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THEME

**Development of a Bioactive Patch Based on (*Marrubium deserti de Noé*)
for Surgical Wound Healing, with Potential Application
to Post-Cesarean Wounds.**

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DEDICATION

In the Name of Allah, the Most Gracious, the Most Merciful All praise is due to Allah, who grants success to every sincere effort and rewards every hard-earned achievement. Peace and blessings be upon our Prophet **Muhammad** (peace be upon him).

I dedicate the fruit of my hard work and this graduation achievement:

To myself... who endured hardships and faced moments of weakness, yet never allowed despair to prevail, and who stands today with pride, saying, "By the grace of Allah, I did it".

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Summary

This study aims to develop a therapeutic approach for post-cesarean section care and to reduce the risk of inflammation and side effects associated with surgical procedures by investigating the medicinal plant *Marrubium deserti* de Noé, commonly known as desert horehound. Plant samples were collected from the Ain Oulmene region in the province of Sétif, Algeria. A methanolic extract was prepared using the maceration method and evaluated through both in vitro and in vivo assays to assess its therapeutic potential against surgical complications resulting from cesarean operations in Wistar rats. The study included the determination of extraction yield and qualitative and quantitative phytochemical analyses to identify major secondary metabolites. In addition, antioxidant activity was evaluated using DPPH and FRAP assays, while anti-inflammatory and antibacterial activities were also investigated. The in vivo experiment was conducted on 25 female Wistar rats divided into five groups of five animals each: a healthy control group, a surgical untreated group, a group treated with *Marrubium deserti* de Noé extract, a group treated with Fusidin®, and a group treated with a combination of the plant extract and Fusidin®. Topical treatments were applied for fifteen consecutive days, and wound healing progression, inflammation, and scar formation were monitored. Qualitative phytochemical screening revealed the presence of several bioactive compounds, including polyphenols, flavonoids, tannins, alkaloids, saponins, anthocyanins, and leucoanthocyanins. The extraction process yielded 13.93%, while quantitative analyses showed high levels of total phenolic and flavonoid contents. The methanolic extract exhibited antioxidant activity in both DPPH and FRAP assays, moderate anti-inflammatory activity, and antibacterial activity against five tested bacterial strains. Furthermore, the in vivo results demonstrated significant improvements in biochemical parameters related to kidney function (GLY, UREA, CREAT, URIC), hematological parameters (WBC, RBC, HGB, PLT, HCT), liver enzyme activities (AST, ALT, GGT, ALP), and inflammatory markers represented by C-reactive protein (CRP). Histological examination also confirmed enhanced tissue repair, accelerated skin regeneration, and restoration of damaged skin structures. These findings highlight the promising therapeutic potential of the methanolic extract of *Marrubium deserti* de Noé, owing to its antioxidant, anti-inflammatory, and antibacterial properties, as well as its ability to accelerate wound healing and reduce scarring and postoperative side effects following cesarean sections. Therefore, this plant may represent a promising natural alternative for the development of effective, safe, and cost-efficient wound-care products.

Keywords: *Marrubium deserti* de Noé, cesarean section, wound healing, antioxidant activity, anti-inflammatory activity, antibacterial activity.

Résumé

Développement d'un patch bioactif à base de Marrubium deserti de Noé pour la cicatrisation des plaies chirurgicales, avec une application potentielle aux plaies post-césariennes.

Cette étude vise à développer une approche thérapeutique pour les soins postopératoires après une césarienne et à réduire les risques d'inflammation ainsi que les effets secondaires associés aux interventions chirurgicales, en évaluant les propriétés médicinales de la plante Marrubium deserti de Noé, connue localement sous le nom de « plante de suture ». Les échantillons végétaux ont été récoltés dans la région d'Aïn Oulmène, wilaya de Sétif (Algérie). Un extrait méthanolique a été préparé par macération puis évalué à travers des essais in vitro et in vivo afin d'étudier son potentiel thérapeutique contre les complications chirurgicales résultant des opérations de césarienne chez des rats Wistar. L'étude a porté sur la détermination du rendement d'extraction ainsi que sur des analyses phytochimiques qualitatives et quantitatives visant à identifier les principaux métabolites secondaires. En outre, l'activité antioxydante a été évaluée à l'aide des tests DPPH et FRAP, tandis que les activités anti-inflammatoire et antibactérienne ont également été examinées. L'expérimentation in vivo a été réalisée sur 25 rats femelles de souche Wistar répartis en cinq groupes de cinq animaux chacun: un groupe témoin sain, un groupe opéré non traité, un groupe traité par l'extrait de Marrubium deserti de Noé, un groupe traité par la pommade Fusidine, et un groupe traité par une association de l'extrait végétal et de la Fusidine. Les traitements topiques ont été appliqués pendant quinze jours consécutifs, avec un suivi de l'évolution de la cicatrisation, de l'inflammation et de la formation des cicatrices. Le criblage phytochimique qualitatif a révélé la présence de plusieurs composés bioactifs, notamment les polyphénols, les flavonoïdes, les tanins, les alcaloïdes, les saponines, les anthocyanes et les leucoanthocyanes. Le rendement d'extraction obtenu était de 13,93 %, tandis que les analyses quantitatives ont montré des teneurs élevées en composés phénoliques totaux et en flavonoïdes. L'extrait méthanolique a présenté une activité antioxydante dans les tests DPPH et FRAP, une activité anti-inflammatoire modérée ainsi qu'une activité antibactérienne contre cinq souches bactériennes testées. Les résultats obtenus in vivo ont également montré une amélioration significative des paramètres biochimiques liés à la fonction rénale (GLY, UREA, CREAT, URIC), des paramètres hématologiques (WBC, RBC, HGB, PLT, HCT), des enzymes hépatiques (AST, ALT, GGT, ALP) ainsi que des marqueurs inflammatoires représentés par la protéine C-réactive (CRP). L'examen histologique a confirmé une amélioration de la réparation tissulaire, une accélération de la régénération cutanée et une restauration des structures endommagées de la peau. Ces résultats mettent en évidence le potentiel

thérapeutique prometteur de l'extrait méthanolique de Marrubium deserti de Noé, grâce à ses propriétés antioxydantes, anti-inflammatoires et antibactériennes, ainsi qu'à sa capacité à accélérer la cicatrisation et à réduire les cicatrices et les effets secondaires postopératoires liés à la césarienne. Cette plante pourrait ainsi constituer une alternative naturelle prometteuse pour le développement de produits de soin des plaies efficaces, sûrs et économiquement accessibles.

Mots-clés: Marrubium deserti de Noé, césarienne, cicatrisation, activité antioxydante, activité anti-inflammatoire, activité antibactérienne.

ملخص

تطوير لصقة حيوية فعالة قائمة على نبات *Marrubium deserti de Noé* من أجل التئام الجروح الجراحية، مع إمكانية تطبيقها على جروح ما بعد العملية القيصرية.

تهدف هذه الدراسة إلى البحث عن بدائل علاجية طبيعية يمكن أن تسهم في تحسين التئام الجروح الناتجة عن العمليات القيصرية والتقليل من مخاطر الالتهابات والآثار الجانبية المصاحبة لها، وذلك من خلال تقييم الخصائص العلاجية للنبات الطبي *Marrubium deserti de Noé* المعروف محلياً بنبتة الخياطة. وقد جُمعت العينات النباتية من منطقة عين ولما بولاية سطيف، وتم تحضير المستخلص الميثانولي للنبات باستعمال تقنية النقع، ثم تقييم بعض أنشطته البيولوجية في المختبر وفي الجسم الحي من أجل دراسة فعاليته العلاجية ضد التأثيرات الجراحية الناجمة عن العمليات القيصرية لدى جرذان سلالة ويستار. شملت الدراسة تحديد مردودية الاستخلاص وإجراء التحاليل الكيميائية النوعية والكمية للكشف عن أهم مركبات الأيض الثانوية، بالإضافة إلى دراسة النشاط المضاد للأكسدة باستعمال اختباري DPPH وFRAP، والنشاط المضاد للالتهاب، والنشاط المضاد للبكتيريا. أما الدراسة التجريبية في الجسم الحي فقد أجريت على 25 جرّداً من إناث سلالة ويستار قُسمت إلى خمس مجموعات متساوية، تمثلت في مجموعة شاهدة سليمة، ومجموعة مصابة بعملية جراحية وغير معالجة، ومجموعة معالجة بمستخلص نبتة الخياطة، ومجموعة معالجة بمرهم فيوسيديين، ومجموعة معالجة بمزيج من المستخلص النباتي وفيوسيديين، أظهرت نتائج التحليل الكيميائي النوعي وجود العديد من المركبات الفعالة بيولوجياً، من بينها البوليفينولات والفلافونويدات والتانات والقلويدات والصابونينات والأنثوسيانينات والليكوأنثوسيانينات، كما سجلت عملية الاستخلاص مردودية معتبرة بلغت 13.93%، في حين كشفت التحاليل الكمية عن محتوى مرتفع من الفينولات والفلافونويدات. كما أظهر المستخلص نشاطاً مضاداً للأكسدة وفق اختباري DPPH وFRAP، ونشاطاً مضاداً للالتهاب بدرجة متوسطة، إضافة إلى فعالية مضادة لخمس أنواع من البكتيريا المختبرة. وبينت نتائج الدراسة في الجسم الحي تحسناً ملحوظاً في مختلف المؤشرات البيوكيميائية المرتبطة بوظائف الكلى (GLY، UREA، CREAT، URIC) والمؤشرات الدموية (WBC، RBC، HGB، PLT، HCT) والإنزيمات الكبدية (ALT، AST، GGT، ALP). فضلاً عن انخفاض مؤشرات الالتهاب ممثلة في البروتين المتفاعل (CRP) C، كما أكدت الفحوص النسيجية حدوث تحسن واضح في البنية النسيجية للجلد وتسارع عمليات الإصلاح والتجدد الخلوي واستعادة سلامة الأنسجة المتضررة. وتُبرز هذه النتائج الإمكانيات العلاجية الواعدة للمستخلص الميثانولي لنبتة الخياطة، لما يتمتع به من خصائص مضادة للأكسدة والالتهاب والبكتيريا، وقدرته على تسريع التئام الجروح والحد من الندبات والآثار الجانبية المترتبة عن العمليات القيصرية،

