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THEME

**Topical Application of a Plant Extract Mixture for Anti
Inflammatory Therapy: *In Vivo* and *In Vitro* Assessment**

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شكر وتقدير

بسم الله الرحمن الرحيم، الحمد لله الذي بنعمته تتم الصالحات، وبتوفيقه تتحقق الأمنيات. أتقدم بأسمى عبارات الشكر والتقدير إلى الدكتورة القديرة "شيماء بنين" التي كانت لنا مصدر إلهام ، و روحًا داعمة، ويدا حانية، ورفيقة صادقة في كل المراحل، ساندتنا نفسيًا وجسديًا، واحتضنت جهودنا بصبر واهتمام. فلك منا كل التقدير، وجعل الله عطاؤك في ميزان حسناتك. كما لا يفوتني أن أشكر الأستاذة الفاضلة "وفاء بوضبية"، التي أثرتنا بعلمها وتوجيهاتها الدقيقة، وكانت مثالًا للعلم والخلق والتفاني في العمل

ولا يسعني إلا أن أعبر عن امتناني العميق للأستاذة الكرام: الدكتور "العازز الحفناوي"، صاحب الكلمة الصادقة والتوجيه النابع من خبرة وحكمة، الدكتور "العربي بن عمر"، الذي لم يتوان عن تقديم المساعدة والنصيحة بروح طبية وعلم غزير، كما نتوجه بخالص التقدير والاحترام إلى الأستاذ "حسين شعوة"، الذي كان لنا مصدر دعم وتوجيه الأستاذ "لعويد حسام" الذي كان بخلقه وعلمه نبراسًا مضيئًا في مسارنا، الأستاذ "خوفه عمر"، صاحب الكلمة الطيبة والدعم المستمر، فله منا كل الشكر والتقدير.

وأخص بالشكر أيضًا البيطري "صالح شويخ"، الذي كان له دور فعال في تقديم الدعم العملي والميداني، بتفانيه واحترافيته ساعدنا على تجاوز العديد من التحديات البحثية. والشكر موصول " لطبيب مخبر الفردية" الذي قدم لنا من وقته وخبرته، وكان سندًا طبيًا وعلميًا حقيقيًا خلال مراحل البحث.

نتقدم بجزيل الشكر وعظيم الامتنان للطلبة "أحمد، محمد، ورائيا" من الفرقة الثانية – شعبة البيولوجي، على ما بذلوه من جهد مخلص وتعاون صادق، خاصة في أوقات الشدة والضغط، أثر بالغ في إنجاز هذا العمل، ولا يسعنا إلا أن نشكر على روح التعاون والمسؤولية التي تحلوا بها. راجين لهم دوام التوفيق والنجاح في مسيرتهم العلمية. ولا يفوتنا أن نعرب عن بالغ شكرنا وامتناننا للجنة المناقشة الموقرة، التي تكّرت بقبول مناقشة هذا العمل وتقييمه، فتوجيهاتهم وملاحظاتهم القيمة يُستكمل مسعانا العلمي نحو الأفضل. فلكم جميعًا منّا أسمى عبارات الشكر والعرفان، وجزاكم الله خير الجزاء.

إهداء

بسم الله الرحمن الرحيم الحمد لله الذي بنعمته تتم الصالحات، وبفضله تنزل البركات، وبصبرٍ منه وعونٍ، بلغت هذه...الخطوة من طريقي

الى من غرست في قلبي حب العلم والعمل، إلى من كانت لي العون والسند في كل خطوة

، إلى نبع الحنان وضيء الدرب، إلى...إلى جنتي في الدنيا...إلى من كانت دعواتها سرّ توفيقِي، وسهرها نور دربي. من سكبت في روحي عطر الحكمة ونور البركة، إلى أمي "الطيفة"، لك كل الحب، وكل العرفان، وكل نبض في قلبي وإلى من غرس في روحي معنى الثبات، الذي كان لي دعمًا لا ينضب، وعونًا لا يكل، إلى من علمني أن الرجال مواقف، أبي "كمال"، دمت فخراً لي ما حييت

زوجي الحبيب، شكراً لأنك كنت في كل خطوة معي، لقد كنت...إلى نصفي الآخر، رفيق تعب الأيام وفرحة الإنجاز رفيق مشواري، آمنت بي، وساندتني في كل خطوة، ولولاك ما كان هذا الطريق ليكتمل، لك مني كل الحب والامتنان، "نصر الدين" "سراج الدين"، إلى من : وإلى نوريين الصغيرين، ألمي وبهجة عمري. فأنت نجاحي وسندي في الحياة أجلهم يسهل التعب، ويُصبح الإنجاز له طعم ومعنى، إلى من أرجو أن يكونوا فخورين بي كما أفخر بهم، لأجلكما أطمح لأن أكون أفضل دائماً

خولة" نبض العائلة وأمان العمرالى من تنبض روحي إلى من كانت لي أمًا حين غابت أمي، وسندًا حين ثقلت الأيام" بحبها، إلى أختي الصغيرة "صفاء"، زهرة قلبي ورفيقة دربي، يا من كان وجودك في حياتي نعمة تفيض صفاءً كما وصفاء، لا تكفيك كلمات، لكنّ .اسمك، أهديك هذا العمل عربون محبة واعتزاز، ودعاء بأن يحقق الله لك كل خير إلى إخوتي وأخواتي، يا أجنحة الروح وعزوة القلب، يا أنسام العطف حين يشتد الغيظ، ويا . القلب يشهد بمحبتكن لكم جميعاً، أهدى ثمرة جهدي، ممزوجة بعقب الامتنان وعبير الوفاء، متمنية أن تظل . صدى الأمل في ربيع الحياة محبتنا سارية كجدول لا ينضب، ومشعة كبدر لا يافل

الى " معلمة ابني" من كانت لأبني أمًا ثانية، بحبك وحنانك خففت عني أعباء الطريق، شكري لك لا توفيه الكلمات، وامتناني لك لا يسعه الورق، فلك مني كل التقدير، ولك في هذا الإنجاز نصيب كبير

إلى صديقاتي الوفيات، إلى أولئك الذين كانوا السند والرفيق في رحلة العلم والسعي، إلى من تقاسموا معي لحظات التعب والفرح، إلى الذين لم يبخلوا عليّ بكلمة دعم، أو بلمسة تشجيع، أهديك ثمرة جهدي وتعب سنواتي، فبوجودكم من كنّ بلسماً في زمن الجراح. كان للطريق طعمٌ آخر، وللنجاح معنى أجمل

أهدى هذه المذكرة المتواضعة إلى كل من ساندني، دعمني، آمن بي، أو حتى دعا لي بظهر الغيب

دعاءً متجددًا بأن يبارك الله في أعماركم، وأن يجعل ما خطّه قلمي علمًا نافعا يُنتفع به...فالحمد لله أولاً، وآخرًا، وظاهرًا، وباطنًا



مروة.

إهداء

بسم الله الرحمن الرحيم "وأخر دعواهم أن الحمد لله رب العالمين" ومن قال "أنا لها" تالها، وأنا — بعون الله — نلتها، رغم ما واجهته من مشقة، نلتها رغمًا عنها. فالحمد لله الذي بلّغني هذا المقام، وما ذلك إلا بفضلته ومَنّته.

ما سلكنَا البدايات إلا بتيسيره، وما بلّغنا النهايات إلا بتوفيقه، وما صفقنا الغايات إلا بفضلته. الحمد لله على التمام، وحسن الختام.

إلى أمي الغالية "آمال" يا من غرست في قلبي حب العلم والصبر، بدعائك وحنائك وصلت، وبدفئك اجتزت الصعاب. لك كل الشكر والحب ما حييت. إلى أبي العزيز "مسعود" يا من كنت السند والقُدوة، دعمتني في كل خطواتي، ووجهتني بحكمتك، فلك مني كل الوفاء والتقدير.

إلى زوجي الحبيب "العربي" رفيق دربي، ورفيقي في الحلم، صبرك، دعمك، ومساندتك كان لها الأثر الكبير، فشكرًا لقلبك الذي احتواني في كل حين.

إلى ابني الغالي "أريب" يا نور عيني، وأعلى هدية من ربي، كنت الحافز الأجل في طريقي، أسأل الله أن يحفظك ويبارك لك في عمرك.

إلى إخوتي الأحباء: "جنان"، "سارة"، "عبد الباري"، "وليد" كنتم دومًا العون والسند، ضحكاتكم ودعمكم خفف عني الكثير، فلكم في القلب محبة لا تنطفئ.

إلى جدتي العزيزة "عائشة"، وجدتي الغالية "حدي"، وجدتي الكريم "عبد الرزاق"، وجدتي الحنون "خليفة" أنتم جذر العائلة ودفؤها، دعواتكم ونظرات رضاكم كانت النور في طريقي، أطال الله في أعماركم وحفظكم لنا نذرًا. إلى أم زوجي "مريم" أمّ ثانية بحنائك، ورفيقة درب بدعواتك، شكرًا من القلب على وجودك ودعمك الدائم.

إلى صديقتي العزيزتين "مروة" و"فتيحة" كنتمما الضوء في لحظات العتمة، والضحكة في أوقات التعب، رفقتكما كانت نعمة، ودعمكما الصادق كان بلسمًا في كل مراحل الطريق، فلكما مني كل الشكر والامتنان، ومحبة خالدة في القلب.... وختامًا اللهم اجعل هذا الإنجاز شاهدًا لي لا علي، وانفعني بما علمتني، وزدني علمًا وعملاً ورفعةً في الدنيا والآخرة.

اللهم اجعل هذا العلم حجة لي في حياتي، ونورًا في قبوري، وسببًا في رضوانك.

واجعل ما مضى تعبًا، وما بقي فرحًا وسعة، وما أتممته بداية لا نهاية. الحمد لله أولاً وآخرًا، ظاهرًا وباطنًا، عدد ما كان، وعدد ما يكون، وعدد الحركات والسكون



اهـداء

قال تعالى «وَ كَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا» الحمد لله هو المستحقُّ للحمد والثناء إلى من يبقى أُملي رضاه وغايتي حبه و رجائي غفرانه.... الله رب العالمين .إلى من بَلَغَ الرسالة و أدى الأمانة..... ونصح الأمة..... إلى نبي الرحمة ونور العالمين..... سيدنا مُحَمَّد صَلَّى اللهُ عَلَيْهِ وَسَلَّمَ .إلى من أذهب الله عنهم الرجس وطهرهم تطهيراً..... ساتدتي ومعمدي أهل بيت النبوة (عليهم السلام) .أهدي مجهود بحثي لأجمل مافي الوجود إلي عائلتي التي وثقت بي ودفعتني لمواصله المشوار وعلمتني أن الطموح لا حدود له وأنه بين الواقع والحلم إرادة لم يطلق سراحها وأن القمم لا تُنال بالتمني بل بالعمل الجاد والإيمان بالله والذات، عائلتي هي مدرستي الأولى وما أنا وأخوتي وأخواتي إلا شهادات نجاح ووسام عظمة لأمنا المعلمة ولأبينا .إلى القلب الذي ينبض في لحظة بالحب والرحمة إلى التي أثارت قلبي وكتبت إسمي على حدقات عيونها والتي أفنت عمرها علي كي تجعل مني مثالية إلى التي جعل الله الجنة تحت قدمها "أمي " إلى "أبي" دمت لي أباً وظهراً و قلباً لا يميل إلى من قيل فيهم «سَنَشُدُّ عَضُدَكَ بِأَخِيكَ». إخوتي "إسماعيل"، "عمر"، "محمد"، "علي" إلى أخواتي وسندي في هذه الحياة " زكية"، "ريحانة"، "زهراء". إلى من كانت دوماً السند والملاذ، خالتي العزيزة" فاطمة دبار " التي منحنتني حباً لا يُقاس، واهتماماً لا يُنسى، كانت كلماتها تشد من أزري، ودعواتها تسبق خطاي في كل درب،أهدي هذا الإنجاز إليك، لأنك كنتِ جزءاً من كل خطوة، وكل تعب، وكل لحظة انتصار إلى "جدتي" الغالية رحلت بجسدك، لكن حبك ودعواتك ما زالت ترافقني، رحمك الله وأسكنك فسيح جناته .إلي هؤلاء "المعلمين" الذين اختاروا لي طريق العلم وساندوني في لحظات ضعفي وفرحوا معي في كل لحظة فرح وتمنوا لي الخير، وزرعوا في قلبي حب العلا وجعلوني مختلفة عن سائر بنات قريتي، فكنت أول من تنال شهادة البكالوريا، وها أنا اليوم والله الحمد أول خريجة لشهادة الماجيستر ، ولمساعيهم بقية، كما أختص بالشكر أستاذي الفاضل" قريشة الطيب".لهؤلاء أهدي كل ثمرة أجنيتها من بساتين مجهوداتي العلمية .إلى نفسي التي تعبت وأكملت المسير مشيا وزحفا وهرولة، إلى فتيحة التي سعت للإختلاف، وعانت في صمت دون دموع ولا كلمات، إلى قلبي الذي أرى الخير من خلاله في كل بقاع العالم، إلى عقلي الذي يقودني إلى المكان الذي تستحقه عائلتي، إلى أنا التي ترفض الفشل وتردد عبارتها الرائعة: أما أنا فإنني لا أسعى أن أتحول إلى نسخة أحد، بل الذي أسعى إليه أن أرى العالم يتحول إلى نسختي .إلى رفاق الدرب ومن مهدوا طريق النجاح وخاضوها معي بكل شغف وحب....صديقاتي إلى من شاركني هذا العمل أصحاب الفضل العظيم بعد الله تعالى صديقات الرحلة والنجاح. "مروة"، أسماء .إلى من يُحسنون الصُحبة ويكرمون الرُفقة، فلا يشقى في صُحبتهم أحد لله دُرُّ أفئدة أناس تحمل من الطُهر والنقاء ما لا قدر له...."تسرين". "نعيمة" إلى كل قلب أحبني بصدق وإخلاص وكل يد امتدت لمساعدتي والدعاء لي بكل خطوة... أهدي ثمرة جهدي .إلى كل من ذكرهم قلبي ونسيهم قلبي .لا يسعني وأنا أختتم جهدي هذا، إلا أن أقف بين يدي الله متضرعةً وشاكرةً لنعمه التي أنعم بها علي، متوجهةً إليه بالحمد والشكر، اعترافاً بفضله، وطلباً للقبول، فهو نعم المولى ونعم النصير



فُتِيحَةٌ

Abstract

This study aimed to evaluate the biological efficacy of a compound plant extract prepared from a group of traditionally widely used medicinal and aromatic plants, including saffron (*Crocus sativus L.*), thyme (*Thymus vulgaris L.*), clove (*Syzygium aromaticum*), walnut bark (*Juglans regia L.*), as well as coarse salt and alum. The extracts of these plants were prepared by hydrolyzing and used in the formulation of a topical cream. The cream was tested on an animal model to evaluate its therapeutic properties, particularly in accelerating wound healing and reducing inflammation and oxidative stress. The methodology was based on three main axes: a field study to investigate popular practices associated with medicinal plants among the population of El Oued Province; physiological experiments to evaluate the cream's effect on wound healing; and laboratory analyses covering hematological, immunological, inflammatory markers, and oxidative stress aspects, in addition to histological studies. The results showed that ZASSC cream has physical properties suitable for skin use (yellow color, pH = 4.93, wet texture, and 100% stability). Animal experiments also demonstrated its high effectiveness in accelerating wound healing, with a healing rate of 79.12% by day 14, outperforming MEBO cream and the base cream. Furthermore, BSA Denaturation Test analyses revealed the extract's ability to inhibit inflammation by 83.89% compared to 60.36% for diclofenac, with a low IC₅₀ value (145.31 µg/mL), reflecting its strong anti-inflammatory activity. Regarding hematological parameters, the extract contributed to a reduction in white blood cell and platelet counts, as well as lower CRP, ESR, and ASL values, reinforcing the hypothesis of its immunoregulatory effect. The extract also demonstrated significant efficacy in reducing MDA levels and increasing antioxidant activity (GSH, SOD, CAT) in the skin and lymphoid tissues (spleen and thymus). Histological analyses confirmed these results through tissue remodeling and regularity of cellular structure in the ZASSC group compared to the other groups. Together, these results highlight the effectiveness of ZASSC cream as a multifunctional natural product, with anti-inflammatory and antioxidant properties, and an effective role in accelerating wound healing and regulating immune balance, making it a promising candidate for dermatological and cosmetic applications.

Keywords: Medicinal plants, ZASSC cream, wound healing, antioxidants, inflammation, hematological parameters, histological analysis.

Résumé

Cette étude visait à évaluer l'efficacité biologique d'un extrait végétal composé préparé à partir d'un groupe de plantes médicinales et aromatiques traditionnellement largement utilisées, notamment le safran (*Crocus sativus L.*), le thym (*Thymus vulgaris L.*), le clou de girofle (*Syzygium aromaticum*), l'écorce de noyer (*Juglans regia L.*), ainsi que le gros sel et l'alun. Les extraits de ces plantes ont été préparés par hydrolyse et utilisés dans la formulation d'une crème topique. Cette crème a été testée sur un modèle animal afin d'évaluer ses propriétés thérapeutiques, notamment pour accélérer la cicatrisation des plaies et réduire l'inflammation et le stress oxydatif. La méthodologie reposait sur trois axes principaux : une étude de terrain visant à étudier les pratiques populaires associées aux plantes médicinales au sein de la population de la province d'El Oued ; des expériences physiologiques visant à évaluer l'effet de la crème sur la cicatrisation des plaies ; et des analyses en laboratoire portant sur les aspects hématologiques, immunologiques, les marqueurs inflammatoires et le stress oxydatif, ainsi que des études histologiques. Français Les résultats ont montré que la crème ZASSC possède des propriétés physiques adaptées à une utilisation cutanée (couleur jaune, pH = 4,93, texture humide et stabilité à 100 %). Les expériences sur les animaux ont également démontré sa grande efficacité pour accélérer la cicatrisation des plaies, avec un taux de cicatrisation de 79,12 % au jour 14, surpassant la crème MEBO et la crème de base. De plus, les analyses du test de dénaturation de la BSA ont révélé la capacité de l'extrait à inhiber l'inflammation de 83,89 % contre 60,36 % pour le diclofénac, avec une faible valeur de CI_{50} (145,31 $\mu\text{g/mL}$), reflétant sa forte activité anti-inflammatoire. Concernant les paramètres hématologiques, l'extrait a contribué à une réduction du nombre de globules blancs et de plaquettes, ainsi qu'à des valeurs plus faibles de CRP, de VS et d'ASL, renforçant l'hypothèse de son effet immunorégulateur. L'extrait a également démontré une efficacité significative dans la réduction des taux de MDA et l'augmentation de l'activité antioxydante (GSH, SOD, CAT) au niveau de la peau et des tissus lymphoïdes (rate et thymus). Les analyses histologiques ont confirmé ces résultats grâce au remodelage tissulaire et à la régularité de la structure cellulaire dans le groupe ZASSC par rapport aux autres groupes. Ces résultats soulignent l'efficacité de la crème ZASSC en tant que produit naturel multifonctionnel, doté de propriétés anti-inflammatoires et antioxydantes, et jouant un rôle efficace dans l'accélération de la cicatrisation des plaies et la régulation de l'équilibre immunitaire, ce qui en fait un candidat prometteur pour des applications dermatologiques et cosmétiques.

Mots-clés : Plantes médicinales, Crème ZASSC, Cicatrisation des plaies, Antioxydants, Inflammation, Paramètres sanguins, Analyse histologique.

ملخص

تهدف هذه الدراسة إلى تقييم الفعالية البيولوجية لمستخلص نباتي مركب محضّر من مجموعة من النباتات الطبية والعطرية ذات استخدام تقليدي واسع، من بينها الزعفران (*Crocus sativus L.*) ، الزعتر (*Thymus vulgaris L.*) ، القرنفل (*Syzygium aromaticum*) ، لحاء الجوز (*Juglans regia L.*) ، إلى جانب الملح الخشن والشبة. تم تحضير مستخلص هذه النباتات بطريقة الغلي المائي، واستخدامه في تركيب كريم موضعي اختُبر على نموذج حيواني لتقييم خصائصه العلاجية، لا سيما في تسريع التئام الجروح والحد من الالتهاب والإجهاد التأكسدي. اعتمدت المنهجية على ثلاث محاور أساسية: دراسة ميدانية لاستقصاء الممارسات الشعبية المرتبطة بالنباتات الطبية لدى سكان ولاية الوادي، تجارب فيزيولوجية لتقييم تأثير الكريم على التئام الجروح، وتحاليل مخبرية همّت الجوانب الدموية، المناعية، المؤشرات الالتهابية، والإجهاد التأكسدي، بالإضافة إلى دراسات نسيجية. أظهرت النتائج أن كريم **ZASSC** يمتاز بخصائص فيزيائية ملائمة للاستعمال الجلدي (لون أصفر، $pH = 4.93$ ، قوام رطب، وثباتية بنسبة 100%). كما بيّنت التجارب الحيوانية فعاليته العالية في تسريع التئام الجروح، بنسبة شفاء بلغت 79.12% في اليوم الرابع عشر، متفوقاً على كريم **MEBO** والكريم القاعدي. من جهة أخرى، أظهرت تحاليل **BSA Denaturation Test** قدرة المستخلص على تثبيط الالتهاب بنسبة 83.89% مقارنة بـ 60.36% للديكلوفيناك، مع قيمة IC_{50} منخفضة ($145.31 \mu g/mL$)، مما يعكس فعالية مضادة للالتهاب قوية. أما على مستوى المؤشرات الدموية، فقد ساهم المستخلص في تقليل تعداد الكريات البيضاء والصفائح، وانخفاض قيم **CRP** و **ESR** و **ASLO**، مما يعزز فرضية تأثيره المنظم للمناعة. كما أظهر المستخلص فعالية واضحة في تقليل مستويات **MDA** ورفع نشاط مضادات الأكسدة (**GSH**) ، **SOD** ، (**CAT**) على مستوى الجلد والأنسجة اللمفاوية (الطحال والغدة الزعترية). وأكدت التحاليل النسيجية هذه النتائج من خلال إعادة التكوين النسيجي وانتظام البنية الخلوية لدى مجموعة **ZASSC** مقارنة بباقي المجموعات. تُبرز هذه النتائج مجتمعة فعالية كريم **ZASSC** كمستحضر طبيعي متعدد الوظائف، بخصائص مضادة للالتهاب والأكسدة، ودور فعّال في تسريع شفاء الجروح وتنظيم التوازن المناعي، مما يجعله مرشحاً واعداً في الاستخدامات العلاجية الجلدية والتجميلية.

الكلمات المفتاحية: النباتات الطبية، كريم **ZASSC** ، التئام الجروح، مضادات الأكسدة، الالتهاب ، مؤشرات الدم،

التحليل النسيجي.

