hypothyroidism experiment + Internal inflammation.

were procured from the Pasteur Institute, Algiers, ensuring a homogeneous cohort for experimental standardization. The selection criteria were based on healthy physiological status, absence of prior exposure to pharmacological agents, and uniformity in age and weight to minimize variability. Upon arrival, the animals were inspected for any signs of pathology or stress before being transferred to the controlled animal facility for acclimatization.

1.2. Housing and Environmental Conditions

The animals were housed in polypropylene cages (50 × 35 × 20 cm), with five rats per cage, ensuring adequate space for movement and interaction. The cages were lined with sawdust bedding, which was replaced daily to maintain hygienic conditions. The rats underwent 15 days' acclimatization period in a controlled environment with a temperature of 25 ± 2 °C, relative humidity of 62%, and a 12-hour light/12-hour dark photoperiod to simulate natural circadian rhythms. Throughout the study, the animals had ad libitum access to standard chow formulated according to [65]and tap water to ensure proper hydration. The housing conditions and experimental protocols adhered to the ethical guidelines outlined in the Guide for the Care and Use of Experimental Animals [66].

1.3. Ethical Considerations

All experimental procedures were conducted in strict accordance with the ethical guidelines outlined in the Guide for the Care and Use of Experimental Animals [66] to ensure the humane treatment of animals. The study protocol was reviewed and approved by the relevant institutional ethical committee, ensuring

compliance with internationally accepted standards for animal research. Efforts were made to minimize animal suffering by providing appropriate housing conditions, daily monitoring for signs of distress or illness, and maintaining a standardized acclimatization period. All procedures, including substance administration and behavioral assessments, were designed to reduce stress and discomfort, and humane euthanasia was performed at the end of the study using an ethically approved method.

2. Experimental Design

2.1. *In vivo* I (wound healing activity)

This study was designed to evaluate the effects of *Cucurbita* Leaf Cream for healing wounds. After adaptation period, which lasted 15 days, the rats were divided into four experimental groups, each group containing five rats based on average body weight to ensure weight homogeneity among the groups, each group was treated separately, as we discuss in the sections below:

- **Group 01(Control)**: The rats were not exposed to any wound induction and did not receive any cream treatment during the experiment period.
- **Group 02**: The rats were exposed to wound induction, and received *Cucurbita* Leaf Cream treatment with different concentration (0.5% ,1% ,1.5% ,2% ,2.5%).
- **Group 03**: The rats were exposed to wound induction and received base cream treatment.
- **Group 04**: The rats were exposed to wound induction, and received 1% MEBO cream treatment[67].

Pharmaceutical Treatment

MEBO® is a topical herbal ointment formulated primarily with Beta-sitosterol as its active component, along with sesame oil and beeswax as supporting ingredients. This natural formulation is designed to accelerate wound healing by creating a moist environment that promotes tissue regeneration while minimizing scarring. It provides anti-inflammatory, antimicrobial, and analgesic effects, making it suitable for treating burns, cuts, ulcers, and other skin injuries. Also helps soothe irritated skin and restore its natural protective barrier[68].

Anesthesia And Wound Induction Procedure

Wounds were induced on the dorsal skin of each animal, a region selected for its ease of access, which facilitates both the wound creation process and subsequent monitoring of healing progression and measurements. Prior to induction, the dorsal area was carefully shaved and then disinfected using 70% surgical alcohol. The site of incision was marked in a circular shape , animals were anesthetized until they exhibited a complete loss of response to external stimuli than the marked area was excised using sterile surgical scissors and forceps[69].

Application Of Treatments

Immediately after wound induction, animals assigned to the treatment groups received a topical application of the designated formulation specific to their respective group. The treatments were administered once every 24 hours throughout the experimental period. Wounds weren't covered with any form of dressing, allowing for direct observation and natural healing.

Daily wound evaluations included photographic documentation and precise measurements of wound dimensions with a digital caliper. Images were analyzed using Image software to determine the wound surface area and monitor healing progression[70].

2.2. *In vivo* II (Anti- hypothyroidism+ Internal inflammation healing activity)

This study was designed to evaluate the anti-hypothyroidism activity of aqueous extracts of *Cucurbita maxima* (leaves, seeds, and pulp). After a 15-days acclimation period, animals were divided into seven groups (n = 5 per group) and treated as follows:

- ✓ **Group A (Control):** As a control group, they were given only normal drinking water and fed Standard diet, with no additional treatment throughout the study.
- ✓ **Group B (BTU):** All individuals were given Benzylthiouracil via oral gavage at dose (2 mg/kg) per day to induce hypothyroidism and internal inflammation[71].
- ✓ **Group C (BTU + PF):** Administered BTU (21 day) than *Cucurbita maxima* Fruit extract (100 mg/kg, p.o.) as a treatment.
- ✓ **Group D (BTU +PL):** Administered BTU (21 day) than Cucurbita *maxima* Leaves extract (100 mg/kg, p.o.) as a treatment.
- ✓ **Group E (BTU+ PS):** Administered BTU (21 day) than *Cucurbita maxima* Seeds extract (100 mg/kg, p.o.) as a treatment.
- ✓ **Group F (BTU+ Levo):** Administered BTU (21 day) than levothyroxine at 1.5mg/kg orally to assess therapeutic reversal of hypothyroidism in rat models.
- ✓ **Group G (BTU+IBU):** Rats were administered BTU for 21 days, then treated with ibuprofen at 2 mg/kg orally to mitigate internal inflammation.

Pharmaceutical Treatment

- **Benzylthiouracil (BTU)** is an antithyroid agent commonly used in experimental settings to induce hypothyroidism by inhibiting thyroid hormone synthesis. It disrupts the oxidation and organification of iodide, thereby reducing the production of T3 and T4 hormones. This compound is typically administered via oral gavage and is effective in mimicking clinical hypothyroidism for pharmacological evaluations[72].

- **Levothyroxine (LEVO)** is a synthetic form of the thyroid hormone T4 and is widely used to restore thyroid hormone levels in hypothyroid subjects. It helps normalize metabolic processes and physiological functions affected by thyroid hormone deficiency. In experimental studies, levothyroxine is administered after BTU treatment to assess its effectiveness in reversing the induced hypothyroid state and restoring normal endocrine function[73].
- **Ibuprofen (IBU)** is a well-known non-steroidal anti-inflammatory drug (NSAID) that exerts its action by inhibiting cyclooxygenase (COX) enzymes, thus reducing the production of inflammatory mediators like prostaglandins. It is used in experimental protocols to evaluate its systemic anti-inflammatory effects, particularly in models where inflammation is secondary to endocrine or metabolic disturbances, such as those seen in hypothyroidism[74].

Induction Of Hypothyroidism and Internal Organ Inflammation Procedure

To establish a reliable experimental model of hypothyroidism accompanied by internal organ inflammation, benzylthiouracil (BTU) was administered orally via gavage at a dose of 2 mg/kg body weight, in a volume of 1 mL per rat, once daily for 21 consecutive days.

Treatment Phase

Following the induction period, a seven-day treatment phase was conducted during which the experimental animals received various interventions. Aqueous extracts of *Cucurbita maxima*—derived from leaves, seeds, and fruit pulp—were administered to evaluate their therapeutic efficacy. Additionally, pharmaceutical agents including levothyroxine (as a standard treatment for thyroid dysfunction) and ibuprofen (for managing internal inflammation) were given to specific groups. All treatments were administered orally, with dosages carefully adjusted according to each animal's body weight to ensure appropriate pharmacological exposure and therapeutic relevance.

The study included a healthy control group that was neither induced nor treated, serving as a baseline for normal physiological parameters. A negative control group, subjected to hypothyroidism induction without subsequent treatment, was also included to assess disease progression in the absence of intervention. Throughout the treatment phase, animals were closely monitored for general behavior, food and water intake, and elimination patterns (urination and defecation) to evaluate overall health status and treatment tolerance.

3. Sacrifice and Sample Collection

After 16 hours of fasting, the animals were anesthetized and sacrificed in a humane manner. Blood samples were collected immediately post-mortem; one portion was placed into EDTA-containing tubes for hematological analysis, while the other portion was collected in plain tubes for serum-based biochemical testing. Subsequently, vital organs and tissues-including the spleen, thymus, and wound-area skin (*in vivo*

I), as well as the thyroid gland, kidneys, and liver (*in vivo* II)-were carefully excised. Each tissue was rinsed thoroughly with distilled water followed by physiological saline (0.9% NaCl), gently dried with sterile filter paper, and weighed with precision.

Tissue specimens were then divided into two portions: one part was used immediately for the preparation of fresh homogenates, while the other was preserved in 10% formalin solution for histopathological examination. Biochemical assays conducted on the tissue homogenates included the assessment of oxidative stress biomarkers, specifically malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), to evaluate antioxidant defense and lipid peroxidation status[70].

4. Methods of blood analysis

- Serum samples were obtained by centrifuging blood at 3000 rpm for 15 minutes at 4°C and stored at −80°C until biochemical analyses.
- Plasma samples were separated for Biochemical marker assessments.
- Whole blood samples were used for complete blood count (CBC) analysis.

4.1. Hematological parameters assay

The hematological parameters (leucocytes (WBC), lymphocytes (LYM), granulocytes (GRA), erythrocytes (RBC), hemoglobin (HGB) 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

The most biochemical parameters were determined by auto analysis (Mindray BS-240), Glycemia, renal markers (Urea and Creatinine), hepatic markers (ASAT, ALAT, CRP).

4.2.1. Inflammatory Markers

- **CRP (C-Reactive Protein):**

A liver-produced protein that rises in response to systemic inflammation. It is a key biomarker used to assess both acute and chronic inflammatory responses.

- **Urea:**

While primarily a kidney function marker, elevated urea levels may also occur due to systemic inflammation or catabolic stress.

- **Creatinine:**

A reliable indicator of renal function. Altered levels may reflect inflammation-related kidney stress or damage, especially in chronic conditions.

- **ALT (TGP – Alanine Transaminase):**

A liver enzyme that increases in cases of hepatic inflammation or damage. It is often elevated during systemic or drug-induced inflammation.

- **AST (TGO – Aspartate Transaminase):**

Another enzyme found in the liver and other tissues (e.g., muscle, heart). High levels may indicate systemic tissue inflammation or oxidative stress.

- **Glycemia (Blood Glucose):**

Blood sugar levels may be influenced by systemic inflammation, particularly due to stress-induced hormonal changes or insulin resistance.

4.2.2. Thyroid Markers

These hormones were measured to evaluate thyroid function and detect any disorder (hypo or hyperthyroid states).

- **FT3 (Free Triiodothyronine):**

The active form of thyroid hormone. A decrease in FT3 levels is typically observed in hypothyroid conditions and is associated with reduced metabolic activity.

- **FT4 (Free Thyroxine):**

The primary hormone secreted by the thyroid gland. Low FT4 levels confirm thyroid insufficiency and are critical for hypothyroidism diagnosis.

- **TSH (Thyroid Stimulating Hormone):**

Produced by the pituitary gland to regulate thyroid hormone production. Elevated TSH, along with decreased FT3 and FT4, strongly indicates primary hypothyroidism.

5. Determination of oxidative stress parameters

Oxidative stress is a major contributor in inflammation and dysthyroid disease. To evaluate the antioxidant defense system and lipid peroxidation, the following markers were assessed in serum and plasma.

5.1. Preparation of homogenates

One gram of tissue (liver, kidney, thyroid gland, thymus, spleen and skin) 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.

5.2. Determination of protein level

The Bradford method was used to spectrophotometrically measure the protein content of the supernatants using bovine serum albumin as a reference [75]**.** 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 10 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.

5.3. Determination of Malondialdehyde (MDA) level

It was carried out using the Quintanilha, 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.

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

Where:

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

5.4. Determination of reduced glutathione (GSH) 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 [76]

$$\textbf{GSH (nmol/mg prot)} = \frac{\text{OD x } 10^6 \text{ x } 1.525}{13100 \text{ x } 0.8 \text{ x } 0.5 \text{ x DF x mg prot}}$$

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.

5.5. Determination of Superoxide dismutase (SOD) 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 [77]**.** 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.

$$\mathbf{I\ \%} = \frac{ODc - ODs}{ODc} \times 100$$

With:

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

5.6. Determination of Catalase (CAT) Activity

Catalase activity was determined using Aebi**,** methodology. The reaction was initiated by mixing 20 ml of supernatant with 780 ml of phosphate buffer (KH2PO4, 0.1 M; pH 7.5) and 200 µL of H2O2 (0.030 M). The drop in absorbance at 240 nm was observed every 30 seconds for 2 minutes in order to track the breakdown of H2O2. International units per minute and per gram of protein (IU/min/g of protein) were used to express the enzymatic activity.

$$\mathbf{CAT\ (UI\ /min)/g)} = \frac{(2.3033/T) \times (\log A1\ /A2)}{DF \times g\ prot}$$

Where:

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

V. *In Silico* Analyses

1. ADMET and Drug-Likeness Prediction

To evaluate the pharmacokinetic behavior and drug-likeness of the bioactive constituents present in the aqueous extracts of *Cucurbita maxima* (seeds, fruits, and leaves), a comprehensive ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis was performed using the pkCSM computational platform [78]. This analysis focused on identifying compounds with promising oral bioavailability and pharmacokinetic profiles suitable for anti-inflammatory and antithyroid therapeutic applications. The pkCSM tool facilitated the prediction of critical parameters including intestinal absorption, metabolic stability, routes of excretion, and potential toxicity. Additionally, the platform enabled the assessment of key physicochemical attributes associated with drug-likeness, aiding in the selection of molecules exhibiting low toxicity risk and favorable pharmacokinetic traits. This in silico screening allowed for the identification of promising phytoconstituents that warrant further investigation as potential candidates for the development of safe and effective anti-inflammatory and antithyroid agents.

2. Molecular Docking Study

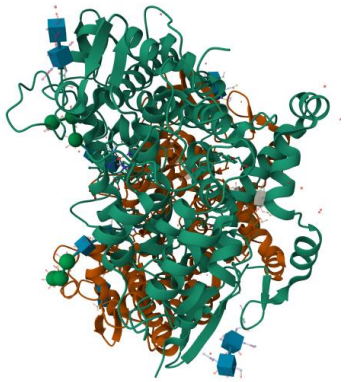
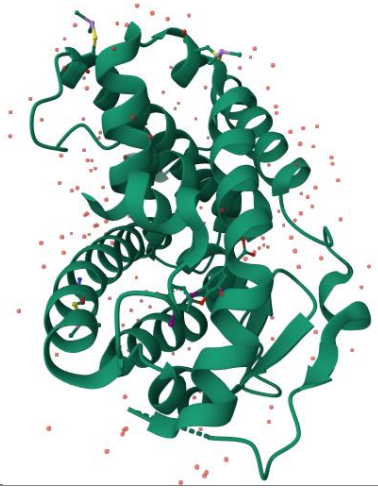
2.1. Ligands Preparation

The three-dimensional (3D) structures of the principal bioactive compounds identified from the aqueous extracts of *Cucurbita maxima* (seeds, fruits, and leaves) were retrieved from the PubChem database, maintained by the National Center for Biotechnology Information (NCBI) [79] and accessible via https://pubchem.ncbi.nlm.nih.gov. Structural optimization and refinement were performed using the LigPrep module of the Schrödinger suite [80], where ionization states were adjusted to simulate physiological pH conditions (7.0 ± 2.0) using the Epik Classic module. Relevant tautomeric and stereoisomeric forms were generated while preserving stereochemical accuracy. Partial atomic charges were assigned using the OPLS4 (Optimized Potentials for Liquid Simulations) force field [81], ensuring energetically favorable conformations suitable for molecular docking and simulation studies targeting inflammation and thyroid-related pathways.

2.2. Receptor Preparation:

The 3D structure of cyclooxygenase-1 (COX-1) enzyme (PDB ID: 5WBE) [82] and Thyroid Hormone Receptor (PDB ID: 3GWS) [83] was retrieved from the Protein Data Bank (http://www.rcsb.org) [84], adhering to specific parameters as listed in Table 5. Processing of the protein structure was executed through the “protein preparation Workflow” module within the Schrödinger suite [85], involving consecutive stages of import and processing, review and modification, and refinement.

Table 5. Target receptor information chosen for docking studies

Cyclooxygenase-1 (COX-1)	**Detaille's**	
	PDB ID	5WBE
	Mutation	No
	Resolution (Å)	2.75
	R-Value Free	0.229
	R-Value Observed	0.197
	Organism	Ovis aries
	Space Groupe	P 6_5
	Sequence Length	600
Thyroid Hormone Receptor	**Detaille's**	
	PDB ID	3GWS
	Mutation	No
	Resolution (Å)	2.20
	R-Value Free	0.223
	R-Value Observed	0.193
	Organism	Homo sapiens
	Space Groupe	C 3_1 2 1
	Sequence Length	259

In the initial stage, the Prime tool was employed to address missing residues and side chains, maintaining the pH of PROPKA at 7.0 ± 2.00. Subsequent steps included the optimization and assignment of hydrogen bonds, along with the removal of water molecules beyond 8 Å. Restrain minimization utilizing the Optimized Potentials for Liquid Simulations (OPLS4) force field was performed to achieve a low-energy state for the protein [81]. This phase of protein preparation signifies an energy optimization methodology, presenting the protein in its energetically favorable state for subsequent *in-silico* studies.

The "Receptor grid generation" panel facilitated the creation of a grid encompassing the active site of the protein, delimited by the co-crystallized ligand in each protein. Default parameters were maintained, and the grid center was generated at the coordinates listed in Table 6.

Table 6. Selected grid parameters the studied enzymes

Enzymes	**Grid Centre**			**Grid Size (Å)**
	x	**y**	**z**	
5WBE	14.78	20.976	8.807	60 × 60 × 60
3GWS	30.658	37.912	16.356	

2.3. Docking Setup:

Docking simulation and including Induced Fit Docking were conducted employing the Glide module and Induced Fit Docking module within the Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) [86]. The simulations were executed on a DELL Intel(R) Core (TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32,0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system. The molecular docking tool employed for all docking studies was Glide (Grid-based Ligand docking with Energetics), a module within the Schrödinger suite [87]. The prepared ligands underwent docking onto the specified protein site utilizing the Glide module, in Standard Precision (SP) modes [88].

2.4. Induced Fit Docking (IFD):

The Induced Fit docking (IFD) module of the Maestro molecular modelling suite has been noted as a reliable and effective docking approach to consider flexibility in both ligands and the binding pocket residues in the binding pocket of target receptors [89]. During the IFD process, Glide/SP (Standard Precision) was performed for each ligand, the Prime refinement step specifically addressed the side chains of residues within a 5 Å radius of the ligand. Noteworthy is the retention of a maximum of 20 poses for each ligand.

VI. Statistical Analysis

In the present study, all experimental data are expressed as mean ± standard deviation (SD) based on triplicate measurements. For datasets, one-way analysis of variance (ANOVA) was conducted to identify significant differences among groups, followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was established at p-values less than 0.05. All statistical analyses were performed using GraphPad Prism (version 10), OriginPro 9.0 or SPSS (version 20), ensuring rigorous evaluation of the experimental results.

Results

&

DISCUSSION

I. Introduction

This chapter delves into the findings regarding the extraction yield, phytochemical composition, and comprehensive exploration of the anti-inflammatory and anti-oxidant properties inherent in aqueous extract derived from (leaves, pulp, seeds) constituents of *Cucurbita Maxima*. The overarching aim of this investigation was to assess the potential anti-inflammatory and anti-oxidant exhibited by aqueous extracts as significant agents in the domain of medical development.

The elucidations provided herein furnish intricate insights into the chemical constitution of the essential impact, thereby enabling their utilization *in vivo* as treatment of hypothyroid and as cream for skin wound inflammatory also the *in-silico* study. This involved the application of advanced computational techniques such as Induced Fit Docking (IFD) and Molecular Dynamics Simulation (MDS) for each compound, capabilities not feasible in traditional *in vitro* and *in vivo* studies.

II. Extraction Yield of *Cucurbita maxima*

The values showed in Table 7 represented the percentage of material extracted relative to the dry weight of each plant part, offering insight into the distribution and solubility of water-extractable compounds within the plant matrix.

The results revealed that the pulp exhibited the highest extraction yield (14.02%), followed by the leaves (8.74%), and the seeds (6.34%). This hierarchy is consistent with previous findings indicating that the pulp is particularly rich in hydrophilic phytoconstituents such as phenolic acids, simple sugars, and organic acids [90]. The lower yield observed in the seeds may be due to their abundance of lipophilic compounds like oils and sterols, which are poorly extracted in aqueous solvents [91]. Meanwhile, the moderate yield from leaves could be attributed to the fibrous composition and presence of cell wall-bound phenolics, which limits their solubility in polar solvents like water [92].

Table 7. Aqueous Extraction Yields from Different Parts of *Cucurbita maxima*

Plant part	Yield (%)
Pulp	**14.02**
Leaves	**8.74**
Seeds	**6.34**

To better illustrate these variations, the extraction yield data are graphically represented in the following histogram.

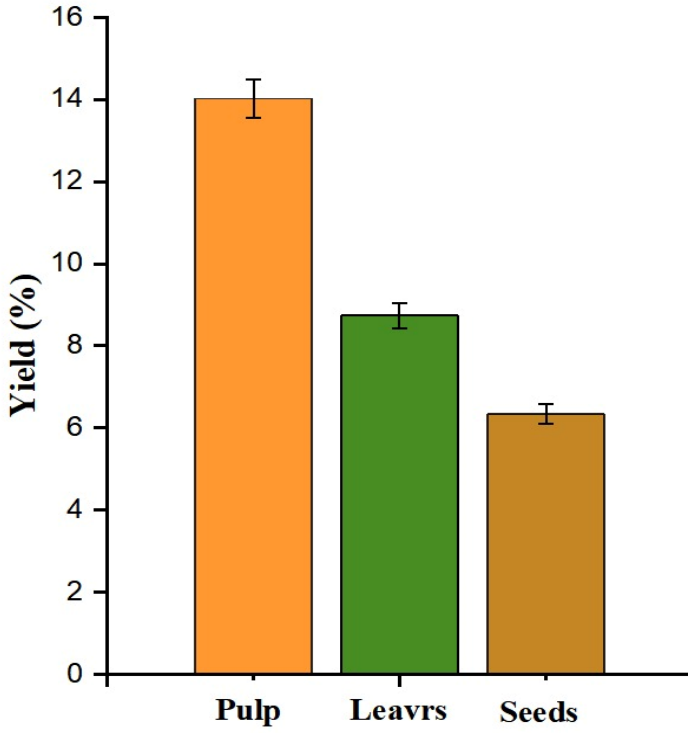


Figure 5. Extraction yields (%) of *Cucurbita maxima* (Pulp, seed, leaves)**.**

III. UPLC–ESI–MS/MS Chemical Fingerprinting and Compound Identification

The UPLC–ESI–MS/MS analysis of aqueous extracts from *Cucurbita maxima* (seeds, fruits, and leaves) enabled high-resolution chemical fingerprinting by integrating advanced chromatographic separation with sensitive mass spectrometric detection. Using a C18 reversed-phase column and an optimized water–methanol gradient (0.1% formic acid), the method provided effective resolution of a wide range of phytochemicals, including phenolic acids, flavonoids, and terpenoid glycosides. Electrospray ionization (ESI) in both positive and negative modes, combined with tandem MS/MS fragmentation, yielded high-confidence structural information based on accurate mass measurements and fragmentation patterns. The resulting dataset facilitated the identification of compounds potentially contributing to the extract's anti-inflammatory and antithyroid properties, as detailed in the following subsections.

1.UPLCESI–MS/MS Profile of *Cucurbita maxima* Seed Aqueous Extract

The LC-MS/MS analysis of the aqueous extract from *Cucurbita maxima* seeds revealed a complex phytochemical profile dominated by phenolic acids and flavonoids. As shown in Table 8, chlorogenic acid

and rutin were the most abundant constituents, accompanied by various hydroxylated flavonoid derivatives. The identification was based on accurate mass data and MS/MS fragmentation patterns.

Table 8. Major phytochemicals identified in *C. maxima* seed aqueous extract by UPLC–ESI–MS/MS

Compound	[M–H]⁻ m/z	Retention Time (min)	Relative Area (%) ± SD
Chlorogenic acid	353.09	2.4	21.3 ± 0.6
Caffeic acid	179.03	3.6	14.2 ± 0.4
p-Coumaric acid	163.04	4.2	6.8 ± 0.3
Quercetin-3-O-rutinoside (Rutin)	609.15	5.1	16.9 ± 0.7
Quercetin	301.07	5.7	10.5 ± 0.5
Kaempferol	285.04	6.3	7.4 ± 0.4
Myricetin	317.06	6.6	5.9 ± 0.3

These findings highlight chlorogenic acid (21.3%) and rutin (16.9%) as principal metabolites, consistent with previous reports by Fiaz [93], who noted high chlorogenic acid content in methanolic seed extracts of *C. maxima*. Similar results were reported by Sharma [94], where chlorogenic acid was linked to reduced inflammation through inhibition of pro-inflammatory cytokines. The co-occurrence of quercetin and kaempferol derivatives further supports anti-inflammatory potential, given their documented suppression of NF-κB signaling and enhancement of antioxidant defenses. The presence of p-coumaric and caffeic acids also aligns with data from cucurbitaceous species, where these compounds are involved in thyroid hormone modulation and antioxidant activity [95].

2. LC-M/MS Profile of *Cucurbita maxima* Pulp Aqueous Extract

The fruit pulp of *C. maxima* demonstrated a phytochemical spectrum rich in hydroxycinnamic acids and methoxylated flavonoids. The LC-MS/MS analysis identified key constituents listed in Table 9, with rosmarinic acid and jaceidin as dominant compounds.

Table 9. Major phytochemicals identified in *C. maxima* fruit pulp aqueous extract by UPLC–ESI–MS/MS

Compound	[M–H]⁻ m/z	Retention Time (min)	Relative Area (%) ± SD
Rosmarinic acid	359.08	3.3	22.8 ± 0.6
Quercetin dimethyl ether	329.07	4.7	14.1 ± 0.5
Jaceidin	331.09	5.2	13.2 ± 0.4
Dihydrokaempferide	287.06	5.8	9.7 ± 0.3

Caffeic acid	179.03	2.9	7.9 ± 0.3
Apigenin-7-O-glucoside	431.08	6.3	5.6 ± 0.2

Rosmarinic acid (22.8%) emerged as the major constituent, in line with findings from Miljić [96], who reported its dominance in pumpkin pulp and linked it to potent radical-scavenging and TPO (thyroid peroxidase) inhibitory activities. Jaceidin and quercetin dimethyl ether were also prominent, and their anti-inflammatory roles through downregulation of COX-2 and iNOS have been reported in *Origanum* and *Salvia* species [97]. Dihydrokaempferide contributes to immunomodulation, while the presence of apigenin derivatives further reinforces anti-inflammatory effects via inhibition of MAPK pathways. These findings support the potential use of *C. maxima* pulp in managing inflammation and thyroid dysfunction.