مما يجعله مرشحاً طبيعياً واعدًا للتطوير في مجال المستحضرات الطبية المخصصة للعناية بالجروح الجلدية وتحسين عملية الشفاء بتكلفة أقل وفعالية جيدة مقارنة بالبدايل التقليدية.

الكلمات المفتاحية: *Marrubium deserti de Noé*، نبتة الخياطة، العمليات القيصرية، التئام الجروح، مضاد للأكسدة، مضاد للالتهاب، مضاد للبكتيريا.

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List of Abbreviations

Abbreviation	Meaning
<i>ATCC</i>	American Type Culture Collection
BSA	Bovine Serum Albumin
CAT	Catalase
CFU	Colony Forming Unit
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ETC	Electron Transport Chain
FRAP	Ferric Reducing Antioxidant Power
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
IC ₅₀	Half Maximal Inhibitory Concentration
MDA	Malondialdehyde
MHA	Mueller–Hinton Agar
NF-κB	Nuclear Factor Kappa B
NO•	Nitric Oxide Radical
ONOO ⁻	Peroxynitrite
O ₂ • ⁻	Superoxide Anion Radical
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
rpm	Revolutions Per Minute
SDA	Sabouraud Dextrose Agar
SOD	Superoxide Dismutase
TBS	Tris-Buffered Saline
TCA	Trichloroacetic Acid
TFC	Total Flavonoid Content

TPC	Total Phenolic Content
UV–Vis	Ultraviolet–Visible Spectrophotometry

List of Symbols

Symbol	Meaning
•	Bullet Point
•	Radical (Unpaired Electron)
–	Anion
O ₂	Molecular Oxygen (Dioxygen)
Fe ²⁺ / Fe ³⁺	Ferrous/Ferric Iron
→	Reaction Arrow
⇌	Equilibrium Arrow
ΔG	Gibbs Free Energy Change
g	Gram
mg	Milligram
mL	Milliliter
μL	Microliter
mm	Millimeter
°C	Degree Celsius
h	Hour
rpm	Revolutions Per Minute
nm	Nanometer
%	Percentage

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INTRODUCTION

The biological properties of (*Marrubium deserti de Noé*) are mainly attributed to its richness in phenolic compounds and flavonoids. These secondary metabolites are well known for their potent antioxidant activity, their capacity to scavenge free radicals, and their ability to modulate inflammatory responses. In addition, these compounds contribute to tissue protection and promote the healing process through their antimicrobial and regenerative effects. Wound healing is a complex biological process involving a sequence of overlapping phases, including inflammation, cell proliferation, collagen synthesis, and tissue remodeling. Oxidative stress and persistent inflammation are among the major factors that interfere with this process, particularly in deep wounds and post-surgical wounds. Among common surgical procedures, cesarean section is frequently performed when vaginal delivery is not possible or may endanger the health of the mother or fetus. Despite significant advances in surgical techniques and medical care, cesarean section remains associated with several potential complications, including postoperative pain, local infections, delayed wound healing, scar formation, and bacterial infections, all of which may negatively affect maternal health and prolong the recovery period (Betrán *et al.*, 2021). In this context, medicinal plants emerge as promising therapeutic alternatives due to their richness in natural compounds capable of combating inflammation, oxidative stress, and microbial infections, which are key factors associated with surgical wound complications. Therefore, it is hypothesized that the high content of phenolic compounds and flavonoids in (*Marrubium deserti de Noé*) may confer significant antioxidant and anti-inflammatory activities capable of enhancing

tissue regeneration and accelerating the healing of deep wounds and cesarean section wounds. Accordingly, the present study was designed to evaluate the therapeutic potential of the methanolic extract of (*Marrubium deserti de Noé*) through the investigation of its chemical and biological properties and the assessment of its effectiveness in improving the healing of cesarean section wounds in Wistar rats using biochemical, hematological, and histological parameters related to the wound-healing process. Based on the above considerations, the main research question can be formulated as follows: To what extent can the methanolic extract of (*Marrubium deserti de Noé*) contribute to accelerating the healing of cesarean section wounds and reducing the associated inflammation and complications through its antioxidant, anti-inflammatory, and antibacterial properties?

PART ONE

Bibliographic Review

CHAPTER I

Bibliographic Review — (*Marrubium
deserti de Noé*)

1.Introdaction

This chapter presents a comprehensive bibliographic review centred on (*Marrubium deserti de Noé*), a medicinal plant of the Lamiaceae family distributed across arid and semi-arid regions of North Africa. We begin by contextualising the plant within a broader botanical framework through a detailed presentation of the Lamiaceae family and the genus *Marrubium*, encompassing systematics, geographic and botanical distribution, economic interest, and therapeutic applications documented in the ethnobotanical literature.

A dedicated section then examines the secondary metabolites identified in the genus *Marrubium*, employing a structured classification that covers phenolic compounds, flavonoids, saponins, tannins, alkaloids, terpenes, sterols, and anthraquinones—metabolic classes whose structural diversity underpins a wide spectrum of biological activities. The chapter subsequently addresses the oxidant system, integrating current mechanistic understanding of oxidative stress, the nature and generation of reactive oxygen species (ROS) and other free radicals, and the multiple layers of antioxidant defence.

Throughout, we synthesise published evidence critically, highlighting convergences and contradictions in the literature and situating (*Marrubium deserti de Noé*) within the wider scientific discourse on plant-derived antioxidants and bioactive compounds.

This review serves as the theoretical foundation for the experimental investigations reported in subsequent chapters of this thesis.

1.1 Presentation of the Lamiaceae Family

1.1.1 Generalities about the Lamiaceae Family

The Lamiaceae Martinov (1820)—commonly designated the mint family or, by its former name, Labiatae—constitutes one of the most economically and medicinally significant families within the Angiosperms. Encompassing approximately 236 genera and between 6,900 and 7,200 accepted species, the family ranks among the largest of all flowering plant families and has attracted sustained scientific attention from systematists, pharmacognosists, and toxicologists alike (Harley *et al.*, 2004; APG IV, 2016).

The family's circumscription has been substantially revised following molecular phylogenetic analyses that incorporated previously segregated families, including Verbenaceae (in part) and Chloanthaceae, within an expanded Lamiaceae sensu lato (Judd *et al.*, 2016).

Morphologically, Lamiaceae members display a constellation of diagnostic characters. The stem is typically quadrangular (four-angled) in cross-section, a feature attributable to collenchymatous strand development at each corner. Leaves are arranged in opposite or whorled pairs, are generally simple, and frequently bear trichomes—both glandular and non-glandular—that secrete volatile terpenoid compounds responsible for the characteristic aromatic properties of the family (Harley *et al.*, 2004). Flowers are almost invariably zygomorphic (bilaterally symmetrical), grouped in condensed cymose inflorescences that often appear as whorled verticillasters around the stem. The corolla is characteristically bilabiate, comprising an upper (adaxial) lip of two fused petals and a lower (abaxial) lip of three fused petals. The calyx is typically tubular with five teeth, and the androecium usually contains four didynamous (two long, two short) stamens, occasionally reduced to two, attached to the corolla tube (Cantino, 1992).