LIST OF ABBREVIATIONS

ANOVA: Analysis of Variance

ASLO: Anti-Streptolysin O

BHT: Butylated Hydroxytoluene

BSA: Bovine Serum Albumin

CAT: Catalase

CRP: C-Reactive Protein

DMSO: Dimethyl Sulfoxide

ESR: Erythrocyte Sedimentation Rate

FBS: Fasting Blood Sugar ---

GRA: Granulocytes ---

GSH: Glutathione

HGB: Hemoglobin

IC₅₀: Inhibitory Concentration 50%

IU: International Unit SD: Standard Deviation

LYM: Lymphocytes

MDA: Malondialdehyde

MEBO: Moist Exposed Burn Ointment

NS: Non-Significant

PLT: Platelets

RBC: Red Blood Cells

SD: Standard Deviation n: number (sample size)

SOD: Superoxide Dismutase

TBA: Thiobarbituric Acid

TBS: Tris-Buffered Saline OD: Optical Density

TCA: Trichloroacetic Acid DTNB: 5,5'-Dithiobis-(2-nitrobenzoic acid)

VS: Erythrocyte Sedimentation Rate (Vitesse de Sédimentation)

WBC: White Blood Cells

ZASSC: Zaatar, Alum, Salt, Saffron, Clove

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Introduction

Introduction

In recent years, global interest in medicinal plants has grown due to their diverse therapeutic benefits, making them a promising source for discovering new compounds that could contribute to the development of future medicines. With the increasing emergence of new diseases and the difficulty of treating them using traditional methods, the need for natural alternatives has become more urgent, especially since many plants contain biologically active compounds with multiple therapeutic effects. Algeria is a country rich in biodiversity, particularly in the Mediterranean region, and is home to approximately 146 plant species used for medicinal purposes, among thousands of species that grow naturally in its environment, making it a leading country in this field (**Boughazala et al., 2024**). Reliance on medicinal plants has increased in recent years, particularly following the World Health Organization's recommendations to return to natural treatments and reduce reliance on synthetic chemicals, due to their negative long-term side effects. Despite the widespread use of synthetic drugs, a large portion of the population still prefers natural remedies. The World Health Organization reports that there are more than 20,000 species of medicinal plants worldwide, with approximately 25% of the active ingredients in currently available medicines derived from plant sources (2024، عيساوي وحمه). Phytochemistry plays a pivotal role in extracting active ingredients from plants using various physical, chemical, and analytical techniques, followed by biological and pharmacological evaluation to test the efficacy of these compounds. A prominent example is thyme (*Thymus vulgaris*), which contains essential oils such as thymol and carvacrol, known for their antibacterial and anti-inflammatory properties, making it suitable for treating wounds and skin infections. Saffron (*Crocus sativus*) is characterized by its antioxidant properties and is traditionally used to promote cell regeneration and accelerate wound healing. Walnut bark (*Juglans regia*) is a natural source of antibacterial compounds such as sulfur, making it beneficial for oral health and accelerating the healing of superficial wounds. Cloves (*Syzygium aromaticum*) are also rich in eugenol, a compound with analgesic and antibacterial properties. It is used to relieve toothaches and accelerate wound healing. Recent studies and medical conferences emphasize the importance of reducing reliance on chemical drugs and highlighting medicinal plants as safer and more effective sources of treatment (**Chadou et al., 2023**). It is well known that wounds have been and continue to be a major health challenge facing humanity. Their prevalence has increased in the modern era, especially with the rise in life expectancy and changing lifestyles, which has led to an increase in the incidence of chronic

wounds. The wound healing process includes four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Local or systemic immune dysfunction may prolong the inflammatory phase and delay the healing process. This is influenced by several factors, including wound size, blood flow, and the presence of infection or foreign bodies **(Benin et al., 2024)**. In this context, medicinal plants play an important role in reducing inflammation and accelerating wound healing, thanks to their antioxidant and anti-inflammatory compounds that contribute to pain relief and stimulating tissue regeneration. The presence of growth factors extracted from white blood cells or platelets is also essential for accelerating wound healing. This highlights the importance of medicinal plants as natural and safe sources for promoting healing, reducing inflammation, and stimulating the repair of damaged tissue, making them a key focus for future research in the field of regenerative medicine **(Pedra et al., 2024)**. This research is divided into two main sections: Part One: Theoretical Study; Chapter One: Overview of Medicinal Plants; Chapter Two: Study of Wounds and Inflammation; Part Two: Experimental Study; Chapter Three: Materials and Methods; Chapter Four: Results and Discussion. This study aims to evaluate the anti-inflammatory and wound-healing properties of a compound plant extract, comprising *Thymus vulgaris*, *Juglans regia* L., *Syzygium aromaticum*, and *Crocus sativus*, after its transformation into a topical cream. The study seeks to verify its effectiveness as a safe and natural alternative to topical chemical preparations used to treat skin infections and accelerate wound healing, by studying its biological effects on experimental models. In this context, can this plant extract, in its topical form, demonstrate anti-inflammatory properties and accelerate skin wound healing, qualifying it as a natural and safe alternative to commonly used chemical preparations ?

First part

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Chapter I

Generalities about Medicinal Plants

I. Saffron

1. Generalities and History about *Crocus sativus* L

Crocus sativus L. is an ancient spice with a botanical origin that remains unclear despite its long history of cultivation, dating back to at least 3,500 years ago during the Sumerian and Babylonian eras. Studies indicate that saffron may have originated along the Mediterranean shores of Asia Minor and in the valley between the Tigris and Euphrates rivers. Other potential origins include Southeast Asia, Central Asia, or India, while some researchers suggest Persia (Iran), ancient Greece, or Crete as possible starting points. It was demonstrated that saffron was selected and domesticated in Crete during the Late Bronze Age. From there, it spread to India, China, and Middle Eastern countries, eventually reaching the Mediterranean basin through the Arabs, likely being introduced to Morocco in the 9th century. (Lachguer et al.,2024)

2. Botanical traits

Saffron (*Crocus sativus* L.) is a monocotyledonous, hysteranthous, herbaceous perennial plant that typically reaches a height of 25 to 40 cm. Its high adaptability facilitates widespread cultivation in the Mediterranean region and western Asia. The species grows between longitudes 10 °W and 80 °E and latitudes 30–50°N. The corm, a short, thick underground stem, is composed of parenchyma cells rich in starch and covered with fibrous tunics. Corms vary in shape and size, typically measuring 0.5 to 6.5 cm in diameter and weighing between 0.5 and 70 g. Mature corms have 1 to 3 dominant apical buds that germinate in the following season to produce floral axes and daughter corms. Saffron has two types of roots: thin, fibrous roots responsible for water and nutrient absorption, and contractile, thick roots that help maintain soil depth (Lachguer et al.,2024)

3. Plant Description

Saffron is a perennial, bulbous plant that can reach up to 30 cm in height. It has long, thin leaves and purple to violet flowers. During its flowering period between September and November, saffron produces one to three flowers the first year and then four to twelve flowers in subsequent years.

The flowers each consist of three yellow pistils with three orange-red stigmas, 2.5 to 3.5 cm long. These stigmas, which emit a strong aromatic odor, constitute the most expensive spice in the world (Crozet et al., 2012).

Saffron bulbs, also called horns, are 3 to 5 cm in diameter and brown or orange in color. They are underground and accumulate the stored substances necessary for flowering and budding. The flowers do not produce viable seeds; the plant is dependent on humans for its reproduction. There are one to three flowers per bulb and two to three bulbs per plant (**Winterh and Straubinger, 2000**)

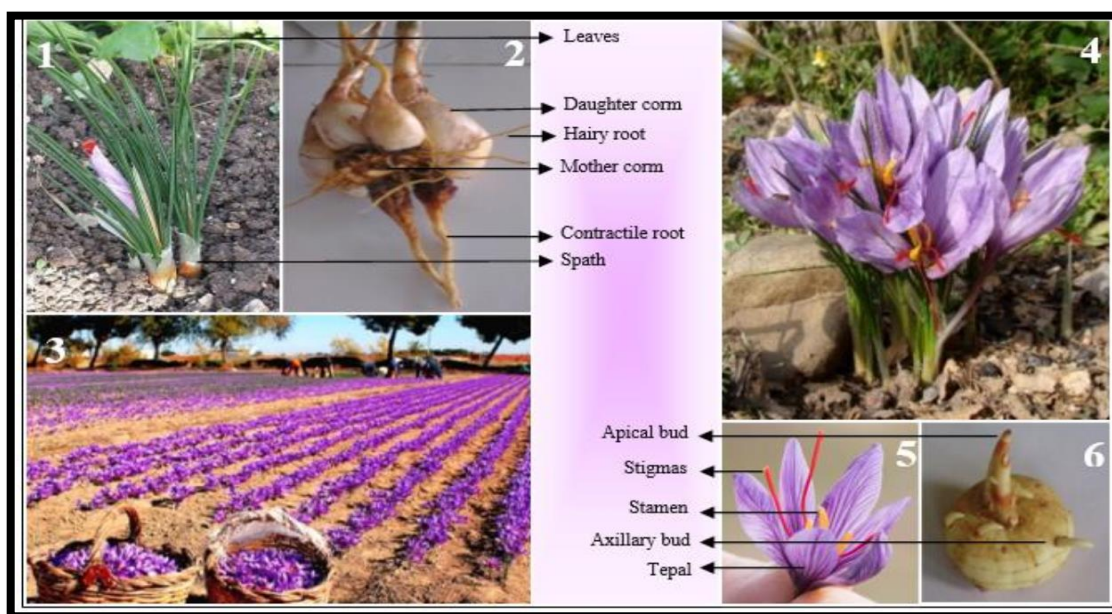


Figure 1 Saffron leaves (1), Saffron corms and roots (2), saffron farming (3), Saffron plant (4), saffron flower (5) and (Lachguer et al., 2024)

4. Taxonomy and Classification

According to Cronquist's 1981 botanical classification, which is based on anatomical, morphological, and chemical criteria to differentiate angiosperms, *Crocus sativus* L. belongs to:

- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiosperms (Magnoliophyta)
- Class: Monocots (Liliopsida)
- Subclass: Liliidae
- Order: Liliales
- Family: Iridaceae
- Subfamily: Crocoideae
- Genus: *Crocus*

- Species: *Crocus sativus* L. (ZBIRI et al.,2021)

5. SYNONYMS

Hindi - kesar, zaffran; Sanskrit - avarakta, saurab, mangalya, agnishikha, kumkuma, mangal, kusrunam; English- saffron; Arab and Persian - zafrah, zipharana; Ben jafran; Bom - safran, kessar; Mah - kecara; Guj - keshar; Tel - kunkuma-purva, kunkumma-purru; Tam. and Mal. - kunkumappu; Can. and Kon kunkuma-kesara; Fr.and Ger. - safran.

Scientific Name and Key Compounds of Saffron *Crocus sativus* L.

6. CHEMICAL CONSTITUENTS

Saffron has been widely studied for its medicinal properties, leading to the identification of key active compounds: crocin (color), picrocrocin (bitter taste), and safranal (aroma). It contains over 150 volatile and non-volatile compounds, including carotenoids like crocetin, zeaxanthin, and lycopene. The main coloring agent, α -crocin, is a water-soluble carotenoid formed by the esterification of crocetin with two gentiobiose sugars, making up more than 10% of the saffron's dry weight.(Srivastava et al., 2010)

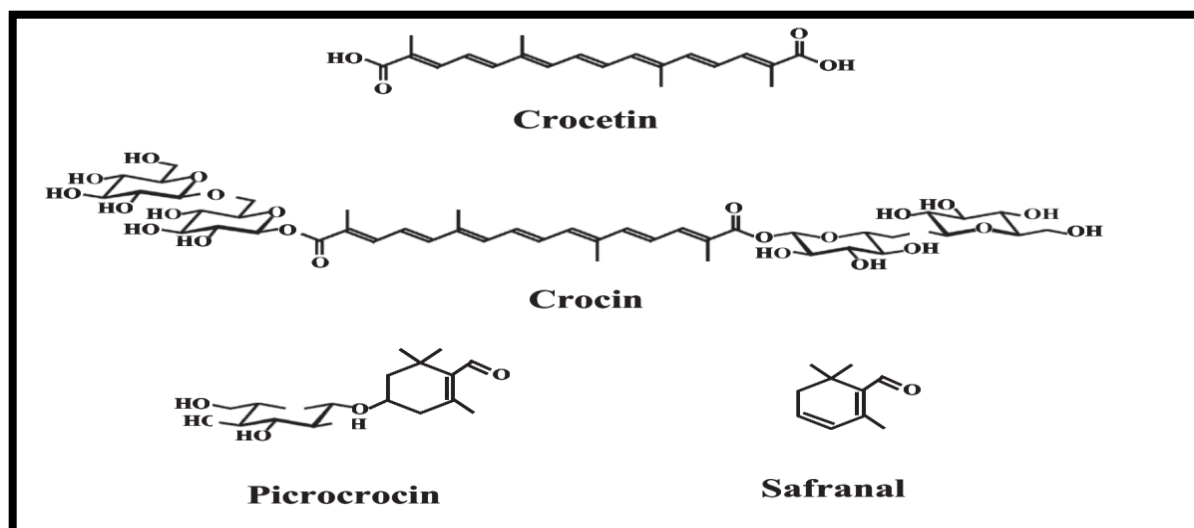


Figure 2 Structures of the chemical constituents (Srivastava et al., 2010)

7. Major uses of saffron

Saffron has long been recognized for its medicinal properties, historically utilized by local populations to treat a variety of health conditions, including poisoning, cold symptoms, respiratory disorders, and to alleviate childbirth pain and teething discomfort

in children. It has also been applied topically to promote the healing of scars following circumcision.

In recent years, numerous scientific studies, including 14 recent research papers, have provided evidence supporting the potential therapeutic benefits of saffron. These studies suggest that saffron may be effective in managing conditions such as depression, Alzheimer's disease, premenstrual syndrome (PMS), and certain eye disorders, as well as aiding in weight management.

Modern medicine has acknowledged some of saffron's therapeutic properties, particularly its role in stimulating the nervous and digestive systems. (ZBIRI et al., 2021)

II. Thyme

1. ORIGIN AND DISTRIBUTION

Thyme is the general name for the many herb varieties of the *Thymus* species, all of which are native to Europe and Asia. Common or garden Thyme is considered the principal type, and is utilized commercially for flowering and ornamental purposes. Thyme is native to the Western Mediterranean region, extending to south-eastern Italy.

The name Thyme, in its Greek form, was first given to the plant by the Greeks as a derivative of a word which meant 'to fumigate,' either because they used it as incense, for its balsamic Odour, or because it was taken as a type of all sweet-smelling herbs. Others derive the name from the Greek words *thyo*, meaning perfume or *Thymus*, signifying courage, the plant being held in ancient and medieval days to be a great source of invigoration, its pleasant qualities inspiring courage. Another source quotes its use by the Sumerians as long ago as 3,500 BC and to the ancient Egyptians who called it *tham* (Eqbal et al., 2017)

2. Thyme Botanical Aspects

T. vulgaris is a perennial, evergreen subshrub with a generally upright, woody-based stem. The leaves are arranged as whorls around the stem, evergreen and simple. They have an ovate shape, fine texture and a pleasant scent. They mainly constitute the edible part for humans. The flowers are of a cyme type, purple and white in color, bisexual and two-lipped with a hairy glandular calyx giving a pleasant scent. Their blooming occurs during spring and summer. *T. vulgaris* has a moderate growth rate. At maturity, it reaches 0.5 to 1 m in height and spreads over 0.5 to 1 m and sometimes 15 m on the ground as a green cover. It takes 2 to 5 years to

reach its maximum height . It can tolerate frost and drought and poor, salty environments. It lives in loamy and sandy soils, having a neutral and alkaline pH. In Lebanon, *T. vulgaris* grows in groups on calcareous rocks. It does not need a lot of water but requires full sunlight. It has no invasive potential and can live for a maximum of 25 years . *T. vulgaris* is most affected by root rot disease. Finally, thyme can be propagated from seeds, cuttings or by layering (Halat et al., 2022)

3. Description

Thymus vulgaris commonly known as “thyme”, A perennial wild shrub from the *Lamiaceae* family and native to the Mediterranean region. The name thyme derives from the Greek word ‘thymos’ which means courage or strength. In the first century AD, thyme was used mainly as a medicinal plant, which was mentioned in Dioscorides’ work. However, in the Mediterranean region, it was used mainly as spice and then spread all over the world (Halat et al., 2022)

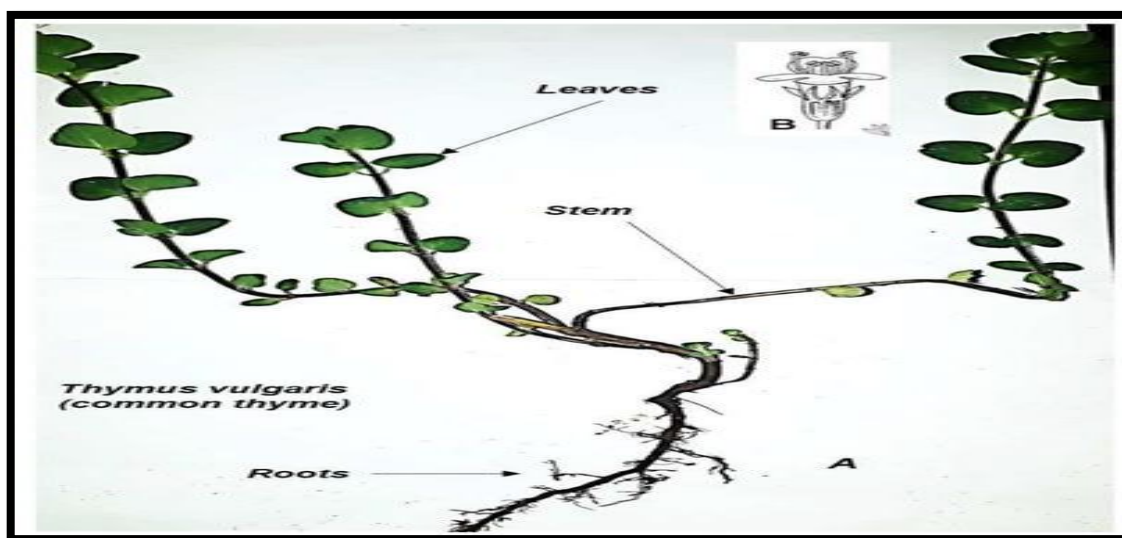


Figure 3 *Thymus vulgaris* (common thyme), sketch drawing (Halat et al., 2022)

4. Taxonomy

Thyme, scientifically known as *Thymus vulgaris*, is a well-known aromatic and medicinal herb that belongs to the plant kingdom. Its botanical classification places it within the group of flowering plants and highlights its position among dicotyledons. Below is the detailed taxonomic hierarchy of thyme (Halat et al., 2022)

- Kingdom: Plantae

- Subkingdom: Tracheobionta
- Superdivision: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Asteridae
- Order: Lamiales
- Family: Lamiaceae
- Genus: *Thymus* L.
- Species: *Thymus vulgaris* L.

3. Main Active Compounds

Thyme leaves and its aromatic oil contain 40 active phenolic and terpenoid compounds, the concentrations of which vary according to the types of thyme, including phenols such as Thymol and Carvacrol 51.34% and Pinen, Limonene, Cymen, monoterpene hydrocarbons , oxygenated monoterpenes (56.53%).(TaHER et al., 2021)

5. Medicinal Parts Used

Medicinal preparations of common Thyme are made from the leaves and the flowers. Thyme essential oil also has medicinal applications. (TaHER et al., 2021)

6. Health Benefits of Thyme

Against microbes

The main component of the essential oil of Thyme, thymol, is active against Salmonella and Staphylococcus bacteria. The antiseptic and tonic properties of Thyme make it a useful tonic for the immune system in chronic, especially fungal, infections as well as an effective remedy for chest infections such as bronchitis, whooping cough, and pleurisy (Dauqan and Abdullah, 2017)

Thyme has been thought to be antiseptic, antimicrobial, astringent, anthelmintic, carminative, disinfectant, medicinal drug and tonic (Dauqan and Abdullah, 2017)

III. Clove

1. Demography

Clove requires damp tropical and sub-tropical environments for growth. It has been cultivated in the following countries: Indonesia, Sri-Lanka, India, Tanzania, Malaysia, Madagascar, and Pakistan (Maggini.,2024)

2. Botany

The clove plant is a medium-sized tree that can reach heights of 8-12 meters. It has a straight trunk with smooth grey bark. The leaves are simple, opposite, and elliptical in shape, with a glossy dark green colour. The flowers are small and numerous, with characteristic crimson colour. These flowers are grouped into terminal clusters and have a strong, aromatic fragrance. The most valuable part of the clove plant is the unopened flower buds, which is harvested for culinary and medicinal purposes.

The flowers are bisexual and have a cup has numerous stamens and a single pistil. develop from the swollen floral tube after fertilization. These fruits are single seeded.