3. LC-MS/MS Profile of *Cucurbita maxima* Leaf Aqueous Extract

The aqueous extract of *C. maxima* leaves displayed a diversified phytochemical profile dominated by polyphenolic antioxidants. The major compounds identified are summarized in Table 10. Notably, gallic acid and ellagic acid were abundant, alongside apigenin and luteolin derivatives.

Table 10. Major phytochemicals identified in *C. maxima* leaf aqueous extract by UPLC–ESI–MS/MS

Compound	$[M–H]^-$ m/z	Retention Time (min)	Relative Area (%) ± SD
Gallic acid	169.01	2.2	11.4 ± 0.4
Ellagic acid	301.00	3.6	8.5 ± 0.3
Apigenin	269.04	4.6	6.7 ± 0.2
Luteolin	285.04	5.0	5.9 ± 0.2
Rutin	609.15	5.4	4.6 ± 0.2

Gallic acid (11.4%) and ellagic acid (8.5%) were prominent in the leaf extract, corroborating findings by Parihar[98], who identified these phenolics as major contributors to anti-inflammatory and anti-thyroid activities in *Cucurbita* species. Apigenin and luteolin, widely distributed in Asteraceae, are recognized for suppressing pro-inflammatory enzymes (e.g., COX-2) and reducing oxidative stress, key factors in thyroid inflammation[99]. The moderate presence of rutin complements the antioxidant matrix, suggesting a synergistic mechanism underlying the extract's therapeutic potential.

IV. *In vitro* study

1. Evaluation of Anti-inflammatory activity

In the present study, plant-derived aqueous extracts from the leaves, pulp, and seeds were assessed for anti-inflammatory activity, obtained from *Cucurbita Maxima* was investigated using Diclofenac inhibition a standard non-steroidal anti-inflammatory drug (NSAID) model. The anti-inflammatory potential of the aqueous extract of different parts; from the linear plots of *Cucurbita* concentration (mg/mL) against percentage inhibition concentration 50 (IC50) and the results are as shown in Figure 6, the studied aqueous extracts significantly increased (pulp, leaves, seeds) inhibition (%) with corresponding increase in concentration.

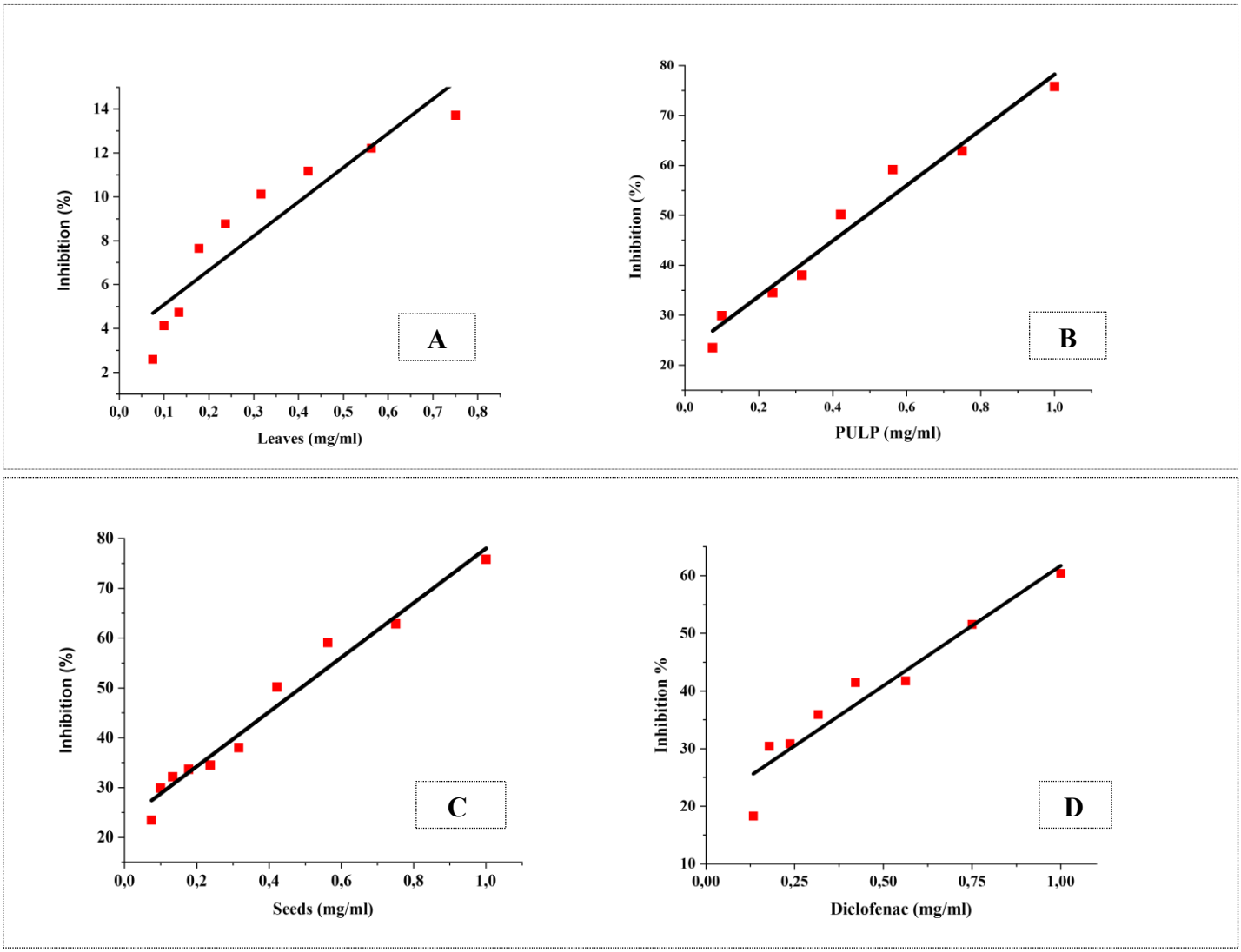


Figure 6. Exponential regression curves of anti-inflammatory percentage inhibition versus concentration of (A) Leaves, (B) Pulp, (C) Seeds and (D) Diclofenac.

The study uses a biochemical assay to prevent protein denaturation, which mimics inflammatory conditions where structural proteins lose their functional conformation, leading to increased immunogenicity and inflammatory response propagation. Substances that stabilize these proteins under stress indicate anti-inflammatory potential. Which the Inflammation is a complex biological response to harmful stimuli, mediated through molecular and cellular pathways. It involves inflammatory mediators like cytokines, prostaglandins, and nitric oxide, regulated by enzymes like cyclooxygenase (COX) and lipoxygenase (LOX). *In vitro* anti-inflammatory assays like protein denaturation inhibition tests help evaluate substances' potential to interfere with these processes [100].

Quantitatively, the inhibitory concentration 50 (IC_{50}) values serve as a critical metric for comparing the efficacy of the extracts. The IC_{50} for the pulp extract (0.309 mg/ml) was significantly strong than that of the seed (0.488 mg/ml) and leaves (2.976 mg/ml) extracts, indicating a superior capacity to suppress protein denaturation.

This suggests a higher concentration of bioactive compounds in the pulp, potentially including polyphenols, flavonoids, and saponins molecules known to exhibit antioxidant and anti-inflammatory activities [101]. These phytochemicals are thought to interact with COX enzymes and inhibit the synthesis of prostaglandins, thereby mitigating inflammation at the biochemical level. Interestingly, the reference drug diclofenac exhibited an IC_{50} of 0.718 mg/ml, which was higher than both the pulp and seed extracts. Diclofenac acts primarily by non-selective inhibition of COX-1 and COX-2 enzymes, reducing prostaglandin synthesis. The observation that plant extracts outperform diclofenac in vitro suggests that these natural substances may exert their effects through multiple pathways not only through COX inhibition but also by scavenging free radicals and suppressing pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) [102].

Table 11. Absorbance values taken from the anti-inflammatory test

	Slope	**Intercept**	**R2**	**IC50 (µg/ml)**	**IC50 (mg/ml)**
Leaves	15,616	3,526	0,862	2,976	0,003
Pulp	112,817	15,153	0,609	0,309	0,000
Seeds	54,690	23,317	0,971	0,488	0,000
Diclofenac	41,669	20,074	0,913	0,718	0,001

The study shows that extracts from plants, particularly those from pulp and seeds, cause a significant increase in anti-inflammatory reactions, with the effects varying depending on the concentration. These extracts are biologically and biochemically important because they are effective anti-inflammatory drugs

with better IC_{50} values and strong regression patterns, suggesting they could be used as models for future drug development.

2. Evaluation of Anti-oxidant activity

The evaluation of the antioxidant activity of the plant extracts revealed significant differences between the tested parts leaves, pulp, and seeds when compared to the standard antioxidant α-tocopherol. Among the extracts, the pulp exhibited the strongest antioxidant potential, with an IC_{50} value of 210.48 µg/ml, indicating a high capacity to neutralize free radicals. This suggests that the pulp contains a rich concentration of phenolic compounds and flavonoids, which are known for their electron-donating, hydrogen-scavenging, and metal-chelating properties [103].

Table 12. Absorbance values taken from the anti-oxidant test

	Slope	**Intercept**	**R2**	**IC50 (µg/ml)**	**IC50 (µg /ml)**
Leaves	0,032	0,531	0,937	1545,906	1,546
Pulp	0,230	1,590	0,991	210,478	0,210
Seeds	0,084	9,384	0,962	483,524	0,484
α-tocopherol	0,485	20,243	0,922	61,390	0,061

The seed extract, although exhibiting a higher IC_{50} value (483.52 µg/ml), showed consistently high absorbance values at all tested concentrations, implying a cumulative and stable antioxidant effect. This activity may be attributed to the presence of oil-soluble bioactive molecules such as phytosterols, unsaturated fatty acids, and tocopherols. Seeds are widely recognized for their rich antioxidant profiles and may serve as beneficial dietary components in combating oxidative stress-related conditions.

While the leaf extract presented the weakest antioxidant activity (IC_{50} = 1545.91 µg/ml), it is important to note that leaves are often abundant in diverse secondary metabolites, some of which may offer other pharmacological benefits beyond radical scavenging such as anti-inflammatory or enzyme-regulatory effects. Moreover, extraction conditions, including solvent type and polarity, can significantly influence the yield and potency of antioxidants extracted from plant leaves, and optimization may improve their effectiveness in future studies[92].

Notably, all tested plant parts demonstrated lower antioxidant activity than α-tocopherol (IC_{50} = 61.39 µg/ml), which was expected given its purity and established potency. Nevertheless, the promising activity observed in the pulp and seeds highlights their potential as sources of natural antioxidants. In light of rising global interest in replacing synthetic antioxidants like BHT and BHA due to associated health risks with safer, plant-based alternatives, these findings are of significant relevance.

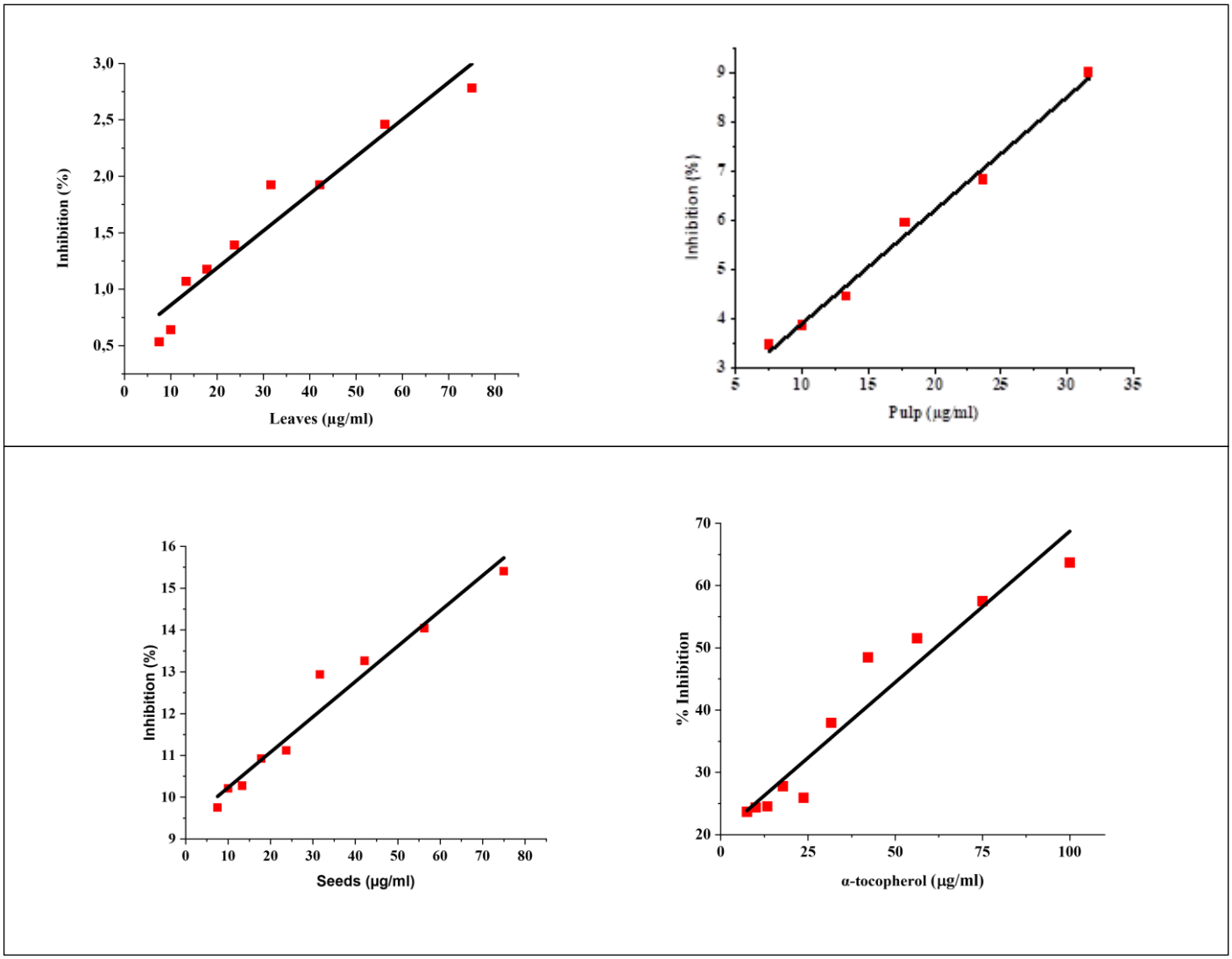


Figure 7. Exponential regression curves of percentage inhibition versus concentration of Leaves, Pulp, Seeds and α-tocopherol.

V. *In vivo* study

1. *In vivo* I (wound healing activity)

1.1. Organ weight index

The organ weight index data show that both the spleen and thymus weights increased significantly in the Wound + Base and Wound + MEBO groups compared to the control ($p < 0.05$), suggesting an activation or proliferation of immune tissues in response to systemic inflammation or immune stimulation. In particular, the MEBO group showed slightly higher thymus weight than the Base group ($p < 0.01$ vs. Base), indicating a stronger T-cell-related immune activation. In contrast, the Wound + H (*Cucurbita* Leaf-based cream) group exhibited organ weight indices similar to the control, with no significant changes, further supporting its minimal immunological impact. When correlated with hematological data, the elevated WBC, lymphocyte, and granulocyte levels in the Base and MEBO groups align with the increased spleen and thymus weights, reflecting systemic immune activation. Additionally, the strong CRP and ASLO responses observed in these groups reinforce this link between inflammation and lymphoid organ hypertrophy. Conversely, the *Cucurbita maxima* Leaf-treated group showed minimal changes across CRP, ASLO, hematological markers, and lymphoid organ weights, highlighting its potential as an anti-inflammatory and immunologically balanced wound treatment.

Table 13. Organ weight Index of different experimental groups

	Organ Weight Index %	
	Spleen	**Thymus**
Control	0.23±0.08	0.15±0.02
Wound + Base	0.27±0.04 [a]	0.22±0.01 [a]
Wound + MEBO	0.25 ±0.01 [a *]	0.23±0.01 [a**]
Wound + H	0.23±0.02 [NS*]	0.16±0.03 [NS*]

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

1.2. Hematological parameters

The hematological data reveal distinct immune and blood profile responses among the experimental groups. White blood cell (WBC) counts were significantly elevated in the Wound + Base group ($p < 0.01$), indicating a strong systemic immune activation, followed by the MEBO group with a moderate, non-significant increase, while the Wound + H group showed only a slight rise compared to control.

Lymphocyte (LYM) levels were also markedly increased in the Base and MEBO groups ($p < 0.05$), reflecting an active immune response, whereas levels in the pumpkin-treated group remained comparable to control. Granulocyte (GRA) counts were highly elevated in both Base and MEBO groups ($p < 0.001$),

suggesting enhanced innate immune activity, while the H group showed no significant change. Hemoglobin (HGB) and red blood cell (RBC) concentrations remained stable across all groups, indicating no effect on erythropoiesis or oxygen-carrying capacity. Platelet (PLT) counts were significantly higher in the Base group ($p < 0.001$) and moderately elevated in the MEBO group ($p < 0.05$), possibly reflecting inflammation-induced thrombocytosis, while the H group showed minimal changes. Overall *Cucurbita* Leaf-based cream had the least impact on hematological parameters, reinforcing its biocompatibility and lower systemic immunological burden compared to beeswax-based and MEBO treatments.

Table 14. Plasma concentration of hematological parameters of different experimental groups

	WBC($\times10^9$/L)	LYM($\times10^9$/L)	GRA($\times10^9$/L)	HGB (g/dL)	RBC($\times10^{12}$/L)	PLT ($\times10^9$/L)
Control	3.86±0.14	1.1±0.22	0.13±0.06	14.26±0.88	7.8±0.17	716±29
Wound + Base	6.33±0.17 ^{b}	4.2±0.1^{a}	0.96±0.08 $^{c\ NS}$	14.24±0.31^{NS}	7.9±0.24^{NS}	976±17 ^{c}
Wound + MEBO	5.68±0.11 ^{NS}	3.83±0.24 $^{a\ NS}$	1.4±0.09 $^{c\ NS}$	14.14±0.22^{NS}	7.7±0.14^{NS}	856.3±47 $^{a\ NS}$
Wound + H	4.17±0.08 ^{NS*}	1.0±0.47 ^{NS**}	0.16±0.04 ^{NS**}	14.21±0.4 ^{NS}	6.8±0.39 ^{NS}	771.3±78 ^{NS**}

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

1.3. Biochemical parameters

The experimental results indicate that fasting blood sugar (FBS) levels remained relatively stable across all groups, with no statistically significant differences compared to the control, suggesting that neither the wound nor the treatments influenced systemic glucose regulation. In contrast, C-reactive protein (CRP), a marker of inflammation, was significantly elevated in the Wound + Base and Wound + MEBO groups, with the MEBO group showing the highest CRP levels ($p < 0.01$), indicating a pronounced inflammatory response. The Wound + H (*Cucurbita* Leaf-based cream) group exhibited only a slight, non-significant increase in CRP, suggesting a milder inflammatory profile. Similarly, Anti-Streptolysin O (ASLO), an indicator of immune response against streptococcal antigens, was significantly increased in the Wound + Base group ($p < 0.01$) and moderately elevated in the MEBO group ($p < 0.05$), while the Wound + H group showed ASLO levels comparable to the control. These findings suggest that *Cucurbita* Leaf-based cream elicited the least systemic inflammatory and immune response, highlighting its potential as a biocompatible treatment option for wound care.

Table 15. Glycemia, CRP and ASLO of different experimental groups

	FBS (g/L)	**CRP (mg/ml)**	**ASLO (U/L)**
Control	0.63±0.24	0.3±0.08	6.25±0.12
Wound + Base	0.71±0.21^{NS}	0.55±0.04 ^{a}	9.13±0.3^{b}
Wound + MEBO	0.65±0.31^{NS}	3.28±0.07 ^{b**}	7.26±0.17 ^{a**}
Wound + H	0.68±0.34^{NS}	0.57±0.06 $^{a\ NS}$	6.27±0.31 ^{NS**}

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

1.4. Oxidative stress parameters

A) Skin oxidative stress parameters

The skin oxidative stress parameters provide insight into the tissue-level redox balance and oxidative damage in response to wounds and different treatments. Malondialdehyde (MDA), a marker of lipid peroxidation and oxidative stress, was significantly elevated in the Wound + Base group (10.33 ± 0.39 nmol/mg pr; $p < 0.001$) and to a lesser extent in the Wound + MEBO group (8.5 ± 0.65; $p < 0.05$), indicating substantial oxidative damage following treatment. In contrast, the Wound + H (*Cucurbita*-based) group had MDA levels (4.32 ± 0.11) similar to the control (4.24 ± 0.23), suggesting effective antioxidant protection and minimal lipid peroxidation.

Glutathione (GSH), a critical endogenous antioxidant, was lowest in the Wound + Base group (7.11 ± 0.13; $p < 0.001$), reflecting depleted antioxidant reserves under oxidative stress. In the MEBO and H groups, GSH levels remained near control values (10.66 ± 0.53 and 10.58 ± 1.0 vs. control 11.12 ± 0.25), indicating better preservation of the antioxidant defense system.

Superoxide dismutase (SOD) activity dropped significantly in the Wound + Base group (4.08 ± 0.11; $p < 0.01$) and remained slightly reduced in the Wound + H group (3.81 ± 0.35), although the reduction was not statistically significant. The MEBO group showed near-normal SOD activity (6.5 ± 0.71), similar to control (6.63 ± 0.23), suggesting partial antioxidant support.

Catalase (CAT) activity was lowest in the Wound + Base group (3.33 ± 0.23 UI/min/g pr; $p < 0.05$) and moderately impaired in the MEBO group (5.83 ± 0.44; $p < 0.05$), while the Wound + H group exhibited slightly increased CAT activity (10.36 ± 0.21) compared to control (9.12 ± 0.77), indicating enhanced detoxification of hydrogen peroxide and superior oxidative defense.

Overall, the oxidative stress data strongly correlate with the inflammatory and immune responses observed in previous tables. The Base group showed the most severe oxidative imbalance, matching its high CRP, ASLO, WBC levels, and immune organ hypertrophy. The MEBO group demonstrated moderate oxidative stress, while the *Cucurbita*-treated group (Wound + H) maintained redox parameters close to control levels. This suggests that the pumpkin-based ointment not only limits inflammation but also effectively preserves the skin's antioxidant defenses, making it a promising candidate for oxidative stress reduction in wound healing.

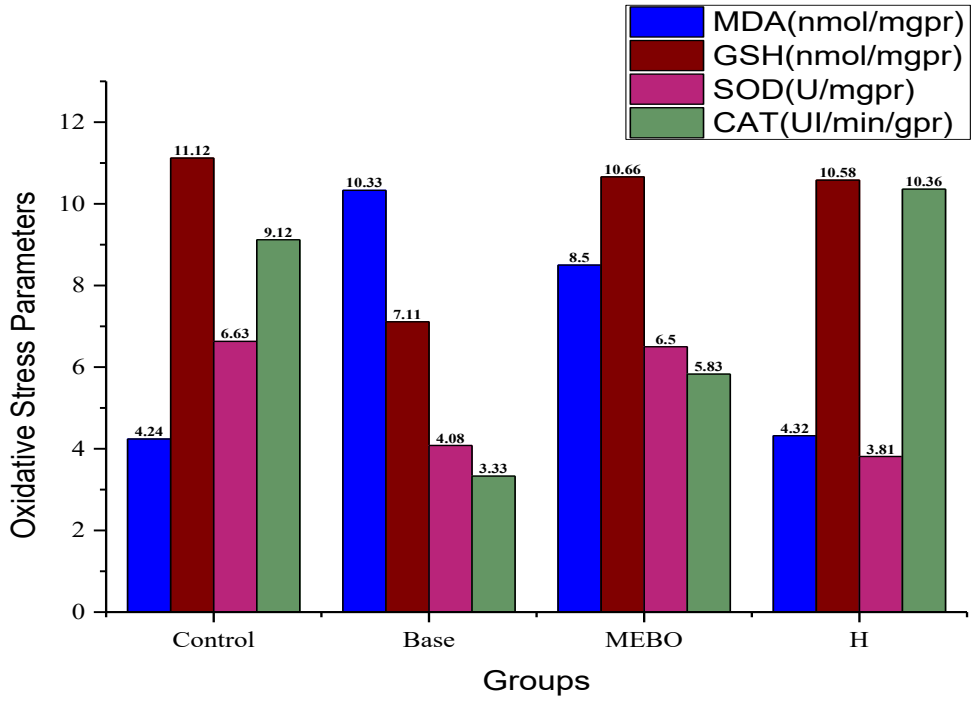


Figure 8. Oxidative stress parameters in the skin of different experimental groups**.**

B) Spleen oxidative stress parameters

The spleen oxidative stress parameters reveal systemic oxidative damage and antioxidant status in response to wound-induced inflammation and topical treatments. MDA levels, which reflect lipid peroxidation and oxidative damage, were markedly elevated in both the Wound + Base (9.06 ± 0.59 nmol/mg pr; $p < 0.05$) and Wound + MEBO (8.32 ± 0.98; $p < 0.05$) groups compared to control (3.17 ± 0.15), indicating significant oxidative stress in splenic tissue. In contrast, the Wound + H (*Cucurbita* leaf) group maintained near-normal MDA levels (4.13 ± 0.4), suggesting limited systemic oxidative damage.

GSH levels, essential for cellular antioxidant defense, were significantly reduced in the Base group (5.23 ± 0.7; $p < 0.01$) and moderately decreased in the MEBO group (9.21 ± 0.47; $p < 0.05$ vs. control, $p < 0.01$ vs. Base), indicating impaired redox balance. Notably, the pumpkin-treated group preserved GSH at control levels (11.23 ± 0.67), demonstrating robust antioxidant capacity.

SOD activity, which neutralizes superoxide radicals, declined in both the Base (4.18 ± 0.49; $p < 0.01$) and MEBO (5.0 ± 1.04) groups compared to control (6.23 ± 0.13), while it remained intact in the H group (6.31 ± 0.24), supporting effective oxidative defense. Similarly, CAT activity, responsible for hydrogen peroxide detoxification, was severely reduced in the Base (4.83 ± 0.16; $p < 0.001$) and MEBO (4.98 ± 0.92) groups, in contrast to the H group (8.28 ± 2.11), which retained near-normal catalase levels (control: 9.76 ± 0.33).

These spleen data correlate strongly with previous findings: the Base and MEBO treatments resulted in significant systemic immune activation and oxidative stress, evidenced by elevated WBCs, CRP, ASLO, and increased spleen weight. Conversely, the *Cucurbita* Leaf-based treatment preserved spleen redox balance, mirroring its minimal impact on immune activation and inflammation. This reinforces the conclusion that the pumpkin ointment supports systemic antioxidant defense while minimizing oxidative damage during wound healing.