The gynoecium is composed of two fused carpels, resulting in a deeply four-lobed ovary and a gynobasic style—a feature considered synapomorphic for the core Lamiaceae. The fruit is characteristically a schizocarp separating into four one-seeded nutlets (mericarps), though fleshy drupes occur in some lineages (Judd *et al.*, 2016). The prevalence of glandular trichomes, elaborate pollinator-attracting floral architecture, and an exceptionally diverse secondary chemistry collectively suggest that the family's evolutionary success has been shaped substantially by co-evolutionary pressures with pollinators and herbivores (Paton *et al.*, 2004).

From a phytochemical perspective, Lamiaceae members are characterised by an unusually rich secondary metabolite profile.

Monoterpenoids and sesquiterpenoids dominate the essential oil fraction, with compounds such as menthol (*Menthaspp.*), thymol and carvacrol (*Thymus* and *Origanumspp.*), and camphor (*Salviaspp.*) representing archetypal examples (Baser & Buchbauer, 2010). Beyond volatile terpenoids, the family is a prolific producer of phenolic acids—particularly rosmarinic acid, widely regarded as a marker compound for Lamiaceae—as well as labdane, clerodane, and abietanediterpenoids, iridoidglycosides, and a variety of flavonoids (Harley *et al.*, 2004; Petersen & Simmonds, 2003). The biosynthetic versatility of the family renders it a

valuable pharmacological resource; indeed, several Lamiaceae genera are among the most commonly studied plant groups globally for antimicrobial, anti-inflammatory, and antioxidant properties.

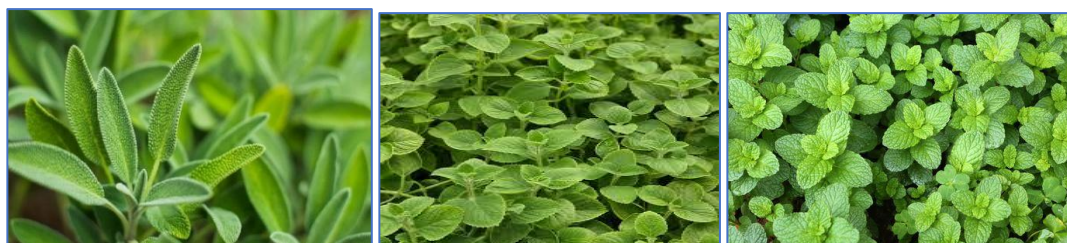


Figure (1): Representative species of the Lamiaceae family (Eidi *et al.*, 2013).

1.1.2 Geographic Distribution

The Lamiaceae exhibits a cosmopolitan distribution, with representatives documented on every continent except Antarctica, yet it achieves its greatest species richness and ecological dominance in specific biogeographical regions. The centre of maximum diversity is generally recognised as the Mediterranean Basin and the Near East, encompassing the Irano-Turanian phytogeographic region, where environmental conditions—characterised by hot, dry summers, mild wet winters, and typically calcareous, shallow soils—appear to have driven the speciation of numerous genera, particularly *Salvia*, *Thymus*, *Stachys*, *Phlomis*, and *Marrubium* (Heywood, 1993; Harley *et al.*, 2004).

A secondary centre of diversity is recognised in the highlands of tropical and subtropical Africa, especially East Africa and the Great Rift Valley region, where genera such as *Clerodendrum*, *Plectranthus*, and *Coleus* exhibit high endemism (Paton *et al.*, 2004). The Americas constitute a third centre, with *Salvia* alone contributing over 500 neotropical species, many restricted to montane cloud forests of Mexico and the Andes (Walker *et al.*, 2004). In contrast, the representation of the family in humid equatorial forests and high-latitude boreal zones is comparatively sparse, consistent with a broad ecological preference for seasonally dry, open habitats.

Within North Africa and the Algerian phytogeographic context, the Lamiaceae assumes particular prominence. Algeria's floral biodiversity, documented extensively by Quezel & Santa (1963) in their authoritative *Nouvelle Flore de l'Algérie*, recognises numerous native Lamiaceae taxa distributed across a steep gradient of bioclimatic zones—from the humid northern Tell Atlas to the hyperarid Saharan interior. Genera with notable representation include *Thymus*, *Rosmarinus*, *Salvia*, *Lavandula*, *Origanum*, *Teucrium*, and *Marrubium*, the last of which shows a marked affinity for desert margins and pre-Saharan steppes (Quezel & Santa, 1963; Boudjelal *et al.*, 2012). This geographic context is directly relevant to the present study, as (*Marrubium deserti de Noé*) is principally a North African–Saharan taxon whose ecological niche corresponds closely to this transitional zone.



Figure (2): Semi-arid Mediterranean and pre-Saharan habitat representing the geographic distribution of certain Lamiaceae species (Ghedadba *et al.*, 2021).

1.1.3 Botanical Description

Lamiaceae members span an extraordinary range of growth forms, from diminutive annual herbs (e.g., *Salvia aegyptiaca*) to large woody shrubs and small trees (e.g., *Vitex agnus-castus*, *Tectona grandis*—teak). The majority of species are herbaceous perennials or dwarf subshrubs, with the perennial habit frequently associated with geophytic adaptation to drought and fire regimes characteristic of Mediterranean-type ecosystems (Heywood, 1993).

Within the genus *Marrubium*—the focal genus of this study—plants are perennial aromatic subshrubs or herbs, densely covered with woolly-tomentose indumentum of branched or stellate trichomes that serve multiple adaptive functions: reduction of transpirational water loss, reflection of excess solar radiation, and deterrence of generalist herbivores (*Pottier-*

Alapetite, 1979). Stems are woody at the base, freely branched, and typically 20–80 cm in height. Leaves are petiolate, ovate to sub-orbicular, crenate or dentate at the margins, and strongly rugose (*wrinkled*) on the adaxial surface—a texture attributable to the dense venation network visible in dried specimens. Inflorescences are arranged in globose verticillasters, each containing numerous small white to pale-lilac flowers subtended by spinescent bracteoles. The calyx bears 5–10 unequal teeth that become indurate and reflexed at maturity, facilitating epizoochorous seed dispersal (*Quezel & Santa, 1963; Harraz et al., 2019*). These botanical characters are broadly consistent across the genus, with (*Marrubium deserti de Noé*) distinguished from congeners principally by calyx morphology, indumentum density, and leaflet shape—characters elaborated in Section 1.2.

1.1.4 Economic Interest and Therapeutic Uses of Lamiaceae

The Lamiaceae occupies an exceptional position among plant families from economic, cultural, and pharmacological perspectives. The culinary contribution of the family is immeasurable: species of *Ocimum* (basil), *Mentha* (mint), *Thymus* (thyme), *Origanum* (oregano), *Rosmarinus* (rosemary), *Salvia* (sage), and *Lavandula* (lavender) collectively constitute a disproportionate share of global spice and condiment markets (*Baser & Buchbauer, 2010*). Essential oil industries derived from Lamiaceae genera—particularly *Mentha*, *Lavandula*, *Thymus*, and *Salvia*—represent multi-billion-dollar sectors of the flavour, fragrance, and nutraceutical industries worldwide.

Therapeutically, the family has been employed in virtually all traditional medical systems across its geographic range. In Unani, Ayurvedic, and traditional Chinese medicine, species of *Salvia*, *Mentha*, and *Perilla* are employed for respiratory, gastrointestinal, and neurological conditions (*Petersen & Simmonds, 2003*). In North African and Saharan ethnomedicine, Lamiaceae herbs—including *Thymus algeriensis*, *Rosmarinus officinalis*, and *Marrubium* species—are widely used for the management of respiratory infections, digestive disorders, diabetes, hypertension, and febrile illnesses (*Boudjelal et al., 2012; Lazreg-Aref et al., 2012*). *Marrubium vulgare* (white horehound) merits particular mention: its expectorant properties, mediated principally by the diterpenoid lactone marrubium, have been validated in multiple pharmacological studies and the plant is listed in several European pharmacopoeias (*European Scientific Cooperative on Phytotherapy [ESCOP], 2003; Gruenwald et al., 2007*).

From a toxicological standpoint—pertinent to the disciplinary framework of this thesis—certain Lamiaceae members have demonstrated genotoxic, hepatotoxic, or cytotoxic effects at elevated doses or following prolonged administration. Pulegone, a monocyclic monoterpenoid abundant in *Mentha pulegium* essential oil, is metabolically activated to reactive intermediates capable of inducing hepatocellular damage, a toxicity documented in both experimental animal models and human cases (*Gordon et al., 2003*).

Similarly, the pyrrolizidine alkaloid content of some Lamiaceae species (e.g., *Symphytum officinale*) presents hepatotoxic and potentially carcinogenic risks that necessitate careful risk–benefit assessment in medicinal contexts (*EFSA, 2017*). This toxicological dimension underscores the importance of systematic phytochemical and safety characterisation of under-investigated species such as (*Marrubium deserti de Noé*).