3. Description

Clove is a spice name representing a small reddish brown dried flower buds of *Syzygium aromaticum* (Synonym *Eugenia caryophyllata*) of the family Myrtaceae. (Habtemariam., 2019)



Figure 4 Representation of *S. aromaticum* (Maggini et al., 2024)

4. Taxonomic classification

- Kingdom – Plantae
- Sub kingdom – Tracheobionta

- Super division – Spermatophyta
- Division – Magnoliophyta
- Class – Magnoliopsida
- Subclass – Rosidae
- Order – Myrtales
- Family – Myrtaceae
- Genus – Syzygium
- Species – aromaticum(L.) (Zahoor .,2022)

5. PHYTOCHEMICAL CONSTITUENTS OF CLOVE

Various studies have been carried out to find various constituents of *S. aromaticum* [10-12]. Clove buds contain 15–20% essential oil, which is dominated by eugenol (70–85%), eugenyl acetate (15%) and β -caryophyllene (5–12%). Other essential oil ingredients of clove oil are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin and triterpenoids like oleanolic acid. The constituents of the oil also include methyl amyl ketone, methyl salicylate, α and β -humulene, benzaldehyde, β -ylangene and chavicol. The minor constituents like methyl amyl ketone, methyl salicylate etc., are responsible for the characteristic pleasant odour of cloves. Gopalakrishnan *et al.* (1984) characterized six sesquiterpenes, namely: α -cubebene (1.3%), α -copaene (0.4%), β -humulene (9.1%), β -caryophyllene (64.5%), γ -cadinene (2.6%) and δ -cadinene (2.6%) in the hydrocarbon fraction of the freshly distilled Indian clove bud oil. (Zahoor ., 2022)

IV. Juglans Regia

1. ORIGIN AND DISTRIBUTION

Derem is the common name for the bark of the walnut tree (*Juglans regia*) in some parts of the Middle East and North Africa. Although the native range of the walnut tree traces back to European and Balkan regions around 2,500 to 3,000 years ago, it has since spread widely in recent centuries to areas such as the Himalayas, China, Africa, South America, East Asia, and North America. Over the past century, the use of derem as a chewing tool has become increasingly popular in regions like North Africa, Saudi Arabia, Kuwait, and the Indian subcontinent. A study conducted in Saudi Arabia found that the prevalence of derem use among

Saudi women was approximately 31.9%, with the highest usage observed in the age group of 25 to 39 years. The Riyadh region recorded the highest usage rate at 20.6%.(Zahrani, 2024)

2. Botany

The common walnut tree (*Juglans regia* L.) is a large deciduous species, typically reaching heights of 25 to 35 meters, with trunk diameters occasionally exceeding two meters under exceptional conditions. It is known for its longevity, commonly living between 100 and 200 years, with rare specimens surpassing a thousand years. The bark is initially smooth and silver-gray in young trees, but as the tree matures, it becomes thicker, rougher, and deeply fissured with wide cracks and prominent ridges. This bark serves crucial protective functions, acting as a thermal insulator and enhancing resistance to pests and harsh environmental conditions.(Martínez et al.,2010)

3. Description

Juglans regia, commonly known as walnut or Akhrot in India, is a member of the Juglandaceae family. Its bark is notable for its resinous and aromatic properties. Walnut bark is known to exhibit a wide range of biological activities, including anti-inflammatory, antioxidant, antibacterial, antiplaque, antifungal, anthelmintic, blood-purifying, anticancer, depurative, diuretic, and laxative effects (Ibrahim et al.,2023)



Figure 5 dried bark of the walnut tree, in its commercially available natural form (Zahrani.,2024)

4. Scientific Classification of the Walnut Tree Bark (*Juglans regia*):

- Kingdom: Plantae
- Phylum: Angiosperms
- Class: Eudicots
- Order: Fagales
- Family: Juglandaceae
- Genus: Juglans
- Species: Juglans regia (Missouri Botanical Garden ,s.d)

5. Composition:

Understanding the chemical composition of *Juglans regia* (walnut) is essential due to the presence of various bioactive compounds in all parts of the plant. While walnuts are primarily cultivated for their kernels, other parts such as the husk and bark are also utilized because of their valuable constituents. The main component of the walnut is fat, which includes saturated, monounsaturated, and polyunsaturated fatty acids. In addition, walnuts contain proteins, sugars, dietary fiber, and a broad spectrum of vitamins including folate, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, and vitamins A, C, E, and K. They are also rich in minerals such as potassium, phosphorus, calcium, magnesium, sodium, iron, copper, manganese, zinc, and aluminum. The starch content, however, is relatively low, at approximately 2.8%. (Khattak et.,2024)

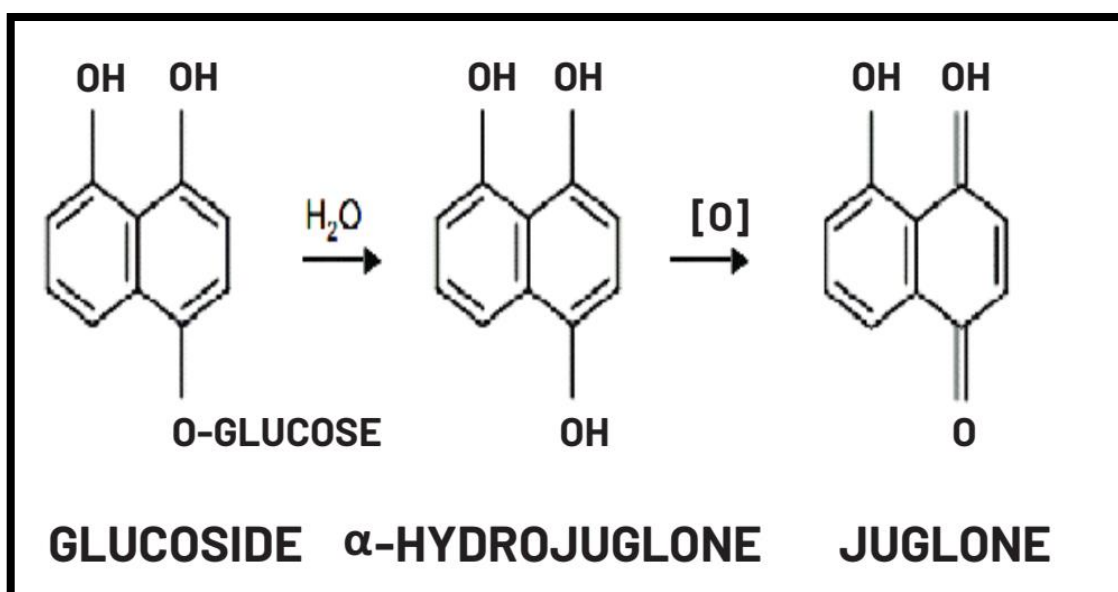


Figure 6 Chemical structure of Juglone-C₁₀H₆O₃ (5-hydroxy-1,4, naphthoquinone). It is formed from glucoside by hydrolysis followed by oxidation(Khattak et al.,2024)

6. Uses

Extracts from the bark and leaves of certain plants, such as *Juglans regia* L., are widely used in traditional medicine due to their important therapeutic properties. Studies have shown that these extracts possess antifungal activity, particularly against *Candida* species, which are responsible for various skin and mucosal infections. They have also been found effective in inhibiting the growth of bacteria involved in the formation of dental plaque, thereby helping to prevent tooth decay and gum inflammation. Moreover, some research suggests that these extracts may promote insulin secretion, making them potentially beneficial as a natural aid in regulating blood sugar levels in diabetic patients(**de Rigo.,2016**)

Chapter II

Wound and inflammation

I. Skin

The skin is the largest and most externally visible organ of the human body, comprising approximately 16% of the total body weight. It is composed of multiple layers, which collectively serve as a protective barrier against the external environment. In addition to protection, the skin fulfills several essential physiological functions. The medical field dedicated to the study and treatment of skin and its disorders is called dermatology.

1. Chemical Composition of the Skin (Average)

- 70% water
- 27.5% proteins
- 2% lipids
- 0.5% minerals and trace elements

2. Structurally, the skin is organized into three primary layers

- The epidermis (outermost layer)
- The dermis (middle supportive layer)
- The hypodermis (deepest layer, also known as the subcutaneous tissue)

(Tortora et al., 2017)

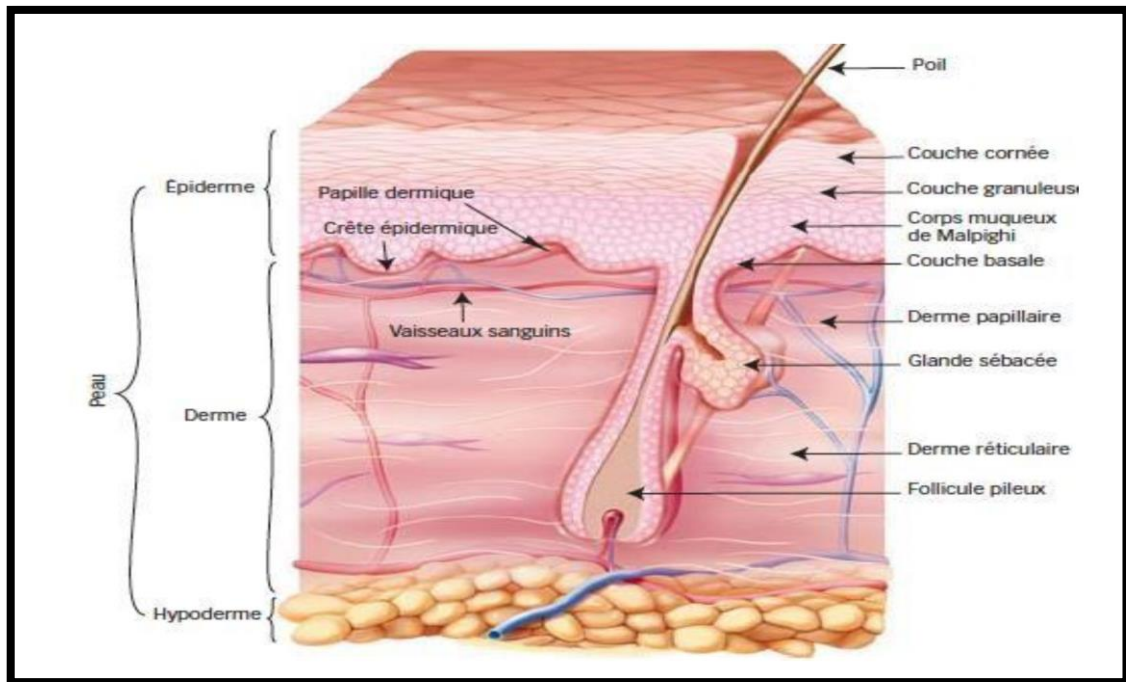


Figure 7 The document represents the components of the skin. (Moussaoui et al., 2020)

II. Wounds

1. Wounds Definition :

A wound is an injury that causes disruption or damage to the body's normal anatomical structure and function.

This damage can range from a minor break in the skin's outer layer to a deeper injury affecting subcutaneous tissues, potentially involving critical structures such as tendons, muscles, blood vessels, nerves, internal organs, and even bones.

Wounds may result from external or internal factors within the affected organ, and they may have accidental (e.g., trauma), intentional (e.g., surgery), or pathological origins.

Regardless of the cause or type, a wound leads to tissue damage and disturbs the local cellular environment. The body responds to this injury through a series of physiological processes, starting with bleeding, followed by blood vessel constriction, activation of coagulation pathways, complement system stimulation, and an inflammatory response.

A wound is defined as a disruption of normal anatomic structure and function" (Velnar et al., 2009)

2. Wound Classification

Wounds can be classified based on various clinical and pathological criteria. Accurate classification helps determine the optimal treatment strategy and predict healing outcomes. The main criteria include etiology, depth, contamination, and injury mechanism:

2.1. Etiology-Based Classification:

-Acute wounds result from a sudden injury and typically progress through the normal stages of healing without complications (**Guo & DiPietro, 2010**).

-Chronic wounds fail to proceed through an orderly and timely reparative process, often due to underlying conditions like diabetes or vascular diseases (**Velnar et al., 2009**).

2.2. Depth-Based Classification:

-Superficial wounds affect only the epidermis.

-Partial-thickness wounds involve both the epidermis and parts of the dermis.

-Full-thickness wounds extend through the entire dermis and may reach subcutaneous tissues, muscles, or bones (**Kumar et al., 2014**).

2.3. Contamination-Based Classification:

-Clean wounds are free of microbial contamination and are commonly observed in surgical incisions.

-Contaminated wounds contain microorganisms without overt signs of infection.

-Infected wounds present clinical signs of infection such as erythema, warmth, pain, swelling, and purulent discharge (**Guo & DiPietro, 2010**).

2.4. Mechanism-Based Classification:

-Incised wounds result from sharp objects like knives.

-Crush wounds are caused by compressive forces.

-Lacerated wounds result from blunt trauma with irregular tearing.

-Puncture wounds are due to sharp, pointed instruments.

-Bite wounds are inflicted by animals or humans and are prone to infection (**Velnar et al., 2009**).

3. The Mechanism of Wound Healing

The body has the ability to heal its tissues through two main processes: regeneration or repair. Regeneration involves replacing damaged tissues with identical cells, but this process is more limited than repair. In humans, full regeneration occurs only in certain types of cells, such as skin, liver, and nerve cells. Repair is the more common mechanism, where damaged tissue is replaced with connective tissue, which eventually forms a scar. Wound healing can be defined as the physiological process by which the body restores and recovers the function of damaged tissues.

4. Stages of Wound Healing

Wound healing occurs in four key, overlapping phases, during which the body works in a coordinated manner to repair the damaged tissue and restore its integrity:

a. Hemostasis Phase:

Timeframe: Immediately after injury

In this phase, the body responds quickly to stop bleeding:

Blood vessels constrict to reduce blood loss.

Platelets aggregate to form a clot that seals the wound.

Clotting factors are activated, laying the foundation for healing.

b. Inflammatory Phase:

Timeframe: A few hours to the third day

The body clears the wound of debris and pathogens:

White blood cells (initially neutrophils, later macrophages) migrate to the site.

Macrophages phagocytose bacteria and damaged cells.

Growth factors are released to initiate tissue repair

c. Proliferation Phase:

Timeframe: Day 3 to around the second week

New tissue begins to form to replace damaged areas:

Keratinocytes migrate across the wound to cover the surface.

Fibroblasts proliferate and produce collagen and extracellular matrix.

New blood vessels form (angiogenesis), creating granulation tissue to nourish the repair area.

d. Remodeling (Maturation) Phase:

Timeframe: From the third week to months (or even a year)

The tissue is reorganized for strength and functionality:

Temporary collagen is replaced with stronger type I collagen.

Tissue strength gradually increases (up to ~80% of normal skin strength).

A scar forms, which may lack skin appendages like hair follicles or glands. (Singer et al.,1999)

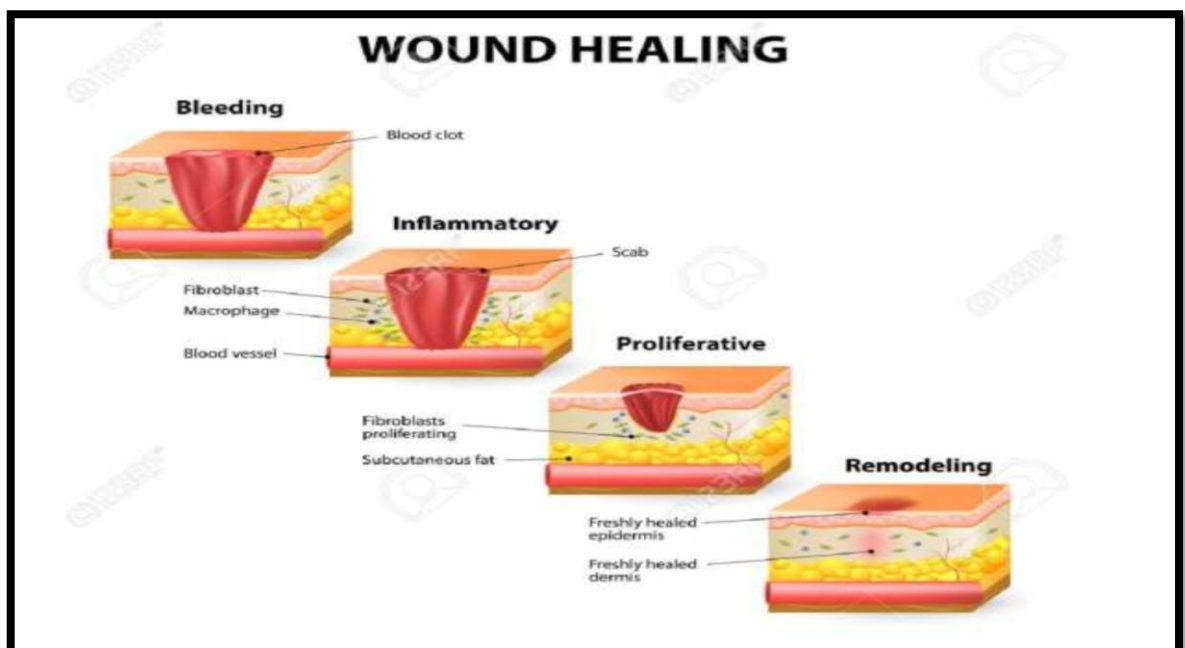


Figure 8 Stages of wound healing (Singer et al.,1999)

III. Inflammation

Inflammation is a well-organized and complex biological response of vascular tissues to harmful stimuli, and it constitutes a fundamental component of the body's immune defense mechanisms. This process is activated when white blood cells attempt to counter infectious agents such as bacteria and viruses. Inflammation may also result from physical injuries, such as muscle tears or strains during physical activity, often leading to localized symptoms including pain, swelling, and redness. Inflammation is generally classified into two types: acute and chronic. Each involves a series of biochemical reactions that activate the local vascular and immune systems, with the participation of various cell types within the affected tissues (Sá et al., 2013).

1. Types of Inflammation

According to the referenced academic document, inflammation is categorized into the following types.

a. Acute Inflammation.:

A rapid and short-term response (lasting a few days to weeks..

Characterized by redness, swelling, heat, pain, and loss of function.

- It occurs as an immediate response to injury or infection.

b. Chronic Inflammation:

A prolonged inflammatory response (lasting weeks to year

Often results from the failure of acute inflammation to resolve the issue or due to persistent immune activation.(Bedra ;et al 2024)

Second part

Experimental part

Chapter I

Material & Method

I. Material and Methods

1. Plant Materials

Clove (*Syzygium aromaticum*), thyme (*Thymus vulgaris*), saffron (*Crocus sativus*), desert miswak (*Juglans Regia*), coarse salt (sodium chloride), and alum (aluminum potassium sulfate) were obtained from certified herbalists. The botanical identity of the plant species was confirmed by experts in the Department of Biology at El-Oued University, officially named Université Echahid Hamma Lakhdar d'El Oued (Algeria).

- 1. coarse salt (sodium chloride)** Coarse salt (sodium chloride) possesses osmotic and antimicrobial properties that contribute to inhibiting microbial growth and reducing local inflammation, making it useful in traditional and topical therapeutic preparations (**Wijnker et al., 2006**).
- 2. alum (aluminum potassium sulfate)** Alum (Potassium aluminum sulfate) is widely known for its astringent, antiseptic, and hemostatic properties, making it valuable in traditional medicine for treating wounds and reducing local inflammation (**Olajide et al., 2012**).



Figure 9 Plant that make up the extract (original picture)

2. study by survey

a) Aim of the survey

Evaluation of the effectiveness of a plant extract formulated into a cream for treating inflammation and wound healing .

b) Description and Definition of the Study Area

The study area is located in the Wilaya of El Oued, one of the main oases in the northern Algerian Sahara. It includes the following areas : El Oued, Bayadha, Hassi Khalifa, Mih Ouensa, Ar rabah, Taghzout, Magrne, Kouinine, Debila and Guemar.

c) Administrative Boundaries of El Oued:

To the north: Tebessa and Biskra provinces

To the east: The Tunisian Republic

To the south: Ouargla province

To the west: Biskra and Ouargla provinces This area spans approximately 120,000 square kilometers, geographically bounded by the following coordinates:

Longitude: 06° 20' to 07° 50' East

Latitude: 32° 50' to 34° 30' North

The region is characterized by a dry desert climate, with extremely high summer temperatures reaching up

to 50°C and scarce annual rainfall ranging from 80 to 100 mm. The nights are notably cooler compared to other areas due to the extensive stretches of sand.

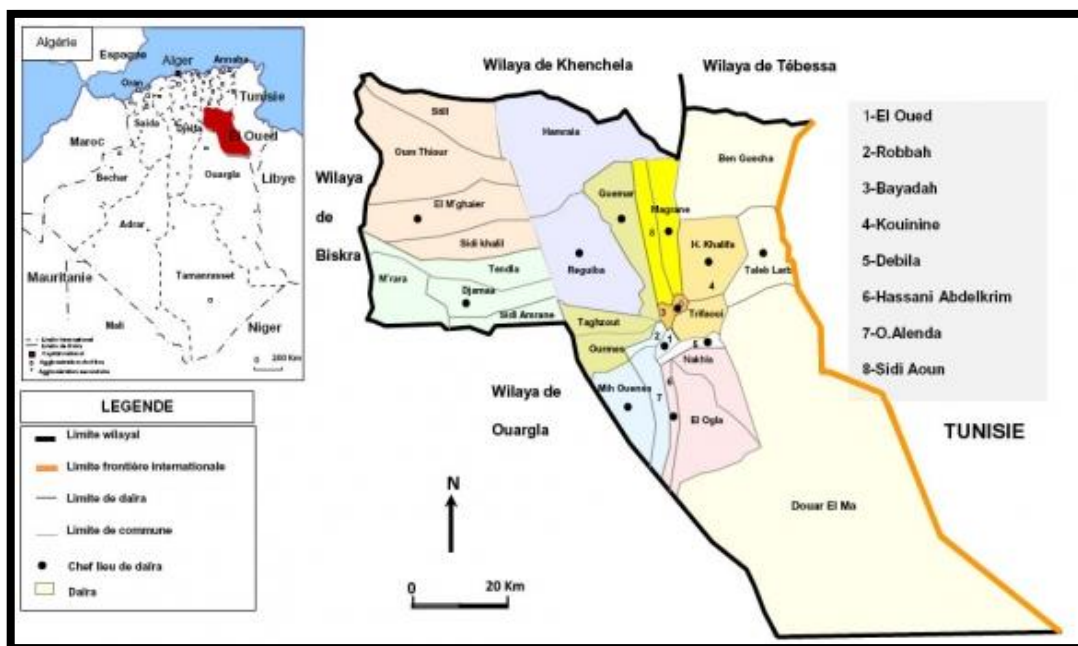


Figure 10 Carte du découpage administratif de la wilaya d'El Oued (Medarag ,H et al ;2019)

d) Study Population

This field study was conducted as part of a graduation dissertation focused on a traditional plant-based extract (Zassc extract) with potential anti-inflammatory properties. The survey was carried out across several region within the Wilaya of El Oued, selected as a representative sample. The questionnaire targeted various segments of the population, including users, traditional practitioners, vendors, and even individuals who do not use traditional remedies, in order to gather comprehensive data on the knowledge, practices, and perceptions related to the use of the plant extract in traditional medicine. The survey was carefully designed and distributed in to ensure broad participation to ensure broad participation. The distribution of respondents and analysis of their answers will be detailed in the Results and Discussion section. (Boudebia2023)

e) User Section

The user section consists of 71 questions, divided into two main groups:

1. Personal Information – This part collects basic demographic and personal details about the user.
2. Active Compounds and Usage – This part covers information about the most important active compounds for each plant, usage methods, and the diseases they can treat.

Each interview is designed to take approximately 25 minutes.

f) Survey Process

Survey Process Before the start of the survey, the finalized questionnaire was tested on around twenty people in order to: Ensure that the questions were clear and unambiguous; Confirm, support, and enrich the questionnaire, especially to clarify it; Eliminate some unnecessary questions; Add or remove some options in the case of multiple-choice questions; Rephrase and modify incomprehensible questions. Our field study was conducted from November to May 2025. The online questionnaire was distributed through social media, while the paper questionnaire was administered directly to the target individuals.

Difficulties Encountered During the Field Survey:

One of the main difficulties encountered during the field survey was the reluctance of some individuals to answer the questions posed. We observed a clear hesitation from several local residents, particularly when discussing plants with traditional uses or those associated with popular beliefs, which made it challenging to collect complete and accurate information. Another difficulty we faced was the limited availability of certain native plants in the study area. Specifically, obtaining authentic saffron was difficult, despite its presence in some areas, as it was extremely rare in the locations visited. This scarcity of samples affected the process of field data collection.