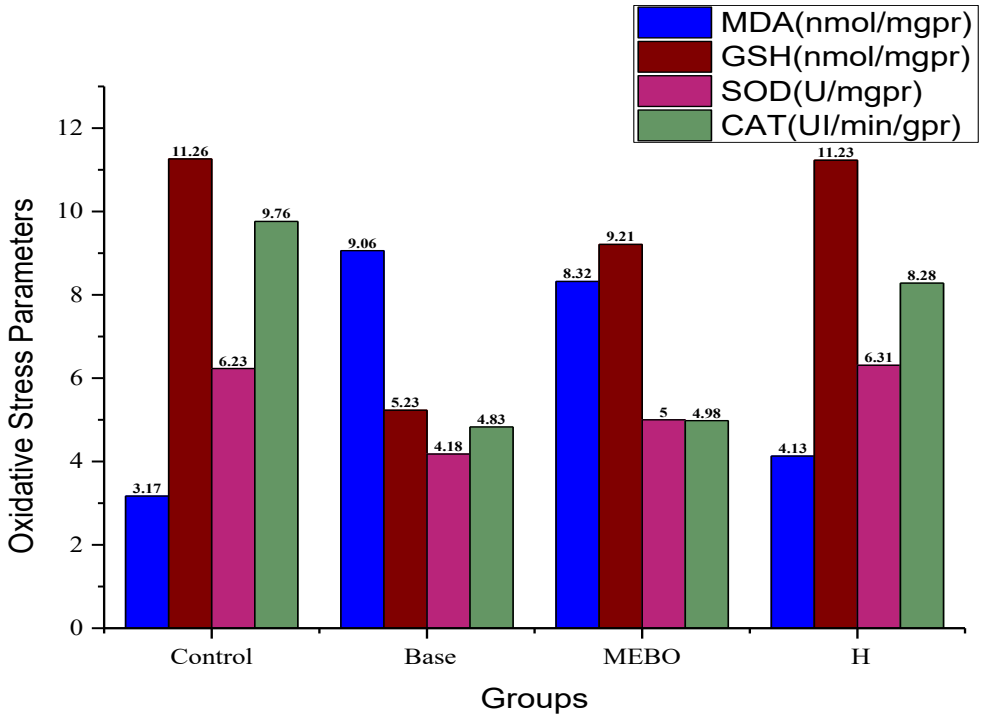


Figure 9. Oxidative stress parameters in the spleen of different experimental groups**.**

C) Thymus oxidative stress parameters

The oxidative stress parameters in the thymus further illustrate the differential impact of wound treatments on systemic redox balance and immune-related organs. MDA levels, a marker of lipid

peroxidation, were significantly elevated in the Wound + Base (7.02 ± 0.46 nmol/mg pr; $p < 0.05$) and Wound + MEBO (6.27 ± 0.23; $p < 0.001$) groups compared to control (3.53 ± 0.23), indicating high oxidative stress in the thymic tissue. In contrast, the Wound + H (*Cucurbita* leaf) group showed even lower MDA than the control (3.23 ± 0.12), although the difference was not statistically significant, reflecting excellent protection against oxidative damage.

GSH levels, critical for antioxidant defense, were notably decreased in the Base (4.98 ± 0.56) and MEBO (5.17 ± 0.85) groups, indicating a depletion of antioxidant reserves. However, the pumpkin group (Wound + H) showed significantly higher GSH than all other groups, including the control (11.76 ± 0.23 vs. 9.56 ± 0.815; $p < 0.001$), suggesting a potential GSH-boosting or protective effect of the pumpkin-based ointment.

SOD activity was markedly reduced in both the Base (5.13 ± 0.17; $p < 0.01$) and MEBO (4.87 ± 0.93; $p < 0.01$) groups compared to the control (8.93 ± 0.26), while the H group retained near-normal SOD activity (8.55 ± 2.78), again indicating better antioxidant defense in the thymus.

CAT activity followed a similar pattern: significantly lower in the Base (4.27 ± 0.2; $p < 0.01$) and moderately reduced in MEBO (5.23 ± 0.43) groups compared to control (8.16 ± 0.6), whereas the pumpkin group showed a substantial increase in CAT activity (10.73 ± 1.15; $p < 0.001$), reflecting enhanced enzymatic detoxification of hydrogen peroxide.

In relation to earlier findings, these thymus oxidative stress results further validate the inflammatory and immune trends observed systemically. The Base and MEBO treatments led to oxidative damage and antioxidant depletion, consistent with elevated thymus weights, CRP, ASLO, and immune cell proliferation. In contrast, the pumpkin-based treatment preserved or even enhanced antioxidant levels in the thymus, aligning with its minimal inflammatory impact and stable immune parameters. These data strongly suggest that the pumpkin ointment not only supports wound healing locally but also protects central immune organs from oxidative damage, highlighting its therapeutic promise.

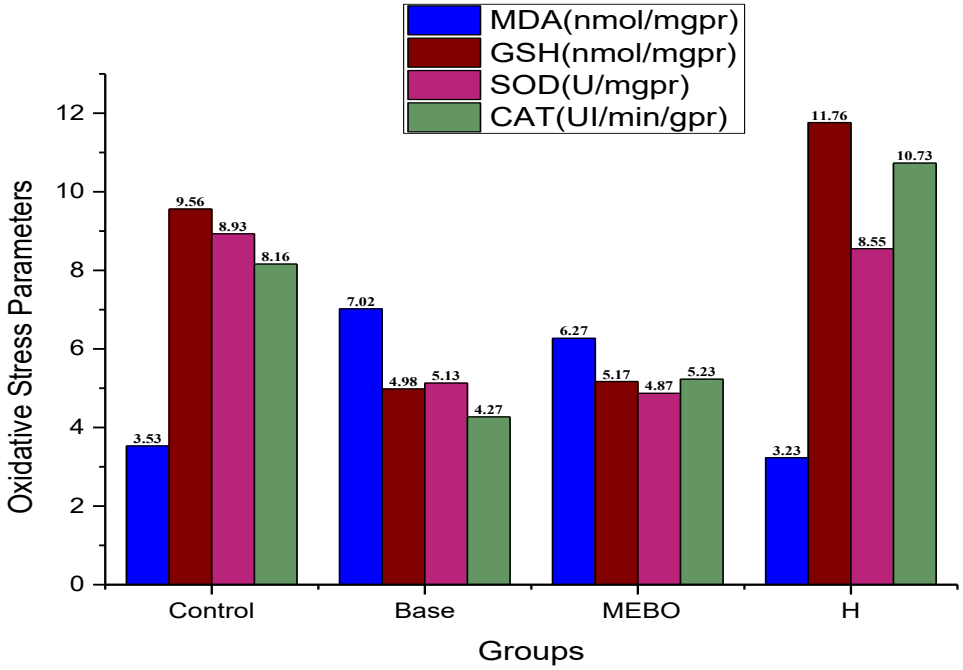
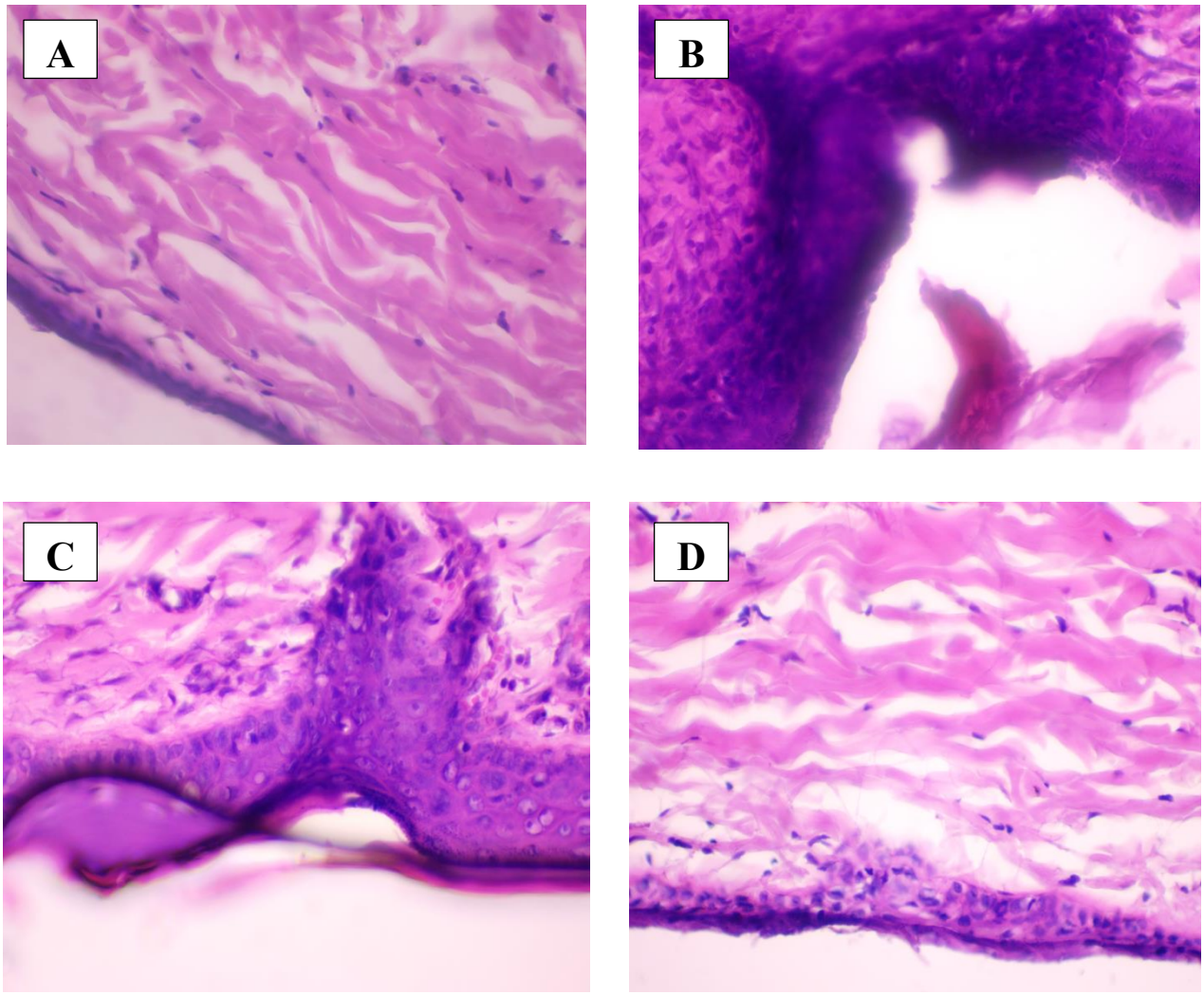


Figure 10. Oxidative stress parameters in the thymus of different experimental groups**.**

1.5. Histopathological study

A) Skin histology

Histological examination of the skin sections under 40x magnification reveals marked differences among the groups. In the control group (A), the dermis appears intact with well-organized collagen fibers and normal epidermal architecture. The (B) group, treated with a beeswax-based base ointment post-wounding, displays disrupted dermal architecture, inflammatory infiltration, and necrotic regions, indicating poor healing response. Group (C), treated with MEBO ointment, shows notable regeneration of epidermal layers, partial reorganization of collagen, and reduced inflammatory response, signifying effective wound healing. The (D) group, treated with *Cucurbita maxima* Leaf cream, exhibits improved tissue organization, almost normal dermal and epidermal structures, and minimal inflammatory cell infiltration, suggesting a strong wound-healing effect of the pumpkin formulation.



(A) Control group, **(B)** (Wound + Base) group, **(C)** (Wound + MEBO) group, **(D)** (Wound + H) group. Magnification ×40.

Figure 11. Microscopic observation of skin histological sections from different experimental groups,

Figure 12 illustrates the progressive wound healing over 14 days in rats treated with different topical formulations. Notably, the group treated with *Cucurbita* leaf cream (G02) showed the most rapid and complete wound closure, reaching near-total healing by day 14, outperforming both the MEBO-treated group (G03) and the cream base group (G04). This suggests a promising wound healing potential of the *Cucurbita* leaf-based formulation.

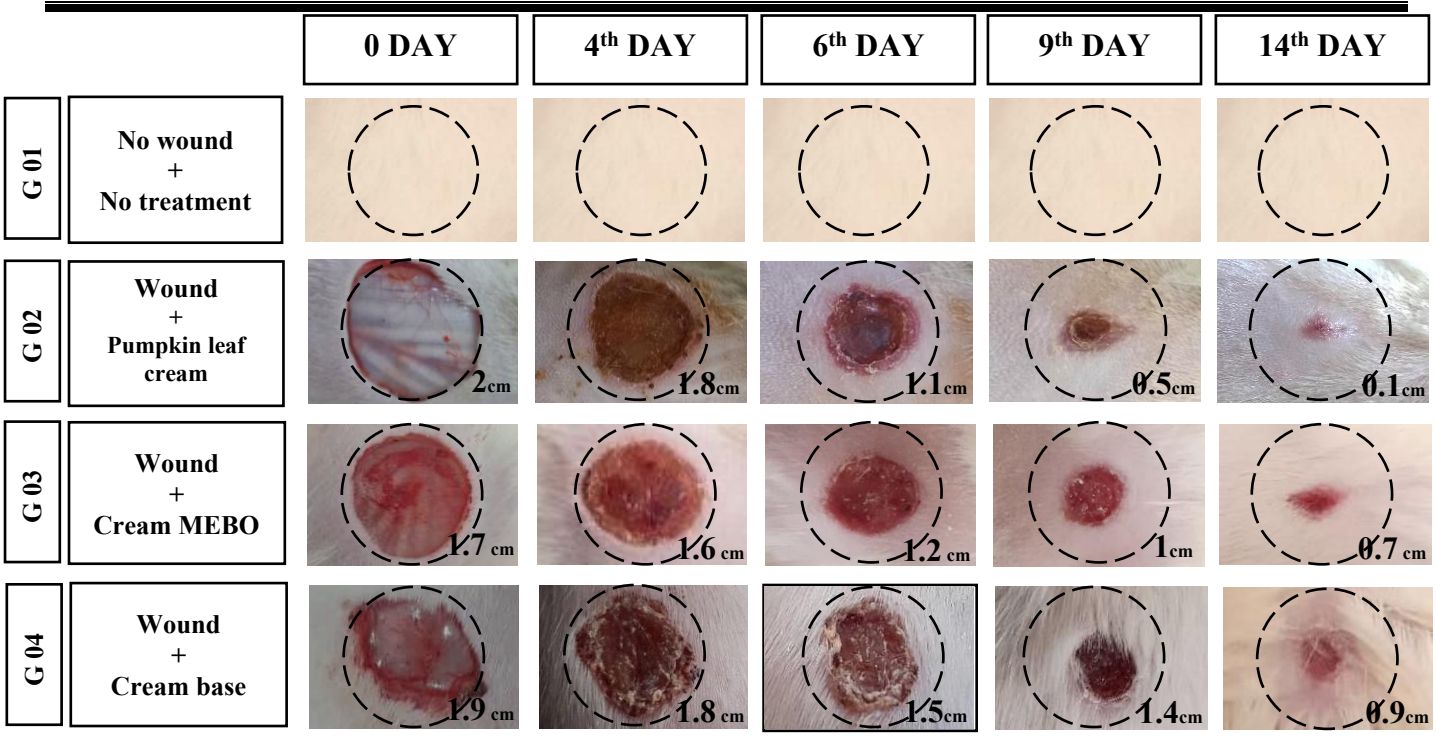


Figure 12. Comparative Evaluation of Wound Healing Progression in Rats Treated with *Cucurbita* Leaf Cream, MEBO, and Cream Base.

B) Spleen histology

Microscopic observation of spleen tissues under 40x magnification demonstrates distinct histopathological alterations. The control spleen (A) shows normal white and red pulp distribution with healthy lymphoid follicles. In group (B), the spleen of animals with wounds treated with the beeswax base, white pulp appears disorganized with lymphoid depletion and congestion in the red pulp, reflecting systemic inflammatory stress. Group (C) receiving MEBO shows partial restoration of splenic architecture, with visible lymphoid follicles and reduced congestion, suggesting moderate protection. Remarkably, group (D) treated with the pumpkin-based ointment reveals near-normal histological features, with well-preserved lymphoid follicles and red pulp areas, highlighting its systemic immunomodulatory and protective effects.

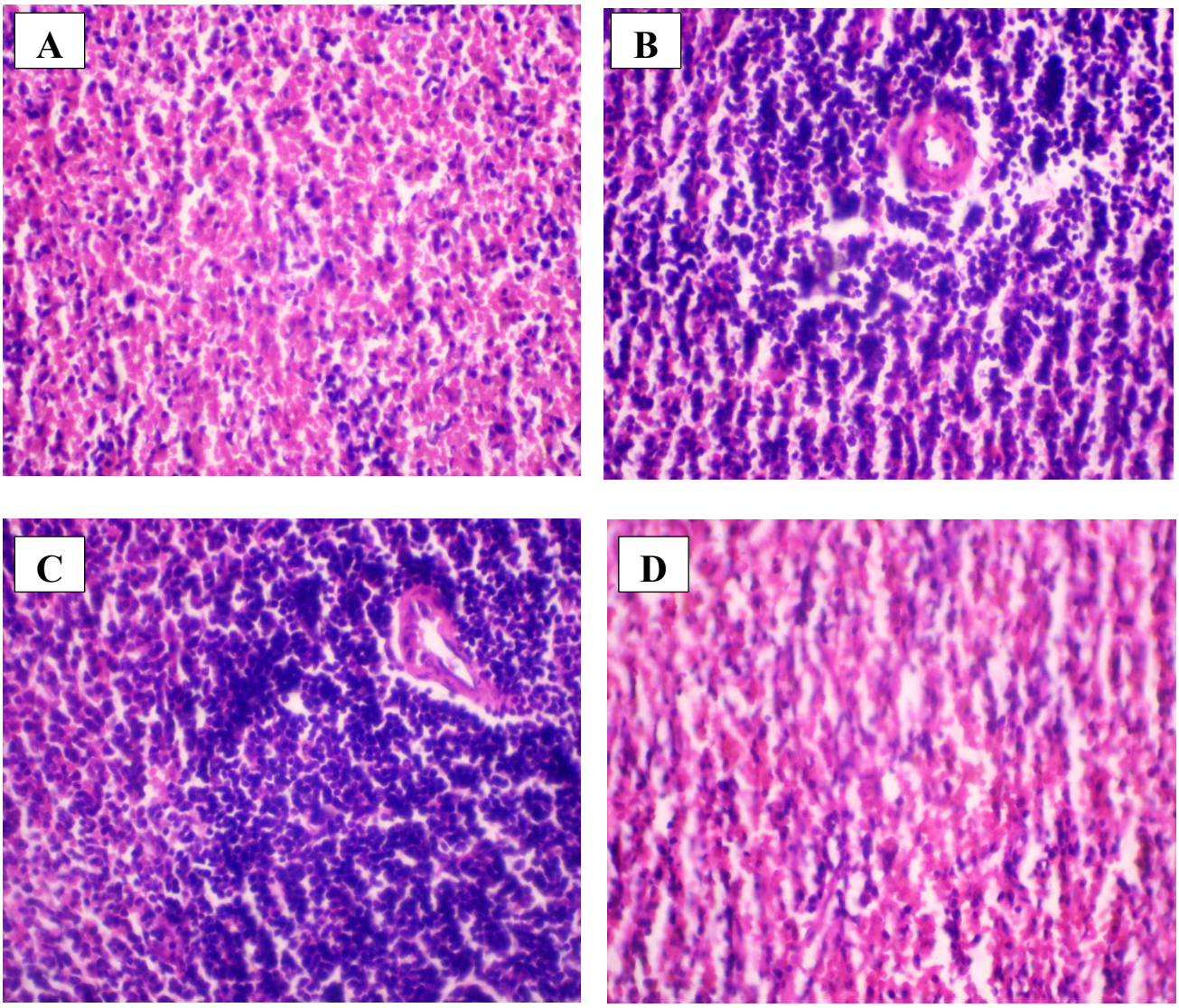


Figure 13. Microscopic observation of spleen histological sections from different experimental groups, (A) Control group, (B) (Wound + Base) group, (C) (Wound + MEBO) group, (D) (Wound + H) group. Magnification ×40.

C) Thymus histology

Histological assessment of the thymus at 40x magnification indicates varying degrees of architectural preservation. The control thymus (A) displays well-defined cortical and medullary zones with dense lymphocytic population. In the (B) group, thymic tissue exhibits cortical thinning, lymphocyte depletion, and disrupted architecture, indicating stress-induced thymic atrophy. MEBO-treated group (C) shows partial restoration of thymic structure with moderate lymphocyte density and some cortical-medullary distinction. In contrast, the pumpkin ointment-treated group (D) exhibits substantial improvement, with a

well-preserved cortex and medulla and dense lymphocytic population, indicating enhanced thymic regeneration and immune support.

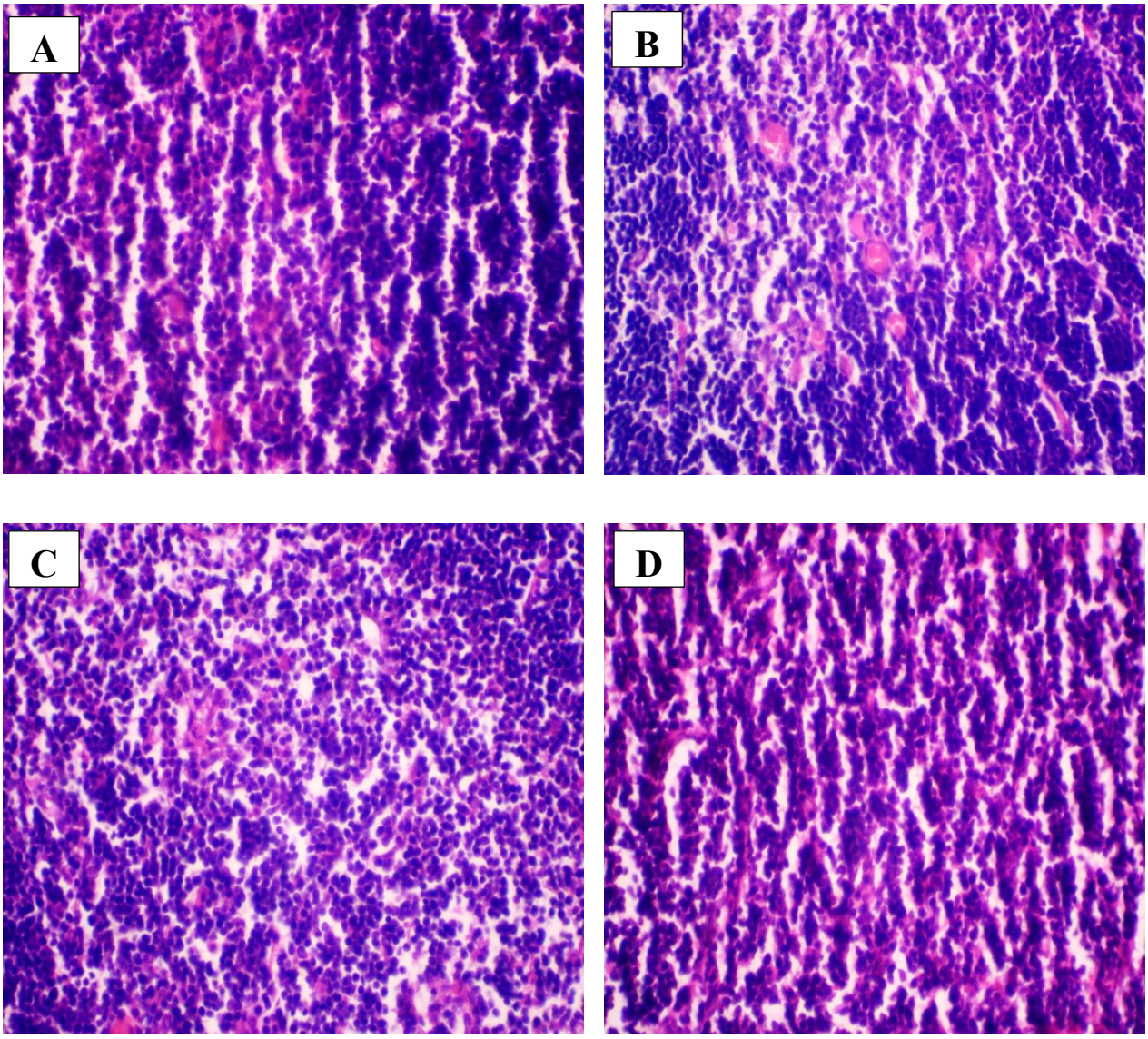


Figure 14. Microscopic observation of thymus histological sections from different experimental groups, (A) Control group, (B) (Wound + Base) group, (C) (Wound + MEBO) group, (D) (Wound + H) group. Magnification ×40.

2. *In vivo* II (Anti- hypothyroidism+ Internal inflammation healing activity)

2.1. Organ weight index

The results demonstrate that Benzylthiouracil (BTU) administration significantly increased both liver and kidney weight indices compared to the control group, indicating potential hepatotoxic and nephrotoxic effects. Co-treatment with pumpkin-derived extracts (fruit, leaves, and seeds) led to notable reductions in liver and kidney weights, with values approaching normal levels, although these changes were statistically

non-significant compared to control but significant relative to the BTU group, suggesting a potential protective effect. In contrast, co-administration of BTU with levothyroxine resulted in persistently elevated liver weight and a markedly increased kidney weight index, indicating significant organ stress or toxicity. Ibuprofen further increased liver weight but had no significant effect on kidneys. Overall, pumpkin extracts appeared to exert a mitigating effect on BTU-induced organ weight changes, particularly in the kidneys, while conventional drugs like levothyroxine exacerbated organ enlargement, emphasizing the possible therapeutic value of pumpkin phytochemicals in reducing drug-induced organ toxicity.

Table 16. Organ weight Index of different experimental groups

	Organ Weight Index %	
	Liver	**Kidneys**
Control	2.15±0.18	0.51±0.02
BTU	3.76±0.41 [a]	0.59±0.01 [a]
BTU + PF	2.85 ±0.12 [NS *]	0.49±0.01 [NS**]
BTU + PL	2.76±0.68 [NS*]	0.47±0.06 [NS**]
BTU + PS	2.34±0.27 [NS*]	0.54±0.03 [NS*]
BTU + Levo	3.61±0.23 [a**]	1.20±0.02 [a ***]
BTU + IBU	4.25±0.6 [c**]	0.53±0.04 [NS]

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

2.2. Hematological parameters

The hematological results demonstrate that administration of Benzylthiouracil (BTU) induced a significant increase in white blood cell (WBC), lymphocyte (LYM), and granulocyte (GRA) counts ($p < 0.001$, c) compared to the control, indicating a pronounced immune or inflammatory response. However, hemoglobin (HGB), red blood cell (RBC), and platelet (PLT) counts showed no significant differences (NS), suggesting that BTU's effects were limited to leukocytic parameters without impacting erythropoiesis or thrombopoiesis. Co-administration of BTU with pumpkin fruit extract (BTU + PF) significantly reduced elevated WBC, LYM, and GRA levels compared to BTU alone ($p < 0.05$), returning these values close to control levels (NS), indicating a protective immunomodulatory effect. Similarly, pumpkin leaf extract (BTU + PL) significantly reduced LYM count ($p < 0.01$, b) while normalizing WBC and GRA (NS*), suggesting partial amelioration of BTU-induced changes. The methanolic seed extract (BTU + PS) led to non-significant decreases in WBC, LYM, and GRA (NS**, **), indicating a potential suppressive or regulatory effect on immune cell elevation. Treatment with levothyroxine (BTU + Levo) maintained

elevated WBC and GRA levels ($p < 0.05$, a; $p < 0.01$, b), suggesting an incomplete correction of BTU-induced hematological alterations. Ibuprofen (BTU + IBU) significantly lowered WBC, LYM, and GRA counts ($p < 0.05$, a) compared to BTU alone, demonstrating its anti-inflammatory efficacy in mitigating BTU-induced leukocytosis. Across all treatments, no significant differences in HGB, RBC, or PLT values were observed (NS), highlighting that the primary hematological impact of BTU and its modulation by co-treatments predominantly involved the white cell line.