1.2 Botanical Description of the Genus *Marrubium*

1.2.1 Systematics

The genus *Marrubium* L. was formally described by Linnaeus in *Species Plantarum* (1753) and belongs to the tribe Marrubieae within the subfamily Lamioideae of the Lamiaceae (*Harley et al., 2004*). The tribe Marrubieae, as circumscribed in recent molecular phylogenetic treatments (*Bendiksby et al., 2011*; *Bräuchler et al., 2010*), is characterised by the combination of a strongly spiny calyx, actinomorphic or only weakly zygomorphic flowers, and comparatively reduced (two-lipped) corollas. *Marrubium* is monotypic in its tribe or grouped with a small number of closely related genera depending on the systematic treatment adopted.

The genus comprises approximately 40–50 accepted species, distributed primarily around the Mediterranean Basin, extending east through the Caucasus, Iran, and Central Asia, and south into North Africa and the Canary Islands (*Pottier-Alapetite, 1979*; *Harraz et al., 2019*). The Algerian flora recognises several native or naturalised taxa, notably *Marrubium vulgare* L., (*Marrubium deserti de Noé*) de Noé, *M. alysson* L., and *M. supinum* L., with (*Marrubium deserti de Noé*) de Noé being the most strongly adapted to xeric conditions (*Quezel & Santa, 1963*). (*Marrubium deserti de Noé*) de Noé — hereinafter (*Marrubium deserti de Noé*) — is the species of primary concern in this study. The epithet 'deserti' (of the desert) reflects its ecological affinity for hyperarid and semi-arid substrates. The species was first described by the French

botanist Xavier de Noé in the nineteenth century based on specimens collected from Algerian desert margins (Noé, 1852, as cited in Pottier-Alapetite, 1979).

Its distinguishing morphological characters include: densely white-tomentose stems and leaves; broadly ovate to sub-orbicular leaves (2–5 cm) with deeply crenate margins; calyx with 5 prominent, straight, hooked teeth (not recurved as in *M. vulgare*); and corolla white with a minutely two-lobed upper lip (Quezel & Santa, 1963). These features differentiate (*Marrubium deserti de Noé*) from congeners and have been confirmed in recent field studies conducted in southern Algeria (Boudjelal *et al.*, 2012; Harraz *et al.*, 2019).

Phytogeographically, *Marrubium deserti de Noé* belongs to the Saharo-Mediterranean element of the North African flora, occurring primarily in the transitional zone between the pre-Saharan steppe and the northern desert proper, at altitudes ranging from approximately 400 to 1,200 m above sea level. In Algeria, the species is documented across the provinces of Biskra, El-Oued, Laghouat, and Ouargla—a distribution pattern that places it within the bioclimatic ambit of the University of Echahid Hamma Lakhdar El-Oued, where the present research is conducted. This local availability, combined with the plant's established use in Saharan folk medicine, motivates its selection as the focus of this toxicological investigation.

Table (1): Taxonomic classification of (*Marrubium deserti de Noé*) within the Lamiaceae family.

Taxonomic Rank	Taxon
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	<i>Marrubium</i>
Species	<i>Marrubium deserti de Noé</i>

Source: Adopted from Bentham (1848) and The Plant List (2013), as cited in Harraz *et al.* (2019).

1.2.2 Secondary Metabolites of the Genus *Marrubium*

Research into the phytochemistry of the genus *Marrubium* has been ongoing since the isolation of marrubiin by Thümmel in 1862 (as reviewed by El-Seedi *et al.*, 2009), and the corpus of literature has expanded considerably since the 1990s with the widespread adoption of high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Collectively, the genus is now recognised as a rich source of structurally diverse secondary metabolites, with diterpenoids, flavonoids, phenolic acids, and iridoids representing the most intensively studied compound classes.

Among diterpenoids, the labdane-type lactone marrubiin (C₂₀H₂₈O₃) stands as the most celebrated and widely documented compound across the genus. Isolated from *M. vulgare* with particularly high concentrations, marrubiin has also been reported in (*Marrubium deserti de Noé*), *M. peregrinum*, *M. alysson*, and *M. supinum*, suggesting a biosynthetically conserved pathway within the genus (El-Seedi *et al.*, 2009; Mrabti *et al.*, 2019). The compound's pharmacological profile includes expectorant, antinociceptive, anti-inflammatory, antiarrhythmic, and vasodilatory activities, well-documented in rodent models (Schlemper *et al.*, 1996; Pukalskas *et al.*, 2012). Related diterpenoids—including marrubenol, marrubasch, marrubiinic acid, and premarrubiin—have also been identified, with their relative abundances varying between species and between plant organs and phenological stages (Harraz *et al.*, 2019).

The flavonoid complement of *Marrubium* species encompasses flavones and flavonols, primarily as O- and C-glycosides. Luteolin, apigenin, diosmetin, and their 7-O-glucuronide conjugates are consistently reported across multiple taxa, while quercetin and kaempferol derivatives appear with lower frequency (Boudjelal *et al.*, 2012; Mrabti *et al.*, 2019). Importantly, specific flavonoid profiles appear to distinguish (*Marrubium deserti de Noé*) from the more widely studied *M. vulgare*; for instance, (Harraz *et al.* 2019) reported elevated concentrations of luteolin-7-O-glucoside and apigenin-7-O-glucoside in (*Marrubium deserti de Noé*) aerial parts, whereas marrubin-derived diterpenoids were proportionally more abundant in *M. vulgare*. These compositional differences may have significant implications for the comparative bioactivity and toxicity profiles of the two species.

Phenolic acid profiling of (*Marrubium deserti de Noé*) extracts has revealed the presence of rosmarinic acid, caffeic acid, chlorogenic acid, and protocatechuic acid as the dominant

constituents, with rosmarinic acid typically contributing the highest proportion of the total phenolic content (Boudjelal *et al.*, 2012; Lazreg-Aref *et al.*, 2012). The iridoid glycoside fraction, less extensively characterised in (*Marrubium deserti de Noé*) specifically, includes ajugol and harpagide—compounds associated with sedative and anti-inflammatory activities in related Lamiaceae genera (Ghia *et al.*, 2011). Alkaloids of the pyrrolizidine and imidazole classes, though recorded at low concentrations in some *Marrubium* taxa, require systematic evaluation in (*Marrubium deserti de Noé*) given their potential toxicological relevance (Lazreg-Aref *et al.*, 2012).

Table (2): Principal secondary metabolite classes reported in the genus *Marrubium*.

Metabolite Class	Representative Compounds	Species Reported	Key Activities
Diterpenoids	Marrubiin, marrubenol, marrubasch	<i>M. vulgare</i> , (<i>Marrubium deserti de Noé</i>)	Expectorant, anti-inflammatory
Flavonoids	Luteolin, apigenin, quercetin glycosides	Multiple spp.	Antioxidant, antimicrobial
Phenolic acids	Rosmarinic acid, caffeic acid	Multiple spp.	Antioxidant, anti-inflammatory
Iridoids	Harpagide, ajugol	<i>M. vulgare</i>	Sedative, anti-inflammatory
Alkaloids	Stachydrine, betonicine	Multiple spp.	Hypotensive
Tannins	Condensed and hydrolysable	Multiple spp.	Astringent, antimicrobial
Saponins	Triterpenoid saponins	Multiple spp.	Haemolytic, expectorant
Essential oils	Thymol, carvacrol, limonene	<i>Marrubium deserti de)</i> (<i>Noé</i>)	Antimicrobial, antifungal

Source: Compiled from El-Seedi *et al.* (2009), Boudjelal *et al.* (2012), Mrabti *et al.* (2019), and Harraz *et al.* (2019).

1.2.3 Uses and Biological Activities

The genus *Marrubium* occupies a prominent position in the ethnobotanical pharmacopoeias of the Mediterranean and North African regions. In Algeria, (*Marrubium deserti de Noé*)—known locally as murriyya or qettaf—is employed by traditional healers in the treatment of respiratory conditions (coughs, asthma, bronchitis), gastrointestinal disorders (flatulence, dyspepsia, hepatic pain), fever, rheumatic pain, and wound infections (Quezel & Santa, 1963; Boudjelal *et al.*, 2012). Preparations typically involve aqueous decoctions or maceration in warm water of the aerial parts, with less frequent use of the root.

The congruence of these traditional uses with the documented pharmacological activities of isolated compounds lends support to the ethnopharmacological validity of the species.

Antimicrobial activity represents one of the most consistently documented biological properties of *Marrubium* extracts across different studies and solvent systems. Boudjelal *et al.* (2012) demonstrated that methanolic extracts of (*Marrubium deserti de Noé*) exhibited significant inhibitory activity against clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, with minimum inhibitory concentrations (MICs) comparable to those of reference antibiotics in the case of the Gram-positive bacterium.