3. Animals

Preparing the rats for the Experiment: A group of female rats was acquired from the Pasteur Institute, with initial weights ranging between 110 and 120 grams. The mice were housed in suitable conditions in the animal facility of the institute, where they were provided with regular natural lighting, a suitable temperature (between 22 and 25°C), good ventilation, and a balanced diet and clean drinking water available ad libitum. The adaptation period lasted for 15 day, during which the mice's health and behavior were regularly monitored until they reached a weight between 160 and 200 grams, the ideal

weight for the experiment. After ensuring their health was stable and free of any apparent disease, experimental incisions were performed on 15 rats, according to a standardized protocol that respects ethical considerations and scientific standards for animal experimentation. The

incisions were performed under sterile conditions using sterile surgical instruments, ensuring minimal pain through appropriate anesthesia.

4. Preparation of Raw Materials:

All components were thoroughly washed with tap water followed by rinsing with distilled water. Specific parts of each plant were selected as follows: Clove: Only the dried flower buds (heads) were used. Thyme: The upper flowering part (capitulum) was used. Saffron: Dried stigma threads were used. Desert miswak: Cut into small pieces without grinding. Coarse salt and alum were lightly crushed to facilitate dissolution. The components were weighed in the following proportions (adjustable based on preliminary testing): Clove heads: 0,1430 g Thyme capitula: 0,1366 g Saffron threads: 0,3214g Desert miswak pieces (*Juglans Regia*):0,2030 g Coarse salt: 0,105g Alum:0,105 g

5. Extraction Method (Aqueous Decoction):

The entire mixture was added to 200 mL of boiling distilled water in a glass beaker. The mixture was gently boiled for 10 minutes with occasional stirring. After boiling, it was allowed to cool at room temperature for 1 hour. The extract was first filtered through sterile gauze, then through Whatman filter paper to remove solid residues. The filtrate (aqueous extract) was collected in sterile dark glass bottles.

The final extract was stored at 4°C in a refrigerator until use in biological experiments.

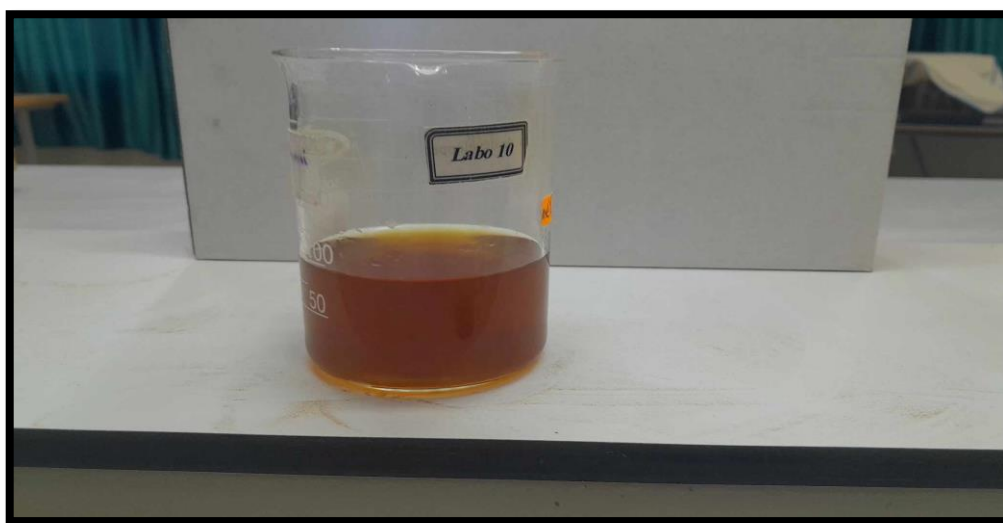


Figure 11 Final form of the plant extract (original picture)

6. Preparation of the Experimental Cream:

A therapeutic cream was prepared consisting of two phases: Aqueous phase: Contained a water-based extract of medicinal plants known for their anti-inflammatory and tissue-regenerating properties. Oily phase: Composed of sweet almond oil, which is recognized for its moisturizing and nourishing effects on the skin. The two phases were emulsified using Emulsifying Wax E, resulting in a stable oil-in-water (O/W) emulsion. Vitamin E was added for its role in promoting cellular and tissue regeneration, along with sodium citrate, which may act as an antioxidant or pH regulator. Regarding storage conditions, it recommend storing the cream in a cool, dry place, away from direct sunlight, based on the nature of its ingredients

6.1. Physical Analyzes of Formulated Cream

- a) **pH Test.** The pH measurement of the cream is measured by diluting a quantity of the samples with water, then filtered with Watman N°4 filter paper. The pH analysis is carried out at a temperature of 20 °C. using a pH meter (**HANNA-HI 8424**).
- b) **Stability Test.** This test was carried out on the sample after centrifugation at 3000 rpm, for 30 min and at room temperature. The degree of separation of the two phases is expressed by the total percentage of stability, ie (100 = stable, 0 = unstable). The cream demonstrated a shelf life of more than 12 months, which indicates its stability over an extended period. This was confirmed through preliminary stability tests, where the cream maintained its consistency, pH, and effectiveness without any signs of phase separation or ingredient degradation during this period. .(**Benine ,2025**)

7. In vivo study:

7.1. Animals and experimental batches

A total of 20 adult female *Rattus norvegicus* (weighing approximately 160–200 g) were used in this study. The animals were housed under standard laboratory conditions (temperature 22±2°C, 12/12 h light/dark cycle, with free access to food and water) for an acclimatization period of 15 days.

The rats were randomly divided into four groups (n = 5 per group)

Control group (T0): Received no treatment

Test group (T): Treated with a standard wound healing cream

Negative control group (T-): base cream treatment was applied after wounding

Positive control MEBO group (T+): Treated with the commercial MEBO cream

After the 15 day adaptation period, a circular excisional wound (2 mm in diameter) was created on the dorsal region of each rat using a sterile scalpel under anesthesia. Treatment began immediately after the injury and continued daily for a defined period

A. Anesthetics and Preparation

A formulated MEBO-based cream.

70% ethanol for skin disinfection prior to wound induction. Sterile cotton for cleaning and disinfecting the skin surface. Sterile surgical instruments, including a scalpel, scissors, and forceps. Sterile gloves to maintain aseptic working conditions.

An analytical balance for precise weighing of substances and samples.

A digital camera for daily photographic documentation of wound progression.

A precision ruler or digital caliper for accurate wound size measurement.

A computer equipped with ImageJ software for wound image analysis and surface area calculation. Individual cages for housing the rats separately, to prevent interference and ensure reliable results.

Chloroform – for inhalation anesthesia..

Glass container with a lid – for safely holding the mouse and chloroform.

Hair removal device – for removing hair from the wound area.

Antiseptic solution (such as Betadine or 70% alcohol) – for sterilizing the skin before incision.

B. Incisional Instruments

Sterile scalpel – for making the incision.

Small surgical scissors – for cutting or adjusting.

Surgical forceps – for helping to hold the skin in place.

Sterile gauze – for cleaning the wound or stopping minor bleeding.

7.1.1. Mechanism of incision of 15 mice under stable conditions

A. Preparation and Anesthesia:

Chloroform was used to anesthetize the mice via inhalation (in a sealed glass container with a saturated cotton swab).

The researcher ensured that the mouse reached the appropriate level of anesthesia (loss of response to touch or pinching).

The hair on the back was removed using an epilator (not shaving) to provide a clear working area.

B. Sterilization and preparation of the wound site:

The area was sterilized using an antiseptic solution such as alcohol or betadine.

The mouse was placed in a suitable position (ventral prone) on a sterile surface.

C. Incision Execution:

Using a sterile scalpel, an incision of a fixed length and thickness (e.g., 1 cm long x 2 mm deep) was made in the back of each mouse .

The wound was not sutured, as the goal was to study the effect on extract plant (Zassc extract) inflammatory

The rats underwent treatment for 14 days, during which wound dimensions were regularly measured, alongside the provision of daily care and monitoring of their behavioral responses to the applied cream.

7.1.2. Sacrifice of Rats and Collection of Blood and Tissues

After a fasting period of 16 hours, the rats were anesthetized using chloroform at a concentration of 94%, followed by humane sacrifice. Blood samples were collected immediately at the time of sacrifice: part of the blood was transferred into EDTA-containing tubes for hematological analyses, and another part into dry tubes for biochemical assays. Following dissection, the spleen, thymus gland, and a piece of skin at the site of the wound were carefully excised. The tissues were first rinsed with distilled water to remove excess blood, followed by washing with physiological saline solution (0.9% NaCl). The samples were then gently dried using sterile filter paper and accurately weighed. Portions of the collected tissues

were used for the preparation of fresh homogenates, while other portions were preserved appropriately for histological processing. The tissue homogenates were utilized for the evaluation of oxidative stress markers, including the measurement of malondialdehyde (MDA) levels, reduced glutathione (GSH) content, and the activities of superoxide dismutase (SOD) and catalase (CAT). (Boudabia ,2023)

8. In Vitro Bioassays

8.1. Hematological study

Hematological and Biochemical Assessment On day 15 post-treatment and wound closure, blood samples were collected from all experimental groups for a comprehensive evaluation of hematological and biochemical parameters. The analyses included a complete blood count (CBC) to determine the main cellular components of blood, and C-reactive protein (CRP) levels to assess the systemic inflammatory response. Additionally, the erythrocyte sedimentation rate (VS) was measured, and antistreptolysin O (ASLO) titers were determined as indicators of streptococcal infection or immune activity. Blood glucose levels (glycemia) were also measured to evaluate potential metabolic changes associated with the treatment. (Boughezala, 2023)

8.2. 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

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

8.3.1. Overview

Protein denaturation is a key process in inflammatory disorders (Farooq et al., 2025), making BSA denaturation inhibition an effective model for assessing anti-inflammatory potential (Smati et al., 2025). This assay evaluates the ability of ZASSC extracts to prevent protein denaturation, which may indicate their therapeutic potential for inflammation-related diseases.

8.3.2. Chemicals and Reagents

- Bovine serum albumin (BSA, 5% w/v) from Sigma-Aldrich
- deionized water Plant extracts (0.1–1 mg/mL)
- Diclofenac sodium (positive control, anti-inflammatory drug)

8.3.3. Procedure

In the present study, the assay was conducted following established methodologies (Gangadharan et al., 2025) with slight modifications to optimize experimental conditions. The reaction mixture was prepared by combining 500 μ L of a 5% BSA solution with 250 μ L of the test sample at varying concentrations (100 – 1000 μ g/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 500 μ L with deionized water before measuring their absorbance at 660 nm using a UV-Vis spectrophotometer. Blank was prepared with mixing: 1mL water + 250 μ L DMSO. The percentage inhibition of protein denaturation was calculated using the Equation 4:

$$\% \text{ Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \quad (1)$$

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 \pm standard deviation (SD).

8.4. Stress parameters

➤ Homogenate Preparation and Oxidative Stress Marker Assessment:

One gram of tissue from the skin, spleen, and thymus was collected from each experimental animal in the studied groups. The tissues were homogenized in Tris-buffered saline (TBS) consisting of 50 mM Tris and 150 mM sodium chloride at pH 7.4. The resulting homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was immediately preserved on ice for subsequent analysis of oxidative stress markers.

➤ Determination of Total Protein Content:

Protein concentration in the supernatants was measured using the Bradford method (Bradford, 1976), based on absorbance at 595 nm, with bovine serum albumin (BSA) used as a standard. The Bradford reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of ethanol, followed by mechanical stirring for 2 hours. Then, 850 mL of distilled water and 100 mL of phosphoric acid were added to make up a final volume of 1 liter. The reagent was filtered using Whatman filter paper.

For the assay, 40 μ L of the sample or standard was added to 2 mL of Bradford reagent in dark tubes. After 5 minutes of incubation at room temperature, absorbance was measured at 595 nm. Protein concentrations were determined by comparing the readings to a standard curve prepared under the same conditions using BSA. (**Boudebia , 2023**) .

➤ Determination of Malondialdehyde (MDA) Concentration:

The concentration of malondialdehyde (MDA) was determined following the method described by Quintanilla (1981), which is based on the reaction between MDA and thiobarbituric acid (TBA). Briefly, 1 mL of the diluted tissue homogenate was mixed with 20 μ L of 2% (w/v) ethanolic butylated hydroxytoluene (BHT) to prevent lipid oxidation. Then, 2 mL of MDA reagent was added, consisting of 15% (w/v) trichloroacetic acid (TCA), 0.375% (w/v) TBA, and 0.25 N hydrochloric acid (HCl).

The mixture was incubated in a boiling water bath for 15 minutes to promote the MDA–TBA adduct formation, then cooled to room temperature and centrifuged to remove precipitates. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer.

MDA concentration was calculated in nanomoles per milligram of protein using the following equation:

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

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 .(**Boudebia , 2023**)

➤ Determination of Reduced Glutathione (GSH) Concentration

The concentration of reduced glutathione (GSH) was determined based on the formation of a yellow-colored chromophore upon reaction of sulfhydryl (-SH) groups with 5,5'-dithiobis-

(2-nitrobenzoic acid) (DTNB). Specifically, 0.8 ml of tissue homogenate was mixed with 0.2 ml of 0.25% sulfosalicylic acid, vortexed thoroughly, and incubated at 4 °C for 15 minutes. The mixture was then centrifuged at 1000 rpm for 5 minutes. For the assay, 0.5 ml of the resulting supernatant was combined with 1 ml of Tris-buffered saline (TBS, pH 7.4) and 0.025 ml of 0.01 M DTNB solution. After 5 minutes of incubation at room temperature, the absorbance was measured at 412 nm using a spectrophotometer. The total GSH content was calculated and expressed as nanomoles of GSH per milligram of protein (nmol GSH/mg protein) using the following equation, as described by Weckbecker and Cory (1988):

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

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 .(**Boudebia , 2023**)

➤ Determination of Superoxide Dismutase (SOD) Activity

The activity of superoxide dismutase (SOD) was determined based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) by superoxide anions ($\text{O}_2^{\cdot-}$), as originally described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 500 μL of EDTA-methionine solution, 900 μL of phosphate buffer (pH 7.8, 25 μL of the sample, and 50 μL of NBT solution. This mixture was incubated in a water bath at 25 °C for 5 minutes.

Subsequently, 25 μL of riboflavin solution was added to initiate the reaction. The mixture was then exposed to light for 20 minutes to allow the generation of superoxide radicals. A control sample was prepared using the same procedure, with the exception that the 25 μL of sample was replaced by an equal volume of phosphate buffer.

Following light exposure, the absorbance was measured at 560 nm. The percentage of inhibition of NBT reduction by SOD in the sample was calculated using the following equation:

$$\text{I\%} = (\text{ODC} - \text{ODS}) / \text{ODC} \times 100$$

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. .(**Boudebia , 2023**)

Determination of Catalase Activity:

Catalase activity was assessed following the method described by Aebi (1984). The reaction was initiated by combining 20 μ L of the supernatant with 780 μ L of phosphate buffer (KH_2PO_4 , 0.1 M, pH 7.5), followed by the addition of 200 μ L of hydrogen peroxide (H_2O_2) at a concentration of 0.030 M. The decomposition of H_2O_2 was monitored by measuring the decrease in absorbance at 240 nm at 30-second intervals over a period of 2 minutes. Enzymatic activity was expressed in international units per minute per gram of protein (IU/min/g protein) using the following formula:

$$\text{Catalase ((UI/min)/g)} = (2.3033 / T) \times (\log A1 / A2) / (DF \times \text{g prot}$$

Where: **A1**: Absorbance at the first minute, **A2**: Absorbance at the second minute, **T**:Time interval in minutes. .(**Boudebia , 2023**)

8.5. Statistical Analysis

Data were statistically analyzed using one-way analysis of variance (One-way ANOVA) to compare the means among more than two groups. Results are expressed as mean \pm standard deviation (SD), based on six replicates per group. The analysis was performed using SPSS software (version 26) and Microsoft Excel (version 2010). A significance level of $\alpha = 0.05$ was set, and differences were considered statistically significant when the P-value was less than 0.05, leading to the rejection of the null hypothesis of equal means among groups

8.6. Histological study

Skin tissue samples were collected from the wound sites of all Wistar albino rats to prepare histological sections for monitoring the stages of wound healing. The excised skin fragments were previously fixed in 10% neutral buffered formalin, placed into tissue cassettes, and then processed using an automated tissue processor. The histological preparation involved gradual dehydration of the samples by immersion in ethanol solutions of increasing concentrations (60%, 70%, 80%, and 100%), followed by two xylene baths to remove residual ethanol and facilitate paraffin infiltration, as xylene is hydrophobic. Subsequently, the samples were immersed in two baths of melted paraffin for 24 hours. The tissues were then embedded in metallic molds using an embedding device and allowed to cool, forming solid blocks ready for

sectioning. The paraffin blocks were sectioned using a microtome to obtain 3 µm-thick slices. Using fine forceps, the sections were transferred to glass slides. Paraffin was removed by placing the slides in an oven at an appropriate temperature for 20 minutes, preparing them for rehydration and staining. To visualize the skin layers, the sections were rehydrated gradually by immersion in a xylene bath for 5 minutes, followed by 5 minutes in ethanol, and rinsed in distilled water for 5 minutes. The rehydrated sections were then stained with hematoxylin for 5–6 minutes to highlight cell nuclei. Excess stain was removed by washing with water containing a few drops of ammonium hydroxide (NH₄OH). The sections were then immersed in eosin for 5 minutes to stain the cytoplasm, and excess dye was removed using ethanol. Finally, the slides were covered with cover slips, making them ready for microscopic examination. (**Benine, 2025**)

Chapter II

Results & Discussion

I. Results

1. Results of Survey

1.1. Statistical study

1.1.1. Description of the study community

The survey was conducted across 14 municipalities in the state of El Oued, with a total population of 99 people from various categories, as shown in the table below.

The data shows that the municipality of Rabah recorded the largest percentage of participants, at 21% (21 participants), followed by the municipality of Hassi Khalifa at 15% (15 participants), then El Oued at 13% (13 participants). On the other hand, there are municipalities such as Hassani Abdelkrim, Trifawi, and outside the state of El Oued, which recorded low participation rates of only 1% (one participant each). It is also noted that the remaining municipalities were distributed in varying percentages, as the percentages ranged between 3% and 11%.

Table 1 Distribution of the surveyed population by municipality

Municipality	Frequency	Percentage (%)
Bayadha	3	3
Debila	3	3
Robbah	21	21
Reguiba	3	3
Zorg	6	6
Magrane	7	7
Nakhla	7	7
El Oued	13	13
Mih Ouansa	11	11

Taghzout	4	4
Hassi Khalifa	15	15
Hassani Abdelkrim	1	1
Trifaoui	1	1
Guemmar	4	4
Outside El Oued Province	1	1

1.1.2. Description of study population

The results showed that the number of female participants was greater than that of males, with females accounting for 64% and males for 36%. The predominant age group within the sample was 18–under 25 years, representing 63% of the total participants. This was followed by 25–under 35 years at 18%, 35–under 45 years at 8%, and 45–under 55 years at 6%. The age group 55 years and older represented only 5%. The educational level of the sample was university, accounting for 78% (78 individuals out of 100). This was followed by secondary education at 12%, and then postgraduate studies at 5%. The lowest percentages were primary and intermediate education, at 3% and 2%, respectively.

Table 2 socio-demographic characteristics of the people surveyed

Characteristic	Number of respondents	P%
Sex		
Men	36	36
Women	64	64
Age		
18-25 years	63	63
25-35 years	18	18
35-45 years	8	8

45-55 years	6	6
55 years and above	5	5
Educational Level		
Elementary Education	3	3
Middle school Education	2	2
Secondary Education	12	12
University Education	78	78
Postgraduate Studies	5	5

1.1.3. Have you ever used plants for health purposes?

The data showed that 77% of participants had previously used the miswak for health purposes such as oral or eye care, while 23% responded that they had not. The results indicate that the vast majority of participants had previous experience using the miswak in this health context. As for thyme, 87% had previously used it in cooking or medicine. In contrast, only a small percentage (13%) had never used it. These percentages represent a clear indication of the prevalence of thyme in the daily lives of the respondents. Regarding saffron, 69% of respondents had previously used or consumed it in their food or natural remedies, while 31% had never used saffron. As for those who had previously used cloves topically or internally (such as drinking it or using it as oil), 82% had previously used it, while only 18% had never used it.