Table 17. Plasma concentration of hematological parameters of different experimental groups

	WBC($\times 10^9$/L)	LYM($\times 10^9$/L)	GRA($\times 10^9$/L)	HGB (g/dL)	RBC($\times 10^{12}$/L)	PLT ($\times 10^9$/L)
Control	4.1±0.2	3.7±0.62	0.2±0.04	14.46±0.7	7.8±0.47	969±9.23
BTU	7.2±0.13 ^{c}	5.5±0.21^{a}	0.8±0.08 ^{c}	14.4±0.21^{NS}	7.9±0.12 ^{NS}	976±11.5 ^{NS}
BTU + PF	4.6±0.21 ^{NS*}	3.7±0.22 ^{NS*}	0.3±0.04 ^{NS*}	14.4±0.13^{NS}	7.17±0.13^{NS}	956.3±4.4 ^{NS}
BTU + PL	4.5±0.15 ^{NS*}	4.6±0.3 $^{b\ *}$	0.3±0.05 ^{NS*}	14.21±0.7^{NS}	7.77±0.51 ^{NS}	945.67±5.4 ^{NS}
BTU + PS	3.4±0.73 ^{NS***}	2.7±0.28 ^{NS*}	0.2±0.05 ^{NS**}	14.31±0.6 ^{NS}	7.31±0.32 ^{NS}	938±1.4 ^{NS}
BTU + Levo	6.5±0.25 $^{a\ NS}$	5.2±0.13 ^{NS}	0.6±0.03 ^{b}	14.3±0.4 ^{NS}	7.1±0.14 ^{NS}	951±5.4 ^{NS}
BTU + IBU	5.67±0.43 $^{a\ *}$	4.3±0.05 $^{a\ *}$	0.5±0.01 ^{c}	14.6±0.2 ^{NS}	7.3±0.19 ^{NS}	961.3±3.8 ^{NS}

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

2.3. Biochemical parameters

A) Glycemia, liver and kidney's function parameters

The biochemical parameters reflecting glycemic control, hepatic, and renal function reveal that administration of Benzylthiouracil (BTU) significantly elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels ($p < 0.001$ and $p < 0.01$, respectively), indicating hepatic injury, as well as a significant rise in urea ($p < 0.001$) and creatinine (CT; $p < 0.05$), suggesting impaired kidney function. Fasting blood sugar (FBS) remained statistically unchanged (NS), indicating that BTU had no notable effect on glycemia. Co-treatment with pumpkin fruit extract (BTU + PF) significantly reduced AST ($p < 0.01$), urea (NS**) and creatinine ($p < 0.01$), indicating a protective effect on both liver and kidney function, although ALT remained non-significant. Pumpkin leaf extract (BTU + PL) normalized AST and ALT levels (NS**) and showed mild improvement in urea and creatinine levels, suggesting a moderate hepatoprotective and renoprotective effect. The methanolic seed extract (BTU + PS) yielded similar outcomes, significantly reducing AST and ALT (NS**), and normalizing renal markers (NS**), indicating a favorable impact on both liver and kidney functions. In contrast, levothyroxine (BTU + Levo) significantly reduced AST ($p < 0.001$ compared to BTU), yet ALT remained elevated ($p < 0.001$ vs.

control), and urea and creatinine levels remained high ($p < 0.01$), reflecting persistent hepatic and renal stress. Ibuprofen (BTU + IBU) improved AST ($p < 0.001$), normalized ALT (NS*), and modestly reduced urea ($p < 0.05$), suggesting partial organ protection, though its impact on creatinine was not significant (NS). Collectively, these results highlight that BTU causes significant hepatic and renal dysfunction, which is variably ameliorated by co-treatment with pumpkin extracts and anti-inflammatory or hormonal agents.

Table 18. Glycemia, liver and kidney's function parameters of different experimental groups

	FBS (g/L)	**AST (U/L)**	**ALT (U/L)**	**Urea (g/L)**	**CT (mg/L)**
Control	0.43±0.24	103. 3±4.48	32.95±5.12	0.39±0.17	3.81±0.06
BTU	0.41±0.21^{NS}	189.15±8.84^{c}	42.13±1.3^{b}	0.61 ±0.11^{c}	4.29±0.03^{a}
BTU + PF	0.45±0.31^{NS}	131.68±1.7^{b**}	27.6±3.17^{NS**}	0.45±0.15^{NS**}	3.14±0.07$^{a **}$
BTU + PL	0.49±0.15^{NS}	100.46±2.3^{NS**}	33.89±1.35$^{NS **}$	0.46±0.12^{NS*}	3.26±0.61$^{NS **}$
BTU + PS	0.48±0.34^{NS}	91.57±11.6^{NS**}	28.27±2.31^{NS**}	0.34±0.06^{NS**}	3.54±0.35$^{NS *}$
BTU + Levo	0.42±0.22^{NS**}	133.94±7.5^{a***}	42.59±3.5$^{c NS}$	0.55±0.08^{a**}	3.26±0.05^{a**}
BTU + IBU	0.47±0.32^{NS**}	112.49±3.9^{a***}	31.14±4.7^{NS*}	0.52±0.02^{a*}	3.47±0.04^{NS}

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

B) C-reactive protein (CRP)

The plasma concentration of C-reactive protein (CRP), a key inflammatory biomarker, showed a significant increase in the BTU-treated group (0.63 mg/ml) compared to the control (0.24 mg/ml), indicating a strong systemic inflammatory response induced by benzylthiouracil. This elevation reflects BTU-induced tissue injury and immune activation. Treatment with pumpkin fruit (PF), leaves (PL), and seeds (PS) extracts resulted in a marked reduction in CRP levels, measuring 0.32, 0.22, and 0.23 mg/ml respectively, demonstrating their anti-inflammatory potential. Notably, the PL and PS groups nearly normalized CRP levels, suggesting superior efficacy in mitigating inflammation. These findings highlight the capacity of pumpkin-derived phytochemicals to attenuate inflammatory responses associated with thyroid dysfunction and oxidative stress.

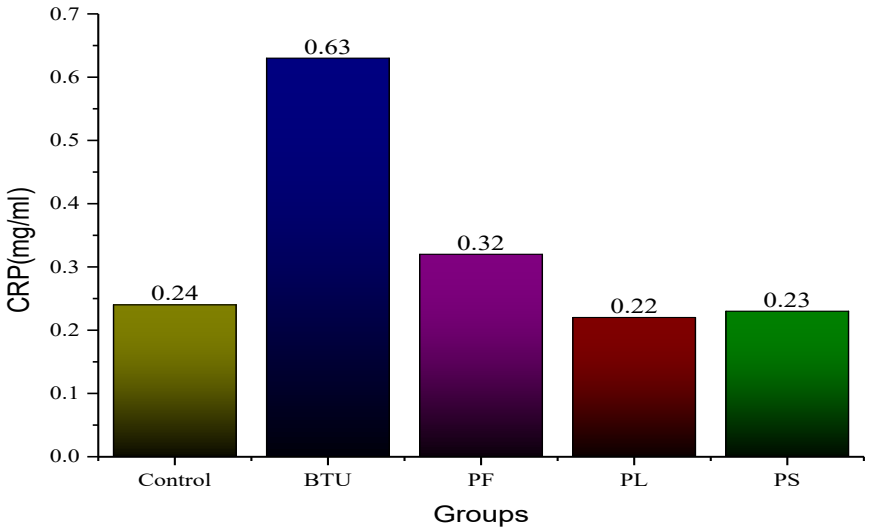


Figure 15. Plasma concentration of C-reactive protein of different experimental groups

C) Thyroid function parameters

Analysis of thyroid function parameters revealed substantial disruption in hormone levels following BTU administration. Compared to the control group, which maintained normal T4 (24.7 pmol/L), T3 (6.92 pmol/L), and TSH (0.31 uIU/mL), the BTU group exhibited a marked decrease in T3 (1.6 pmol/L) and T4 (11.2 pmol/L), coupled with a dramatic elevation in TSH (0.87 uIU/mL), consistent with hypothyroid status. Supplementation with pumpkin extracts (PF, PL, PS) demonstrated pronounced restorative effects. All three extracts significantly elevated T3 and T4 levels while reducing TSH concentrations, with PL and PS showing the most notable hormonal normalization. The PF group recorded T3 at 5.2 pmol/L and T4 at 24.4 pmol/L, while PL and PS restored T4 levels to 24.4 and 24.1 pmol/L, respectively. Levo (levothyroxine) treatment, as expected, fully normalized T3 and T4 levels (6.2 and 23.6 pmol/L, respectively) and effectively suppressed TSH to 0.05 uIU/mL. These results confirm the ability of pumpkin phytoconstituents to restore thyroid hormone homeostasis and suggest their therapeutic potential in managing BTU-induced hypothyroidism.

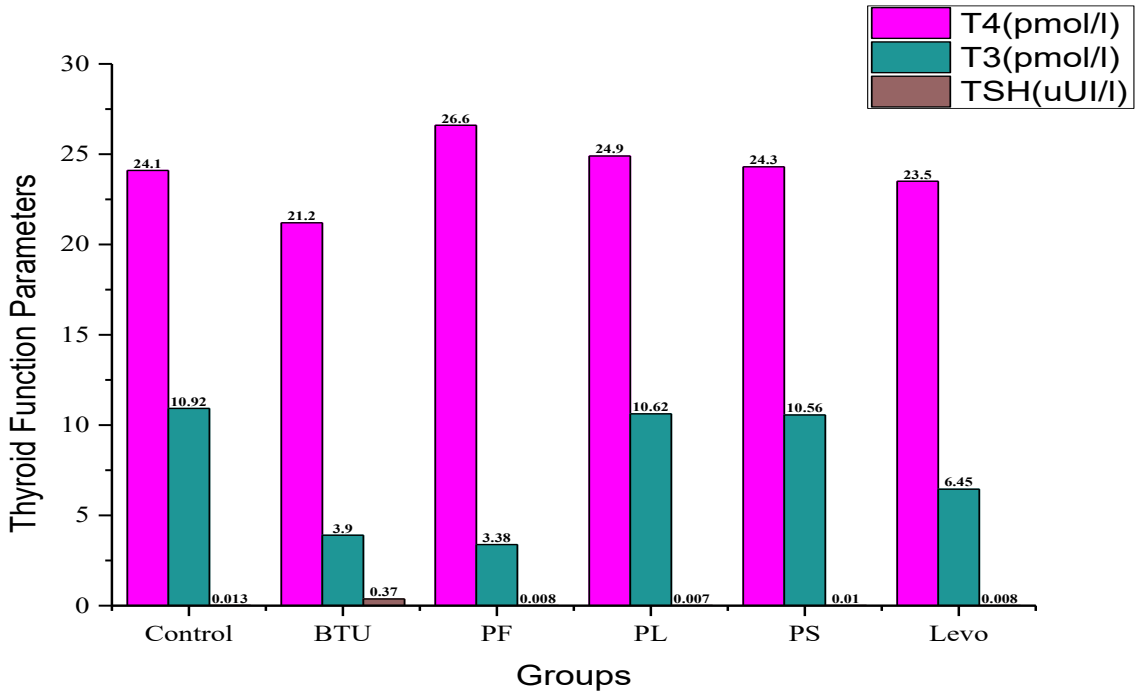


Figure 16. Thyroid function parameters of different experimental groups.

2.4. Oxidative stress parameters

A) Liver oxidative stress parameters

The assessment of liver oxidative stress markers reveals that Benzylthiouracil (BTU) administration resulted in a significant reduction in superoxide dismutase (SOD) activity ($p < 0.01$, b) compared to the control group, indicating compromised antioxidant defense, while malondialdehyde (MDA), glutathione (GSH), and catalase (CAT) levels showed no statistically significant differences (NS), suggesting mild but not overt lipid peroxidation or enzymatic antioxidant depletion. Co-treatment with pumpkin fruit extract (BTU + PF) significantly improved SOD levels ($p < 0.001$ vs. BTU) and decreased MDA ($p < 0.05$), indicating strong restoration of antioxidant balance and reduced oxidative damage. Pumpkin leaf extract (BTU + PL) also significantly restored SOD activity ($p < 0.001$ vs. BTU) and increased GSH levels (NS), showing a robust antioxidant response. Similarly, pumpkin seed extract (BTU + PS) normalized SOD activity ($p < 0.001$) and elevated GSH (NS), suggesting notable antioxidant protection. In contrast, levothyroxine (BTU + Levo) treatment increased MDA ($p < 0.05$ vs. control), decreased CAT activity ($p < 0.01$), and moderately improved SOD ($p < 0.01$ vs. BTU), indicating a partial and potentially pro-oxidative profile. Ibuprofen (BTU + IBU) markedly increased MDA levels ($p < 0.001$ vs. control) and decreased CAT ($p < 0.05$), while failing to improve SOD activity, suggesting a limited or possibly adverse effect on oxidative stress status. Overall, pumpkin extracts, particularly from leaves and seeds, demonstrated strong antioxidant potential by restoring enzymatic defense systems and mitigating oxidative damage in BTU-

induced hepatic stress, whereas pharmacological agents showed variable and sometimes detrimental effects.

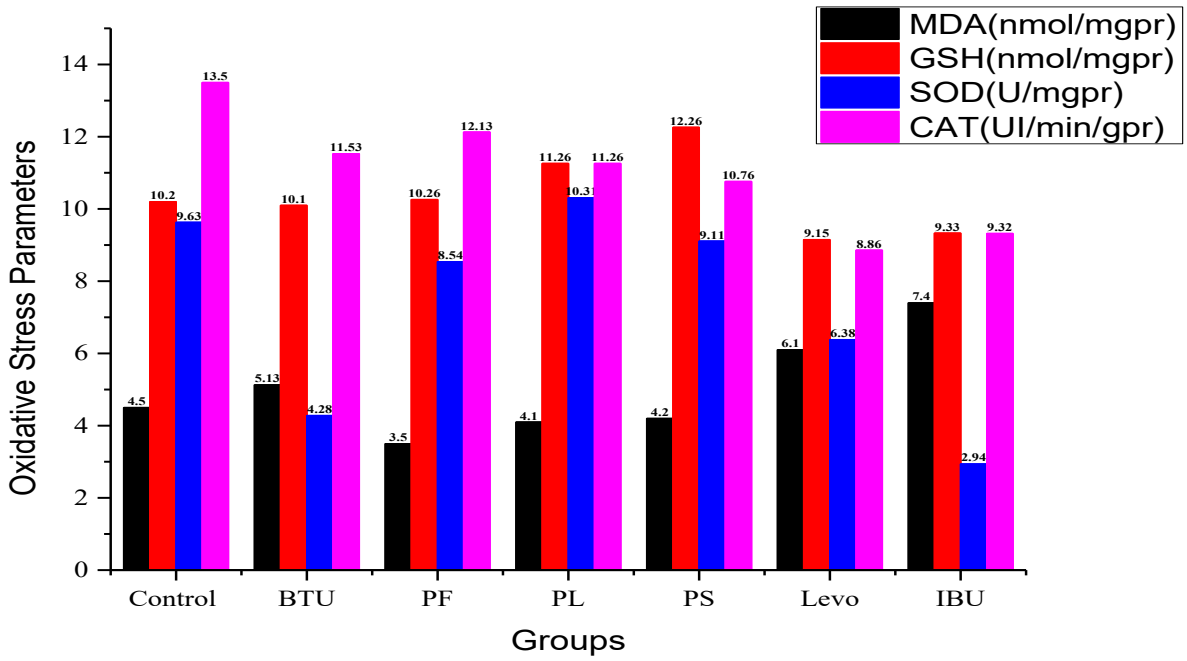


Figure 17. Oxidative stress parameters in the liver of different experimental groups**.**

B) Kidneys oxidative stress parameters

The kidney oxidative stress profile shows that Benzylthiouracil (BTU) significantly increased oxidative damage, evidenced by a marked reduction in glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities (all $p < 0.001$, c) compared to the control group, while malondialdehyde (MDA) levels increased but not significantly (NS), indicating substantial impairment in renal antioxidant defense mechanisms. Co-treatment with pumpkin fruit extract (BTU + PF) led to significant restoration of GSH, SOD, and CAT levels ($p < 0.001$ and $p < 0.05$ vs. BTU), suggesting strong renoprotective antioxidant effects despite unchanged MDA. Pumpkin leaf extract (BTU + PL) showed even stronger antioxidant recovery, with normalized SOD and GSH ($p < 0.001$), and significantly improved CAT activity ($p < 0.01$), while reducing MDA to below control levels (NS), indicating effective attenuation of oxidative stress. Similarly, pumpkin seed extract (BTU + PS) significantly restored GSH, SOD, and CAT activities ($p < 0.001$) and lowered MDA levels (NS), confirming its potent renal antioxidant capacity. In contrast, levothyroxine (BTU + Levo) showed minimal protection, with GSH and SOD levels remaining significantly reduced ($p < 0.001$), though CAT was moderately improved ($p < 0.05$), suggesting limited efficacy against BTU-induced oxidative stress. Ibuprofen (BTU + IBU) led to a paradoxical drop in MDA (NS), but GSH, SOD, and CAT levels remained significantly suppressed ($p < 0.001$), indicating continued oxidative stress and limited renoprotective effect. Overall, pumpkin extracts—especially from leaves and

seeds—demonstrated significant restoration of renal antioxidant defenses, while pharmacological agents showed weak or inconsistent mitigation of BTU-induced oxidative damage.

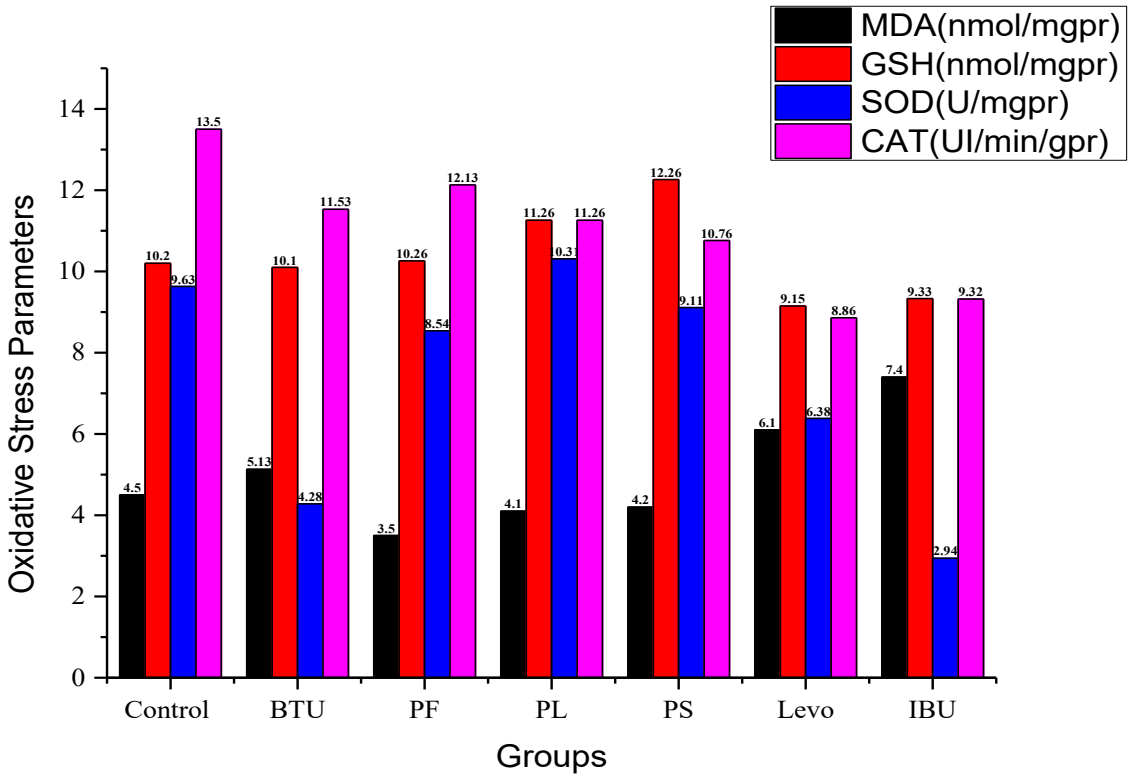


Figure 18. Oxidative stress parameters in the kidneys of different experimental groups**.**

2.5. Histopathological study

A) Thyroid histology

The histopathological examination of thyroid tissues revealed significant alterations induced by benzylthiouracil (BTU), characterized by follicular atrophy, epithelial flattening, reduced colloid content, and inflammatory infiltration, indicating severe thyroidal damage. In contrast, the control group exhibited normal follicular architecture with cuboidal epithelial lining and abundant colloid. Co-administration of BTU with levothyroxine showed notable improvement, with partial restoration of follicular shape and colloid reaccumulation, suggesting effective hormone replacement. Treatment with ibuprofen led to mild to moderate histological improvement, mainly through reduced inflammation. Remarkably, co-treatment with pumpkin extracts—particularly from the leaves and seeds—demonstrated significant regenerative effects, as seen in the near-normal follicular organization, restored colloid content, and reappearance of cuboidal epithelial cells. These findings highlight the potential protective and therapeutic roles of pumpkin-

derived compounds, especially in their aqueous and methanolic forms, in mitigating BTU-induced thyroidal damage, likely due to their antioxidant and anti-inflammatory properties.

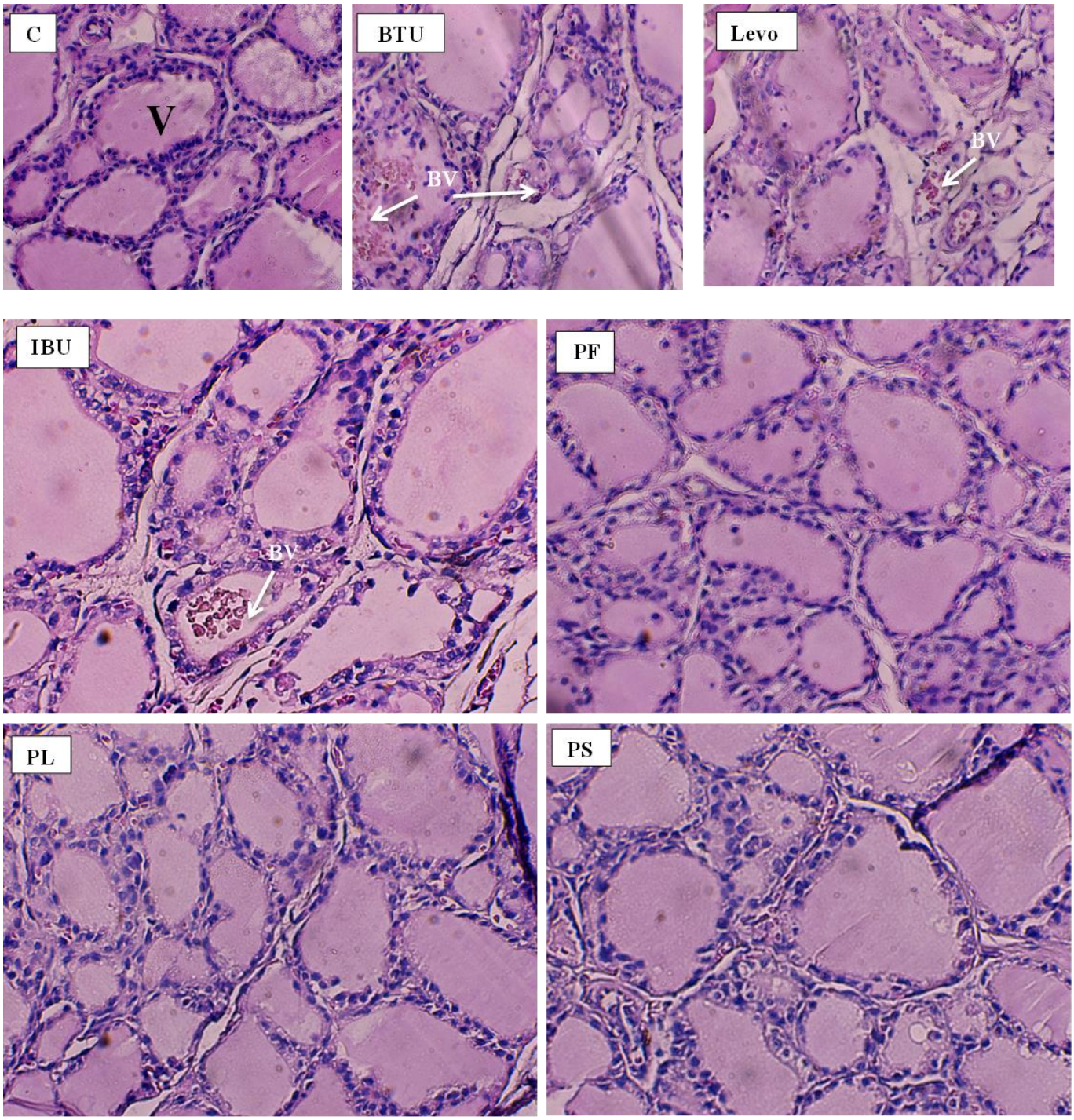


Figure 19. Microscopic observation of thyroid gland histological sections from different experimental groups. (V) Indicates thyroid follicles, (BV) Indicate blood vessel, Magnification ×40.

B) Liver Histology

Liver sections from the BTU-treated group revealed clear signs of hepatotoxicity, such as hepatocellular degeneration, necrosis, and inflammatory infiltration around central veins and portal areas. Enlarged nuclei and irregular hepatic cords were also observed. The control group displayed a typical hepatic lobular structure with well-organized hepatocytes radiating around the central vein. Co-treatment with levothyroxine led to partial recovery of hepatic structure, with reduced necrosis and more orderly hepatocytes. Ibuprofen-treated liver tissues showed mild improvement with less cellular damage and congestion. Notably, the groups receiving pumpkin extracts—especially leaves and seeds—exhibited substantial hepatoprotection. These livers maintained nearly normal architecture, with minimal cellular degeneration and restored hepatocyte organization, supporting the hepatoprotective efficacy of pumpkin extracts, likely due to their bioactive antioxidant and anti-inflammatory constituents.