The antimicrobial action has been attributed to the combined effect of phenolic acids, particularly rosmarinic acid—a potent inhibitor of bacterial DNA gyrase—and terpenoid compounds that disrupt membrane integrity (Pukalskas *et al.*, 2012).

Antioxidant capacity is the biological property most extensively investigated in recent literature on *Marrubium*, driven partly by the growing recognition of oxidative stress as a pathomechanism common to chronic diseases prevalent in North African populations. Harraz *et al.* (2019) and Mrabti *et al.* (2019) independently demonstrated that methanolic and ethyl acetate fractions of (*Marrubium deserti de Noé*) aerial parts exhibited substantial DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and ferric reducing antioxidant power (FRAP), with IC₅₀ values in the range of 40–120 µg/mL depending on extraction conditions. These values were generally comparable to, though somewhat higher than, the synthetic antioxidant butylated hydroxytoluene (BHT), suggesting that (*Marrubium deserti de Noé*) extracts possess biologically relevant antioxidant capacity, albeit inferior to the synthetic standard in the tested assay systems.

Anti-inflammatory activity, evaluated both in vitro (inhibition of cyclooxygenase and lipoxygenase enzymes) and in vivo (rodent models of carrageenan-induced paw oedema and acetic acid-induced peritonitis), has been reported for *M. vulgare* and *M. alysson* extracts, with marrubiin identified as the primary active constituent (Schlemper *et al.*, 1996; Rodrigues *et al.*, 2012).

Whether the diterpenoid profile of (*Marrubium deserti de Noé*) confers equivalent anti-inflammatory potency remains incompletely characterised, and this constitutes a scientifically meaningful research gap that the present thesis begins to address through in vitro toxicological and biological activity assays.

Cytotoxic and antiproliferative activities of *Marrubium* extracts have been investigated in cancer cell line models. Mrabti *et al.* (2019) reported that hexane and dichloromethane fractions of *M. vulgare* displayed moderate cytotoxicity against HeLa (cervical carcinoma) and MCF-7 (breast adenocarcinoma) cells, with IC₅₀ values of 85–200 µg/mL—a pharmacological range generally considered modest but worthy of further fractionation to identify active sub-fractions.

Diterpenoids, particularly marrubiin and its synthetic analogues, have emerged as candidate antiproliferative agents; their mechanism appears to involve induction of apoptosis via mitochondrial pathway activation and downregulation of Bcl-2 family proteins (Pukalskas *et al.*, 2012). Toxicological evaluation of (*Marrubium deserti de Noé*)—the principal objective of the present study—therefore extends this body of work by examining the dose–response relationship and mechanistic basis of any cytotoxic effects at the cellular level.

Table (3): Geographic distribution of selected *Marrubium* species.

Species	Native Range	Preferred Habitat
<i>Marrubium deserti de Noé</i>	Algeria, Tunisia, Libya	Desert margins, rocky slopes
M. vulgare L.	Europe, North Africa, W. Asia	Roadsides, waste places
M. alysson L.	Mediterranean basin	Dry grasslands
M. peregrinum L.	Eastern Mediterranean, Balkans	Rocky hillsides
M. supinum L.	Iberian Peninsula	Mountain pastures

Source: Compiled from Pottier-Alapetite (1979), Quezel & Santa (1963), and Harraz *et al.* (2019).

1.3 Secondary Metabolites

1.3.1 Classification of Secondary Metabolites

Secondary metabolites, also designated natural products or specialised metabolites, are organic compounds synthesised by organisms that are not directly involved in normal growth, development, or reproduction the primary metabolic processes essential for survival. Unlike primary metabolites (sugars, amino acids, fatty acids, nucleotides), secondary metabolites have historically been conceived as the biochemical innovations of individual lineages, shaped by selective pressures including herbivory, microbial pathogen attack, competition, and environmental stress (Hartmann, 2007).

This functional diversity is reflected in the extraordinary structural heterogeneity of the secondary metabolome: tens of thousands of distinct structures have been characterised from the plant kingdom alone, with new compounds continuing to be isolated at a substantial rate (Wink, 2010).

The classification of plant secondary metabolites presents taxonomic and biochemical challenges, and multiple classification schemes coexist in the literature. Biosynthetically, compounds are broadly categorised according to their metabolic origin: (i) the shikimate pathway (phenylpropanoids, including phenolic acids, lignins, and many flavonoids); (ii) the

mevalonate and methylerythritol phosphate (MEP) pathways (terpenoids and steroids); (iii) the malonate/acetate pathway (polyketides, including certain flavonoids and anthraquinones); and (iv) amino acid catabolism (alkaloids, non-protein amino acids, glucosinolates) (Dewick, 2009). A more practically oriented classification groups compounds by their core chemical skeleton and functional properties, as adopted in the present review. The major classes discussed below—phenolic compounds, flavonoids, saponins, tannins, alkaloids, terpenes, sterols, and anthraquinones—represent those with documented or probable occurrence in (*Marrubium deserti de Noé*) or related *Marrubium* taxa.

1.3.1.1 Phenolic Compounds

Phenolic compounds molecules bearing at least one aromatic ring carrying one or more hydroxyl substituents constitute the largest and most structurally diverse class of plant secondary metabolites, with more than 8,000 structures identified (Dai & Mumper, 2010). Their biosynthesis proceeds primarily via the shikimate/phenylpropanoid pathway, with phenylalanine serving as the principal aromatic amino acid precursor. Phenylalanine ammonia lyase (PAL) catalyses the deamination of phenylalanine to trans-cinnamic acid, the pivotal branch-point intermediate from which C₄, C₆–C₁, C₆–C₃, and C₆–C₃–C₆ structural types are elaborated through successive hydroxylation, methylation, glycosylation, and esterification reactions (Vogt, 2010).

Simple phenolic acids including hydroxybenzoic acids (e.g., gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic acids) and hydroxycinnamic acids (e.g., caffeic, p-coumaric, ferulic, sinapic, rosmarinic acids) are the most widely distributed phenolic monomers in the plant kingdom.

Their biological significance is multifaceted: they serve as building blocks for polymeric phenolics (lignins, tannins), as signalling molecules in plant–pathogen interactions, and as potent antioxidants capable of donating hydrogen atoms to neutralise reactive free radicals (Rice-Evans *et al.*, 1996). Rosmarinic acid, a caffeic acid ester of 3,4-dihydroxyphenyllactic acid, deserves particular mention in the *Marrubium* context, as it represents a dominant phenolic acid in multiple species of this genus and contributes substantially to total antioxidant capacity as measured by spectrophotometric assays (Boudjelal *et al.*, 2012).

The physiological and pharmacological activities of phenolic compounds extend beyond antioxidant action to encompass anti-inflammatory (via inhibition of prostaglandin-

synthesising enzymes), antimicrobial, antiviral, antimutagenic, and antitumour effects, as reviewed comprehensively by Dai & Mumper (2010). Their bioavailability in the human gastrointestinal tract is contingent upon their molecular weight, glycosylation status, and structural complexity; simple phenolic acids are generally more readily absorbed than polymeric forms (Manach *et al.*, 2005).

1.3.1.2 Flavonoids

Flavonoids represent a structurally defined sub-class of phenolic compounds characterised by a C6–C3–C6 diphenylpropane skeleton, consisting of two aromatic rings (A and B rings) interconnected by a three-carbon bridge that typically forms an oxygenated heterocyclic ring (C ring). This core flavone scaffold undergoes extensive biological diversification through hydroxylation, methoxylation, glycosylation, acylation, and prenylation, generating in excess of 9,000 identified structures distributed across six principal sub-classes: flavones, flavonols, flavanones, flavanonols (dihydroflavonols), isoflavones, and anthocyanins (Winkel-Shirley, 2001; Harborne & Williams, 2000).

Biosynthetically, the A ring is derived from three acetate (malonyl-CoA) units via the polyketide route, while the B ring is of shikimate origin via 4-coumaroyl-CoA, the reaction being catalysed by chalcone synthase (CHS) to form the universal C15 precursor naringenin chalcone. Subsequent stereospecific isomerisation by chalcone isomerase (CHI), followed by a branching network of cytochrome P450-dependent hydroxylases and transferases, generates the diverse sub-class structures (Winkel-Shirley, 2001). This biosynthetic architecture means that flavonoid profiles can serve as chemotaxonomic markers, a principle that has been exploited in differentiating *Marrubium* species (Harraz *et al.*, 2019).