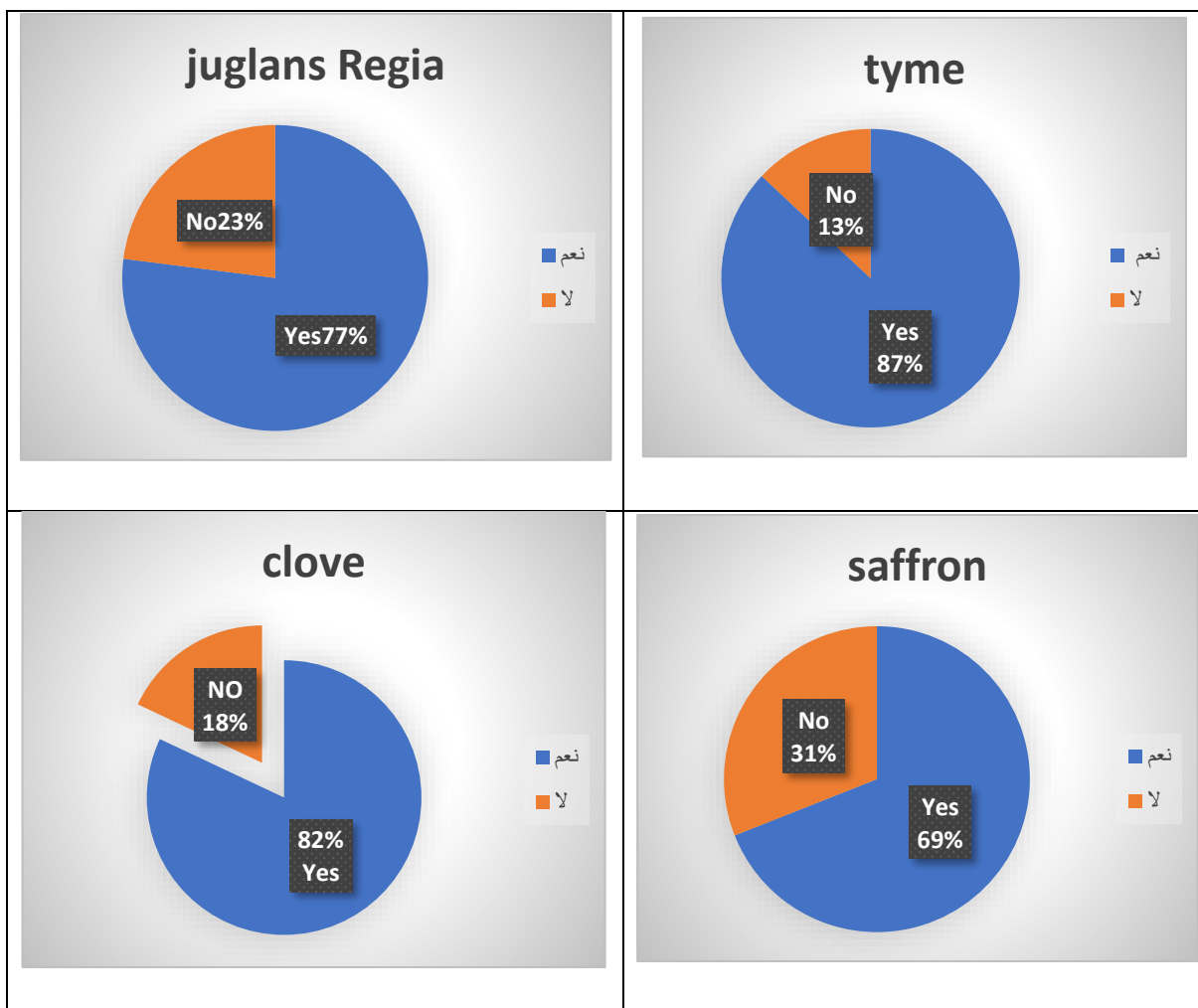
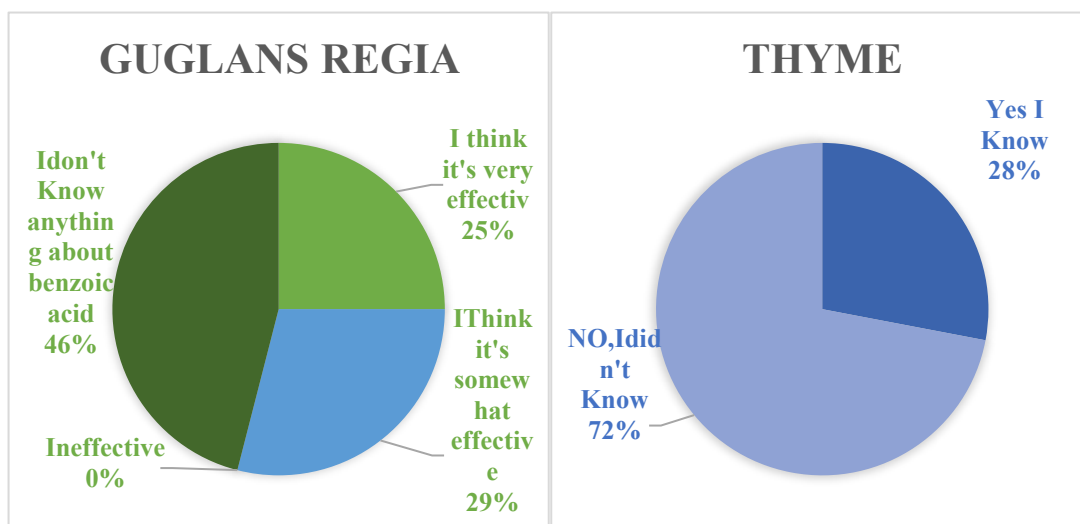


Figure 12 Using plants for health purposes

1.1.4. How much do you know about the effectiveness of plants?

The following statistical study revealed that 80% of the public is unaware of the active ingredients in saffron, while only 20% confirm that it contains a substance known for its positive effect on the retina and eye protection. Seventy-two percent are unaware of thyme, while 28% are aware of its thymol compound (known for its antibacterial and antifungal



properties). Forty-six percent are unaware of the effectiveness of benzoic acid in the miswak as an antimicrobial and antibacterial agent, followed by 29% who confirm it is somewhat effective, while 25% believe it is very effective. Forty-nine percent believe that eugenol in cloves is safe for use in the eyes, while 41% confirm they are unaware of its effectiveness. Eight percent confirm it is very effective, while only 2% believe it is safe.

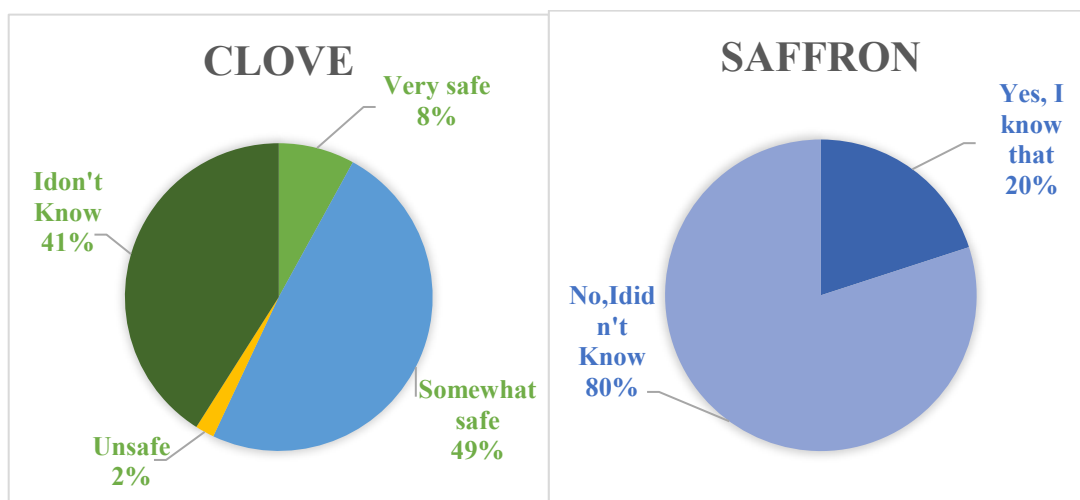


Figure 13 The effectiveness of the substances contained in plants

1.1.5. Do you have any previous experience using salt and alum?

It indicates that 79% of participants had previous experience using salt for health purposes, while 21% reported no previous experience. The results reflect the widespread use of salt for health purposes among the sample studied, such as sterilizing eyes or cleaning wounds. Regarding alum, 93% of participants answered "yes," indicating they had previous experience using alum in various products. In contrast, 7% had no previous experience. These results reflect the widespread use of alum in traditional health and beauty applications, which rely on natural products for personal care and treatment.

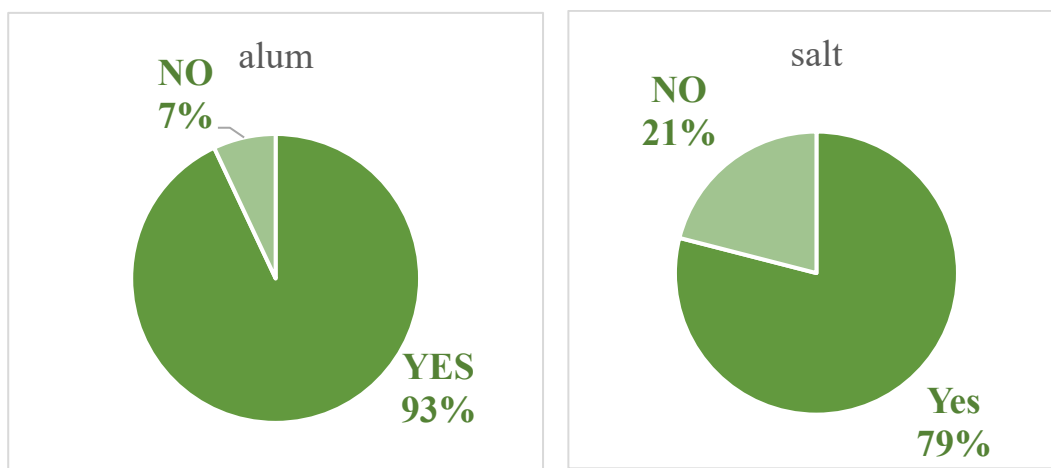


Figure 14 Uses of alum and salt for health purposes

1.1.6. What are your biggest concerns that might make you hesitant to use a natural eye drop containing these ingredients?

The results illustrate the relative and absolute distribution of participants' responses to the three concerns raised. It is clear that potential side effects were the greatest concern for the majority of participants, at 67%. This was followed by the lack of sufficient scientific studies, at 34%, while the least significant concern was the lack of product effectiveness, at 15%. These results clearly indicate that potential consumers of natural eye drops containing these ingredients place safety at the forefront of their priorities. Fear of potential side effects is a significant barrier to adoption of this type of product.

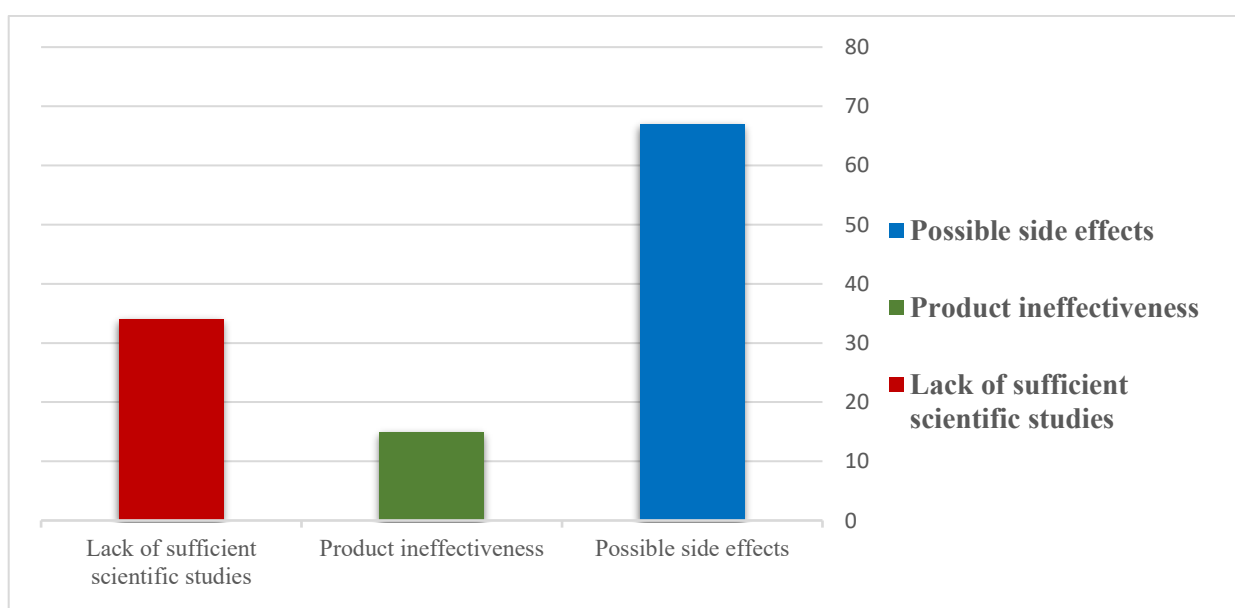


Figure 15 Concerns associated with hesitation in using natural eye drops containing these ingredients.

1.1.7. In your opinion, how can the development of natural therapeutic products for eye treatment be improved?

Increasing scientific research on active ingredients This factor received the highest support at 54%, indicating that the majority of participants believe in the importance of strengthening the scientific foundations for developing these products. Using advanced extraction techniques This factor ranked second at 23%, reflecting a significant portion of participants' awareness of the role of modern technologies in improving the quality and efficacy of natural extracts. Improving formulation and concentration This factor received 13%, representing the lowest support among the proposed factors. This may indicate that participants believe the current focus on raw ingredients or extraction methods is more important. Ensuring safety through laboratory testing This factor received 10% support, underscoring the importance participants place on ensuring the safety of natural therapeutic products through reliable laboratory testing

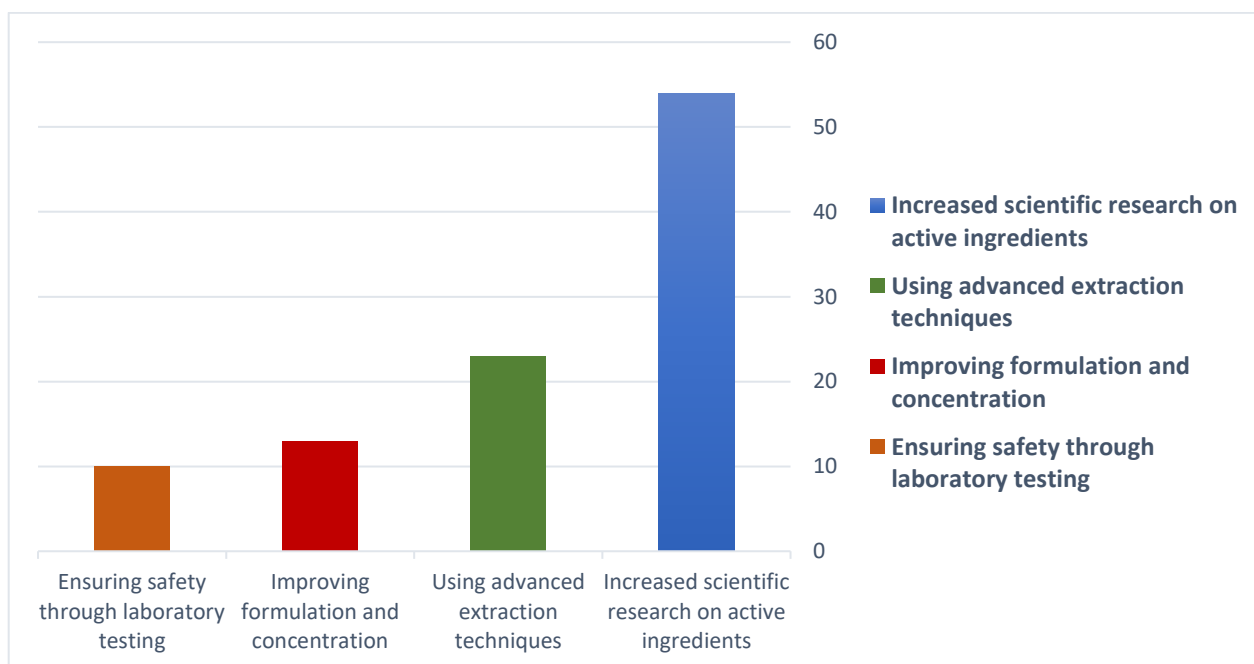


Figure 16 Scientific foundations and methodologies used in developing natural therapeutic products for eye diseases

1.2. Physical Analyzes of Formulated Cream

Table 2 presents the results of the physical analyses of the formulated cream used in this study. The pH value was measured at 25°C using a pH meter (HANNA-HI 8424). The phase separation percentage was assessed as an indicator of the physical stability of the formulation, where a value of 100% denotes complete stability, and 0% indicates total instability. The results show that the cream is characterized by a Light yellow color and the absence of any odor,

indicating that it is free from added fragrances. This makes it suitable for individuals with sensitivities to scented products. The cream exhibits a "moist" consistency, reflecting its high moisture content and potential to provide effective hydration and comfort upon application. The pH of the cream was recorded at 4.93, placing it within the mildly acidic range, which is physiologically compatible with the skin's natural pH and helps minimize the risk of irritation. Furthermore, the cream demonstrated excellent physical stability, with a recorded stability value of 100%.

These properties indicate that the cream is robust and resistant to degradation over time, which supports its sustained efficacy and prolonged shelf life. Collectively, these characteristics make the cream well-suited for dermatological applications, offering effective hydration, user comfort, and consistent stability for routine use.

Table 3 Physical Analyzes of Formulated Cream.

Color	Odor	Texture	pH	Stability
Light yellow	No	Humid	4,93	100

1.3. Wound Healing Activity

Wounds are considered a major health issue due to the burden they place on morbidity and mortality rates, making the acceleration of their healing process essential for restoring the anatomical and functional integrity of the skin. Effective wound healing requires rapid wound contraction, accelerated re-epithelialization, and adequate recovery of skin tensile strength. In this context, herbal preparations are emerging as an increasingly important therapeutic option within healthcare systems, owing to their diverse biological and medicinal properties. The findings of the present study, which employed an animal model to evaluate the efficacy of various topical formulations, revealed significant differences in healing rates depending on the active ingredient content of each formulation. Based on clinical visual monitoring, ZASSC CREAM demonstrated clear effectiveness starting from day six, achieving near-complete healing by day fourteen. It outperformed both MEBO cream, which showed slower, more gradual improvement, and the cream base, which exhibited the weakest response. This observation is supported by quantitative data on wound healing rates measured periodically on days 1, 3, 5, 7, 9, 11, 13, and 14. ZASSC CREAM (orange) recorded the highest healing rate at 79.12% on day fourteen, followed by the cream base (gray) at 73.14%, and MEBO (blue) at

70.21%. It is noteworthy that most formulations showed marked improvement after day seven, indicating the onset of the active phase of cellular regeneration. These findings underscore the importance of pharmaceutical composition and the type of active ingredients in determining the effectiveness of topical preparations. They clearly confirm that formulations rich in bioactive or anti-inflammatory agents make a substantial difference in accelerating tissue repair.

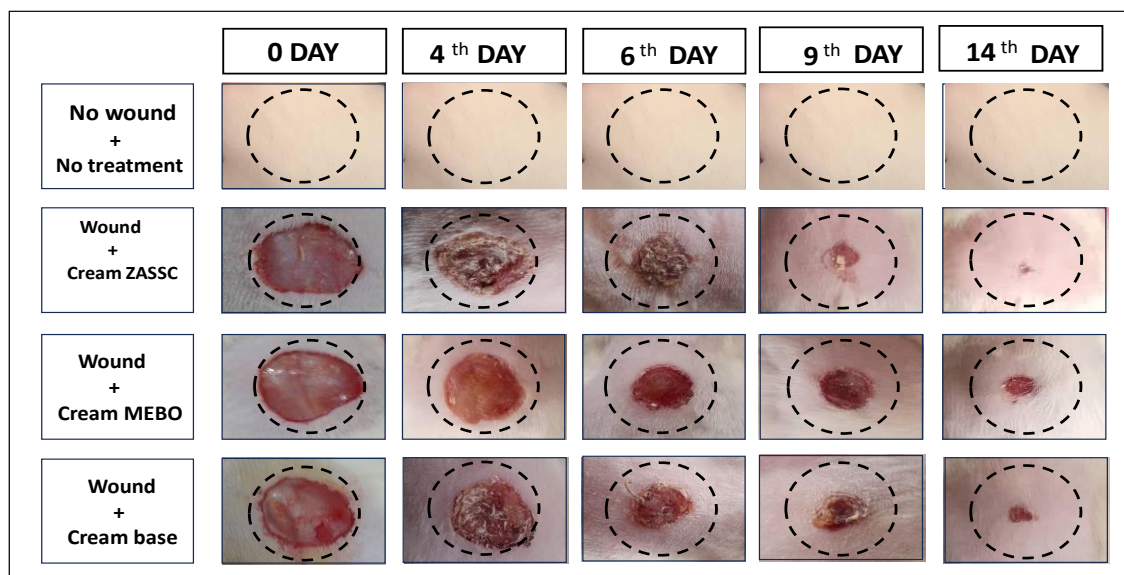


Figure 17 Time schedule of the experiment in vivo wound healing activity of different cream treatment formulations; n =5

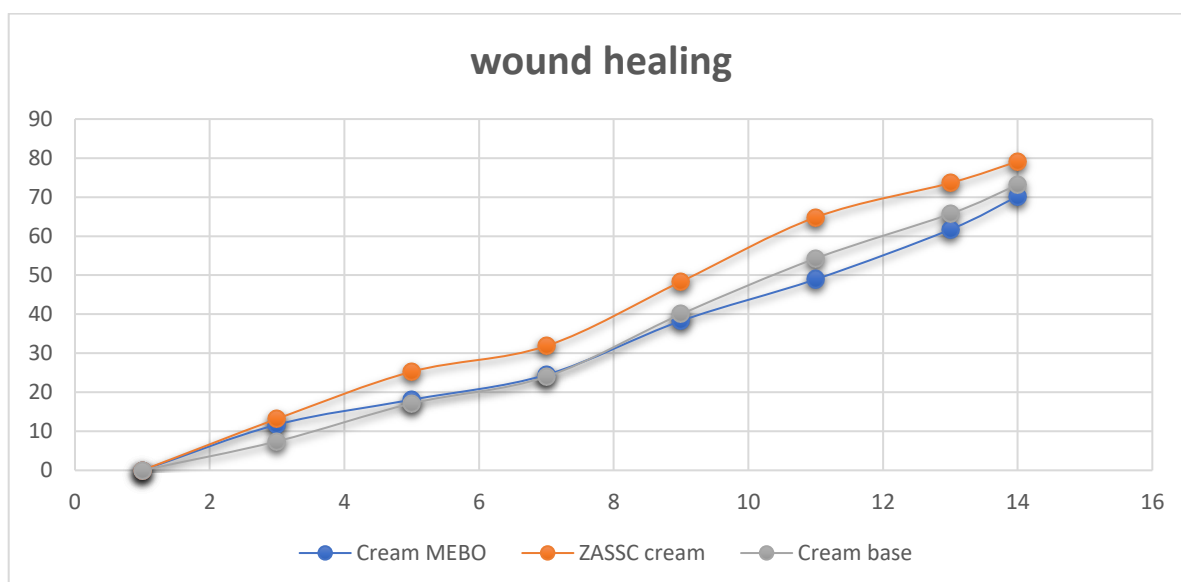


Figure 18 Wound healing rate at day 4, 7, 10, and 14 in vivo treatment of different cream treatment formulations; n = 5

1.4. In vivo anti-wound study

1.4.1. Organ weight index

The spleen and thymus indices showed notable alterations in response to wound induction and subsequent treatment. Compared to the control group, the Base and MEBO treatments significantly increased both spleen and thymus indices ($p < 0.05$), suggesting an immunomodulatory effect potentially linked to local or systemic inflammatory responses. Specifically, MEBO treatment led to further elevation in the thymus index ($p < 0.01$), which may reflect immune activation or lymphoid proliferation. Interestingly, the ZASSC (plant-based ointment) group exhibited spleen and thymus indices that did not significantly differ from the control group but showed significance when compared to the Base treatment ($p < 0.05$ and $p < 0.01$, respectively), suggesting a more balanced or less stimulatory immune response. These findings imply that while MEBO and the base ointment enhance immune organ activity, the plant-based formulation may offer a more regulated immunological profile during wound healing.