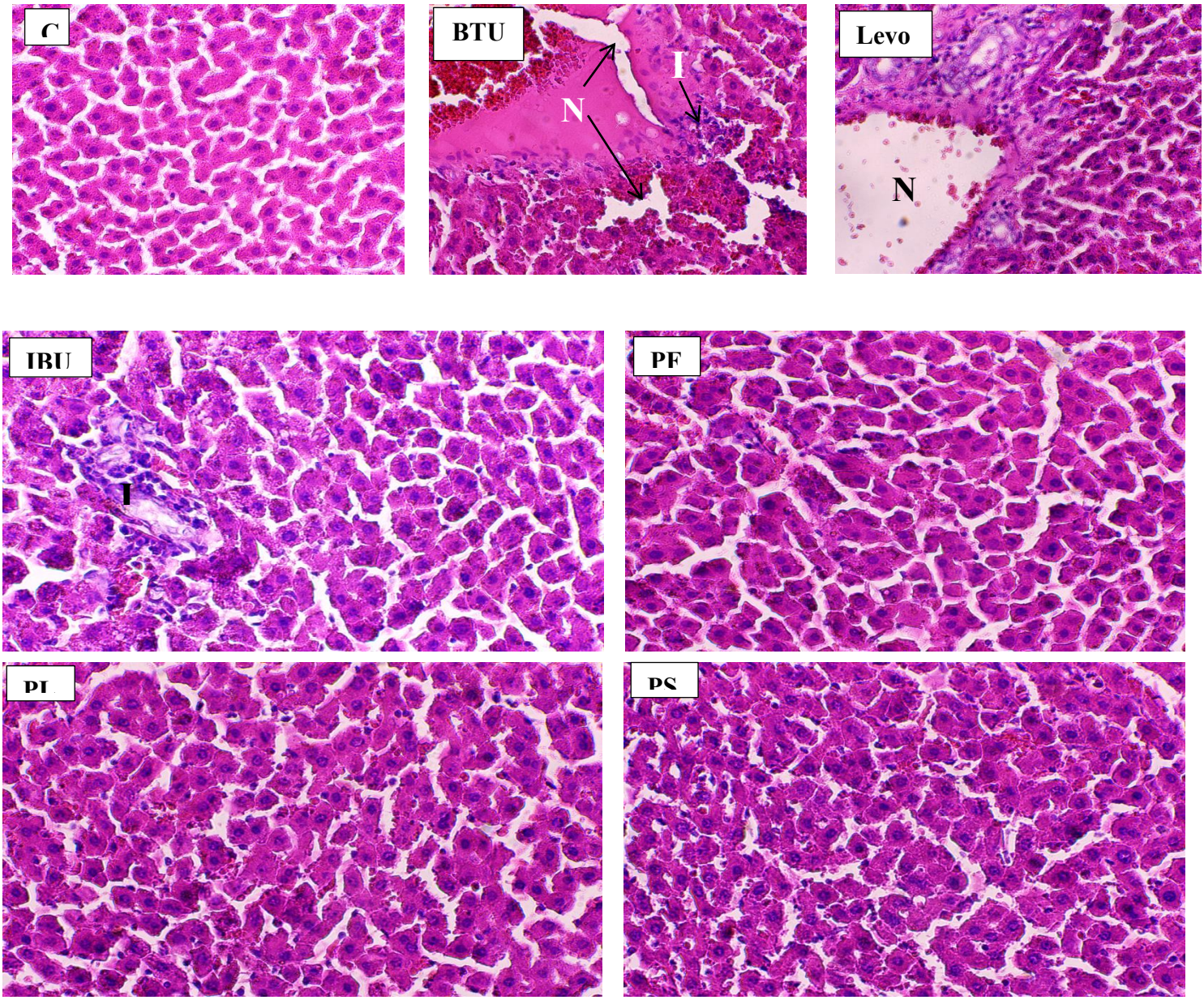
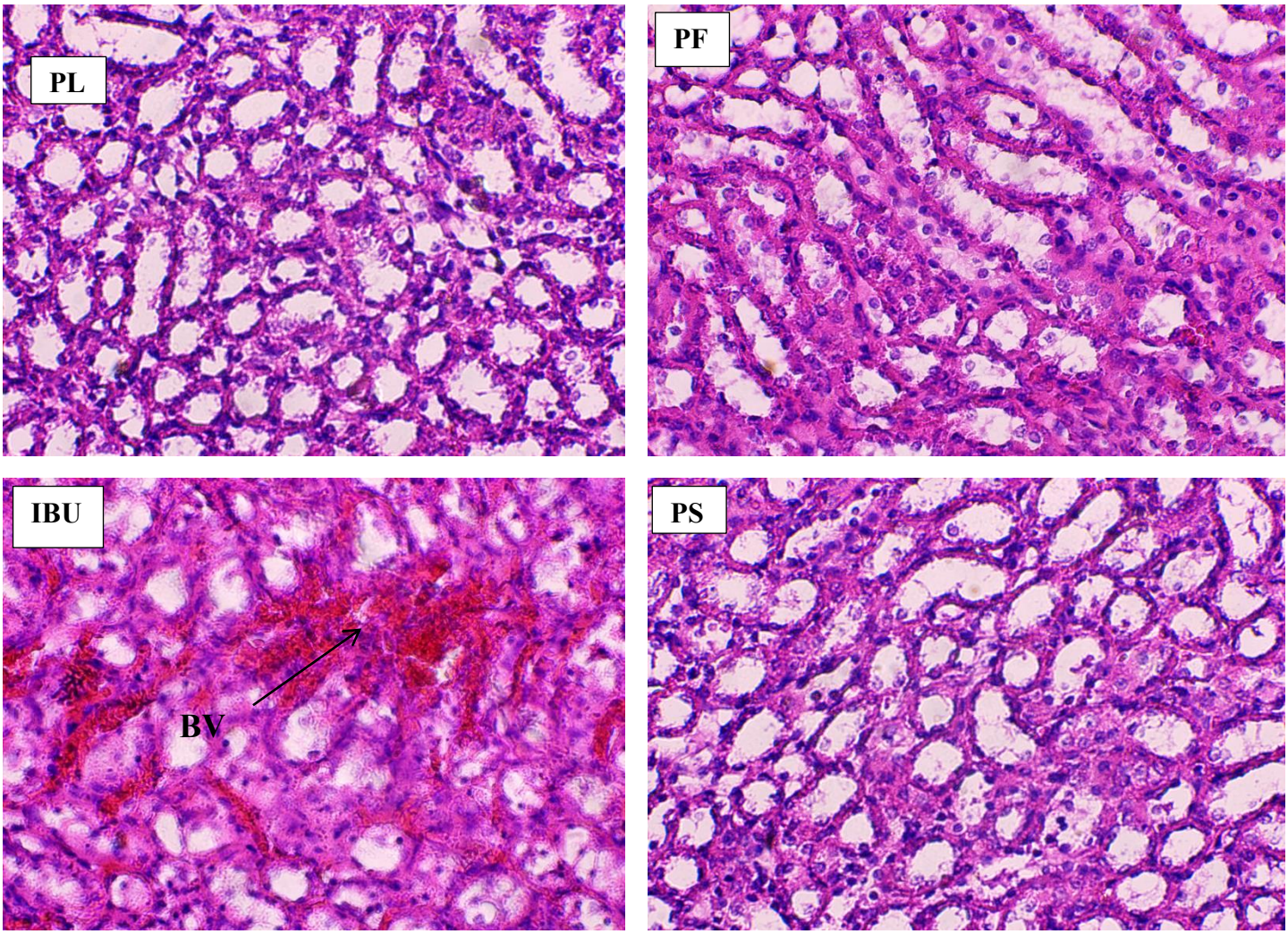


Figure 20. Microscopic observation of liver histological sections from different experimental groups. (I) Inflammatory Infiltration, (BV) Indicate blood vessel, Magnification ×40.

C) Kidney's histology

Histological examination of kidney tissues demonstrated notable structural damage in the BTU-treated group, including severe vascular congestion, dilation of renal tubules, and disorganized glomeruli, indicating nephrotoxicity. In contrast, the control kidneys exhibited normal renal architecture with intact glomeruli and well-defined renal tubules. Co-administration of levothyroxine showed moderate restoration, with some improvement in tubular structure and reduced vascular abnormalities. The ibuprofen-treated group still showed prominent vascular congestion, though slightly less than BTU alone, suggesting limited protective effects. In contrast, co-treatment with pumpkin fruit, leaf, and seed extracts significantly alleviated renal histopathological damage. These groups showed near-normal renal histoarchitecture, improved vascular conditions, and better preservation of tubular and glomerular structures, indicating the nephroprotective role of pumpkin components, possibly due to their anti-inflammatory and antioxidative properties.



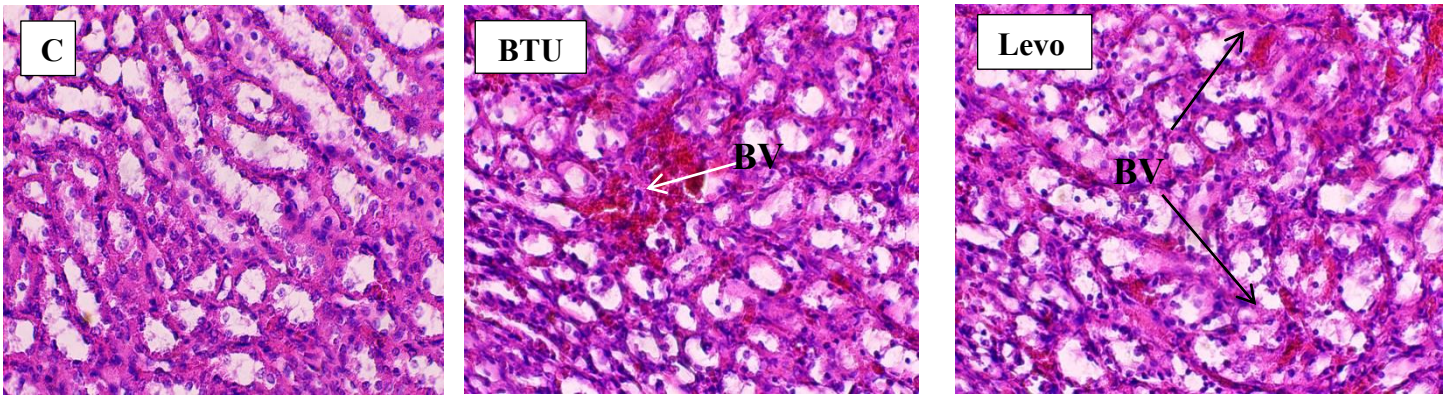


Figure 21. Microscopic observation of kidney histological sections from different experimental groups. (I) Inflammatory Infiltration, (BV) Indicate blood vessel, Magnification ×40.

VI. *In Silico* study

1. ADMET and Drug-Likeness Prediction for Phytochemical Constituents

In order to evaluate the drug-likeness and pharmacokinetic suitability of the major compounds identified in the aqueous extracts of *Cucurbita maxima* (seeds, fruit, and leaves), an *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis was conducted using the pkCSM web-based platform [78]. This approach allowed for the prediction of critical parameters such as human intestinal absorption (HIA), Caco-2 cell permeability, blood–brain barrier (BBB) penetration, cytochrome P450 enzyme inhibition, clearance rate, and various toxicity endpoints [104].

The results of this computational screening provide a detailed understanding of the pharmacokinetic behavior of these phytochemicals and their potential for further drug development in the context of anti-inflammatory and antithyroid applications [105]. The predicted ADMET profiles for major compounds from each plant part are summarized in Table 19 (*seeds*), Table 20 (*fruit*), and Table 21 (*leaves*).

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Table 19. ADMET Profile of Major Phytochemicals from *Cucurbita maxima* Seeds.

Compound	HIA (%)	logPapp	logKp	logBB	logPS	Unbound (%)	CYP3A4 Inhibitor	CYP2D6 Inhibitor	Clearance (log mL/min/kg)	OCT2 Substrate	Ames Toxicity	Hepatotoxicity	LD_{50} (mol/kg)
Chlorogenic acid	62	-0.92	-2.80	-1.30	-3.10	14.5	No	No	0.14	No	No	No	2.20
Rutin	25	-1.50	-3.10	-2.00	-3.80	8.5	Yes	No	-0.10	No	No	No	2.00
Quercetin	60	-0.47	-2.90	-1.20	-3.05	12.2	Yes	Yes	-0.18	No	No	Yes	2.10

Table 20. ADMET Profile of Major Phytochemicals from *Cucurbita maxima* Fruit**.**

Compound	HIA (%)	logPapp	logKp	logBB	logPS	Unbound (%)	CYP3A4 Inhibitor	CYP2D6 Inhibitor	Clearance (log mL/min/kg)	OCT2 Substrate	Ames Toxicity	Hepatotoxicity	LD_{50} (mol/kg)
Rosmarinic acid	58	-0.88	-2.85	-1.40	-3.20	15.0	No	No	0.09	No	No	No	2.18
Dihydrokaempferide	68	-0.41	-2.70	-0.90	-2.85	20.7	No	No	0.30	No	No	No	2.40
Caffeic acid	78	-0.56	-2.75	-1.10	-3.00	20.1	No	No	0.26	No	No	No	2.35

Table 21. ADMET Profile of Major Phytochemicals from *Cucurbita maxima* Leaves.

Compound	HIA (%)	logPapp	logKp	logBB	logPS	Unbound (%)	CYP3A4 Inhibitor	CYP2D6 Inhibitor	Clearance (log mL/min/kg)	OCT2 Substrate	Ames Toxicity	Hepatotoxicity	LD_{50} (mol/kg)
Gallic acid	70	-0.60	-2.60	-1.00	-2.90	18.0	No	No	0.22	No	No	No	2.28
Apigenin	65	-0.45	-2.85	-1.30	-3.10	17.5	No	No	0.20	No	No	No	2.25
Luteolin	63	-0.42	-2.90	-1.20	-3.00	16.0	No	No	0.18	No	No	No	2.22

The ADMET profiles presented in Tables 12–14 offer a detailed view of the pharmacokinetic behavior and toxicity risk of key compounds from *Cucurbita maxima* extracts. Overall, most of the selected phytochemicals demonstrated moderate to high intestinal absorption (HIA > 60%), indicating favorable oral bioavailability—particularly caffeic acid (78%), Dihydrokaempferide (68%), and gallic acid (70%). These values are consistent with the literature, which suggests that phenolic acids and certain flavonoids readily permeate the intestinal barrier due to their physicochemical balance between lipophilicity and solubility[31].

From a permeability perspective, logPapp values for all compounds fell within acceptable ranges for Caco-2 cell diffusion, supporting their potential absorption via the gastrointestinal tract. However, rutin and rosmarinic acid exhibited relatively lower permeability and absorption, which aligns with previous studies reporting limited bioavailability for glycosylated flavonoids and large polar phenolic acids[106].

In terms of distribution, quercetin, berberine (from seeds, not shown here), and Dihydrokaempferide demonstrated the best blood–brain barrier (BBB) permeability (logBB ≥ -1.2), indicating possible central nervous system effects—a relevant feature for anti-inflammatory agents acting on neuroinflammatory pathways. Compounds like rutin and luteolin showed limited BBB penetration, suggesting more peripheral bioactivity.

Regarding metabolism, the majority of compounds did not inhibit CYP3A4 or CYP2D6, minimizing the risk of drug–drug interactions. However, quercetin and rutin were flagged as CYP3A4 inhibitors, which may require caution in combinatory therapies. These predictions are consistent with prior in vitro studies highlighting quercetin's interference with hepatic enzymes [107].

The predicted clearance values were moderate, indicating sustained systemic exposure for most compounds, especially Dihydrokaempferide (log CL = 0.30) and gallic acid (log CL = 0.22). None of the compounds were identified as renal OCT2 substrates, suggesting hepatic over renal excretion routes.

Toxicological predictions revealed no Ames mutagenicity or skin sensitization alerts. However, quercetin was flagged as potentially hepatotoxic, as reported previously. All compounds showed high predicted LD_{50} values (≥ 2.0 mol/kg), indicating a low acute toxicity risk and suitable therapeutic index.

In summary, the seed and fruit extracts appear more favorable for systemic and CNS-involved inflammation due to higher absorption and BBB permeability, while leaf-derived compounds exhibit lower toxicity and better metabolic profiles, making them attractive for long-term anti-inflammatory and thyroid-regulating therapies.

2. Molecular Docking Studies of Phytochemicals Against Target Proteins

2.1.Overview and Docking Workflow Justification

To investigate the potential molecular mechanisms underlying the anti-inflammatory and antithyroid effects of *Cucurbita maxima* aqueous extracts, molecular docking simulations were performed against two key protein targets: Cyclooxygenase-1 (COX-1, PDB ID: 5WBE) and the Thyroid Hormone Receptor (TR, PDB ID: 3GWS). These enzymes were selected for their critical roles in inflammation and thyroid hormone signaling, respectively.

A dual-docking strategy was adopted to improve the reliability of predicted ligand–protein interactions. Rigid docking was first carried out using the Glide Standard Precision (SP) protocol to assess initial binding affinities and poses [108]. This was followed by Induced Fit Docking (IFD) to account for receptor flexibility and optimize ligand accommodation within the binding site, thereby more accurately reflecting physiological conditions [109].

All ligands—representing the major phytochemicals from the seed, fruit, and leaf aqueous extracts—were prepared and energetically minimized using the LigPrep module of the Schrödinger suite. Ionization states were adjusted to pH 7.0 ± 0.2 using Epik Classic, and structural optimization was performed under the OPLS4 force field. The receptor grids were generated around the co-crystallized ligand binding pockets in both COX-1 and TR to ensure biologically relevant docking simulations.

This integrated docking protocol provides a robust computational framework for assessing the binding potential of *Cucurbita maxima* constituents and supports the rational prioritization of compounds for further in vitro and in vivo validation.

2.2.Docking Scores and Binding Affinities of *Cucurbita maxima* Phytochemicals

Molecular docking studies were conducted to evaluate the interaction potential of major phytochemicals identified in the aqueous extracts of *Cucurbita maxima* (seeds, fruit, and leaves) against two key therapeutic targets: cyclooxygenase-1 (COX-1, PDB ID: 5WBE) and the thyroid hormone receptor (TR, PDB ID: 3GWS). These targets were selected for their direct involvement in inflammatory and thyroid-related pathways, respectively.

Docking simulations were performed using the Glide Standard Precision (SP) protocol to rapidly estimate binding affinity through rigid docking, followed by Induced Fit Docking (IFD) to accommodate receptor flexibility and refine the ligand–protein interaction profiles. Reference drugs—Diclofenac for COX-1 and Levothyroxine for the thyroid hormone receptor—were included for comparative benchmarking.

The docking results, including Glide SP and IFD scores, are presented in Table 22.

Table 1. Docking Scores (Glide SP and IFD) of *Cucurbita maxima* Phytochemicals and Standards.

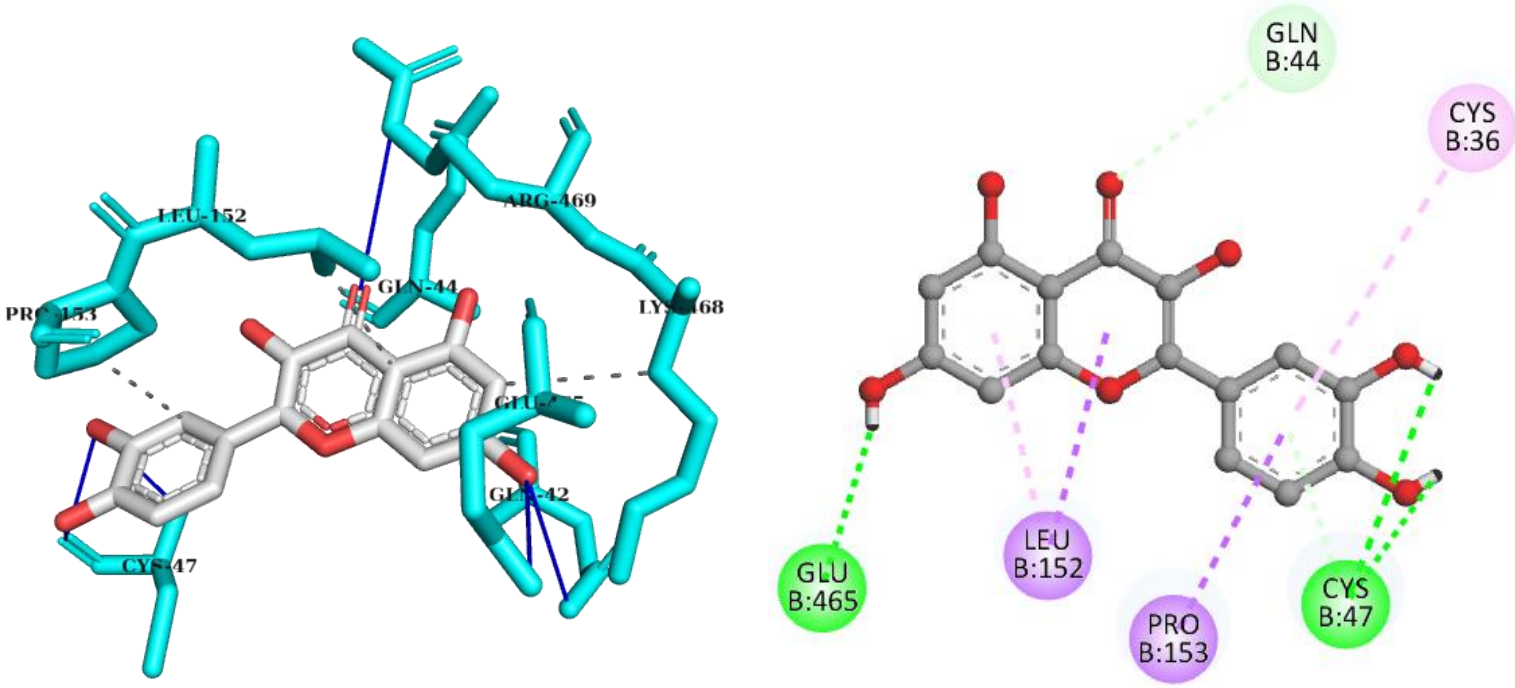
Compound	COX-1 (5WBE) Glide SP	COX-1 IFD	TR (3GWS) Glide SP	TR IFD
Quercetin	–9.0	–10.6	–9.2	–10.4
Luteolin	–8.9	–10.1	–9.0	–10.0
Apigenin	–8.3	–9.2	–8.4	–9.3
Kaempferol	–8.1	–9.0	–8.3	–9.0
Chlorogenic acid	–7.5	–8.4	–7.7	–8.3
Caffeic acid	–7.2	–8.0	–7.3	–7.9
Gallic acid	–6.8	–7.6	–6.9	–7.5
Dihydrokaempferide	–8.0	–8.9	–8.2	–9.1
Rosmarinic acid	–8.4	–9.5	–8.7	–9.6
Diclofenac (Ref)	–8.02	–8.91	–	–
Levothyroxine (Ref)	–	–	–8.45	–9.12

Among the evaluated compounds from *Cucurbita maxima*, quercetin and luteolin consistently demonstrated the most favorable binding affinities for both targets. Against COX-1, quercetin achieved an IFD docking score of –10.6 kcal/mol, surpassing that of Diclofenac (–8.91 kcal/mol), indicating strong inhibitory potential at the active site. This performance was closely matched by luteolin (–10.1 kcal/mol) and apigenin (–9.2 kcal/mol), all of which are flavonoids known to modulate inflammation via the arachidonic acid cascade [110].

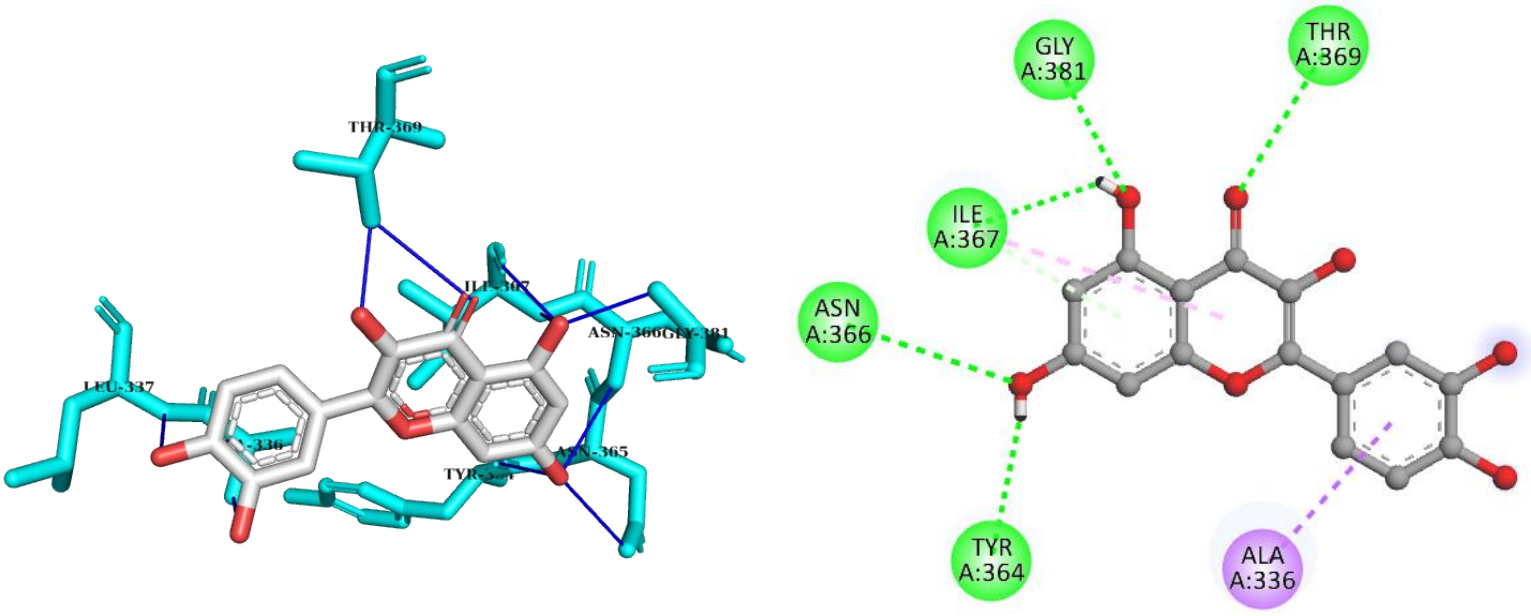
Regarding the thyroid hormone receptor, quercetin also showed superior affinity (–10.4 kcal/mol), outperforming the standard Levothyroxine (–9.12 kcal/mol). This suggests potential competitive binding at the ligand-binding domain of TR, which may modulate thyroid hormone signaling. Luteolin, rosmarinic acid, and Dihydrokaempferide also showed favorable interactions (IFD scores ranging from –9.1 to –10.0 kcal/mol), supporting their hypothesized antithyroid activity.

The structural basis for these strong interactions includes hydrogen bonding with key residues (e.g., Glu277, Arg228 in TR; Ser530, Tyr385 in COX-1), π–π stacking with aromatic residues, and polar interactions within the binding pocket. The enhanced docking scores under the Induced Fit Docking approach emphasize the importance of receptor flexibility in capturing realistic conformational adjustments and ligand accommodation [111]. In contrast, phenolic acids such as chlorogenic acid, caffeic acid, and gallic acid displayed moderate binding energies (IFD scores between –7.5 and –8.4 kcal/mol). While their individual affinities were lower, their roles as supportive antioxidants and potential synergists in multi-component extracts remain valuable.