The pharmacological relevance of flavonoids in *Marrubium* is substantial. Luteolin (3',4',5,7-tetrahydroxyflavone) and apigenin (4',5,7-trihydroxyflavone) are the most consistently identified flavones in *M. vulgare* and have been detected in (*Marrubium deserti de Noé*) extracts (Boudjelal *et al.*, 2012; Mrabti *et al.*, 2019). Luteolin exhibits potent antioxidant activity by virtue of the catechol (o-dihydroxy) moiety in its B ring, which confers exceptional free radical-scavenging capacity through electron or hydrogen atom donation, metal chelation, and inhibition of lipid peroxidation (Rice-Evans *et al.*, 1996). Beyond antioxidant function, luteolin inhibits nuclear factor kappa-B (NF-κB) signalling, a central regulator of inflammatory gene expression, and has demonstrated antiproliferative and pro-apoptotic effects in multiple

cancer cell models (López-Lázaro, 2009). Quercetin, the most extensively studied dietary flavonol, has also been identified in *Marrubium* extracts and shares many of these mechanistic properties.

It should be noted that flavonoid bioavailability is substantially influenced by the degree and pattern of glycosylation: O-glycosides are typically deglycosylated by intestinal lactase phloridzin hydrolase or bacterial β -glucosidases prior to absorption, whereas C-glycosides—where the sugar is attached directly to the ring carbon—are considerably more resistant to enzymatic hydrolysis and consequently may exhibit different systemic bioavailabilities (Hollman & Katan, 1999). This distinction has implications for the interpretation of in vitro antioxidant data: bioassays performed on crude extracts reflect the combined contribution of glycoside and aglycone forms, which may not accurately predict in vivo antioxidant efficacy.

1.3.1.3 Saponins

Saponins are a heterogeneous class of amphiphilic glycosides in which one or more sugar chains (mono- or polysaccharide) are linked via a glycosidic bond to a steroidal (C27) or triterpenoid (C30) aglycone (sapogenin). The term 'saponin' derives from the Latin *sapo* (soap), reflecting the characteristic property of these compounds to produce stable foam in aqueous solution as a consequence of their surfactant-like structure. The steroidal saponins (spirostanol and furostanol types) predominate in monocotyledonous families (Liliaceae, Dioscoreaceae), whereas pentacyclic oleanane, ursane, and lupane triterpenoid saponins are most common in dicotyledonous plants, including Lamiaceae (Hostettmann & Marston, 1995).

The biological activities of saponins are diverse and mechanistically linked to their amphiphilic character. At the cellular membrane level, saponins interact with membrane sterols (principally cholesterol) to form pores that increase membrane permeability, an action underlying their haemolytic activity—a long-recognised property that complicates their parenteral administration but is exploited in adjuvant formulations for vaccines (e.g., Quillaja saponins in ISCOMs) (Rao & Gurfinkel, 2000).

In the gastrointestinal tract, saponins reduce cholesterol absorption by forming insoluble complexes with bile acids, a mechanism proposed to explain the hypocholesterolaemic effects documented in clinical trials with leguminous and *Chenopodium album* extracts (Oakenfull, 1981). Immunostimulatory, anti-inflammatory, antifungal, and expectorant activities have additionally been attributed to specific saponin structures.

In the context of *Marrubium*, triterpenoid saponins though less extensively characterised than diterpenoids and flavonoids have been reported in aerial and root fractions of several species (Lazreg-Aref *et al.*, 2012). Their quantitative significance in (*Marrubium deserti de Noé*) specifically remains to be fully determined; detection in the course of preliminary phytochemical screening (Harraz *et al.*, 2019) suggests that saponins contribute to the overall biological activity profile of this species and may modulate the absorption of co-administered bioactive compounds.

1.3.1.4 Tannins

Tannins are water-soluble, high-molecular-weight polyphenolic compounds defined functionally by their capacity to precipitate proteins, form complexes with metal cations, and exhibit astringent organoleptic properties. They are classified into two major structural groups: hydrolysable tannins (HTs) and condensed tannins (CTs, proanthocyanidins). Hydrolysable tannins are esters of a polyol (typically glucose) with gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins), and are susceptible to acid, alkaline, or enzymatic hydrolysis.

Condensed tannins, in contrast, are oligomeric or polymeric flavanoids assembled from flavan-3-ol units (catechin, epicatechin, and their galloylated derivatives) linked predominantly through C4–C8 or C4–C6 carbon–carbon bonds, rendering them resistant to hydrolysis (Haslam, 1989).

The ecological functions of tannins centre on their protein-precipitation capacity: by denaturing exogenous proteins in the oral cavity and gut of herbivores and inhibiting salivary enzymes, tannins reduce the palatability and nutritional value of plant material, constituting a major anti-herbivore chemical defence (Wink, 2010).

In addition, tannins exhibit broad-spectrum antimicrobial activity through protein complexation with bacterial and fungal enzymes and structural proteins, and their free radical-scavenging capacity particularly that of ellagitannins and highly hydroxylated gallotannins may rival or exceed that of simpler phenolic monomers on a per-gram basis (Bruneton, 1999).

The tannin content of (*Marrubium deserti de Noé*) aerial parts has been detected by standard colorimetric assays (vanillin–HCl method for condensed tannins; FeCl₃ method for total tannins) in several screening studies (Lazreg-Aref *et al.*, 2012; Harraz *et al.*, 2019), though

quantitative data vary markedly between studies a variability attributable to differences in extraction solvent, plant maturity at harvest, geographic provenance, and analytical methodology.

This methodological heterogeneity limits direct cross-study comparisons and underscores the need for standardised extraction protocols and validated analytical methods in future investigations.

1.3.1.5 Alkaloids

Alkaloids constitute a large and heterogeneous class of basic nitrogenous compounds of secondary metabolic origin, predominantly derived from amino acid precursors.

The term encompasses more than 21,000 structures classified into roughly 60 biosynthetically defined sub-types based on their nitrogen-containing ring systems: pyrrolidine, piperidine, tropane, quinoline, isoquinoline, indole, pyrrolizidine, imidazole, and purine alkaloids, among others (Wink, 2010). The nitrogen atom(s) in alkaloids are typically basic (pKa 6–9), confer water solubility in acidic media, and are responsible for the intense physiological effects ranging from potent pharmacological activity to severe toxicity that characterise many members of this class.

Biosynthetically, the most common precursor amino acids are ornithine and lysine (giving pyrrolidine, piperidine, and tropane alkaloids), tyrosine (benzylisoquinoline alkaloids, including morphine and codeine), tryptophan (indole alkaloids, including ergots and vinca alkaloids), and phenylalanine (quinoline and related alkaloids). Purine alkaloids (caffeine, theobromine) derive from the adenine ring of purine nucleotide metabolism.

The diversity of precursors and the multiplicity of biosynthetic tailoring reactions (N-methylation, oxidation, rearrangement) account for the extraordinary structural range of the alkaloid class (Dewick, 2009).

In *Marrubium* and related Lamiaceae genera, the primary alkaloids detected belong to the cyclic betaine sub-type: stachydrine (L-proline betaine) and betonicine (4-hydroxy-stachydrine) are the most frequently reported, their structures being formed by N-methylation of proline or hydroxyproline. Though classified as alkaloids by some authors, these compounds are more precisely described as quaternary ammonium betaines with limited basicity (Bruneton, 1999).

Their physiological roles in the plant are thought to include osmotic adjustment under drought stress consistent with the desert habitat of (*Marrubium deserti de Noé*) as well as roles in nitrogen storage (Rhodes & Hanson, 1993). The potential toxicological significance of alkaloids in (*Marrubium deserti de Noé*) warrants systematic investigation, particularly given that pyrrolizidine alkaloids have been identified in trace quantities in some Lamiaceae species (EFSA, 2017).

1.3.1.6 Terpenes

The terpenoids (terpenes) constitute the most structurally diverse class of natural products, with over 80,000 individual compounds identified, derived biosynthetically from the universal five-carbon building blocks isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) (Croteau *et al.*, 2000). These C5 units are assembled in head-to-tail or head-to-head configurations by prenyltransferases to generate linear prenyl diphosphate intermediates of increasing chain length, which are then cyclised by terpene synthases into the diverse carbon skeletal types characteristic of each sub-class.

Classification is based on the number of C5 isoprene units: monoterpenes (C10, 2 units), sesquiterpenes (C15, 3 units), diterpenes (C20, 4 units), sesterterpenes (C25, 5 units), triterpenes (C30, 6 units), tetraterpenes (C40, 8 units, including carotenoids), and polyterpenes (>C40, e.g., rubber). In Lamiaceae and *Marrubium* specifically, the pharmacologically most significant terpenoids are: (i) monoterpenoids in the essential oil fraction (thymol, carvacrol, menthol, camphor, limonene); and (ii) labdane diterpenoids—particularly marrubiin and related structures—which represent the chemotypic markers of the genus (El-Seedi *et al.*, 2009).