Table 4 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 + ZASSC	0.24±0.06 ^{NS*}	0.15±0.06 ^{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: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

1.4.2. Hematological parameters

Wound induction and treatment significantly influenced hematological profiles. The Base and MEBO groups showed marked leukocytosis, particularly elevated WBC, lymphocyte (LYM), and granulocyte (GRA) counts ($p < 0.01$ to $p < 0.001$), reflecting a robust inflammatory response. Notably, the Base group displayed the highest PLT count (976 ± 17 ; $p < 0.001$ vs. control), likely due to inflammation-induced thrombopoiesis. In contrast, the ZASSC group

demonstrated a significantly lower WBC count than control ($p < 0.05$) and MEBO ($p < 0.01$), along with moderated granulocyte levels, suggesting a reduced systemic inflammatory burden. Hemoglobin (HGB) and RBC levels remained stable across groups, indicating that erythropoiesis was not significantly impacted. These data collectively suggest that while the Base and MEBO treatments activate immune responses, the ZASSC formulation may exert anti-inflammatory or immunoregulatory effects beneficial for wound healing without excessive immune activation.

Table 5 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	3.86 \pm 0.14	1.1 \pm 0.22	0.13 \pm 0.06	14.26 \pm 0.88	7.8 \pm 0.17	716 \pm 29
Wound + Base	6.33 \pm 0.17 ^b	4.2 \pm 0.1 ^a	0.96 \pm 0.08 ^c NS	14.24 \pm 0.31 ^{NS}	7.9 \pm 0.24 ^{NS}	976 \pm 17 ^c
Wound + MEBO	5.68 \pm 0.11 ^{NS}	3.83 \pm 0.24 ^a NS	1.4 \pm 0.09 ^c NS	14.14 \pm 0.22 ^{NS}	7.7 \pm 0.14 ^{NS}	856.3 \pm 47 ^a NS
Wound + ZASSC	2.21 \pm 0.25 ^a **	1.6 \pm 0.26 NS**	0.41 \pm 0.05 NS*	14.16 \pm 0.16 ^{NS}	6.7 \pm 0.45 ^{NS}	816.67 \pm 55 ^b *

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

1.4.3. Biochemical parameters

Metabolic and inflammatory markers revealed important differences among treatment groups. Fasting blood glucose (FBS) levels remained statistically unchanged across all groups, indicating no significant systemic metabolic stress. However, inflammatory markers such as CRP, ASLO, and ESR were markedly elevated in the Base and MEBO groups, with CRP reaching 3.28 U/L in the MEBO group ($p < 0.01$ vs. control and Base), and ESR significantly increased in the Base group ($p < 0.001$). These elevations highlight a strong systemic inflammatory response. In contrast, the ZASSC group showed CRP and ESR levels close to those of the control group, with ASLO even lower than in controls, suggesting an anti-

inflammatory or inflammation-modulating effect. These findings further support the potential of the ZASSC formulation to promote wound healing while minimizing systemic inflammation and associated stress on liver or kidney function.

Table 6 Glycemia, liver and kidneys function parameters of different experimental groups

	FBS (g/L)	CRP (U/L)	ASLO (U/L)	ESR (mm/hr)
Control	0.63±0.24	0.3±0.08	6.25±0.12	2.39±0.17
Wound + Base	0.71±0.21 ^{NS}	0.55±0.04 ^a	9.13±0.3 ^b	6.61 ±0.11 ^c
Wound + MEBO	0.65±0.31 ^{NS}	3.28±0.07 ^{b**}	7.26±0.17 ^{a**}	3.45±0.15 ^{NS**}
Wound + ZASSC	0.69±0.15 ^{NS}	0.46±0.03 ^{aNS}	5.29±0.35 ^{NS**}	2.46±0.12 ^{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 (***)

1.5. In Vitro Evaluation of the Biological Activities

1.5.1. Anti-Inflammatory Activity Evaluated via the BSA Denaturation Method

To assess the anti-inflammatory potential of the extracts and nanoparticles, the ability to inhibit heat-induced denaturation of bovine serum albumin (BSA) was measured across the same concentration range. This assay provides an indication of the samples' capacity to stabilize protein structures under inflammatory conditions, with percentage inhibition values subsequently used to derive IC₅₀ metrics for direct comparison.

The percentage inhibition of BSA denaturation by each extract formulation was determined over the concentration range of 75.085–1000 µg/mL. The resulting I% values for ZASSC, are presented in Table 7 and 8 for direct comparison.

Table 7 Inhibition of BSA denaturation (%) by ZASSC extracts, and diclofenac at selected concentrations (mean \pm SD, n = 3)

Concentration ($\mu\text{g/mL}$)	ZASSC	Diclofenac
1000	83.89 \pm 2.52	60.36 \pm 1.81
750	83.00 \pm 2.49	51.55 \pm 1.55
562.5	80.53 \pm 2.42	41.77 \pm 1.25
421.875	77.33 \pm 2.32	41.48 \pm 1.24
316.406	74.05 \pm 2.22	35.89 \pm 1.08
237.305	67.31 \pm 2.02	30.83 \pm 0.93
177.979	58.20 \pm 1.75	30.41 \pm 0.91
133.484	47.96 \pm 1.44	18.30 \pm 0.55
100.113	35.85 \pm 1.08	12.05 \pm 0.36
75.085	16.39 \pm 0.49	4.76 \pm 0.14

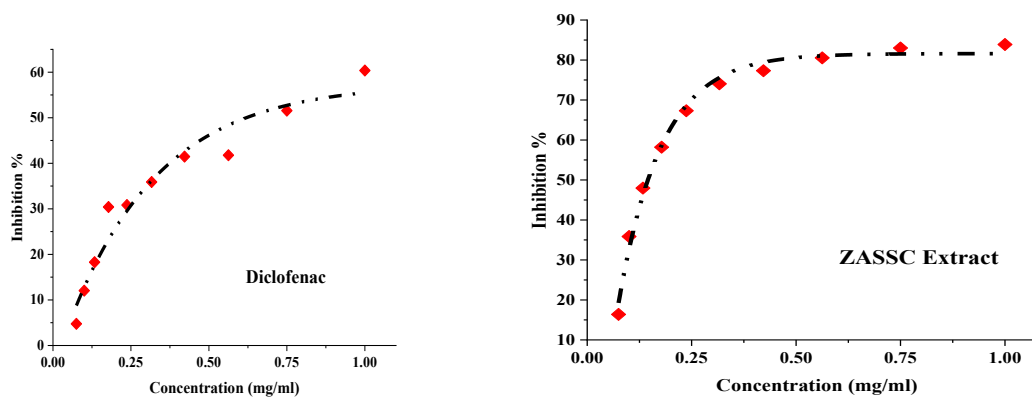


Figure 19 Exponential dose–response curves showing BSA denaturation inhibition (%) of ZASSC extracts across a range of concentrations

The exponential dose–response curves for BSA denaturation inhibition demonstrate that ZASSC extract achieve maximal protein stabilization at 83%. These distinctions underscore the superior anti-inflammatory efficacy of semi-polar phytochemical fractions and highlight the modulatory influence on activity kinetics (Shah et al., 2025).

The half-maximal inhibitory concentrations (IC_{50}) for ZASSC extract were subsequently calculated from the fitted dose–response data, providing a direct measure of their relative anti-inflammatory potencies. These values are summarized in Table 9.

Table 8 IC_{50} values for BSA denaturation inhibition by ZASSC extract, and diclofenac (mean \pm SD, n = 3)

Plant / Sample	IC_{50} ($\mu\text{g/mL}$) \pm SD	IC_{50} (mg/mL) \pm SD
ZASSC Ext	145.31 \pm 4.36	0.145 \pm 0.004
Diclofenac	620.82 \pm 18.63	0.621 \pm 0.019

1.5.2. Oxidative stress parameters

A. Skin oxidative stress parameters

Wound induction significantly increased oxidative stress in skin tissue, as shown by the elevated MDA levels in the Base (10.33 ± 0.39 nmol/mg; $p < 0.001$) and MEBO (8.5 ± 0.65 nmol/mg; $p < 0.05$) groups compared to the control (4.24 ± 0.23 nmol/mg). In contrast, the ZASSC group maintained MDA levels (4.12 ± 0.32 nmol/mg) close to control values, indicating effective lipid peroxidation mitigation. Antioxidant markers (GSH, SOD, CAT) were substantially reduced in the Base group, especially GSH (7.11 ± 0.13 nmol/mg; $p < 0.001$) and CAT (3.33 ± 0.23 UI/min/g; $p < 0.05$), reflecting depleted antioxidant defenses. MEBO partially restored antioxidant levels, particularly GSH and SOD, although not statistically significant compared to control. Importantly, ZASSC significantly restored CAT (10.16 ± 0.23 UI/min/g; $p < 0.05$) and maintained GSH levels above baseline, suggesting a potent antioxidative role. These findings highlight ZASSC's superior protective effect against skin oxidative stress during wound healing.

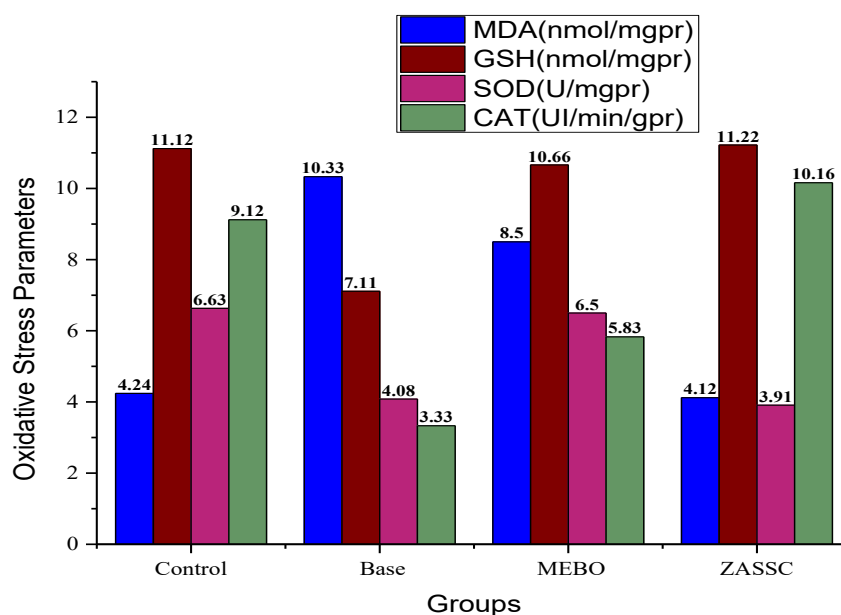


Figure 20 Oxidative stress parameters in the skin of different experimental groups.

B. Spleen oxidative stress parameters

Oxidative stress was markedly elevated in the spleen of wounded animals treated with Base, as evidenced by increased MDA (9.06 ± 0.59 nmol/mg; $p < 0.05$) and a sharp decline in GSH (5.23 ± 0.7 nmol/mg; $p < 0.01$) and CAT (4.83 ± 0.16 UI/min/g; $p < 0.001$) compared to control. The MEBO group showed partial protection, reducing MDA (8.32 ± 0.98 nmol/mg) and slightly improving antioxidant markers, but without statistical significance. Notably, the ZASSC group exhibited oxidative stress profiles comparable to the control, with MDA (3.3 ± 0.28 nmol/mg) and CAT (9.66 ± 0.34 UI/min/g) levels effectively normalized. Furthermore, ZASSC significantly increased SOD activity (7.12 ± 0.8 U/mg; $p < 0.05$ vs. Base) and maintained higher GSH content (10.21 ± 1.75 nmol/mg), indicating a pronounced antioxidant defense response. These data reinforce ZASSC's systemic antioxidant potential, particularly in lymphoid tissues like the spleen.

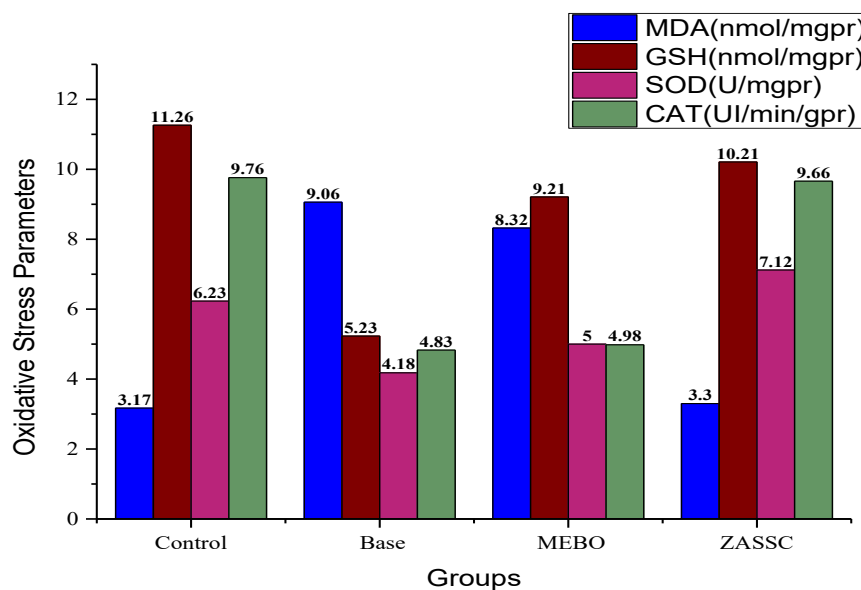


Figure 21 Oxidative stress parameters in the spleen of different experimental groups.

C. Thymus oxidative stress parameters

Wound exposure significantly disrupted thymic redox balance, particularly in the Base and MEBO groups, which exhibited increased MDA (7.02 ± 0.46 and 6.27 ± 0.23 nmol/mg, respectively) and reduced antioxidant markers. The Base group showed significant reductions in SOD (5.13 ± 0.17 U/mg; $p < 0.01$) and CAT (4.27 ± 0.2 UI/min/g; $p < 0.01$), reflecting oxidative damage in thymic tissue. MEBO offered marginal improvements, but oxidative parameters remained impaired compared to control. In contrast, the ZASSC treatment significantly reversed oxidative damage, as evidenced by a reduction in MDA (2.93 ± 0.7 nmol/mg; $p < 0.001$ vs. Base), and restoration of CAT (9.86 ± 0.49 UI/min/g; $p < 0.001$) and GSH (10.53 ± 0.66 nmol/mg; $p < 0.001$). SOD activity also remained high (9.25 ± 1.75 U/mg), similar to control values. These findings underscore ZASSC's strong antioxidant capacity in protecting immune organs from wound-associated oxidative damage.

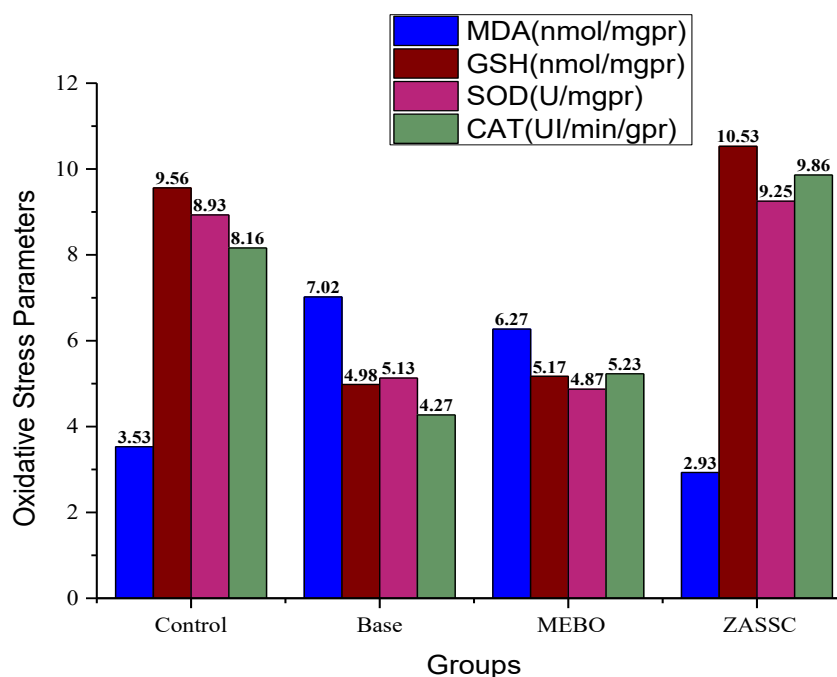


Figure 22 Oxidative stress parameters in the thymus of different experimental groups.

1.6. Histopathological study

A. Skin Histology

Histological examination of skin sections at $\times 40$ magnification revealed distinct differences across the experimental groups. The control group (A) displayed intact epidermal and dermal layers, with well-organized collagen bundles and minimal cellular infiltration, indicative of normal skin architecture. In contrast, the Wound + Base group (B) showed disrupted epidermis, necrotic tissue, and dense inflammatory cell infiltration, suggesting impaired healing and persistent inflammation. The Wound + MEBO group (C) demonstrated partial re-epithelialization, moderate inflammatory infiltration, and granulation tissue formation, indicative of enhanced wound healing compared to the base group. Remarkably, the Wound + Plant-based ointment group (D) exhibited near-complete re-epithelialization, reduced inflammatory cells, and well-organized collagen deposition, reflecting advanced tissue remodeling and effective healing potential of the plant-based treatment.

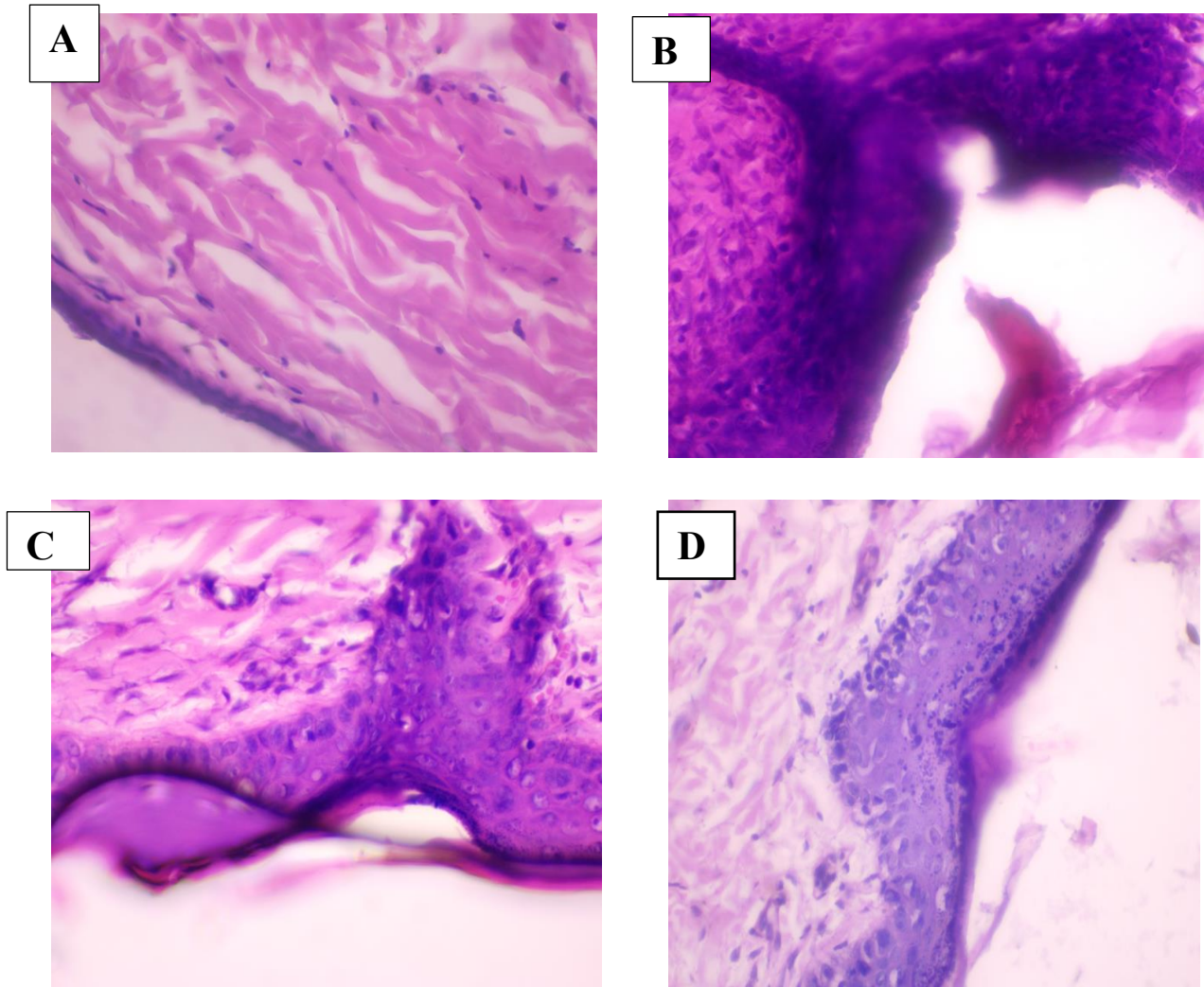


Figure 23 Microscopic observation of skin histological sections from different experimental groups, (A) Control group, (B) (Wound + Base) group, (C) (Wound + MEBO) group, (D) (Wound + ZASSC) group. Magnification $\times 40$.

B. Spleen Histology

Microscopic observations of the spleen at $\times 40$ magnification revealed significant alterations between groups. The control group (A) maintained a normal histological architecture with well-defined white pulp and red pulp regions, and no signs of lymphoid depletion or structural disorganization. In the Wound + Base group (B), disorganization of the splenic architecture was evident, with indistinct white pulp boundaries and marked lymphocytic depletion, suggestive of systemic immune stress or inflammation. The Wound + MEBO group (C) demonstrated partial restoration of splenic structure with identifiable white pulp and moderate lymphocyte presence, indicating a degree of immune modulation. The Wound + Plant-based ointment group (D) exhibited a well-preserved splenic architecture with dense lymphoid follicles, indicating immune recovery and suggesting an immunomodulatory effect of the treatment.