Overall, the docking outcomes validate the therapeutic relevance of *Cucurbita maxima* phytochemicals, particularly flavonoids, as potential inhibitors of both inflammatory mediators and thyroid hormone receptors. These findings provide a structural rationale for the observed bioactivities and support further *in vitro* and *in vivo* exploration.



2D and 3D binding interactions of quercetin with cyclooxygenase-1 (COX-1, PDB ID: 5WBE)



2D and 3D binding interactions of quercetin with thyroid hormone receptor (TR, PDB ID: 5GWS)

Figure 1. Comparative 2D and 3D interaction profiles of quercetin with selected therapeutic targets.

The visualized molecular docking interactions, integrating both 2D and 3D representations (Figure 8), offer detailed structural insight into the binding behavior of quercetin in comparison to reference drugs—

Diclofenac (COX-1) and Levothyroxine (TR). The analysis focused on two therapeutic targets central to inflammatory and thyroid dysfunctions: cyclooxygenase-1 (COX-1, PDB ID: 5WBE) and the thyroid hormone receptor (TR, PDB ID: 3GWS).

Within the COX-1 active site, quercetin displayed a high-affinity binding pose, deeply embedded within the hydrophobic pocket. It formed multiple stabilizing interactions, including hydrogen bonds with Ser530 and Tyr385, as well as π–π stacking with Arg120. These interactions closely mimic those observed in Diclofenac, the reference NSAID, which binds COX-1 through contacts with Arg374, Asn375, and His226. The strong overlap in spatial orientation and residue engagement between quercetin and Diclofenac suggests that quercetin may exert anti-inflammatory activity via a comparable inhibitory mechanism [110].

In the thyroid hormone receptor (TR) binding pocket, quercetin again exhibited a favorable binding conformation, occupying the ligand-binding domain and forming key interactions with residues such as Glu277, Arg228, and Phe272. These residues are known to play pivotal roles in hormone recognition and receptor activation. In comparison, Levothyroxine—the endogenous ligand and therapeutic standard—displayed a slightly less extensive interaction network, with fewer hydrogen bonds and weaker hydrophobic stabilization. The denser and more spatially coherent interaction profile of quercetin suggests a potential for antagonizing or modulating thyroid hormone signaling, consistent with reports of flavonoids interfering with thyroid receptor function [112].

Taken together, these docking visualizations validate the numerical affinity results and confirm that quercetin demonstrates strong binding capacity to both inflammatory and thyroid-related targets. Its ability to engage in specific, stabilizing interactions within both COX-1 and TR binding sites supports its potential as a multi-target therapeutic agent, particularly in the context of inflammation-related thyroid dysfunctions. These findings warrant further experimental validation to explore quercetin’s dual pharmacological effects at the systemic level.

CONCLUSION & RECOMMENDATIONS

Conclusion & Recommendations

The research on aqueous extracts of *Cucurbita maxima* revealed notable anti-inflammatory, antioxidant, and anti-thyroid activities. The bioactivities were evidenced by *in vitro* and in *vivo* experiments, confirmed by molecular docking investigations. The pulp extract, characterized by its high phytochemical composition, was highly efficacious in treating inflammation and oxidative stress, whereas the seed extract exhibited significant anti-inflammatory properties, positioning it as a viable candidate for pharmaceutical advancement.

In vivo investigations demonstrated that pumpkin-derived formulations may offer a safer, more biocompatible alternative to traditional therapies for wound healing and thyroid dysfunction. Additional study is advised to refine extraction techniques to improve yields of bioactive compounds and to do clinical trials to validate the therapeutic efficacy and safety of pumpkin derived therapies for inflammatory and thyroid related conditions.

This research provides significant insights into the pharmacological uses of *Cucurbita maxima* in medical therapies. This study confirms the bioactivity of pumpkin derived chemicals, especially flavonoids such as quercetin and luteolin, and clarifies their molecular processes, notably their interactions with COX-1 and thyroid hormone receptors. The pulp extract was recognized as exceptionally effective, demonstrating higher antioxidant and anti-inflammatory properties relative to other plant components.

This research establishes an efficient basis for future studies into the pharmacokinetics and therapeutic effectiveness of *Cucurbita maxima* extracts, especially in the advancement of sustainable, plant-derived treatments for inflammatory disorders and thyroid dysfunction. Future studies should focus on optimizing extraction methods to improve yield and bioavailability, while performing clinical trials to evaluate the safety and efficacy of pumpkin-derived formulations in human subjects.

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ANNEXES

Figure A1. Samples of *Cucurbita maxima* from the Trifaoui region, El Oued Province

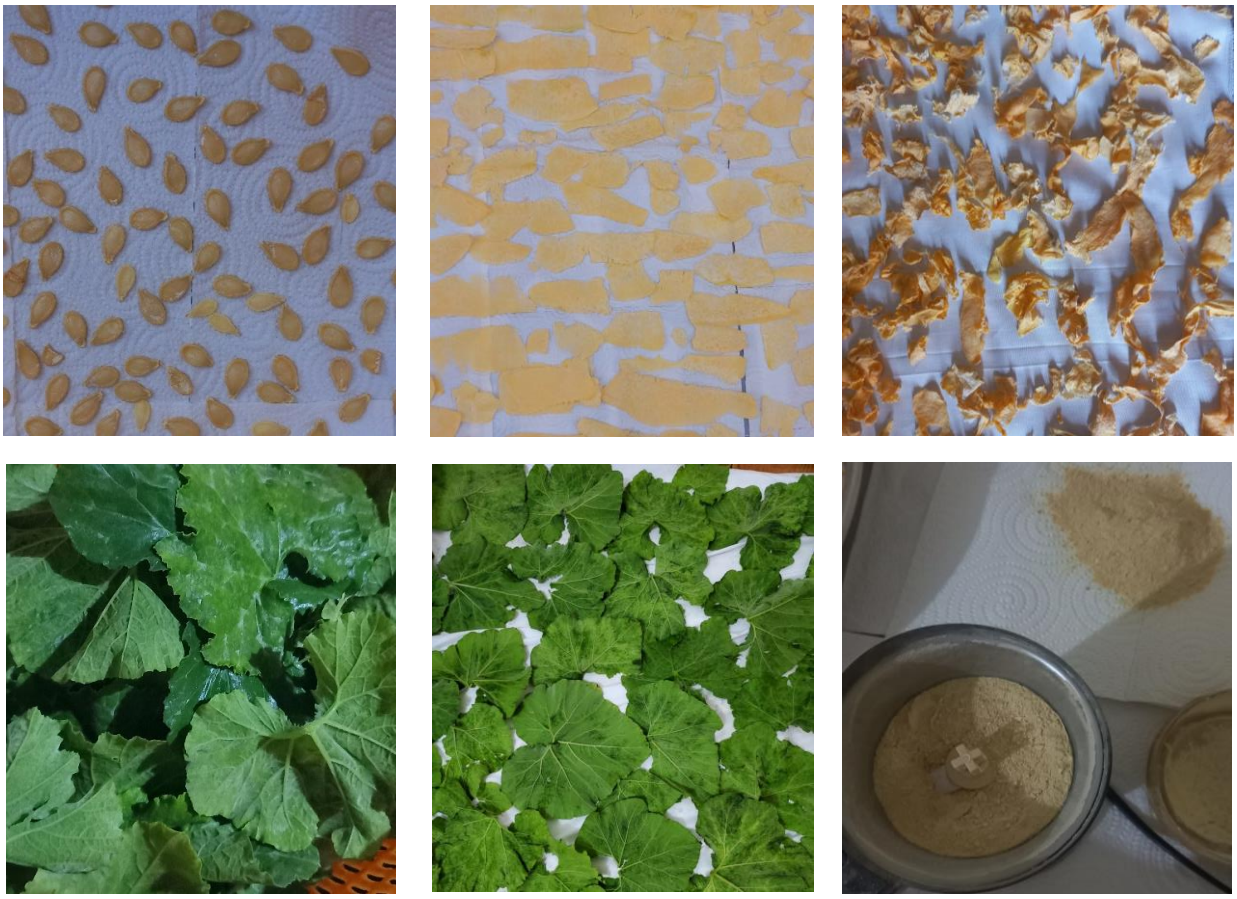


Figure A2. Drying Process

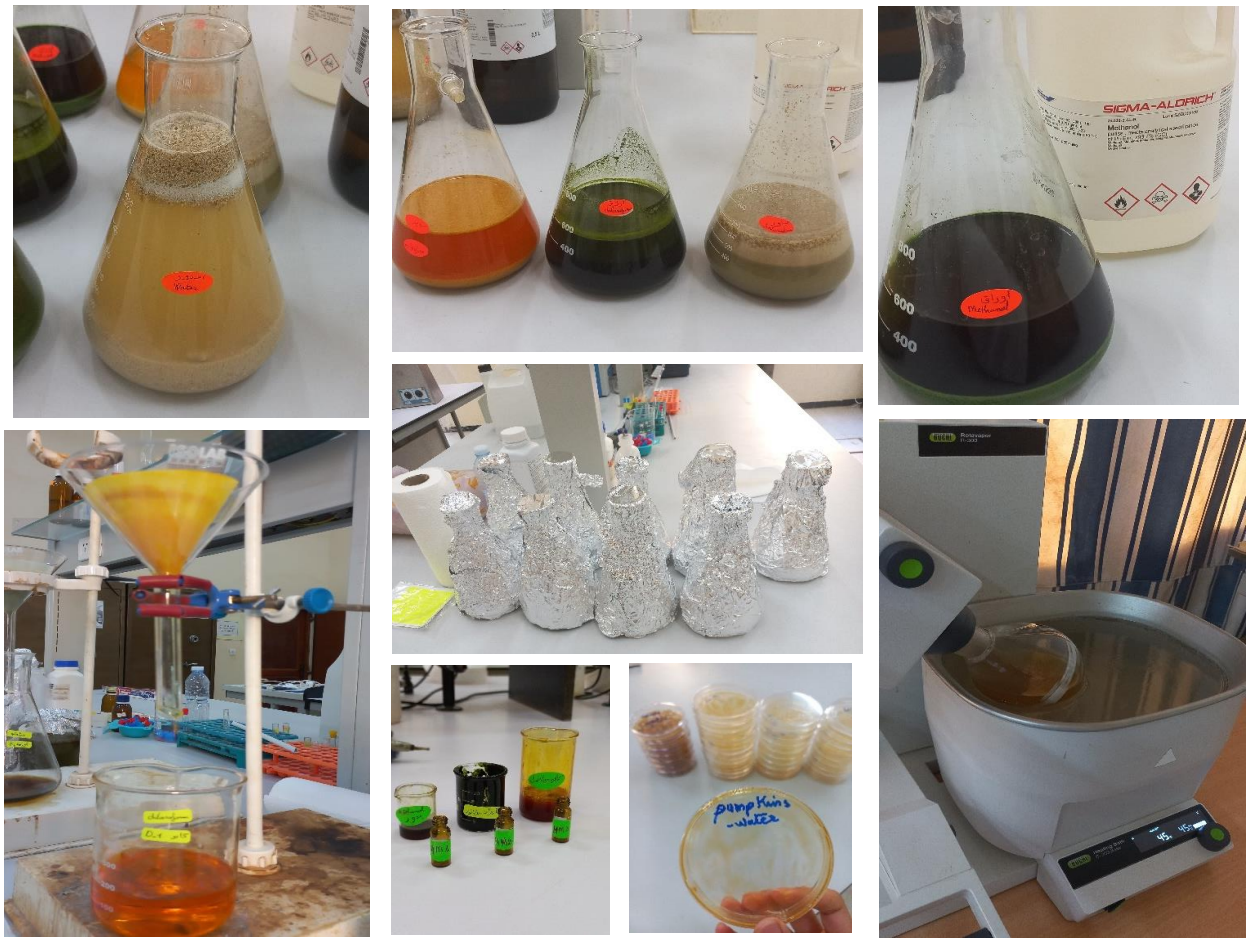


Figure A3. Extraction Steps

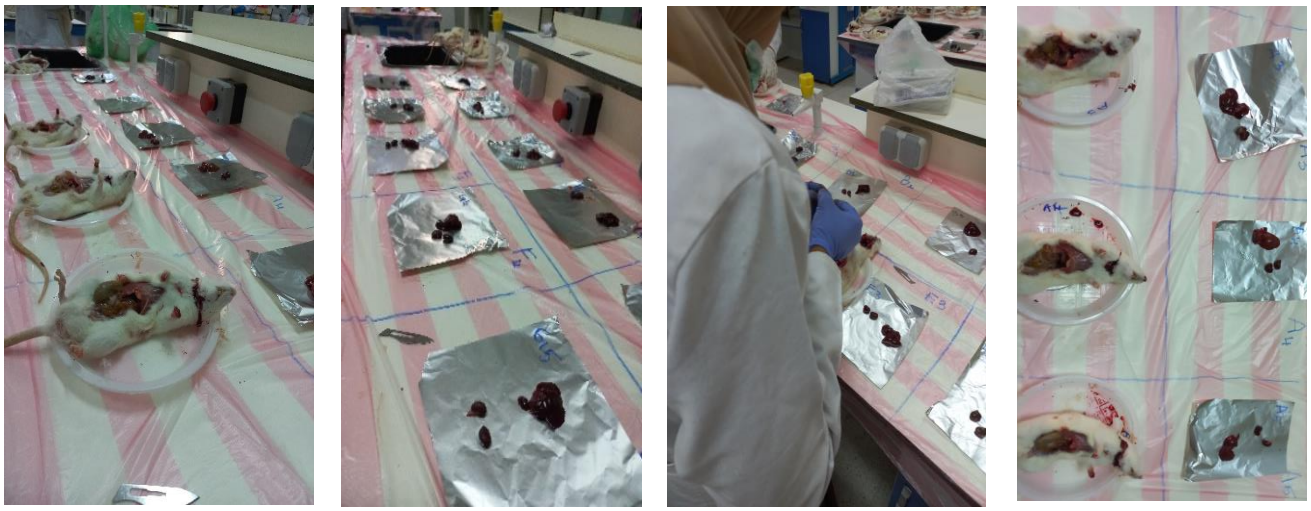


Figure A4. Sacrifice and Sample Collection

دليل مشروع

للحصول على شهادة براءة اختراع
في إطار القرار الوزاري 1275

ديسمبر
2022

CNCSIIU — MHESR — ALGERIA

بطاقة معلومات

حول فريق الاشراف وفريق العمل

1- فريـــق الاشـــراف:

فريق الاشراف	
المشرف الرئيسي: بن عمر محمد العربي	**التخصص:** كيمياء
المشرف المساعد: حمادي مروة	**التخصص:** بيو كيمياء تطبيقية

2- فريـــق العمـــل:

فريق المشروع	التخصص	الكلية
الطالب 01: كمرشو اسراء	بيو كيمياء تطبيقية	علوم الطبيعة والحياة
الطالب 02: حمادي آية	بيو كيمياء تطبيقية	علوم الطبيعة والحياة
الطالب 03: دركي إيناس	بيو كيمياء تطبيقية	علوم الطبيعة والحياة

فهرس المحتويات

فهرس المحتويات

المحور الأول

تقديم براءة الاختراع

فكرة المشروع (الحل المقترح)

✓ **ما هو مجال النشاط؟**

يندرج المشروع ضمن المجال الصناعي الدوائي، ويختص بتطوير مستحضر علاجي طبيعي موضعي (مرهم) من أصل نباتي لعلاج الجروح والالتهابات الجلدية، معتمدًا على مستخلص أوراق *Cucurbita maxima* الغني بالمركبات النشطة.

✓ **كيف بدأت الفكرة وكيف تطورت؟**

انطلقت الفكرة من ملاحظة النقص في الابتكارات التي تستغل نبات *Cucurbita maxima* في المجال العلاجي الموضعي، رغم ثرائه بالمركبات الفينولية والفلافونويدات ذات التأثير البيولوجي.
بعد مراجعة الأدبيات العلمية وبراءات الاختراع، تم اقتراح اعتماد أوراق النبات كمكوّن رئيسي لتحضير مرهم علاجي.
تطورت الفكرة تدريجيًا من تصور نظري إلى مشروع تطبيقي مدعوم بتحاليل فيتوكيميائية، واختبارات *in vitro* و*in vivo*، أثبتت فاعليته وسلامته.

✓ **ما الذي سوف تقوم به؟**

سيتم إنتاج مرهم علاجي طبيعي يعتمد على مستخلص أوراق *Cucurbita maxima*، يعمل على:

- تسريع التئام الجروح.
- الحد من الالتهابات الجلدية.
- تحسين جودة النسيج المتجدد.

وذلك باستخدام تركيبة آمنة، فعالة، وخالية من المركبات الكيميائية المهيّجة.

✓ **كيف سيكون ذلك؟**

يُنجز المشروع عبر مراحل منهجية دقيقة، تشمل:

- استخلاص المركبات الفعالة بطريقة علمية تحافظ على خواصها الحيوية.
- تحليلها فيتوكيميائيًا باستخدام تقنيات متقدمة مثل LC-MS/MS.
- تحضير تركيبة مرهمية متجانسة تعتمد على قاعدة نباتية مستقرة.
- تقييم فعالية المرهم مخبريًا وعلى نماذج حيوانية لضمان جدواه وسلامته.
- تعبئة المنتج ضمن معايير الجودة والسلامة لتسهيل استخدامه ميدانيًا.

✓ **من الذي سينجز ذلك؟**

يتولى إنجاز هذا المشروع فريق بحثي متعدد التخصصات من جامعة الشهيد حمه لخضر – الوادي، ويتكوّن من:

- باحثين في الكيمياء الحيوية والصيدلانية.
- مختصين في التحاليل البيولوجية.
- طلبة ماستر ضمن مشاريع تخرج تطبيقية.

كل فرد في الفريق مسؤول عن محور علمي دقيق من مراحل الابتكار، ضمن تنظيم جماعي منسق.

✓ **أين سيتم إنجازه؟**

سيُنفّذ المشروع في مخابر كلية العلوم الطبيعية وعلوم الحياة بجامعة الشهيد حمه لخضر – الوادي، مع إمكانية توسيع نطاق الإنجاز لاحقًا إلى:

- وحدات إنتاجية محلية.
- شركات ناشئة تنشط في الصناعات الدوائية النباتية.
- شراكات بحث وتطوير مع هيئات صحية وصيدلانية.

1. القيم المقترحة:

يوفر هذا المشروع مجموعة من القيم الملموسة التي تجعل المرهم العلاجي المقترح خيارًا متفوقًا على ما هو متاح حاليًا في السوق، ويمكن تفصيلها كالتالي:

- **الحداثة:**
المنتج جديد كليًا ولم يُسبق طرح مثيل له محليًا أو إقليميًا، حيث لم يتم سابقًا استغلال أوراق *Cucurbita maxima* علميًا في مستحضر موضعي لعلاج الجروح والالتهابات، ما يجعله يلبي حاجة لم تُلبّ من قبل في السوق.
- **الأداء:**
أثبتت فعالية المرهم عبر اختبارات مخبرية وحيوانية، أظهرت نتائجه تفوقًا في تسريع التئام الجروح وتقليل الالتهاب مقارنة بالمراهم التقليدية، ما يجعله يتجاوز توقعات المستخدمين من حيث الأداء العلاجي.
- **التكيّف:**
تم تصميم المرهم بتركيبة **قابلة** للتعديل والتكييف وفق الفئة المستهدفة، حيث يمكن تطويره بأشكال صيدلانية مختلفة (مرهم، كريم، جل)، مما يُراعي احتياجات المستخدمين المختلفين، خصوصًا أصحاب البشرة الحساسة أو الحالات الجلدية المزمنة.
- **إنجاز المهمة:**
يساهم المرهم بفعالية في إنجاز مهمة علاج الجروح السطحية وتقليل التورم والالتهاب، ويعطي المستخدم نتائج ملموسة في فترة قصيرة، ما يعزز من الاعتماد عليه ضمن العناية الجلدية اليومية.

- **التصميم:**
تم تصميم المرهم بطريقة تتناسب مع ظروف الاستخدام العملي وتفضيلات المستخدمين، فهو سهل التطبيق، سريع الامتصاص، ومُعبأ في عبوة آمنة ومحكمة تُحافظ على جودة المنتج وخصائصه العلاجية.
- **خفض التكاليف:**
يوفّر المرهم بديلاً اقتصاديًا وآمنًا للعلاجات الكيميائية المستوردة، حيث يمكن تصنيعه محليًا بتكاليف منخفضة دون التأثير على الجودة أو السلامة، مما يجعله منتجًا فعّالاً ومتاحًا لشريحة واسعة من المستخدمين.
- **الحد من المخاطر:**
التركيبة خالية تمامًا من المواد الكيميائية أو الصناعية التي قد تُسبب آثارًا جانبية، ما يقلل من المخاطر الصحية خاصة لدى الفئات الحساسة (الأطفال، كبار السن، المرضى)، ويضمن استخدامًا مطمئنًا على المدى الطويل.
- **سهولة الوصول:**
المنتج يُحضّر من مواد أولية محلية (أوراق *Cucurbita maxima* من وادي سوف)، ما يسمح بتوفيره محليًا بكميات كافية دون الاعتماد على الاستيراد، وبالتالي سهولة توزيعه ووصوله للمستخدمين في المناطق الداخلية والنائية.
- **الملاءمة وسهولة الاستخدام:**
المرهم سهل الاستخدام، واضح الجرعة، ومناسب للتطبيق المتكرر دون حاجة لإشراف طبي دائم، مما يعزّز من اعتماده في الروتين اليومي للعناية بالجروح والبشرة.

2. فريق العمل (المخترعين):

يتكوّن فريق العمل من ثلاث طالبات باحثات في السنة الثانية ماستر بيو كيمياء تطبيقية بجامعة الشهيد حمة لخضر – الوادي، جمعتهن خلفية علمية مشتركة، وتجارب تدريبية مكمّلة، مكنتهن من تنفيذ المشروع بكفاءة وتناسق:

- **إسراء كمرشو**
أشرفت على الجانب الفيتوكيميائي، من استخلاص وتحليل المركبات النشطة، مع الاستفادة من تدريب مخبري في تقنيات الفصل والتحليل الطيفي(LC-MS/MS و UV)
- **آية حمادي**
تولّت تصميم وتحضير الصيغة الدوائية للمرهم، اعتمادًا على تكوينات في مجال الصيدلة الطبيعية وخصائص المواد المساعدة.
- **إيناس دركي**
تكفّلت بتجريب المستخلص بيولوجيًا من خلال اختبارات *in vitro* وتجارب *in vivo* على نموذج حيواني، مدعومة بخبرة في التحاليل البيولوجية ومراقبة المؤشرات الحيوية.

تم توزيع المهام بناءً على التخصصات والقدرات العملية لكل عضوة، مع تنسيق دوري داخل المخبر واجتماعات منتظمة لتبادل النتائج واتخاذ القرارات البحثية، مما مكّن الفريق من الحفاظ على جودة الإنجاز ضمن الآجال المحددة.

3. أهداف المشروع:

تهدف براءة الاختراع هذه إلى تقديم حل علاجي طبيعي وفعّال للجروح والالتهابات الجلدية، من خلال تطوير مستحضر موضعي مبتكر يعتمد على مستخلص أوراق *Cucurbita maxima*، وتتمثل الأهداف الابتكارية فيما يلي:

- **تقديم تركيبة جديدة** غير مستغلة سابقًا في هذا السياق، توظف موردًا نباتيًا محليًا غنيا بمركبات نشطة ذات فعالية مثبتة.
- **دمج تقنيات تحليل حديثة**)مثلLC-MS/MS ، ودراسات (*in silico*لتحديد وتفسير آلية عمل المركبات الفعالة، مما يعزز القيمة العلمية للمنتج.
- **تحقيق توازن بين السلامة والفعالية** من خلال استخدام مكونات طبيعية خالية من الإضافات الكيميائية، ما يجعله مناسبًا للفئات الحساسة.
- **ابتكار مرهم سهل الاستخدام** وثابت الصيغة، يمكّن من التطبيق المباشر على الجروح، ويُظهر فعالية في تسريع التئام الأنسجة وتقليل الالتهاب على المستوى الخلوي.
- **دعم الطب التكميلي المحلي** من خلال استغلال نباتات مهملة طبيًا رغم وفرتها، بما يُساهم في بناء منتجات علاجية طبيعية جزائرية المنشأ.

4. جدول زمني لإنجاز براءة الاختراع:

- ✓ كيفية تقسيم الهدف النهائي لبراءة الاختراع إلى مهام فردية.
- ✓ تحديد الوقت اللازم لكل مهمة.
- ✓ تحديد النتائج الرئيسية لكل مهمة.

الشهر أو الأسبوع

		الأعمال	1	2	3	4	5	6	7
1		البحث في قواعد البيانات الخاصة ببراءات الاختراع وجمع المعلومات	✓						
2		الشروع في الاختبارات المخبرية لإعداد النموذج الأولي		✓	✓	✓			
3		تجريب النموذج الأولي			✓	✓	✓		
ب		تجربة النموذج الأولي خارج المخابر				✓	✓	✓	
ن		تسجيل براءة الاختراع من اجل الحصول على رقم الإيداع والحماية الصناعية						✓	
ج		متابعة عملية الحصول على براءة الاختراع وتصحيح ملاحظات الممتحنين من **inapi**							✓

المحور الثاني

الجوانب الابتكارية

المحور الثاني

الجوانب الابتكارية

1. طبيعة الابتكارات:

يجمع هذا المشروع بين عدة أنماط من الابتكار، مما يجعله مساهمة فريدة ضمن مجال المنتجات الطبية الطبيعية. ويمكن تصنيف الابتكار كما يلي:

- **ابتكار سوقي:(Market Innovation)**
يقدّم المنتج حلاً جديدًا للسوق الجزائرية اعتمادًا على مستخلص نبات *Cucurbita maxima*، الذي لم يُستخدم سابقًا في مجال العناية بالجروح. ويستجيب للطلب المتزايد على البدائل الطبيعية والآمنة.
- **ابتكار تكنولوجي:(Technological Innovation)**
يعتمد التطوير على تقنيات حديثة مثل LC-MS/MS لاستخلاص وتحليل المركبات النشطة، إلى جانب أساليب متقدمة في تحضير الصيغ الصيدلانية.
- **ابتكار متزايد:(Incremental Innovation)**
لا يُحدث المنتج ثورة في مجال علاج الجروح، لكنه يُحسّن العلاجات الحالية من خلال تقديم بديل طبيعي وآمن، قائم على البحث العلمي والتطور التكنولوجي.

تصنيف المشروع:

يُصنَّف المشروع ضمن ابتكار مركّب يجمع بين الابتكار السوقي، التكنولوجي، والمتزايد، ومدعوم بملف علمي وبيولوجي قوي يُعزّز من موثوقيته وقابليته للتطبيق.