Marrubiin (molecular formula C₂₀H₂₈O₃; molecular weight 320.43 g/mol) is a bicyclic furanoid labdane diterpenoid characterised by a fused furanolactone ring system. Its biosynthesis proceeds from geranylgeranyl diphosphate (GGPP) via copalyl diphosphate and marrubyl diphosphate intermediates, with the final lactone ring closure catalysed by a 2-oxoglutarate-dependent dioxygenase (Ghia *et al.*, 2011). The compound's expectorant mechanism involves reflex stimulation of bronchial mucous glands via sensory nerve activation; anti-inflammatory action proceeds through inhibition of leukotriene synthesis and cyclooxygenase-2 (COX-2) expression; and vasodilatory effects may be mediated by calcium channel blockade in vascular smooth muscle (Schlemper *et al.*, 1996). The relevance of these mechanisms to the toxicological characterisation of (*Marrubium deserti de Noé*)—where

marrubiin is a major constituent and forms part of the investigative rationale of the present thesis.

1.3.1.7 Sterols

Sterols are triterpene-derived lipids characterised by the tetracyclic cyclopentano perhydro phenanthrene (sterane) skeleton with a hydroxyl group at the C-3 position and typically an eight-carbon side chain at C-17. Phytosterols plant-derived sterols include β -sitosterol, campesterol, stigmasterol, and brassicasterol as the predominant representatives in higher plants.

These compounds are essential structural components of plant plasma membranes, fulfilling a role analogous to that of cholesterol in animal membranes: modulating membrane fluidity and permeability (Piironen *et al.*, 2000).

Pharmacologically, β -sitosterol and related phytosterols have attracted considerable research interest for their ability to reduce intestinal cholesterol absorption by competing with cholesterol for incorporation into mixed micelles in the intestinal lumen. Clinical evidence supports a modest but consistent reduction (approximately 5–10%) in LDL cholesterol concentrations following dietary phytosterol supplementation, and this property has led to their incorporation in functional food formulations in several countries (AHA Scientific Statement; Jones *et al.*, 2000). Anti-inflammatory, immunomodulatory, and anti-neoplastic activities have additionally been attributed to β -sitosterol in *in vitro* and *in vivo* models, though the mechanistic basis and clinical significance of these effects require further elucidation (Bouic *et al.*, 1996).

In *Marrubium*, sterols particularly β -sitosterol and ursolic acid (a pentacyclic triterpenoid sharing biosynthetic origins with sterols) have been detected in root and aerial part extracts of *M. vulgare* and related species (Lazreg-Aref *et al.*, 2012). Their presence in (*Marrubium deserti de Noé*) is biologically plausible and consistent with the broad distribution of phytosterols across the Lamiaceae, though specific quantitative data for this species remain limited in the published literature.

1.3.1.8 Anthraquinones

Anthraquinones are polyketide-derived secondary metabolites based on a 9,10-anthracenedione (anthraquinone) core scaffold, often bearing hydroxyl, methoxyl, carboxyl, or glycosyl substituents at C-1, C-2, C-4, C-5, C-6, and C-8 positions.

They are most prominently associated with families such as Rubiaceae (e.g., *Rubia tinctorum*), Polygonaceae (e.g., *Rheum*, *Rumex*), and Rhamnaceae (e.g., *Rhamnus*, *Cascara*), where they occur as the pharmacologically active purgative principles (Bruneton, 1999). Representative examples include emodin, aloe-emodin, rhein, barbaloin, and sennoside A and B.

The cathartic mechanism of anthraquinone glycosides involves colonic transformation by bacterial β -glucosidases to the active aglycone or anthronol form, followed by stimulation of peristaltic contractions and inhibition of electrolyte and water re-absorption in the large intestine. At high doses, anthraquinone-containing preparations may provoke electrolyte imbalances (hypokalaemia), intestinal mucosal damage, and, with chronic exposure, melanosis coli and theoretical genotoxic risks that have led regulatory agencies to impose restrictions on preparations containing certain anthraquinone derivatives (EFSA, 2018).

The presence of anthraquinones in (*Marrubium deserti de Noé*) specifically has not been confirmed by targeted quantitative analyses in the published literature consulted. However, preliminary phytochemical screening employing standard colorimetric and thin-layer chromatographic (TLC) methods has produced equivocal results in some *Marrubium* taxa, and their characterisation in (*Marrubium deserti de Noé*) extracts may be warranted in future work to complete the secondary metabolite inventory. The inclusion of anthraquinones in this review reflects both their broader pharmacological and toxicological relevance and the possibility of their occurrence as minor constituents.

The concept of oxidative stress was formulated definitively by Sies (1985), who defined it as 'a disturbance in the prooxidant–antioxidant balance in favour of the former, leading to potential damage'. This definition has subsequently been refined and expanded: Sies (2015, 2020) proposed a nuanced framework distinguishing between (i) physiological oxidative eustress a moderate elevation in ROS that serves essential signalling functions in cell proliferation, differentiation, and immune responses and (ii) oxidative distress an excessive, prolonged, or localised elevation in oxidant levels that exceeds the capacity of cellular antioxidant defences and results in macromolecular oxidative damage.

This distinction is critical for interpreting the biological effects of plant antioxidants, whose benefits may lie not merely in radical scavenging but in the modulation of redox signalling networks.

At the molecular level, oxidative stress manifests as the accumulation of oxidised forms of key biomolecules: peroxidised lipids (measured as malondialdehyde, MDA, or 4-hydroxynonenal, 4-HNE), oxidised proteins (marked by carbonyl formation), and oxidatively modified DNA bases (measured as 8-hydroxydeoxyguanosine, 8-OHdG). These modifications can impair enzymatic function, compromise membrane integrity, and in the case of DNA introduce mutations, double-strand breaks, and chromosomal rearrangements that may initiate or promote carcinogenesis (Halliwell & Gutteridge, 2015). The physiological consequences of chronic oxidative stress are implicated in the pathogenesis of numerous conditions prevalent in North Africa and globally: type 2 diabetes mellitus, cardiovascular disease, non-alcoholic fatty liver disease, neurodegenerative disorders (Alzheimer's disease, Parkinson's disease), and cancer (Finkel & Holbrook, 2000).

From the perspective of plant-derived medicinal compounds, the relationship between oxidative stress and disease pathogenesis provides the mechanistic rationale for investigating the antioxidant properties of (*Marrubium deserti de Noé*) extracts.

If the total phenolic content (TPC) and total flavonoid content (TFC) of (*Marrubium deserti de Noé*) are substantial, and if in vitro assays confirm significant radical scavenging and metal-chelating capacity, then the plant may be reasonably considered a candidate source of dietary and pharmacological antioxidants relevant to populations experiencing chronic oxidative burden.

However, the translation from in vitro antioxidant data to in vivo efficacy requires careful consideration of bioavailability, metabolic transformation, and the distinction between direct radical scavenging and indirect antioxidant mechanisms such as Nrf2/ARE pathway activation a nuance frequently underemphasised in ethnopharmacological literature (Sies *et al.*, 2017).

A free radical is defined as any chemical species possessing one or more unpaired electrons in its outermost molecular orbital (Halliwell & Gutteridge, 2015). This electronic configuration confers exceptional chemical reactivity: an unpaired electron creates a thermodynamic driving force for reaction with adjacent molecules to achieve electron pairing, typically through hydrogen atom abstraction, electron transfer, or addition reactions. In biological systems, the relevant free radicals are primarily oxygen-centred (reactive oxygen species, ROS) and nitrogen-centred (reactive nitrogen species, RNS), though carbon- and sulphur-centred radicals also participate as intermediates in radical chain reactions.

The most biologically significant oxygen radicals include the superoxide anion radical ($\text{O}_2^{\bullet-}$), the hydroxyl radical ($\text{HO}^\bullet$), and the peroxy radical ($\text{ROO}^\bullet$). Non-radical but highly reactive oxygen species of pharmacological relevance include hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$), and hypochlorous acid (HOCl).

Among nitrogen-centred radicals, nitric oxide ($\text{NO}^\bullet$) enzymatically generated by nitric oxide synthases (NOS) from L-arginine is the primary species, while peroxynitrite (ONOO^-), formed from the rapid diffusion-limited combination of $\text{NO}^\bullet$ and $\text{O}_2^{\bullet-}$, represents a particularly potent nitrating and oxidising agent (Pacher *et al.*, 2007).