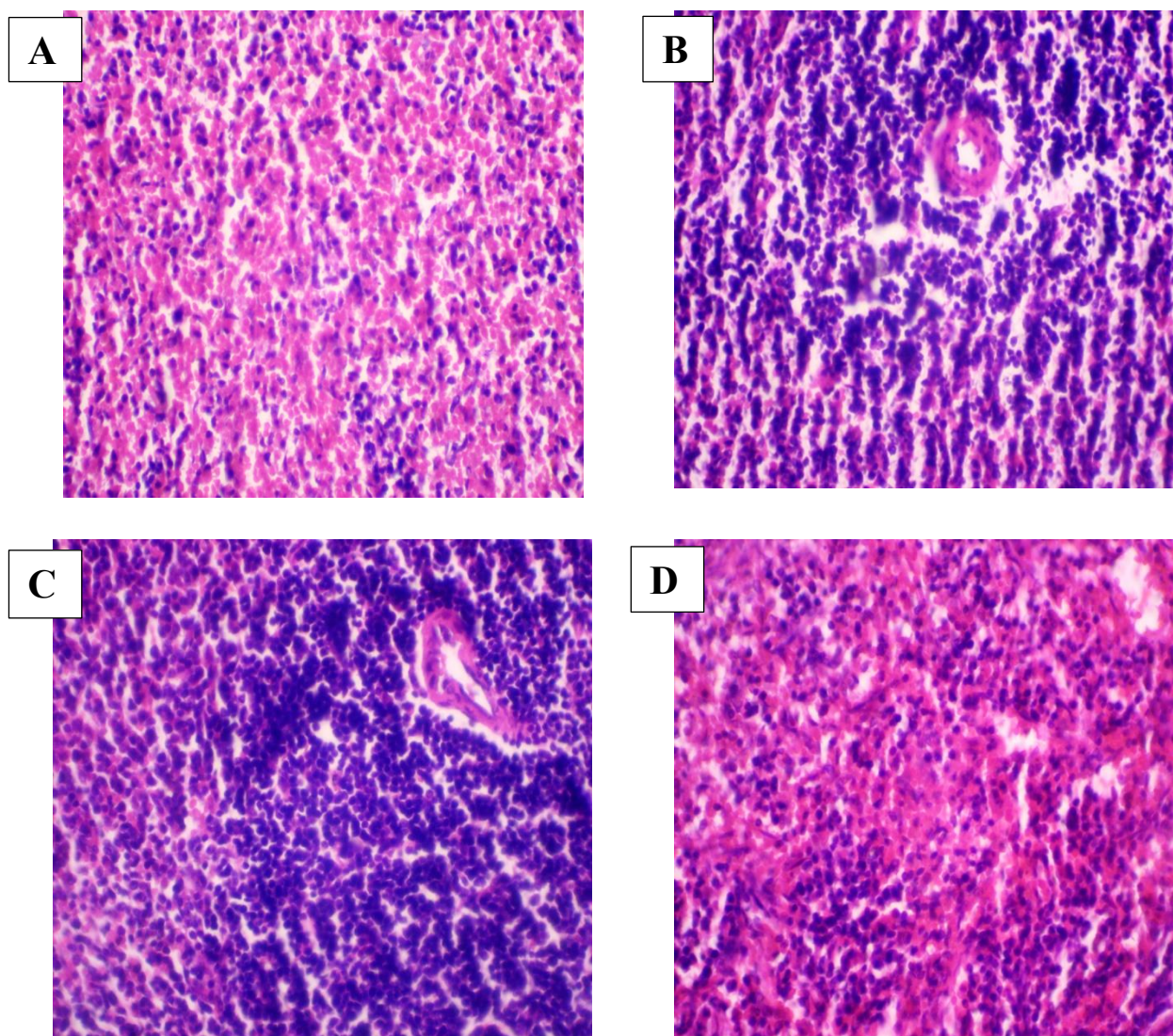


Figure 24 Microscopic observation of spleen histological sections from different experimental groups, (A) Control group, (B) (Wound + Base) group, (C) (Wound + MEBO) group, (D) (Wound + ZASSC) group. Magnification $\times 40$.

C. Thymus Histology

Thymic histological analysis at $\times 40$ magnification showed preserved cortical and medullary architecture in the control group (A), with dense thymocytes and clearly demarcated zones. The Wound + Base group (B) presented with cortical thinning, depletion of thymocytes, and structural disorganization, indicating stress-induced thymic atrophy. In the Wound + MEBO group (C), moderate restoration was noted with partial cortical repopulation and reduced architectural disruption, suggesting regenerative potential. The Wound + Plant-based ointment group (D) showed significant improvement with denser thymocyte populations and

nearly normal corticomedullary demarcation, indicating a protective or restorative effect on thymic integrity, potentially enhancing systemic immune competence.

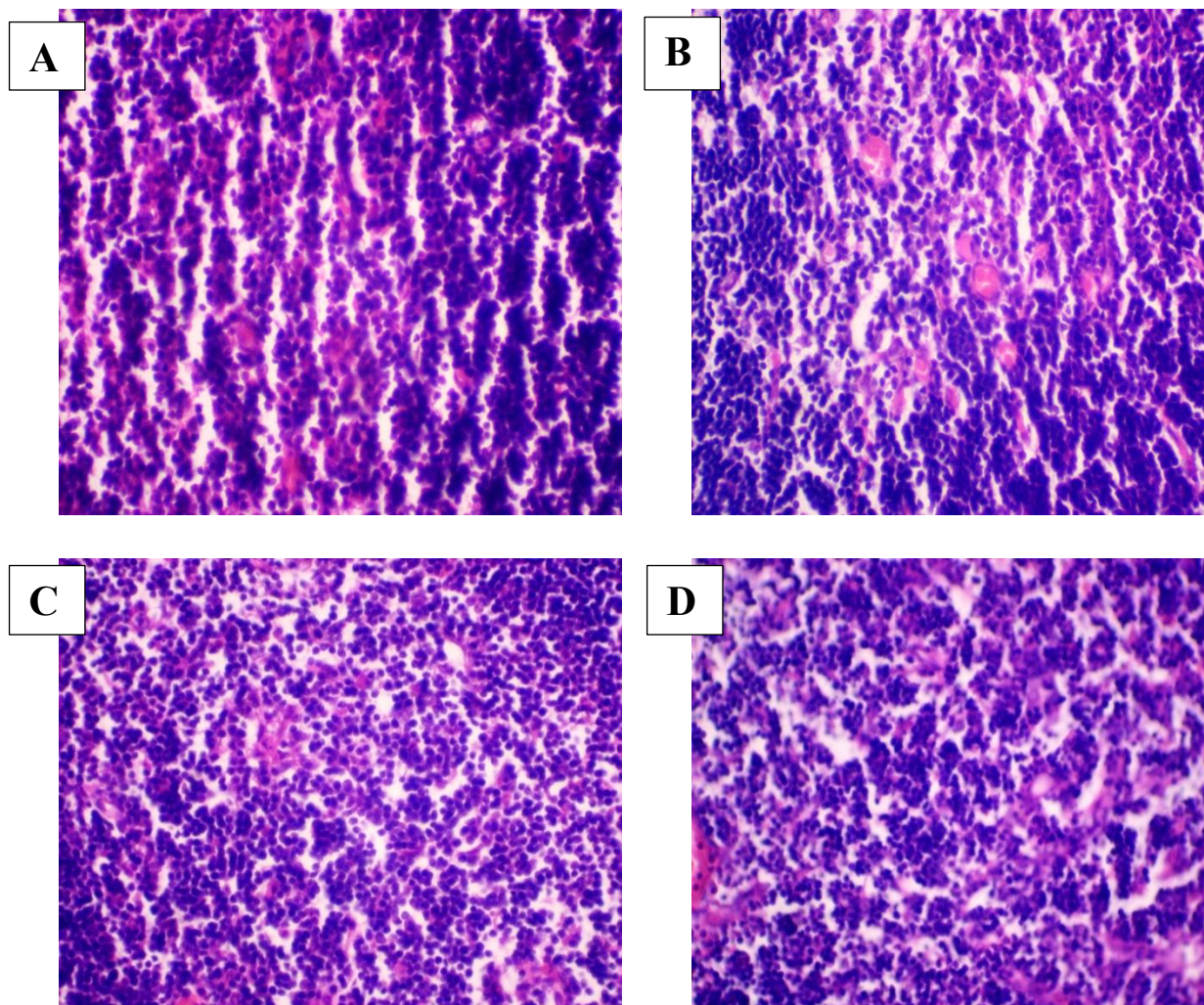


Figure 25 Microscopic observation of thymus histological sections from different experimental groups, (A) Control group, (B) (Wound + Base) group, (C) (Wound + MEBO) group, (D) (Wound + ZASSC) group. Magnification $\times 40$.

The following table summarizes the histopathological findings in the skin, spleen, and thymus tissues from different experimental groups in a wound healing study. The control group (A) exhibited normal tissue architecture without pathological changes. The Wound + Base group (B) showed severe degeneration and disorganization across all organs, including disrupted skin layers with necrosis and heavy inflammatory infiltrates, splenic architectural disorganization with lymphoid depletion, and thymic cortical thinning with thymocyte loss. The Wound + MEBO group (C) displayed moderate improvement, with partial tissue restoration, reduced inflammation, and decreased necrosis, indicating enhanced healing and immune modulation. The Wound + Plant-based ointment group (D) demonstrated the most significant

recovery, with nearly normal tissue structure, minimal inflammatory infiltration, and advanced re-epithelialization and immune tissue regeneration, reflecting its effective therapeutic potential in wound healing and immune support.

Table 9 Histopathological Comparison of Skin, Spleen, and Thymus in Wound Healing Study.

Group	Organ	Degeneration / Disorganization	Hemorrhage	Inflammatory Infiltrate	Necrosis	Vacuolated Cytoplasm
A	Skin	–	–	–	–	–
	Spleen	–	–	–	–	–
	Thymus	–	–	–	–	–
B	Skin	+++ (epidermal disruption)	±	+++	++	±
	Spleen	+++ (architectural loss)	±	++	±	±
	Thymus	++ (cortical thinning, depletion)	–	+	±	±
C	Skin	++ (partial re-epithelialization)	±	++	+	±
	Spleen	+ (partial restoration)	–	+	±	–
	Thymus	+ (moderate cortical repopulation)	–	±	±	–
D	Skin	± (near-complete re-epithelial.)	–	±	±	–
	Spleen	– (well-preserved)	–	±	–	–
	Thymus	± (normal corticomedullary)	–	–	–	–

(–) Absent, (±) Minimal, (+) Mild, (++) Moderate, (+++) Severe

II. Discussion

- **Statistical study**

Based on the content of my project, nature has always been an essential resource for humans since ancient times, as humans have turned to plants, animals, and minerals to treat various ailments (**Haidan et al., 2016**). In this context, this work aims to verify the biological efficacy of Zasse plant extract, which is traditionally used in the valley region to treat several medical conditions. This study included developing a user questionnaire, analyzing the extract's physical and chemical properties, and evaluating its biological activities .

The participation rates across municipalities show clear disparities. Rabah led with 21%, followed by Hassi Khalifa (15%) and El Oued (13%), likely due to factors such as higher population, better infrastructure, or more effective outreach. Conversely, areas like Hassani Abdelkrim, Trifawi, and regions outside El Oued had the lowest rates (1%), possibly due to geographic or logistical challenges and limited awareness. Municipalities with participation between 3% and 11% reflect moderate engagement, influenced by various demographic and administrative factors. These results highlight the need for targeted, context-sensitive strategies to improve inclusivity and ensure equitable participation across all regions.

The results indicate that the sample consisted predominantly of females, which may reflect a greater willingness among women to participate in the study or a higher level of interest in its topic. In terms of age distribution, the majority were young adults (18–under 25 years), which may limit the generalizability of the findings to older age groups. Regarding educational level, most participants had a university education, indicating a high level of awareness that could influence their responses and attitudes toward the study topic. This age and educational distribution should be taken into account when analyzing and interpreting the results.

The results indicate a widespread use of medicinal plants among participants, reflecting a cultural and health-related reliance on them in daily life. The miswak showed a high usage rate (77%), supporting its traditional importance in oral hygiene and possibly other uses. Thyme had an even higher usage rate at 87%, highlighting its common role in both culinary and medicinal practices. Saffron was used by 69% of participants, indicating a moderate presence in traditional remedies. Cloves showed one of the highest usage rates (82%), suggesting their frequent application both topically and internally. These findings reflect a notable interest in natural medicine and underscore the importance of studying it scientifically to verify its efficacy and safety.

These results reveal a limited public awareness of the active compounds in the studied medicinal plants, despite their widespread use. The vast majority (80%) are unaware of saffron's active component that may protect the retina, indicating a knowledge gap regarding its visual health benefits. Similarly, 72% are unaware of thymol in thyme, known for its antibacterial and antifungal properties, reflecting a lack of understanding of its chemical profile. Regarding miswak, participants showed varied perceptions of benzoic acid's effectiveness, with 25% considering it very effective, while 46% were unaware of its antimicrobial role. As for cloves, the findings highlight significant uncertainty: 49% believe eugenol is safe for ocular use, 41% lack knowledge, and only 10% consider it either very effective or safe. These results underscore the need to enhance public health education about the active components of medicinal plants to promote informed and safe use.

The results indicate a widespread use of both salt and alum for health purposes among participants. A total of 79% reported prior experience using salt in health-related practices such as eye disinfection or wound cleaning, highlighting its role as a traditional element in basic first aid. Alum showed an even higher usage rate (93%), reflecting its common application in traditional personal care and cosmetic practices, such as deodorants or wound treatment. These findings emphasize the significance of natural substances in popular health practices and underscore the need for scientific evaluation to ensure their efficacy and safety.

The results indicate that safety is the most influential factor affecting participants' attitudes toward the use of natural eye drops. Sixty-seven percent expressed concerns about potential side effects, making this fear the primary barrier to adopting such products. The lack of supporting scientific studies ranked second at 34%, highlighting a clear need for scientific validation to build trust. Concerns about product effectiveness were the lowest at 15%, suggesting an initial confidence in the efficacy of natural ingredients. Overall, these findings emphasize the importance of focusing on safety and providing scientific evidence to enhance consumer acceptance of these products.

The analysis results show that the majority of participants prioritize enhancing scientific research on the active compounds in natural products. This factor received the highest level of support (54%), reflecting an awareness of the importance of a scientific basis in developing therapeutic products. Forty-three percent of participants also expressed a strong interest in ensuring safety through laboratory testing, reflecting an awareness of the importance of reliability and clinical testing in enhancing confidence in products. Conversely, the use of modern extraction techniques received good support (23%), indicating a growing awareness of

the impact of technology on extract quality. Improving formulation and concentration received the least support (10%), possibly reflecting participants' belief that active ingredients and extraction techniques are more important to a product's effectiveness than its final formulation. These results demonstrate that natural product development must be built on a scientific foundation supported by research and safety before focusing on manufacturing aspects.

The physical characteristics of the formulated cream are essential indicators for evaluating its quality and stability prior to clinical use or market release. The light yellow color of the cream suggests the stability of its components and the absence of undesirable interactions between the herbal extract and the cream base, reflecting the quality of the formulation process (Barry, 2002). The absence of odor is considered a positive attribute, especially for users with sensitive skin or fragrance allergies, enhancing the product's dermatological acceptability (Draelos, 2012). Regarding texture, the "moist" consistency indicates a high water content or the presence of humectant compounds, which contributes to effective skin hydration upon application. This is particularly desirable in topical dermatological products aimed at restoring the skin barrier or alleviating inflammation (Lodén & Maibach, 2000). The recorded pH value of 4.93 falls within the mildly acidic range of normal skin pH (4.5–5.5), which supports the cream's compatibility with the skin's natural environment. This helps inhibit the growth of pathogenic microorganisms and minimizes the risk of skin irritation or disruption of the skin barrier function (Lambers et al., 2006). Therefore, the cream is expected to be safe even for sensitive skin. In terms of physical stability, the 100% stability result reflects the absence of phase separation or physical degradation over the observation period. This indicates the robustness of the formulation and the strength of interactions among its components, which is essential for maintaining the efficacy of the active herbal compounds during storage and use (Nastiti et al., 2017). Overall, these findings suggest that the cream possesses excellent physical properties that make it suitable for daily dermatological use, particularly in therapeutic or preventive contexts requiring long-term stability and high safety standards.

Wound healing remains a complex and dynamic biological process that involves multiple overlapping phases: hemostasis, inflammation, proliferation, and remodeling. A delay or disruption in any of these stages can lead to chronic wounds, which constitute a significant public health burden due to increased morbidity, mortality, and healthcare costs (Guo & DiPietro, 2010). Therefore, accelerating the healing process is critical to ensure proper tissue repair and restore both anatomical integrity and physiological function. The results of this study highlight the promising potential of herbal-based topical formulations, particularly ZASSC

CREAM, in promoting efficient wound healing. Herbal products are gaining considerable attention globally, especially due to their bioactive compounds such as flavonoids, saponins, tannins, and terpenoids, which exhibit anti-inflammatory, antimicrobial, and antioxidant activities that are critical for tissue regeneration (**Mukherjee et al., 2013; Agyare et al., 2016**). The clinical visual monitoring showed that ZASSC CREAM significantly accelerated wound closure starting from day six and achieved near-complete healing by day fourteen. This performance was superior compared to MEBO cream, which demonstrated a slower healing progression, and the cream base, which showed minimal therapeutic effect. These differences can be attributed to the specific bioactive content and formulation characteristics of each cream. It has been well-documented that the presence of phytochemicals with anti-inflammatory and angiogenic properties can modulate cytokine expression, enhance fibroblast proliferation, and stimulate collagen synthesis (**Shanbhag et al., 2019**). Quantitative wound area analysis further confirmed these findings, with ZASSC CREAM achieving a healing rate of 79.12% by day 14, compared to 73.14% for the cream base and 70.21% for MEBO. These results are in line with the temporal dynamics of the wound healing process, where the proliferative phase, which typically intensifies after day 7, plays a pivotal role in epithelialization and granulation tissue formation (**Eming et al., 2014**). Importantly, the notable differences in healing efficiency underline the crucial role of formulation design in topical therapeutics. Previous studies have emphasized that not only the type and concentration of active compounds but also the physical characteristics of the cream base—such as viscosity, occlusiveness, and permeability—significantly influence drug delivery to the wound site (**Boateng & Catanzano, 2015**). In conclusion, the findings of this study support the growing body of evidence that herbal topical preparations, when properly formulated, can significantly enhance wound healing outcomes. ZASSC CREAM, in particular, demonstrates notable efficacy likely due to its rich content of synergistic bioactive agents that promote cellular regeneration, modulate inflammation, and improve tissue remodeling.

- **Organ weight index**

The results of the organ weight index, particularly of the spleen and thymus, indicate a clear variation in immune response depending on the type of treatment applied following wound induction. Treatments with Base and MEBO showed significant increases in the immune organ indices, suggesting immune system activation likely in response to wound-induced inflammation. This aligns with the findings of (**Chen et al;2020**), which demonstrated that MEBO products promote immune and inflammatory responses by enhancing T-cell

proliferation and activating peripheral lymphoid tissues during wound healing. On the other hand, the ZASSC group did not show significant differences compared to the control group, which may indicate that this plant-based extract possesses immunoregulatory rather than strongly immunostimulatory properties. This could be advantageous in the context of chronic or immunologically sensitive wound healing. These findings are consistent with the study by **(Alzahrani et al. 2018)**, which reported that plant extracts, particularly those rich in flavonoids and tannins, contribute to modulating immune responses by reducing oxidative stress.

The results showed that wounding and various treatments significantly affected hematological parameters in the experimental models. Both the "Wound + Base" and "Wound + MEBO" groups showed significantly higher white blood cell (WBC), lymphocyte (LYM), and granulocyte (GRA) counts, reflecting a pronounced and excessive inflammatory response resulting from wound exposure **(Zhao et al., 2016)**. This response is essential in the initial stages of wound healing, as WBCs play a key role in eliminating bacteria and dead cells **(Eming et al., 2007)**. It is worth noting that the "Wound + Base" group showed the highest platelet count (PLT), which may be attributed to the stimulation of platelet production resulting from chronic inflammation associated with untreated wounds or wounds treated with immunostimulating agents **(Semple et al., 2011)**. Platelets not only contribute to clotting but also secrete immune mediators that contribute to the healing process. In contrast, the Wound + ZASSC group showed a significant decrease in white blood cell counts ($p < 0.05$), as well as a decrease in granulocyte counts compared to the Base and MEBO groups. This may indicate an anti-inflammatory or immune-modulating effect of the ZASSC formulation, potentially reducing tissue damage caused by chronic inflammation **(Mantovani et al., 2013)**. These results suggest that ZASSC may contribute to improved healing by reducing immune hyperactivation, promoting the immune balance necessary for tissue repair without excessive inflammatory complications. Hemoglobin (HGB) and red blood cell (RBC) counts remained relatively stable across all groups, indicating that erythropoiesis was not significantly affected by wounds or treatments. This finding is supported by previous studies that have shown that acute inflammation may not have a significant impact on erythrocyte production unless it is chronic or associated with systemic injury **(Means, 2013)**. Overall, these results support the hypothesis that topical treatments such as MEBO and Base activate the necessary inflammatory response in the initial stages of wound healing, while ZASSC appears to possess immunoregulatory properties that may be beneficial in the later stages of healing, or in cases where hyperinflammation or the development of chronic wounds is feared.

The study results showed no significant differences in fasting blood glucose (FBS) levels between the different experimental groups, indicating no general metabolic stress resulting from the injury or the topical treatments used. This is consistent with what Marik and Bellomo (2013) indicated, as the absence of changes in fasting glucose indicates metabolic stability, a positive indicator that the treatments do not cause systemic disturbances. In contrast, inflammatory markers (CRP, ASL, ESR) showed significant changes between groups. The MEBO group recorded a significant increase in CRP (3.28 U/L, $p < 0.01$ compared to the control group), reflecting a strong systemic inflammatory response. CRP is an acute-phase protein secreted from the liver in response to cytokines such as IL-6, and its elevation is often associated with acute or chronic inflammation (**Gabay et Kushner, 1999**). A significant increase in the erythrocyte sedimentation rate (ESR) was also recorded in the Base group ($p < 0.001$), another indicator of general inflammation, as ESR is associated with increased fibrinogen and other inflammatory proteins (**Brigden, 1999**). The ZASSC group showed completely different results, with CRP and ESR levels close to normal values in the control group, while ASLO levels were even lower than in the control group. ASLO is used as an indicator of the immune response to inflammatory agents, particularly streptococci, and its decrease may indicate an inhibitory or regulating effect on inflammation (**Bisno, 2001**). This overall decrease in inflammatory markers in the ZASSC group supports the hypothesis that this extract possesses anti-inflammatory properties and inhibits excessive immune responses. From these results, it can be concluded that the ZASSC extract effectively reduced systemic inflammation resulting from wounding without adverse effects on metabolic functions, which supports its ability to accelerate wound healing and improve the recovery environment. These indicators also suggest that ZASSC may help protect vital organs such as the liver and kidneys from stress associated with chronic or acute inflammation.

All samples demonstrated a clear, concentration-dependent inhibition of BSA denaturation, confirming their anti-inflammatory potential. The aqueous extract of ZASSC showed robust activity ($83.89 \pm 2.52\%$ at $1,000 \mu\text{g/mL}$), outperforming the reference drug diclofenac, which achieved only $60.36 \pm 1.81\%$ inhibition at the same concentration and dropped to $4.76 \pm 0.14\%$ at $75.085 \mu\text{g/mL}$.

These findings indicate that the water extracts of ZASSC contain heat-stable phytochemicals—such as saponins and flavonoids—that effectively stabilize proteins under inflammatory conditions (**Gadakh & Kulkarni, 2025**)

To quantitatively characterize the inhibitory potency of each sample, the percentage inhibition data were fitted to exponential dose–response models. These curves, depicting I% as a function of concentration, facilitate accurate estimation of IC₅₀ values by capturing the full dynamic range of enzyme inhibition. The resulting plots are presented in [Figures 3](#).