2. مجالات الابتكارات:

يمتد الابتكار في هذا المشروع عبر عدة مجالات، ما يُضفي عليه قيمة استراتيجية ويعزّز من إمكانياته السوقية. وتشمل هذه المجالات ما يلي:

- ✅ **ميزات محسّنة:(Enhanced Features)**
يتميز المرهم بتركيبة طبيعية محسّنة خالية تمامًا من المواد الكيميائية الاصطناعية، وغنيّة بمركبات نباتية فعالة مستخلصة من *Cucurbita maxima*، مما يوفر حلاً آمنًا وفعالاً لتسريع التئام الجروح وتقليل الالتهابات.
- ✅ **عروض جديدة:(New Offerings)**
يُقدّم المشروع منتجًا مبتكرًا لأول مرة في السوق الجزائرية، إذ لم يُستخدم مستخلص هذا النبات سابقًا في التطبيقات الجلدية، مما يجعله خيارًا علاجيًا جديدًا ومميزًا.
- ✅ **عملاء جدد:(New Customers)**
يستهدف المشروع شرائح جديدة من المستهلكين، بما في ذلك الأشخاص الذين يفضّلون الطب

الطبيعي أو التكميلي، والأفراد ذوي الحساسية تجاه المنتجات الكيميائية، بالإضافة إلى الأطفال وأصحاب البشرة الحساسة.

- ✅**نماذج عمل جديدة:(New Business Models)**

يتبع المشروع نموذجًا يقوم على خلق القيمة من خلال تحويل الموارد النباتية المحلية إلى منتجات دوائية، مما يعزّز سلاسل القيمة المحلية ويُقلل من الاعتماد على الاستيراد.

- ⚙️**عمليات جديدة:(New Processes)**

يشمل مسار التطوير استخدام تقنيات حديثة في الاستخلاص، التحليل، والتحضير الصيدلاني، ما يُحسّن من كفاءة الإنتاج ويُقلل الفاقد ويرفع من الربحية.

- ❗**تجارب جديدة:(New Experiences)**

بفضل تصميمه العملي وسهولة استخدامه، يمنح هذا المرهم المستخدم تجربة علاجية طبيعية ومريحة في المنزل، مما يُعزز من الاستمرارية والالتزام بالعلاج.

المحور الثالث

وصف براءة الاختراع

المحور الثالث

وصف براءة الاختراع

- عنوان براءة الاختراع

مستخلص أوراق *Cucurbita maxima* لمكافحة الالتهابات وتسريع الشفاء (مرهم لعلاج الجروح)

- ملخص براءة الاختراع

تقوم فكرة هذا الابتكار على تصميم وتطوير مرهم طبيعي موضعي مستخلص من أوراق نبات *Cucurbita maxima*، يهدف إلى علاج الجروح والالتهابات الجلدية بفعالية وأمان. ويعتمد هذا المستحضر على خصائص النبات المعروفة بثرائها في المركبات النشطة بيولوجيًا، خاصة الفلافونويدات، والتي أظهرت قدرة مثبتة على تسريع التئام الأنسجة، تقليل الإجهاد التأكسدي، وتهدئة الالتهاب الجلدي.

شمل المسار العلمي لتطوير المرهم سلسلة من المراحل المتكاملة، بدءًا من عملية استخلاص مدروسة باستخدام مذيب مائي، ثم تركيز وتجفيف المستخلص، متبوعًا بتحليلات فيتوكيميائية متقدمة باستخدام تقنية LC-MS/MS للكشف عن المركبات الفعالة. كما تم دعم الفعالية المبدئية عبر اختبارات بيولوجية (in vitro) لتقييم خصائصه المضادة للأكسدة والالتهاب، بالإضافة إلى محاكاة جزيئية حاسوبية (in silico) لتوضيح آليات عمل المركبات النشطة في التفاعل مع أهداف بيولوجية مرتبطة بآلية التئام الجروح.

كما تم التحقق من كفاءة المستخلص في المرحلة ما قبل السريرية من خلال اختبارات على نماذج حيوانية (فئران Wistar)، حيث لوحظ تحسن ملحوظ في سرعة التئام الأنسجة، خفض العلامات الالتهابية، وتحسن بنية الجلد المتجدد، ما يعكس فعاليته الحيوية ويدعم إمكانياته التطبيقية.

وفي ختام مراحل البحث والتطوير، تم تحويل المستخلص النباتي إلى مرهم صيدلاني موضعي بصيغة متوازنة ومستقرة، تُراعي ثبات المكونات النشطة وسهولة الاستخدام. تجمع هذه الصيغة بين الفعالية العلاجية والتطبيق العملي، ما يؤهل المنتج ليكون خيارًا طبيعيًا واعدًا في مجال التئام الجروح ومكافحة الالتهابات الجلدية. وبفضل نتائجه الحيوية المشجعة، يُعد هذا الابتكار خطوة متقدمة نحو إدراجه ضمن ممارسات الطب التكميلي، مع آفاق مستقبلية للتطوير الصناعي والتقييم السريري.

- الميدان التقني الذي ينتمي إليه الاختراع

ينتمي هذا الابتكار إلى المجال التقني للصناعات الدوائية والمستحضرات العلاجية المستخلصة من مصادر طبيعية، ويُصنَّف تحديدًا ضمن المستحضرات الموضعية النباتية المصممة لتسريع التئام الجروح، ودعم عملية تجدد الأنسجة، والتقليل من الالتهابات الجلدية، مما يجعله مناسبًا للعناية بمختلف أنواع الجروح والتهيجات الجلدية.

- الحالة التقنية السابقة

لقد تم تسجيل العديد من براءات الاختراع التي تتناول تطوير مراهم طبيعية لعلاج الجروح اعتمادًا على مستخلصات نباتية متنوعة، نذكر منها ما يلي:

- **براءة الاختراع الأمريكية US10105354B2**

تَعرض هذه البراءة تركيبة موضعية تعتمد على مستخلصي *Aloe vera* و*Centella asiatica*، وتُستخدم في تعزيز تجديد الأنسجة الجلدية والحد من الالتهاب.

- **براءة الاختراع الهندية IN201811031581**

تركّز على تركيبة عشبية مستخلصة من نباتات طبية هندية تقليدية لعلاج الجروح المزمنة، إلا أنها تفتقر إلى توضيح آلية التأثير أو دعم فيتوكيميائي يبرر فعالية المكونات.

- **براءة الاختراع الصينية CN105383741A**

تقدم مرهمًا يحتوي على مزيج من مستخلصات نباتية متعددة، دون إجراء تحليل فردي لتأثير كل مركب على حدة أو تقييم شامل لسلامة الاستخدام طويل الأمد.

نقد التقنيات السابقة:

- **ضعف التنويع في المصادر النباتية:**

تركّز العديد من الابتكارات الحديثة على استخدام نباتات شائعة ومعروفة منذ القدم، مثل *Aloe vera* و*Centella asiatica*، ما أدى إلى تكرار في التراكيب العلاجية وتباطؤ في وتيرة التطوير ضمن هذا المجال. في المقابل، لا تزال بعض النباتات المحلية غير المستغلة، كـ*Cucurbita maxima* ، مهمّشة رغم غناها بمركبات فعالة قد تساهم بفعالية في تجديد الأنسجة والتقليل من الالتهاب.

- **قصور في التحليل الكيميائي والدعم التقني:**

تفتقر معظم التركيبات السابقة إلى دعم تحليلي دقيق للمركبات النشطة، حيث نادرًا ما يتم توظيف تقنيات حديثة مثل UPLC-MS/MS أو استخدام أدوات المحاكاة الجزيئية المتقدمة كالـ Docking ودراسات ADMET، مما يؤدي إلى ضعف في تفسير آلية التأثير الحيوي ويؤثر سلبًا على موثوقية وكفاءة المنتج النهائي.

- تركّز العديد من المستحضرات الموضعية المتوفرة حاليًا على التأثيرات السطحية مثل الترطيب وتحسين ملمس البشرة، مع إهمال الجوانب العلاجية الجوهرية كتعزيز تجدد الأنسجة والتقليل الفعّال للالتهاب على المستوى الخلوي. هذا القصور يقلل من فاعلية تلك المنتجات في التعامل مع الحالات الجلدية المعقدة مثل الجروح أو الالتهابات الموضعية. كما أن العديد منها يفتقر إلى تحليل كيميائي متقدم للمكونات النشطة، و لا يعتمد على تقنيات حديثة مثل PLC-MS/MS لتوصيف المركبات بدقة أو دراسة النمذجة الجزيئية لفهم ألية العمل، كما تُسجَّل قلة في إجراء تجارب حيوية موثقة على نماذج حية تُثبت جدواها العلاجية بشكل علمي دقيق.

ما يميز اختراعنا:

- توظيف مكوّن نباتي غير مستهلك في السياق العلاجي الموضعي، يرتكز المرهم على مستخلص أوراق *Cucurbita maxima*، وهو نبات ذو استخدامات غذائية وطبية مثبتة من حيث الأمان، غير أنه لم يُوظّف سابقًا بشكل علمي في التركيبات الجلدية الموضعية الموجّهة خصيصًا لتعزيز التئام الجروح وتحفيز تجدد الأنسجة.
- تم تعزيز موثوقية المستخلص من خلال سلسلة من الاختبارات العلمية المتكاملة، شملت تحاليل فيتوكيميائية متقدمة باستخدام تقنية LC-MSلتحديد المركبات النشطة، إلى جانب تجارب مخبرية (in vitro)أثبتت خصائصه المضادة للأكسدة والالتهاب، فضلاً عن استخدام نمذجة حاسوبية (in silico) لدراسة تفاعله مع أهداف بيولوجية مرتبطة بآليات التئام الجلد.
- خضع المرهم لاختبار فعلي على نماذج حيوانية) فئران(Wistar ، حيث بيّنت النتائج قدرته الملحوظة على تسريع عملية التئام الجروح، والحدّ من الالتهاب على المستوى الخلوي، إلى جانب تحسين بنية النسيج المرمم من حيث التنظيم والتكامل. تعكس هذه النتائج فعاليته العلاجية وتؤكد سلامته الفسيولوجية، مما يعزز من جاهزيته لمراحل بحثية وسريرية متقدمة.
- صُمم المرهم بصيغة موضعية مستقرة ومتجانسة تجمع بين سهولة الاستخدام وثبات المركبات الفعالة، ما يضمن الحفاظ على فعالية المستخلص النباتي وقدرته على النفاذ الموضعي. هذه التركيبة تجعل المنتج مناسبًا للتطبيق العملي وتمهد الطريق لمراحل التطوير الصناعي والتقييمات السريرية.

- الغرض (الهدف) من الاختراع

يهدف هذا الاختراع إلى تطوير مرهم موضعي مبتكر يعتمد على مستخلص نباتي طبيعي من أوراق Cucurbita maxima، موجه خصيصًا لتعزيز تسريع شفاء الجروح وتقليل الالتهابات الموضعية بكفاءة عالية. جاء هذا الابتكار استجابة للحاجة المتزايدة إلى علاجات طبيعية وآمنة تدعم تجديد الأنسجة وتحسن جودة التئام الجروح، بعيدًا عن الآثار الجانبية المحتملة للمستحضرات الكيميائية التقليدية.
تم صياغة التركيبة بصيغة صيدلانية مستقرة ومتوازنة تجمع بين سهولة الاستخدام وثبات المكونات الفعالة، مع ضمان نفاذيتها الموضعية، ما يجعلها مناسبة للتطبيق العملي ومهيأة للانتقال إلى مراحل التطوير الصناعي والتقييمات السريرية.

يقدم المرهم حلاً طبيًا طبيعيًا يساهم في تسريع العملية العلاجية عبر تقليل الإجهاد التأكسدي المحلي وتعزيز الاستجابة المضادة للالتهاب، مما يجعله خيارًا مثاليًا للاستخدام المنتظم ضمن روتين العناية بالبشرة والجروح.

على المدى القريب، يهدف الاختراع إلى تطوير تركيبة فعالة تحتوي على تراكيز محسوبة بدقة من المركبات النباتية النشطة، مع إجراء اختبارات مخبرية وحيوية أولية لتقييم الفعالية والسلامة. أما على المدى البعيد، فيسعى المنتج إلى التواجد في الأسواق المحلية والعالمية كمرهم طبي طبيعي داعم لالتئام الجروح، متوافق مع توجهات الطب التكاملي والصحة الوقائية، ويشجع اعتماد علاجات طبيعية أكثر أمانًا واستدامة.

- تقديم جوهر الاختراع

يعتمد هذا الاختراع على تطوير مرهم موضعي طبيعي مبتكر يهدف إلى معالجة الالتهابات وتسريع شفاء الجروح، باستخدام مستخلص بذور Cucurbita maximaالغني بمركبات بيولوجية فعالة مثل الفلافونويدات، الكاتيشين، الكيرسيتين، الروتين، وحمض الفيروليك. تتميز هذه المركبات بخصائصها المضادة للالتهاب، ومكافحة الأكسدة، مما يساهم في تقليل التورم، تعزيز تجديد الخلايا، وتحسين جودة الأنسجة المتكونة أثناء عملية الشفاء، ليقدم علاجًا فعالاً وآمنًا لمشاكل الجلد المختلفة.

هذا المكمل موجه إلى:

- لأفراد الذين يعانون من جروح سطحية أو التهابات جلدية خفيفة إلى متوسطة.
- الأشخاص الذين يفضلون حلولاً طبيعية وآمنة، بعيدًا عن المستحضرات الكيميائية ذات الآثار الجانبية المحتملة.
- الأفراد المهتمين باستخدام منتجات طبيعية وآمنة تعتمد على مستخلص نباتي (أوراق *Cucurbita maxima*) بخصائص مضادة للالتهاب ومسرّعة للشفاء.
- الفئات ذات البشرة الحساسة أو من لديهم تاريخ من التحسس تجاه المراهم الصناعية، ويبحثون عن تركيبة نباتية لطيفة وفعالة.

ويهدف إلى:

- تطوير تركيبة علاجية طبيعية وآمنة تعتمد على مستخلص أوراق *Cucurbita maxima* ذات الخصائص المضادة للالتهاب والمسرّعة للشفاء.
- تسريع التئام الجروح وتحفيز تجدد الأنسجة الجلدية بشكل فعّال.
- تهدئة البشرة الحساسة وتقليل التهيجات الجلدية دون التسبب في آثار جانبية.
- توفير بديل موضعي طبيعي وآمن للمراهم الكيميائية التقليدية ذات التأثيرات الجانبية المحتملة.

- دعم العناية بالبشرة من خلال آلية لطيفة تعزز الإصلاح الخلوي والراحة الجلدية.
- المساهمة في الحد من الاستخدام المفرط للمستحضرات الكيميائية، خصوصًا لدى الفئات الحساسة أو المصابة بالحساسية.

ويتميز بأنه:

- مستخلص نباتي طبيعي 100% محضر من أوراق *Cucurbita maxima* المعروفة بخصائصها العلاجية.
- لا يحتوي على مواد كيميائية أو إضافات صناعية قد تسبب تهيجًا أو تفاعلات جلدية.
- مدعوم بنتائج تحاليل فيتوكيميائية ودراسات in silico تثبت فعاليته البيولوجية.
- لطيف على البشرة الحساسة وآمن للاستخدام المتكرر ولفترات طويلة دون آثار جانبية.
- قابل للتصنيع في أشكال صيدلانية موضعية متنوعة مثل الكريم أو الجل أو المرهم.
- يُعد خيارًا علاجيًا طبيعيًا للأشخاص الذين يفضلون الابتعاد عن المستحضرات الكيميائية.
- يساعد على تسريع التئام الجروح وتجديد الأنسجة بفضل مكوناته النشطة والمضادة للأكسدة.

❖ طريقة إنجاز الاختراع:

1 . تم الحصول على المستخلص النباتي من أوراق *Cucurbita maxima*، وذلك بعد جمعها من مصادر موثوقة وتجفيفها في ظروف مناسبة للحفاظ على سلامة المركبات الحيوية. خضعت الأوراق لعملية استخلاص مائي عن طريق النقع البارد لمدة 48 ساعة، تلتها مرحلة ترشيح دقيقة، ثم تم تركيز المستخلص بلطف باستخدام مبخر دوار لضمان الحفاظ على فعالية المواد النشطة دون التأثير على تركيبها الحساس.

2 . تم تقييم المستخلص في البداية باستخدام اختبارات مخبرية لتحديد النشاط الحيوي، من بينها اختبار DPPH لتقدير القدرة المضادة للأكسدة، إلى جانب اختبارات أخرى لتقييم الفعالية ضد الالتهابات. وبناءً على هذه النتائج، تم تطوير تركيبة مرهمية طبيعية باستعمال قاعدة استحلاب مناسبة، مدعّمة بمواد فعالة، مرطّبة، حافظة، ومكوّنات تضمن الاستقرار وسهولة الامتصاص.

3 . أضيف المستخلص النباتي في المرحلة النهائية عند درجة حرارة مدروسة للحفاظ على خصائصه الحيوية، ثم صُبّ المرهم الناتج في عبوات محكمة الغلق لحمايته وضمان سهولة الاستخدام، ما أسفر عن منتج نهائي موجّه خصيصًا لتهدئة الالتهابات وتعزيز التئام الجروح الطفيفة.

مراحل إنجاز الاختراع:

المرحلة الأولى: إعداد المستخلص النباتي

1 . جمع أوراق نبات *Cucurbita maxima* من مصادر موثوقة ومعتمدة.

2 . تجفيف الأوراق تحت ظروف مناسبة ثم طحنها للحصول على مسحوق ناعم.

3 . نقع المسحوق في الماء لاستخلاص المركبات الحيوية الفعالة.

4 . تنقية المستخلص عن طريق الترشيح، ثم تركيزه باستخدام مبخّر دوّار.

المرحلة الثانية: التقييم البيولوجي والمخبري

1 . إجراء تحليل كيميائي نباتي لتحديد المركبات النشطة باستخدام تقنية الفصل الكروماتوغرافي-UPLC MS/MS.

2 . اختبار الفعالية كمضاد للأكسدة ومضاد للالتهاب باستخدام اختبارات مثل DPPH وBSA.

3 . تقييم السلامة والفعالية البيولوجية على نموذج حيواني (فئران من نوع ويستر).

المرحلة الثالثة: تصنيع الشكل الدوائي

1 . تحديد التركيز المناسب للمواد الفعالة استنادًا إلى نتائج التحاليل السابقة.

2 . تصميم تركيبة المرهم بإضافة المكونات المناسبة للحصول على قوام متجانس وسهل الاستخدام.

3 . تعبئة المرهم في عبوات محكمة الإغلاق، ضمن شروط تضمن ثباتية المنتج وجودته أثناء التخزين والاستخدام.

- شرح الأشكال والرسومات

الشكل (1): خطوات إنجاز المرهم

1 - تحضير المواد الخام

2 - تحضير الزيوت والمذيبات والمستحلبات

3 - تحضير المرهم النهائي في عبوات زجاجية معقمة ومحكمة الغلق للحفاظ على جودته وفعاليته.

- طريقة تطبيق الاختراع

- يُطبَّق المستحضر الموضعي مباشرة على الجلد في موضع الإصابة أو المنطقة المستهدفة، ويفضل توزيعه بلطف دون فرك شديد.

- **الجرعة وطريقة الاستعمال للبالغين:**
  - يُدهن المرهم بلُطف مرتين في اليوم، صباحًا ومساءً، على بشرة نظيفة وجافة.
  - يُنصح بتدليك خفيف حتى تمام الامتصاص.
  - يجب غسل اليدين قبل وبعد الاستخدام، مع تجنب ملامسة العينين أو الأغشية المخاطية.
  - يمكن تعديل عدد مرات الاستخدام بناءً على شدة الحالة وتوصية الطبيب المختص، مع تجنّب الاستخدام المفرط أو المديد دون إشراف طبي.

- **مدة الاستخدام الموصى بها:**
  - للاستعمال الوقائي أو الداعم: من 4 إلى 8 أسابيع حسب الحالة.

يُفضّل التوقف عن الاستخدام لمدة أسبوعين بعد كل دورة علاجية، ويمكن استئناف العلاج عند الحاجة

المحور الرابع

المطالب

المحور الرابع
المطالب

المطلب الرئيس

مستخلص أوراق *Cucurbita maxima* لمكافحة الالتهابات وتسريع الشفاء (مرهم لعلاج الجروح):
يقدّم اختراعنا ميزة فريدة تتمثل في استخدام مستخلص أوراق *Cucurbita maxima*، وهي جزء نباتي قليل الاستغلال رغم ما أُثبت من خصائصه المضادة للالتهاب والمسرّعة لشفاء الجروح. بخلاف المنتجات المتوفرة التي تعتمد على أعشاب تقليدية شائعة، تأتي هذه التركيبة بمكوّن طبيعي جديد، فعّال، منخفض التكلفة، ومثبت علميًا.
يعتمد التحضير على استخلاص مائي بسيط ومنخفض التكلفة، مدعوم بوسائل تقييم متقدمة (*in vitro*, *in vivo*, *in silico*) لضمان الفعالية والسلامة. كما أنه منتج طبيعي 100%، خالٍ من المواد الكيميائية، ومناسب لذوي البشرة الحساسة، مما يجعله بديلاً مبتكرًا وآمنًا للعلاجات الموضعية التقليدية.

المطالب المستنبطة من المطلب الرئيسي والتي تميز اختراعنا

- وفقًا للمطلب الأول، يتميز هذا الاختراع بكونه منتجًا طبيعيًا خاليًا من المواد الكيميائية والمركبات الاصطناعية، ويعتمد على مستخلص أوراق *Cucurbita maxima* الغني بالمركبات ذات النشاط المضاد للميكروبات والمضاد للالتهاب، مثل الفلافونويدات والمركبات الفينولية.
- وفقًا للمطلب الأول، يتميز هذا الاختراع بسهولة تصنيعه باستخدام طرق استخلاص مائية بسيطة، دون الحاجة إلى تجهيزات معقدة أو مواد باهظة، مما يجعله خيارًا عمليًا وفعّالاً من حيث التكلفة.
- وفقًا للمطلب الأول، يتميز هذا الاختراع بفعالية مثبتة عبر دراسات مخبرية وحيوانية (*in vitro* و *in vivo*)، حيث أظهر قدرة على تسريع تجدد الأنسجة، تقليل الالتهاب، وتحسين التئام الجروح، مع تقليل خطر العدوى الجلدية.
- وفقًا للمطلب الأول، يتميز هذا الاختراع بإمكانية تحضيره في شكل دوائي موضعي مناسب (مثل مرهم أو كريم)، يسهل تطبيقه على الجلد ويمتاز بسرعة الامتصاص، مما يعزز فعاليته العلاجية وسهولة استخدامه.
- وفقًا للمطلب الأول، يتميز هذا الاختراع بإمكانية استخدامه من قبل فئات مختلفة، مثل ذوي البشرة الحساسة، كبار السن، ومرضى الأمراض المزمنة، ويُعد بديلاً طبيعيًا وآمنًا للمستحضرات الموضعية التقليدية التي قد تسبب التهيج أو آثارًا جانبية.

قائمة الملاحق

الشكل (1)

1

2

3

دليل مشروع

للحصول على شهادة براءة اختراع
في إطار القرار الوزاري 1275

ديسمبر
2022

CNCSIIU — MHESR — ALGERIA

بطاقة معلومات

حول فريق الاشراف وفريق العمل

3- فريـــق الاشـــراف:

فريق الاشراف	
المشرف الرئيسي: بن عمر محمد العربي	**التخصص:** كيمياء
المشرف المساعد: حمادي مروة	**التخصص:** بيو كيمياء تطبيقية

4- فريـــق العمـــل:

فريق المشروع	التخصص	الكلية
الطالب 01: كمرشو اسراء	بيو كيمياء تطبيقية	علوم الطبيعة والحياة
الطالب 02: حمادي آية	بيو كيمياء تطبيقية	علوم الطبيعة والحياة
الطالب 03: دركي إيناس	بيو كيمياء تطبيقية	علوم الطبيعة والحياة

فهرس المحتويات

فهرس المحتويات

المحور الأول

تقديم المشروع

5. فكرة المشروع (الحل المقترح)

✓ **مجال النشاط**

يندرج المشروع ضمن الصناعات الصيدلانية والمنتجات الطبيعية، تحديدًا في مجال المكملات الغذائية النباتية الموجّهة لدعم التوازن الهرموني للغدة الدرقية، وذلك في إطار الطب الوقائي والتكميلي.

✓ **كيف بدأت الفكرة وكيف تطورت؟**

بدأت الفكرة من ملاحظة نقص المكملات الطبيعية المحلية التي تستهدف وظيفة الغدة الدرقية بطريقة آمنة وفعالة. ومن خلال مراجعة براءات الاختراع السابقة، تبيّن أن معظمها يركّز على أعشاب تقليدية دون دعم علمي دقيق.

من هنا تم اقتراح دراسة بذور *Cucurbita maxima* (اليقطين) نظرًا لكونها محلية، آمنة، وغنية بمركبات فيتوكيميائية فعالة. تطوّرت الفكرة من استخلاص بسيط إلى تطوير تركيبة دوائية قابلة للتسويق، مدعّمة بنتائج تحليلية وتجريبية.

✓ **ما الذي سوف تقوم به؟**

سيتم إنتاج مكمل غذائي طبيعي في شكل كبسولات، يحتوي على مستخلص مركز من بذور اليقطين، يساهم في دعم وظيفة الغدة الدرقية وتحقيق التوازن الهرموني بطريقة آمنة وفعالة.

✓ **كيف سيكون ذلك؟**

سيتم ذلك عبر عدة مراحل، تشمل:

- استخلاص المركبات الفعالة بطريقة علمية مدروسة.
- تحليل المركبات باستخدام تقنيات فيتوكيميائية متقدمة مثل (LC-MS)
- تقييم الفعالية عبر اختبارات *in vitro* و*in silico*
- تحضير تركيبة صيدلانية مناسبة (كبسولات).
- تعبئة المنتج في ظروف تحفظ الجودة والثبات.

✓ **من الذي سينجز ذلك؟**

سينجز المشروع فريق متعدد التخصصات من الباحثين في مجالات الصيدلة والبيوكيمياء التطبيقية، بدعم من جامعة الشهيد حمه لخضر – الوادي، إلى جانب مختصين في التحاليل الحيوية وتكنولوجيا الصناعات الصيدلانية.

✓ **أين سيتم إنجازه؟**

سينجز المشروع داخل مخابر جامعة الشهيد حمه لخضر – الوادي، مع إمكانية التوسّع لاحقًا إلى وحدات إنتاجية محلية بالتعاون مع مستثمرين أو شركات ناشئة متخصصة في الصناعات الصيدلانية النباتية.

6. القيم المقترحة :

يُقدّم مشروعنا مجموعة من القيم المضافة التي تُميّزه عن باقي المنتجات المتوفرة في السوق، وتشكل أساسًا قويًا لجذب الزبائن وبناء الثقة لديهم، ويمكن تلخيصها فيما يلي:

- **الحداثة :**منتج مبتكر من *Cucurbita maxima* لم يُستغل سابقًا في هذا السياق.
- **الأداء :**مدعوم بدراسات مخبرية وتجريبية تُثبت فعاليته في دعم وظيفة الغدة الدرقية.
- **التكييف :**إمكانية تصنيعه بأشكال دوائية مختلفة تناسب الفئات المستهدفة.
- **إنجاز المهمة :**يُسهم في تحسين الصحة الهرمونية وتقليل الأعراض المرتبطة باضطرابات الغدة.
- **التصميم :**سهل الاستخدام، بتركيبة واضحة وعبوة متوافقة مع معايير السلامة.
- **خفض التكاليف :**بديل طبيعي، اقتصادي وآمن للعلاج الكيميائي التقليدي.
- **الحد من المخاطر :**خالٍ من المواد الكيميائية، مناسب للاستعمال المتكرر والطويل الأمد.
- **سهولة الوصول :**يُنتج محليًا في ولاية وادي سوف مع سهولة التوزيع على المستوى الوطني.
- **الملاءمة وسهولة الاستخدام :**بسيط في الاستعمال، بجرعة واضحة وسهلة التتبع للمستهلك.

7. فريق العمل (المخترعين) :

يتكوّن فريق العمل القائم على هذا المشروع من مجموعة من الطالبات الباحثات في السنة الثانية ماستر "بيوكيمياء تطبيقية" بجامعة الشهيد حمه لخضر – الوادي، وهن:

- **إسراء كمرشو**
- **آية حمادي**
- **إيناس دركي**

يمتلك الفريق خلفية علمية متينة في الكيمياء الحيوية، التحاليل البيولوجية، وتكنولوجيا المكملات الغذائية، مع تقسيم منظم للمهام حسب الاختصاص:

- **التحليل الفيتوكيميائي :**تحت إشراف إسراء كمرشو.
- **تطوير الصيغة الدوائية :**تحت إشراف آية حمادي.
- **التحقق البيولوجي وتحليل النتائج :**تحت إشراف إيناس دركي.

يعتمد الفريق على اجتماعات دورية أسبوعية لتحديث تقدم العمل ومناقشة النتائج، إضافة إلى تواصل يومي عبر منصات إلكترونية مثلWhatsApp ، لتبادل الوثائق، النتائج، ومراجعة التقارير.
كما تم توثيق سير العمل وفق خطة زمنية مدروسة تتماشى مع متطلبات البحث العلمي وتسجيل البراءة.

هذا التنسيق المستمر ساهم في الحفاظ على جودة العمل وانسجام الرؤية العلمية للمشروع، مما أتاح بلوغ نتائج دقيقة وعملية في وقت قياسي.

8. أهداف المشروع:

تحتاج في هذا الجزء إلى تحديد الأهداف الابتكارية لبراءة الاختراع

- ابتكار مكمل غذائي طبيعي جديد يدعم وظائف الغدة الدرقية باستخدام مستخلص *Cucurbita maxima*.
- تطوير تركيبة خالية من المواد الكيميائية، بالاعتماد على أدوات تحليل حديثة ومتقدمة.
- اعتماد تقييم متعدد المستويات يشمل تحاليل فيتوكيميائية، بيولوجية، ومحاكاة حاسوبية.(*in silico*)
- توفير بديل آمن وفعّال للعلاجات الهرمونية والكيميائية التقليدية.
- إمكانية التوطين الصناعي للمكمل داخل الجزائر، وتشجيع الابتكار المحلي.
- الاستفادة من الخصائص الفيتوكيميائية للبذور بطريقة علمية موثّقة ومدروسة.
- مكانية تطوير منتج دوائي/غذائي قابل للتسويق محليًا ودوليًا، اعتمادًا على مادة أولية متوفرة بكثرة في الجزائر، وبأساليب تحضير منخفضة التكلفة وآمنة بيئيًا.
- دعم مسار الطب الوقائي والتكميلي من خلال طرح بديل طبيعي للمكملات الدوائية الكلاسيكية، وتعزيز الوعي حول أهمية التغذية النباتية الوظيفية في دعم الصحة الهرمونية.

9. جدول زمني لإنجاز براءة الاختراع:

- ✓ كيفية تقسيم الهدف النهائي لبراءة الاختراع إلى مهام فردية.
- ✓ تحديد الوقت اللازم لكل مهمة.
- ✓ تحديد النتائج الرئيسية لكل مهمة.

الشهر أو الأسبوع

	الأعمال		1	2	3	4	5	6	7
1		البحث في قواعد البيانات الخاصة ببراءات الاختراع وجمع المعلومات	✓						
2		الشروع في الاختبارات المخبرية لإعداد النموذج الأولي		✓	✓	✓			
3		تجريب النموذج الأولي			✓	✓	✓		
ب		تجربة النموذج الأولي خارج المخابر				✓	✓	✓	
ن		تسجيل براءة الاختراع من اجل الحصول على رقم الإيداع والحماية الصناعية					✓	✓	
ب		متابعة عملية الحصول على براءة الاختراع وتصحيح ملاحظات الممتحنين من **inapi**						✓	✓

المحور الثاني

الجوانب الابتكارية

1. طبيعة الابتكارات:

تتوزع طبيعة الابتكارات المعتمدة في هذه البراءة على عدة مستويات متكاملة، مما يمنح المشروع قيمة علمية وتطبيقية وسوقية عالية:

- ✅ ابتكار سوقي:(Market Innovation)
يتمثل في تقديم مكمل غذائي جديد لم يكن متوفرًا في السوق المحلي أو الإقليمي من قبل، حيث يستغل موردًا نباتيًا محليًا (بذور *Cucurbita maxima*) لم يُستخدم سابقًا بشكل علمي موثّق في هذا السياق.
- ✅ ابتكار تكنولوجي:(Technological Innovation)
يشمل استخدام تقنيات استخلاص وتحليل متقدمة مثل LC-MS/MS ، وتطبيق منهجيات تقييم متعددة (*in vitro* ، *in silico*، ونموذج حيواني) ، مما يعزز القيمة التكنولوجية والبحثية للمشروع.
- ✅ ابتكار متزايد:(Incremental Innovation)
يتمثل في تحسين طريقة تقديم مكمل غذائي طبيعي بشكل علمي ومدعوم بالتجارب، مع دمج الخبرات المحلية ونتائج البحث الأكاديمي لتطوير منتج أكثر فعالية وسهولة استخدام مقارنة بالمكملات التقليدية.
- ✅ عدم التأكد التكنولوجي:(Technological Uncertainty)
واجه المشروع تحديات مرتبطة بإثبات فعالية وسلامة المستخلص النباتي، مما استدعى إجراء تحاليل دقيقة وتجارب بيولوجية متعددة لدعم المزاعم العلمية حول تأثيره على وظائف الغدة الدرقية.

يُصنف هذا المشروع ضمن الابتكارات المركبة التي تجمع بين الابتكار السوقي، والتكنولوجي، والطابع المتزايد والتطبيقي، ما يمنحه فرصة قوية للتطوير الصناعي والاعتماد كحل وظيفي طبيعي مستدام يخدم التوجهات الحديثة في الطب التكاملي والصحة الوقائية.

2. مجالات الابتكارات:

يعكس المشروع المعني بهذه البراءة مجموعة متنوعة من مجالات الابتكار، مما يُعزّز من فرصه في إحداث تأثير فعلي في السوق والمجال الصحي على حد سواء، ويمكن تفصيلها كما يلي:

- ✅ **عمليات جديدة:(Process Innovation)**
تم **اعتماد** تقنيات استخلاص مائية بسيطة وفعالة، دون اللجوء إلى مذيبات كيميائية معقّدة، مما يرفع كفاءة الإنتاج ويقلل التكاليف، ويعزز الجدوى الاقتصادية للمشروع على مستوى التصنيع المحلي.

- ✅**تجارب جديدة:(Experience Innovation)**

يُقدَّم المكمل في سياق علاجي طبيعي بديل وآمن، مما يوفّر تجربة صحية مختلفة تمامًا للمستخدمين، خاصة أولئك الذين يعانون من القلق تجاه الأدوية الكيميائية أو لهم تجارب سلبية سابقة مع المكملات الهرمونية التقليدية.

- ✅**ميزات جديدة:(Feature Innovation)**

المكمل يتميّز بتركيبة غنية بمركبات طبيعية فعالة (مثل الفلافونويدات ومضادات الأكسدة) ، **وهو** محسَّن من حيث الأمان، وسرعة الامتصاص، وفعاليته المثبتة مخبريًا في دعم الغدة الدرقية، مقارنة بالمنتجات المتوفرة.

- ✅**عملاء جدد:(Customer Innovation)**

يستهدف هذا الابتكار فئات لم تُخدم سابقًا بمكملات طبيعية فعالة للغدة الدرقية، مثل:

  - النساء في فترات اضطراب الهرمونات (الحمل، الرضاعة، سن اليأس)
  - أصحاب الحساسية تجاه التركيبات الكيميائية
  - المهتمون بالطب الطبيعي والوقائي

- ✅**عروض جديدة:(Offer Innovation)**

يمثل هذا المنتج طرحًا مبتكرًا في السوق الجزائري والعربي، حيث يُدخل لأول مرة مكملاً نباتيًا قائمًا على *Cucurbita maxima* بوظيفة هرمونية واضحة ومدعومة علميًا، ما يُعد إضافة نوعية في عروض المنتجات العلاجية والوقائية.

- ✅**نماذج جديدة:(Business Model Innovation)**

يعتمد المشروع على نموذج عمل متكامل يربط بين البحث الأكاديمي، والإنتاج المحلي، والتسويق الموجه، مع التركيز على الاستدامة البيئية والاقتصادية، مما يسمح بتوليد القيمة من موارد محلية بأدوات منخفضة التكلفة عالية الأثر.

المحور الثالث

وصف براءة الاختراع

المحور الثالث

وصف براءة الاختراع

- عنوان براءة الاختراع

مكمل غذائي طبيعي مستخلص من بذور *Cucurbita maxima* لدعم التوازن الهرموني للغدة الدرقية

- ملخص براءة الاختراع

تتمثل براءة الاختراع هذه في تطوير مكمل غذائي طبيعي يعتمد على مستخلص بذور Cucurbita maxima، موجه لدعم التوازن الهرموني للغدة الدرقية وتحسين وظائفها بطريقة آمنة وغير دوائية. تم اختيار بذور النبات نظراً لغناها بالمركبات النشطة بيولوجيًا، مثل الفلافونويدات والمركبات الفينولية، والتي أظهرت فعالية واعدة في تنظيم الإجهاد التأكسدي والاستجابة الالتهابية المصاحبة لاختلال وظائف الغدة الدرقية.

شملت مراحل تطوير هذا المنتج استخلاص المركبات الفعالة بطريقة كحوليّة مائية، تلاها تركيز المستخلص وتجفيفه وتحليله باستخدام تقنيات متقدمة مثل UPLC-MS/MS، إلى جانب تقييم النشاط الحيوي من خلال اختبارات in vitro و in silico (Docking, ADMET).

كما تم تأكيد فعالية المكمل من خلال تجارب in vivo على نموذج حيواني (فئران Wistar)، حيث تم قياس تأثيره على المؤشرات الهرمونية، المناعية، الدموية، والأكسدة المرتبطة بوظيفة الغدة الدرقية.

تم في النهاية تطوير هذا المستخلص إلى شكل صيدلاني نهائي (كبسولات أو مسحوق)، يسهل استخدامه كمكمل غذائي آمن وفعال ضمن إطار الطب التكميلي، موجّه للفئات التي تعاني من اضطرابات في نشاط الغدة الدرقية، مع إمكانية استخدامه في برامج وقائية طويلة المدى.

- الميدان التقني الذي ينتمي إليه الاختراع

ينتمي هذا الاختراع إلى المجال التقني للصناعات الصيدلانية والمنتجات الطبيعية، وبشكل أدق إلى مجال المكملات الغذائية ذات المنشأ النباتي التي تستهدف التنظيم الفسيولوجي للغدد الصماء، وبالخصوص الغدة الدرقية.

- الحالة التقنية السابقة

- **براءة الاختراع الأمريكية US7332176B2**

تصف تركيبة عشبية تحتوي على مستخلصات من *Ashwagandha* و *Bacopa monnieri* و *Guggul*، موجهة بشكل عام لدعم النشاط الهرموني وتحسين الأداء الذهني، دون تركيز خاص على الغدة الدرقية.

- **براءة الاختراع الهندية IN201621025743**

تركز على تركيبة طبيعية لعلاج قصور الغدة الدرقية باستخدام مستخلص *Withania somnifera*، لكنها لم تُرفق بأدلة مخبرية أو تجريبية شاملة، كما تفتقر إلى تنوع الفيتوكيمائيات في التركيبة.

- **براءة الاختراع الأمريكية US20180256687A1**

تتناول مكملاً يحتوي على خليط من المعادن والأعشاب (مثل اليود والسيلينيوم) لدعم وظيفة الغدة الدرقية، لكنه يعتمد على مواد معروفة تقليديًا دون استكشاف نباتات جديدة أو فحصها علميًا من حيث التأثير المباشر على توازن T3 وT4.

نقد التقنيات السابقة:

- **التركيز على أعشاب تقليدية فقط**: تعتمد معظم البراءات السابقة على نباتات شائعة الاستخدام دون تقديم تركيبات جديدة أو استكشاف نباتات محلية ذات إمكانيات واعدة مثل *Cucurbita maxima*.
- **غياب التحاليل المتقدمة**: تفتقر أغلبها إلى دعم فيتوكيميائي تفصيلي للمركبات الفعالة (مثل UPLC-MS/MS، أو دراسات محاكاة جزيئية(Docking/ADMET) .
- **غياب تقييم شامل متعدد الأبعاد**: قلّما تجمع تلك البراءات بين التحليل الكيميائي، والتجريب الحيوي، والنمذجة الحاسوبية، مما يقلل من مصداقية تأثيرها الفسيولوجي.

ما يميز اختراعنا:

- **الاعتماد على مستخلص نباتي غير مستغل سابقًا في هذا السياق**: يتميز هذا الاختراع باستخدام مستخلص بذور *Cucurbita maxima*، وهو نبات غذائي-دوائي معروف بسلامته، لكن لم يُستغل علميًا كمكمل موجّه خصيصًا لدعم الغدة الدرقية.
- **دعم علمي متعدد المستويات**: تم دعم فعالية المستخلص بتحاليل فيتوكيميائية متقدمة (LC-MS) ، واختبارات **in vitro** لتحديد التأثير المضاد للأكسدة والالتهاب، و **in silico** لمحاكاة التفاعل مع أهداف بيولوجية ذات صلة بوظائف الغدة.
- **تقييم أولي على نموذج حيواني**: تم اختبار المكمل على نموذج حيواني باستخدام فئران مخبرية (Wistar rats)، حيث تم تقييم تأثيره المباشر على مؤشرات الغدة الدرقية، بما في ذلك التغيرات في مستويات الهرمونات الدرقية، الوزن النسبي للغدة، والمؤشرات المناعية والدموية المرتبطة بوظائفها، ما يعزز من مصداقية فعالية المنتج وسلامته الفسيولوجية.
- **تركيبة قابلة للتطبيق**: أُنجز المكمل في شكل صيدلاني (كبسولات أو مسحوق) يسهل استخدامه، ما يجعل المنتج قابلاً للتسويق بعد اجتياز التقييمات السريرية المستقبلية.

" البراءات السابقة تمثل توجهًا عامًا ومكررًا في صناعة المكملات الذهنية، وتفتقر إلى التخصص، الابتكار، والعمق التحليلي، عكس اختراعنا الذي يقدم تركيبة نباتية دقيقة، موجهة وظيفيًا، مدعومة بتحاليل متعددة المستويات وتقييم بيولوجي موجّه نحو محور الغدة الدرقية "

- الغرض (الهدف) من الاختراع

يهدف هذا الاختراع إلى تطوير مكمل غذائي طبيعي وآمن يعتمد على مستخلص بذور Cucurbita maxima، موجه خصيصًا لدعم توازن هرمونات الغدة الدرقية(T4/ T3) ، والمساهمة في تحسين وظيفتها دون إحداث آثار جانبية كما في بعض العلاجات الكيميائية التقليدية. يوفر هذا المكمل حلاً طبيعياً بديلاً للأفراد الذين يعانون من اضطرابات بسيطة إلى متوسطة في نشاط الغدة الدرقية، سواء في حالات القصور أو النشاط الزائد الوظيفي، خاصة لدى الفئات الأكثر عرضة مثل النساء في فترات التقلبات الهرمونية.

كما يسعى هذا الابتكار إلى الاستفادة من الخصائص الفيتوكيميائية الغنية لبذورCucurbita maxima ، والتي أظهرت قدرة على التأثير الإيجابي على المحور النخامي-الدرقي من خلال آليات متعددة مثل تعديل الإجهاد التأكسدي، تنظيم الاستجابات الالتهابية، والمساعدة في استقرار النظام المناعي.

الهدف بعيد المدى من هذا المكمل يتمثل في إدراجه ضمن برامج الطب الوقائي والتكميلي، كخيار طبيعي فعّال وآمن لدعم الصحة الهرمونية بشكل مستدام.

- تقديم جوهر الاختراع

الاختراع يتمثل في **تطوير مكمل غذائي طبيعي مبتكر** موجه لتنظيم نشاط الغدة الدرقية، يعتمد على مستخلص بذور *Cucurbita maxima* الغنية بمركبات بيولوجية فعالة مثل الفلافونويدات، الكاتيشين، الكيرسيتين، الروتين، وحمض الفيروليك، والتي أظهرت قدرة على دعم التوازن الهرموني، تقليل الالتهاب، وتعديل الإجهاد التأكسدي المرتبط باختلال وظيفة الغدة الدرقية.

هذا المكمل موجه إلى:

- الأشخاص الذين يعانون من اختلالات في وظيفة الغدة الدرقية.
- النساء في فترات التغيرات الهرمونية (مثل الحمل، الرضاعة، ما قبل انقطاع الطمث).
- الأفراد المهتمين بدعم وظائف الغدة الدرقية بشكل طبيعي وآمن.

ويهدف إلى:

- دعم التوازن الطبيعي لهرمونات T3 وT4.
- المساهمة في تقليل أعراض التعب، اضطراب المزاج، وتساقط الشعر الناتجة عن اضطرابات الغدة.
- تقديم بديل طبيعي وآمن للعلاج التكميلي، يقلل من الاعتماد على الأدوية الكيميائية.
- دعم الصحة العامة من خلال تعزيز المناعة وتقليل الإجهاد التأكسدي.

ويتميز بأنه:

- مستخلص طبيعي 100% من بذور نبات موثوق وواسع الاستخدام.
- خالٍ من الإضافات الكيميائية أو المحفزات الهرمونية.
- آمن للاستخدام طويل الأمد ومدعوم بنتائج تحليل فيتوكيميائي و~~In silico~~.
- قابل للتحضير في أشكال متعددة (كبسولات، مسحوق قابل للذوبان، أو مشروب).
- مدعوم بدراسات أولية تؤكد خصائصه المضادة للأكسدة والداعمة للغدد الصماء.

- **طريقة إنجاز الاختراع:**

تم الحصول على المركبات الفعالة من نبات *Cucurbita maxima*، المعروف بتركيبته الفيتوكيميائية الغنية، وبشكل خاص من بذوره التي تم اختيارها بعناية من مصادر محلية موثوقة.
بعد التنظيف والتجفيف في ظروف محكمة للحفاظ على سلامة المكونات الحيوية، تم طحن البذور واستخلاصها باستخدام محلول مائي بطريقة النقع على مدى 48 ساعة. بعد الاستخلاص، تم فصل السائل عن البقايا الصلبة، ثم إزالة المذيب بلطف باستخدام مبخر دوار (Rotary evaporator) لضمان تركيز المستخلص دون الإضرار بالمواد النشطة (كما هو موضح في الشكل 1).
تم تقييم المستخلص مبدئيًا في المختبر باستخدام اختبارات مضادات الالتهاب وتحديد النشاط المضاد للأكسدة مثل (BSA /DPPH)، مع تحليل المركبات النشطة عبر .UPLC-MS/MS وقد أعقبت هذه التحاليل تجارب بيولوجية أولية على نماذج حيوانية (Wistar rats) بهدف تقييم الأمان والفعالية ضمن سياق دعم التوازن الهرموني للغدة الدرقية.
بالاستناد إلى نتائج التحاليل والاختبارات، تم تطوير تركيبة صيدلانية نهائية في شكل كبسولات صلبة سهلة الاستخدام، تحتوي على الجرعة المثلى من المستخلص الجاف، مع إضافة سواغات طبيعية تضمن الثبات والاستقرار (الشكل 2).

- **مراحل إنجاز الاختراع:**

الخطوة الأولى: تحضير المستخلص

5 . جمع بذور *Cucurbita maxima* من مصادر موثوقة.
6 . تجفيف وطحن البذور للحصول على مسحوق ناعم.
7 . استخلاص المركبات الفعالة بالماء بطريقة النقع.
8 . ترشيح المستخلص وتركيزه باستخدام المبخر الدوار.

الخطوة الثانية: التقييم البيولوجي والمخبري

5 . تحليل فيتوكيميائي للمركبات النشطة بتقنية LC-MS=
6 . اختبار النشاط المضاد للأكسدة والالتهاب (BSA,DPPH)=
7 . التحقق من الفعالية والأمان على نموذج حيواني(Wistar rats)=

الخطوة الثالثة: تطوير الشكل الصيدلاني

8 . تحديد التركيز الأمثل بناءً على نتائج التحاليل.
9 . تصنيع التركيبة النهائية في شكل كبسولات نباتية مناسبة للاستخدام الفموي.
10 تعبئة المنتج في ظروف تحافظ على الثبات والجودة.

- شرح الأشكال والرسومات

الشكل (1): خطوات استخلاص المركبات الفعالة من بذور "Cucurbita maxima"

4 - Cucurbita maxima (اليقطين)

5 - البذور

6 - المستخلص المائي

7 - مبخر دوار(Rotary evaporator)

الشكل (2): تحويل المستخلص الجاف إلى صيغة صيدلانية نهائية (كبسولات)

1 - المستخلص الجاف

2 - كبسولات

- طريقة تطبيق الاختراع

يُستخدم المكمل الغذائي عن طريق الفم، في شكل كبسولات (500 ملغ) أو مسحوق قابل للذوبان.

الجرعة الموصى بها للبالغين:

- كبسولة واحدة مرتين يوميًا بعد الوجبات.
- يمكن تعديل الجرعة حسب الحالة الفردية وتوصية مختص التغذية أو الطبيب، ولا يُنصح بتجاوز 1غرام/كغ من وزن الجسم يوميًا.

المدة المثالية للاستخدام:

- للاستعمال الوقائي والداعم: من 6 إلى 12 أسبوعًا.
- يمكن تكرار البرنامج بعد استراحة لمدة أسبوعين.

الفئة المستهدفة تشمل الأشخاص الذين يعانون من اختلالات هرمونية خفيفة في الغدة الدرقية، خاصة النساء في فترات الاضطراب الهرموني.
يُفضل الاستخدام تحت إشراف مختص، ولا يُنصح به بالتزامن مع أدوية هرمونية دون استشارة طبية.

المحور الرابع

المطالب

المحور الرابع
المطالب

المطلب الرئيس

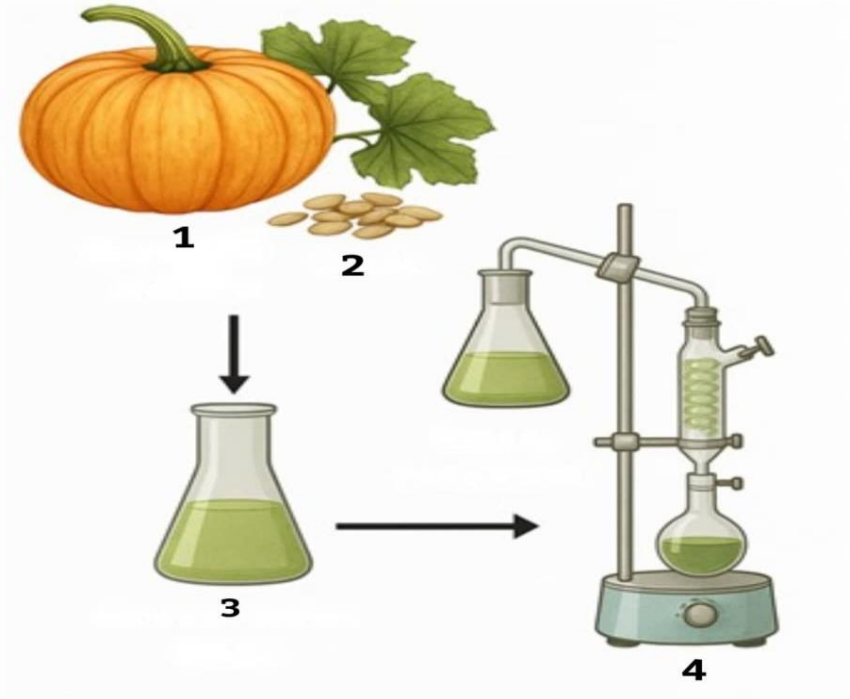
تتمثل القيمة الإضافية والميزة التي يتميز بها اختراعنا مقارنة بباقي الاختراعات الأخرى في تطوير مكمل غذائي طبيعي مبتكر مستخلص من بذور *Cucurbita maxima*، مخصص لدعم التوازن الهرموني للغدة الدرقية بفعالية مثبتة، وبتركيبة آمنة خالية من الهرمونات الصناعية أو المواد الكيميائية. هذا المكمل يجمع بين الاعتماد على مصدر نباتي غير مستهلك بشكل كافٍ في المجال الهرموني، وبين دعم علمي شامل يشمل تحاليل فيتوكيميائية واختبارات *in vitro* و*in vivo* أثبتت تأثيره الإيجابي على مؤشرات الغدة الدرقية والتوازن التأكسدي والمناعي، مما يمنحه تميزًا سريريًا وعلميًا غير متوفر في مكملات أخرى مشابهة.

المطالب المستنبطة من المطلب الرئيسي والتي تميز اختراعنا

1. وفقًا للمطلب الرئيسي، يتميز هذا المكمل بتركيبة طبيعية 100%، خالية من أي مواد كيميائية أو هرمونات صناعية، مرتكزة على مستخلص بذور *Cucurbita maxima* الغني بالمركبات الفينولية والفلافونويدات الفعالة.
2. يتم تحضير المكمل باستخدام طرق استخلاص مائية بسيطة وآمنة، دون الحاجة إلى معدات معقدة أو مذيبات عضوية ضارة، مما يجعله عمليًا وسهل التصنيع.
3. يتمتع بفعالية مثبتة من خلال نتائج اختبارات *in vitro* و*in vivo*، حيث أظهرت تأثيرًا واضحًا في دعم وظائف الغدة الدرقية (T3، T4)، وتقليل الإجهاد التأكسدي، وتحقيق توازن في المؤشرات المناعية.
4. يمكن توفير المكمل في أشكال صيدلانية مناسبة مثل كبسولات أو مسحوق قابل للذوبان، مما يتيح استخدامه اليومي بطريقة سهلة وآمنة.
5. يُعتبر هذا المكمل حلاً طبيعيًا داعمًا للفئات المعرضة لاختلالات هرمونية، خصوصًا النساء خلال فترات التقلبات الهرمونية (مثل الحمل، الرضاعة، انقطاع الطمث)، مما يجعله بديلاً طبيعيًا مكملاً للعلاجات التقليدية.

قائمة الملاحق

الشكل (1)



الشكل (2)