The IC₅₀ values presented in Table 9 afford a rigorous, quantitative comparison of the anti-inflammatory efficacy of ZASSC, and the reference drug diclofenac. In this context, lower IC₅₀ values denote greater inhibitory potency against heat-induced bovine serum albumin (BSA) denaturation, a surrogate marker for anti-inflammatory activity ([Sobhy et al., 2025](#)).

For ZASSC, the aqueous fraction extract exhibited the greatest bioactivity, with an IC₅₀ of 145.31 ± 4.36 µg/mL. Notably, ZASSC preparations surpassed the performance of diclofenac (IC₅₀ = 620.82 ± 18.63 µg/mL), indicating a superior capacity of plant-derived phytochemicals to stabilize protein structures under inflammatory conditions.

- **Oxidative stress parameters**

Scientific Discussion on Skin Oxidative Stress Parameters Following Wound Induction
The biochemical data illustrated in the figure above indicate that wound induction significantly increased oxidative stress in skin tissue, particularly through elevated levels of MDA (Malondialdehyde) a well-known marker of lipid peroxidation and oxidative damage. MDA levels were markedly higher in the Base group (10.33 ± 0.39 nmol/mg) and the MEBO group (8.5 ± 0.65 nmol/mg) compared to the Control group (4.24 ± 0.23 nmol/mg), confirming the occurrence of oxidative injury post-wounding. These findings are in agreement with previous studies highlighting MDA as a sensitive indicator of tissue damage caused by reactive oxygen species (ROS) ([Valko et al., 2007](#)). In contrast, the ZASSC-treated group maintained MDA levels (4.12 ± 0.32 nmol/mg) nearly equivalent to those of the Control, suggesting a strong ability to mitigate lipid peroxidation. This indicates a protective antioxidative potential of the ZASSC formulation. Endogenous Antioxidants (GSH, SOD, CAT): GSH (Reduced Glutathione) showed a notable decrease in the Base group (7.11 nmol/mg), reflecting depleted antioxidant reserves in damaged skin tissue. Both MEBO and ZASSC contributed to restoring GSH levels, with ZASSC showing better efficacy. According to ([Halliwell and Gutteridge 2015](#)), GSH plays a central role in cellular detoxification and redox balance, especially during oxidative stress. SOD (Superoxide Dismutase) activity was moderately improved in the ZASSC group compared to Base, supporting its role in dismutating superoxide radicals into hydrogen peroxide—an important detoxifying step ([Valko et al., 2007](#)). CAT (Catalase) activity, which

breaks down hydrogen peroxide into water and oxygen, was significantly restored in the ZASSC group (10.16 ± 0.23 UI/min/g). This value even exceeded that of the Control group, suggesting that ZASSC may actively stimulate the antioxidant enzymatic system, particularly catalase. These results are consistent with findings that certain herbal extracts can modulate enzymatic antioxidant levels in oxidative stress models (**Mansour, 2006**)

The results of this study showed that oxidative stress was significantly elevated in the spleens of injured and Base-treated animals. Levels of MDA, a marker of lipid damage, were elevated, along with a sharp decrease in GSH and CAT, indicating a redox imbalance in splenocytes. This is consistent with previous studies showing that injury and inflammation stimulate the production of free radicals and lead to the depletion of endogenous antioxidants, contributing to the exacerbation of oxidative damage in lymphoid tissue. (**Wang et al., 2020**)

Although the MEBO group showed some improvement in oxidative stress markers, such as a slight decrease in MDA and a non-significant improvement in GSH and CAT, these changes were not statistically significant. This suggests that MEBO may only provide a partial antioxidant effect in this model, and may require a higher dose or a longer treatment period to demonstrate a clear protective effect, especially in cases of severe oxidative damage. (**Elkomy et al., 2022**)

On the other hand, the ZASSC group showed significant improvement in all oxidative stress indicators, with MDA levels dropping to near normal levels, CAT and SOD levels increasing significantly, and GSH levels maintaining elevated levels. These results support the effectiveness of ZASSC as a potent antioxidant capable of reducing oxidative damage and enhancing the cellular defense system. (**Li et al., 2021**)

These data reflect the potential role of ZASSC in protecting lymphoid tissues, such as the spleen, from damage caused by oxidative stress. Enhancing the activity of antioxidant enzymes such as SOD and CAT helps remove free radicals and reduce the inflammatory response, which is consistent with studies that have indicated the importance of supporting the endogenous antioxidant system in preventing diseases associated with oxidative stress. (**Yang et al., 2020**)

The results presented in the figure above indicate that the ZASSC extract possesses a strong efficacy in counteracting wound-induced oxidative stress in the thymus gland. In the Base and MEBO-treated groups, elevated levels of malondialdehyde (MDA) a direct marker of lipid peroxidation were observed, suggesting intensified oxidative damage in these groups (**Ayala et al., 2014**). In contrast, the ZASSC group showed a marked reduction in MDA levels (2.93

nmol/mg), compared to the Base group (7.02 nmol/mg), indicating that this extract can inhibit lipid peroxidation and protect immune cell membranes.

Furthermore, the antioxidant enzymes SOD and CAT were significantly reduced in the Base group (5.13 and 4.27, respectively), reflecting major disruptions in endogenous antioxidant defenses, while MEBO showed only slight improvement. On the other hand, the ZASSC group exhibited substantial increases in SOD (9.25 U/mg) and CAT (9.86 U/min/g) activity, suggesting restored antioxidant enzyme function and the ability to neutralize harmful free radicals such as H₂O₂ (Valko et al., 2007).

Additionally, the ZASSC group demonstrated a notable increase in glutathione (GSH) levels—a key intracellular antioxidant critical for protecting immune cells—reaching 10.53 nmol/mg compared to 4.98 in the Base group, suggesting that ZASSC may either enhance GSH synthesis or prevent its oxidative depletion (Wu et al., 2004).

Based on these findings, the ZASSC extract demonstrates a potent protective and restorative effect against wound-associated oxidative damage in immune tissues, outperforming both the base treatment and MEBO ointment, making it a strong candidate for topical anti-inflammatory and antioxidant medical applications.

- **Histopathological study**

A) Skin Histology

Histopathological examination of skin sections at ×40 magnification revealed distinct differences among the experimental groups. The control group (A) exhibited normal skin architecture, characterized by intact epidermal and dermal layers, well-organized collagen bundles, and minimal inflammatory cell infiltration (Junqueira & Carneiro, 2005). In contrast, the Wound + Base group (B) showed disrupted epidermis, necrotic tissue, and dense infiltration of inflammatory cells, indicating impaired healing and ongoing local inflammation. The Wound + MEBO group (C) demonstrated partial re-epithelialization, moderate inflammatory infiltration, and granulation tissue formation, consistent with the known properties of MEBO in promoting tissue regeneration and reducing inflammation (Zhao et al., 2016). Notably, the Wound + Plant-based ointment group (D) exhibited near-complete re-epithelialization, a marked reduction in inflammatory cells, and well-organized collagen deposition, suggesting that the plant-based compound effectively enhanced wound healing and tissue remodeling.

B) Spleen Histology

The present study revealed clear histological differences in spleen architecture among the experimental groups, reflecting variations in immune response levels and the therapeutic effects of the topical treatments applied. The control group exhibited a normal and intact spleen structure, with a clear distinction between the white pulp and red pulp, and no signs of lymphocyte depletion, indicating a healthy immune function in the absence of any intervention (**Gartner & Hiatt, 2021; Abbas et al., 2018**). In contrast, the wound group treated with the base only showed severe histological disruptions, with indistinct white pulp boundaries and a marked reduction in lymphocytes, which is likely attributable to systemic immune stress caused by the open wound, potentially leading to immunosuppression or systemic inflammation (**Dhabhar, 2014**). The MEBO-treated group showed partial improvement in tissue architecture, with emerging features of white pulp and a moderate presence of lymphocytes, reflecting the known regenerative and anti-inflammatory properties of this ointment (**Xu et al., 2013; El-Hadidy et al., 2016**). Remarkably, the group treated with the herbal ointment exhibited a nearly normal spleen structure, with dense lymphoid follicles and clearly defined white pulp boundaries, suggesting a significant immunomodulatory effect of the plant-based compounds used, known for their ability to enhance immune responses, regulate cytokine secretion, and protect lymphoid tissues (**Shahrajabian et al., 2019; Ghasemian et al., 2016**). Based on these findings, it can be concluded that the immunological effects of the herbal ointment surpass those of MEBO, supporting the potential integration of plant-based therapies as effective adjuncts in post-injury recovery strategies.

Histological analysis of the thymus at $\times 40$ magnification revealed distinct differences in tissue architecture among the experimental groups, reflecting varying degrees of immune impairment or protection resulting from the different topical treatments. The control group displayed a normal thymic structure, with clearly defined cortical and medullary regions and a dense population of thymocytes, indicating intact central immune function (Turgeon, 2020; Abbas et al., 2018). In contrast, the Wound + Base group showed cortical thinning, significant thymocyte depletion, and structural disorganization, which are hallmark signs of stress-induced thymic atrophy, as described in studies linking systemic stress to thymic degradation (**Dhabhar, 2014; Hale et al., 2006**). The Wound + MEBO group exhibited moderate improvement, with partial cortical repopulation and a relative restoration of corticomedullary demarcation, reflecting the regenerative and anti-inflammatory properties attributed to MEBO (**El-Hadidy et al., 2016; Xu et al., 2013**). Notably, the group treated with the plant-based

ointment showed near-normal thymic architecture, with dense thymocyte populations and well-defined cortico-medullary boundaries, suggesting a strong protective and regenerative role of the herbal extract in maintaining thymic integrity and immune function. This effect is likely mediated through anti-inflammatory action and stimulation of cell proliferation (**Shahrajabian et al., 2019; Ghasemian et al., 2016**). These findings support the hypothesis that plant-based therapies may play a significant role in protecting lymphoid tissues from physical damage and enhancing systemic immune competence.

This section presents the results of a histopathological study comparing the effects of several therapeutic interventions on skin, spleen, and thymus tissues within a wound healing model. The control group (A) exhibited normal tissue architecture, indicating a healthy physiological state without pathological changes. This is consistent with the literature that healthy tissues maintain their architectural integrity unless subjected to injury or pathological stimulation (**Kumar et al., 2021**).

The wound + base group (B) demonstrated severe deterioration and tissue disintegration in all studied organs, accompanied by skin necrosis and a dense inflammatory infiltrate, along with splenic structural disturbance, lymphocyte depletion, and cortical thinning in the thymus. These are indicators of an acute inflammatory response and loss of immune regulation, as reported in studies that address delayed wound healing accompanied by an excessive inflammatory response (**Eming et al., 2007; Gurtner et al., 2008**). Treatment with MEBO ointment (group C) resulted in moderate improvement, including partial tissue repair and a relative reduction in inflammation and necrosis, indicating anti-inflammatory and regenerative effects. These properties are documented in several studies demonstrating MEBO's ability to accelerate wound healing by reducing oxidative stress and stimulating growth factors (**Zhang et al., 2020; Li et al., 2015**). In contrast, the plant ointment group (D) showed the greatest improvement, with a near-normal tissue structure, minimal inflammatory infiltration, and advanced epithelial and immune tissue regeneration, reflecting the high efficacy of the plant treatment in supporting wound healing and activating physiological immune responses. Previous studies have indicated that plant extracts rich in polyphenols and flavonoids promote tissue regeneration and reduce inflammation and necrosis through multiple mechanisms, including cytokine modulation and enhanced keratinocyte proliferation (**Kaur et al., 2018; Rahman et al., 2017**).

Thus, the results of this study highlight the superior effect of plant-based therapies on tissue regeneration and immune regulation compared to conventional options, supporting their potential as effective and safe therapeutic alternatives in the context of wound healing.

Conclusion

Conclusion

This study aimed to evaluate the anti-inflammatory efficacy of a multi-component plant extract known as Zasse extract, which consists of clove (*Syzygium aromaticum*), saffron (*Crocus sativus*), thyme (*Thymus vulgaris*), desert toothpick (*Salvadora persica*), alum, and natural salt. This extract was prepared by aqueous infusion (boiling extraction) and then successfully transformed into a topical cream using suitable lipid components and emulsifiers, while ensuring physicochemical stability.

The results of both *in vitro* and *in vivo* experimental analyses demonstrated that this cream has a significant effect in reducing inflammation and accelerating wound healing, compared to the control group. Positive responses were observed in terms of reduced inflammatory markers and improved skin structure in histological examinations, along with encouraging results in biochemical indicators such as reduced MDA concentrations and increased activity of antioxidant enzymes such as SOD and CAT.

These results confirm that the natural ingredients used, known for their therapeutic properties in traditional medicine, are not only effective when used individually, but also exhibit enhanced efficacy when combined in a single topical formulation. Therefore, Zasse Cream holds promise as a safe and natural preparation for treating skin infections and wounds.

We recommend conducting extensive future studies, including human clinical trials and molecular mechanism studies, to ensure its safety and expand its application in the medical and pharmaceutical fields.

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2024، ص7

Annexes

What do you think of the effectiveness of benzoic acid in miswak as an antimicrobial agent?

I think it's very effective I think it's somewhat effective Ineffective I know nothing about benzoic acid

C. Saffron:

Have you ever used or eaten saffron in food or natural treatments? Yes No

How much do you know about the benefits of saffron for eye health?

Excellent Good Basic I don't know anything about that

Do you know that saffron contains powerful antioxidants that help protect the eye? Yes
No

Do you know that saffron contains a substance known for its positive effect on the retina and protecting the eye from damage? Yes No

If yes, how important do you think this substance is for improving eye health?

Very important Somewhat important Not very important

If you have information about saffron, do you think it can help improve vision or reduce eye problems? Yes No

If yes, please explain why:

D. Thyme:

Have you ever used thyme in cooking or treatment? Yes No

Do you have any scientific knowledge about the use of thyme for eye health? Yes No

If yes, how do you think thyme can contribute to improving eye health?

Antibacterial Anti-inflammatory Improves blood circulation in the eye Other:
.....

Do you know that thyme contains the compound thymol, known for its antibacterial and antifungal properties?

Yes, I know No, I didn't know

If yes, how safe do you think thymol is in natural treatments?

Very safe Somewhat safe Not safe

E. Alum:

Do you know that alum has antibacterial properties that may help clean and protect the eye from infections?

Yes, I know No, I didn't know

If yes, how safe do you think eye drops containing alum are as a natural treatment?

Very safe Somewhat safe Not safe

Do you have any concerns about using alum in natural treatments? Yes No

If yes, what are these concerns?

Causes eye irritation Unsafe for long-term use Can cause dry eyes Other:
.....

Have you used alum in other products (e.g., deodorants or traditional medicine)?

Yes No

F. Salt:

What do you think about the effectiveness of salt as a natural cleaning and disinfecting agent?

Very effective Somewhat effective Ineffective I don't know anything
about salt as a cleaning agent

Do you think salt is effective in purifying the eye when diluted in a sterile solution?

Very effective Somewhat effective Ineffective I don't know anything about salt for
eye purification

If yes, how safe do you think saline solution is for the eye when used in appropriate concentration?

Very safe Somewhat safe Not safe

Did you know that some saline solutions are used as an aid to relieve eye irritation, moisturize, clean from dust and impurities, and reduce allergy symptoms (NaCl = 0.091)?

Yes, I knew that No, I didn't know

Do you have previous experience using salt for health purposes (e.g., wound cleaning or eye washing)? Yes No

Do you think combining these natural ingredients in one drop would increase its effectiveness?

Yes No Not sure

To what extent do you think ingredients like clove and saffron could help fight bacterial and fungal infections in the eye?

Very effective Somewhat effective Ineffective

Have you ever read scientific studies confirming the safety of using these natural ingredients in the eye? Yes No

How interested are you in extraction techniques for active ingredients in natural eye drops?

Very interested Somewhat interested Not interested

Do you think extraction methods affect the effectiveness of natural ingredients?

Yes, greatly Yes, but slightly No

In your opinion, how important is the concentration of active ingredients for the effectiveness and safety of eye drops?

Very important Somewhat important Not very important

What are the most important factors for you when choosing a natural eye treatment?

Scientifically proven effectiveness Safety and no side effects Natural ingredients
Availability and cost Other

Do you believe natural eye treatment with herbs can be an effective alternative to industrial treatments?

Yes, I think it's effective Maybe, but I need more scientific evidence No, I think conventional treatments are more effective

If you answered 'maybe', what kind of studies would you like to see to confirm the effectiveness of natural treatments?

Scientific studies Clinical trials on humans User experiences Comparative research between natural and industrial

If this drop is produced, would you prefer it to be:

A daily treatment A temporary treatment for eye issues A preventive product (to protect from eye problems)

What are your biggest concerns that might make you hesitate to use a natural eye drop containing these ingredients?

Possible side effects Ineffectiveness Lack of sufficient scientific studies Other:
.....

Do you believe that eye drops have benefits? Yes No

What are they?

How likely are these drops to cause side effects if used in the eye?

Very unlikely Unlikely Moderate probability High probability I don't know

If there are side effects, what symptoms do you think might occur?

Eye irritation Redness and itching Dry eyes Bacterial infections Other symptoms

Do you think natural eye drops could negatively interact with other medical eye treatments?

Yes, may cause harmful interactions No, not a big risk Not sure

If yes, what interactions do you expect?

Chemical reactions Increased irritation Reduced treatment effectiveness Other

How important is scientific research in developing natural eye drops for you?

Very important Important Not important

In your opinion, how can the development of natural eye treatment products be improved?

Increase scientific research on active ingredients Use advanced extraction techniques
Improve formulation and concentration Ensure safety through lab testing

If this drop is available on the market, how likely are you to buy it based on this information?

Buy it immediately Might buy it after consulting a doctor Will not buy it

What are your expectations from natural products claiming to treat or improve eye health?

Immediate results Gradual results No tangible results

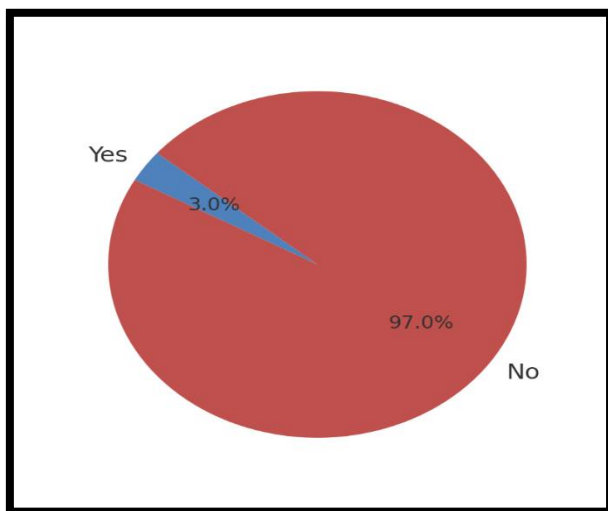
Would you prefer natural products over chemical drugs if they are safe and effective?

Yes No Not sure

Do you have any suggestions for developing or improving the eye drop formula?

.....
.....

If you answered 'Yes', how did you learn about these benefits?



Do you know that cinnamon contains substances that work as anti-inflammatory and pain relief agents?

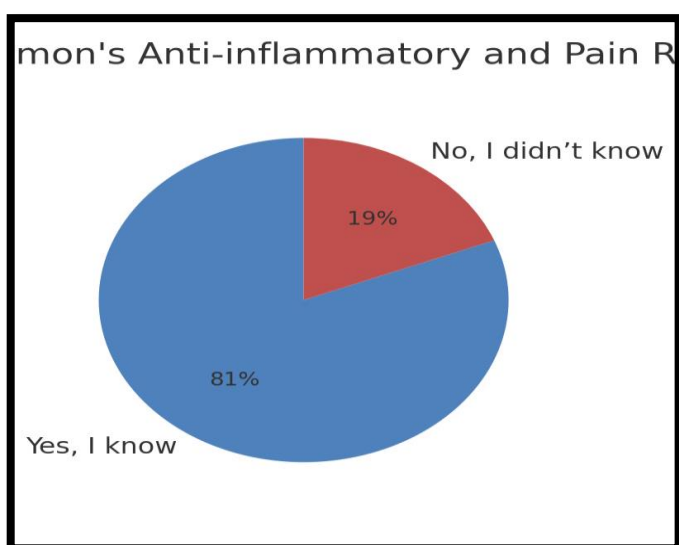
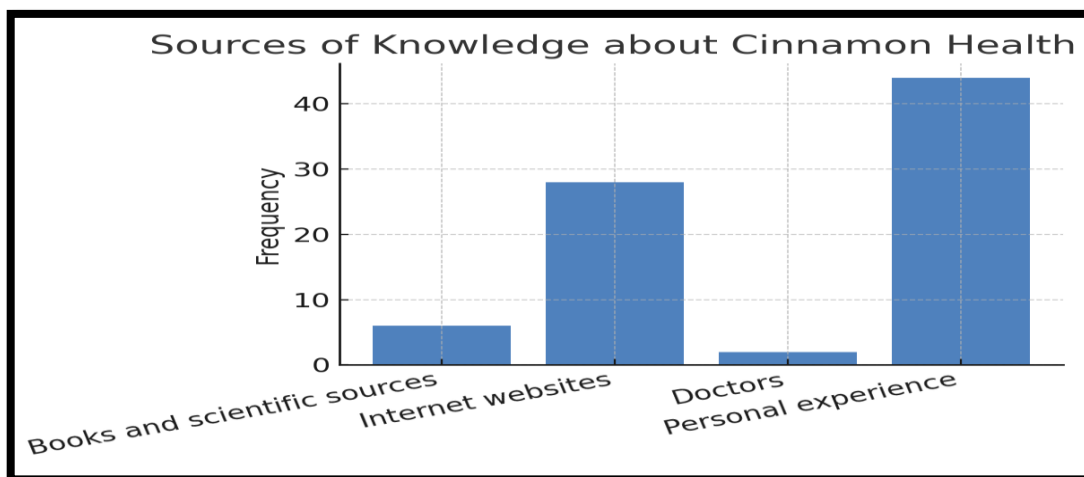


Figure 01 Stages of Wound in Rats



Figure 02: cream MEBO

Vert Medica

ZASSC CREAM

99% Natural , 30 g

مكونات ZASSC CREAM:
يتكون المستخلص من القرنفل، الزعفران، الزعتر، السواك الصحراوي، الشب، والملح الخشن، وهي مكونات ذات خصائص مضادة للالتهاب، مضادة للأكسدة، ومطهرة

طريقة الاستعمال:
يُطبق الكريم موضعياً على الجلد مرتين يومياً، مع تدليك خفيف حتى الامتصاص التام

الاستعمالات:
يستخدم ZASSC CREAM لتخفيف التهابات الجلد، تسريع التئام الجروح، تقليل الألم الموضعي، وعلاج بعض الاضطرابات الجلدية