The chemical reactivity hierarchy among ROS is critical for understanding their differential biological impact. The hydroxyl radical (standard reduction potential $E^\circ = +2.33 \text{ V}$) is among the most reactive species known in aqueous solution, reacting at diffusion-controlled rates with virtually all biological molecules including DNA bases, proteins, lipids, and carbohydrates with second-order rate constants typically in the range of 10^9 – $10^{10} \text{ M}^{-1}\text{s}^{-1}$ (Halliwell & Gutteridge, 2015). Because no enzymatic defence can operate rapidly enough to intercept $\text{HO}^\bullet$ in the immediate vicinity of its site of generation, prevention of $\text{HO}^\bullet$ formation rather than direct scavenging is the physiologically relevant protective strategy. This is achieved primarily by minimising the availability of catalytic transition metal ions (Fe^{2+} , Cu^+) that drive $\text{HO}^\bullet$ generation via Fenton and Haber–Weiss chemistry, and by maintaining efficient H_2O_2 -catabolising enzyme activity (Halliwell & Gutteridge, 2015).

In contrast, superoxide ($E^\circ = -0.16 \text{ V}$ vs. $\text{HO}^\bullet$) is comparatively weak as a direct oxidant but serves as a critical precursor to more damaging species and participates in the reduction of Fe^{3+} to Fe^{2+} , thereby perpetuating the Fenton cycle. Hydrogen peroxide, while not a radical per se, freely permeates cell membranes and participates in Fenton chemistry, justifying its classification within the ROS grouping. The biological significance of each species is thus determined not only by intrinsic reactivity but by compartmentalisation, local concentration, and the availability of catalytic metal ion partners.

Table (4): Major reactive oxygen and nitrogen species: nature, origin, and biological significance.

Species	Radical / non-radical	Primary Source	Biological Significance
Superoxide anion ($O_2^{\bullet-}$)	Radical	Mitochondrial ETC (Complex I & III)	Moderate; precursor to H_2O_2 and $HO^{\bullet}$
Hydrogen peroxide (H_2O_2)	Non-radical ROS	Dismutation of $O_2^{\bullet-}$; oxidases	Membrane-permeable; oxidises thiols
Hydroxyl radical ($HO^{\bullet}$)	Radical	Fenton/Haber–Weiss reaction	Highly reactive; attacks DNA, lipids, proteins
Singlet oxygen ($^1O_2$)	Non-radical ROS	Photosensitisation; peroxidase reactions	Damages unsaturated lipids
Nitric oxide ($NO^{\bullet}$)	Radical (RNS)	Nitric oxide synthases (NOS)	Signalling at low levels; toxic at high levels
Peroxynitrite ($ONOO^-$)	Non-radical RNS	$NO^{\bullet} + O_2^{\bullet-}$ combination	Nitrates tyrosine; damages DNA

Source: Adapted from Halliwell & Gutteridge (2015), Finkel & Holbrook (2000), and Sies (2020).

Endogenous free radical generation occurs as an inevitable by-product of aerobic metabolism and is intrinsically linked to mitochondrial energy transduction. The mitochondrial electron transport chain (ETC) processes approximately 2–3% of consumed oxygen to generate $O_2^{\bullet-}$ under normal physiological conditions, with Complexes I (NADH: ubiquinone oxidoreductase) and III (ubiquinol: cytochrome c oxidoreductase) identified as the primary sites of electron leakage (Murphy, 2009). At Complex I, NADH-bound semiquinone intermediates can react with molecular oxygen to form $O_2^{\bullet-}$; at Complex III, ubisemiquinone radicals generated during the Q cycle similarly transfer single electrons to oxygen. Mitochondrial $O_2^{\bullet-}$ is released on both the matrix side (from Complex I) and the intermembrane space side (from Complex III), where it is dismutated by Mn-SOD and Cu/Zn-SOD, respectively, to H_2O_2 (Finkel & Holbrook, 2000).

Beyond the mitochondria, multiple additional enzymatic and non-enzymatic sources contribute to cellular ROS production. NADPH oxidases (NOX enzymes, particularly NOX2 in phagocytes and NOX4 in vascular endothelium) catalyse the deliberate, receptor-regulated generation of $O_2^{\bullet-}$ as part of the oxidative burst in immune defence and as signalling intermediates in growth factor receptor pathways (Bedard & Krause, 2007). Xanthine oxidase, cyclooxygenases, lipoxygenases, cytochrome P450 monooxygenases (particularly during uncoupled catalysis), and the endoplasmic reticulum oxidative protein folding machinery (Ero1/PDI system) constitute further enzymatic sources of ROS in non-phagocytic cells (Halliwell & Gutteridge, 2015).

Exogenous sources of free radicals are equally diverse and include: ionising radiation (X-rays, γ -rays), which generates $HO^{\bullet}$ through water radiolysis; ultraviolet (UV) radiation, which generates singlet oxygen and triplet-state photosensitisers; environmental pollutants including ozone, nitrogen oxides, and particulate matter; tobacco smoke, which contains an estimated 10^{15} free radicals per puff in the gas phase alone (Pryor & Stone, 1993); transition metal contamination in food and water; and pharmaceutical xenobiotics that undergo redox cycling (e.g., doxorubicin, paraquat).

In the North African context including Algeria's southern desert regions where dust storms may introduce significant particulate and metal ion loads environmental sources of oxidative stress represent a relevant population-level risk factor, situating the investigation of antioxidant plant resources in a meaningful public health context.

The generation of ROS is thus a continuous, multi-sourced, and physiologically ambivalent process. At basal levels, ROS serve as molecular messengers in redox-sensitive signalling pathways that regulate cell cycle progression, apoptosis, autophagy, and inflammatory gene expression pathways collectively designated the 'redox biology' of the cell (Sies *et al.*, 2017).

It is only when their production rate exceeds the combined capacity of enzymatic and non-enzymatic antioxidant defences that the transition to oxidative distress occurs, with consequent accumulation of irreversibly damaged macromolecules.

1.4.3 Antioxidants

1.4.3.1 Definition of Antioxidants

The term 'antioxidant' encompasses a broad and mechanistically heterogeneous category of substances. The most frequently cited definition, proposed by Halliwell & Gutteridge (1989), characterises an antioxidant as 'any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate'. This functional definition is deliberately substrate-independent, thereby including substances that act on proteins, DNA, and lipids, not only those conventionally associated with radical scavenging.

A complementary operational definition, more attuned to biomedical application, defines an antioxidant as any agent that reduces the incidence or severity of oxidative damage in a biological system, whether by direct interception of free radicals, by chelation of redox-active metal ions, by regeneration of primary antioxidants, by suppression of ROS-generating enzymes, or by upregulation of the cell's endogenous antioxidant enzyme systems (Sies *et al.*, 2017). This broader definition accommodates indirect antioxidant mechanisms—such as the activation of the Nrf2/Kelch-like ECH-associated protein 1 (Keap1)/antioxidant response element (ARE) transcriptional pathway by electrophilic and pro-oxidant plant compounds—that are not captured by simple *in vitro* radical scavenging assays.

The practical measurement of antioxidant activity has been dominated by a suite of *in vitro* assay systems, each with different mechanistic bases and thus providing partially complementary information. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS•+ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) decolourisation assay, FRAP (ferric reducing antioxidant power), CUPRAC (cupric reducing antioxidant capacity), and ORAC (oxygen radical absorbance capacity) assays represent the most widely used methods in plant extract characterisation (Prior *et al.*, 2005). However, these assays suffer from significant methodological limitations: they employ artificial radical species (DPPH•, ABTS•+) not present in biological systems; they are performed in non-physiological solvents; they do not account for bioavailability or metabolism; and they may yield discordant results depending on reaction conditions and incubation times (Huang *et al.*, 2005). Consequently, the results of *in vitro* antioxidant assays should be interpreted as indicative of antioxidant potential rather than determinative of *in vivo* antioxidant efficacy.

1.4.3.2 Classification of Antioxidants

Antioxidants may be classified according to several complementary criteria: their chemical nature and origin (endogenous/enzymatic, endogenous/non-enzymatic, exogenous/dietary); their mechanism of action (chain-breaking primary antioxidants, preventive secondary antioxidants, repair tertiary antioxidants); their compartmental location within the cell (aqueous phase: ascorbic acid, GSH; lipid phase: α -tocopherol; membrane-associated); and their molecular weight (high-molecular-weight enzyme systems vs. low-molecular-weight small molecules). These classification schemes are not mutually exclusive and are most usefully deployed in combination to describe the integrated antioxidant network of a cell or organism (Halliwell & Gutteridge, 2015).

Enzymatic antioxidants comprise the first line of cellular defence. Superoxide dismutase (SOD, EC 1.15.1.1) catalyses the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 at a rate ($k \approx 2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$) approaching the diffusion limit, and is present in three isoforms distinguished by their metal cofactor and subcellular localisation: Mn-SOD (mitochondrial matrix), Cu/Zn-SOD (cytoplasm and intermembrane space), and extracellular Cu/Zn-SOD (Fridovich, 1995). Catalase (CAT, EC 1.11.1.6), a haem-containing peroxisomal enzyme, decomposes H_2O_2 to water and oxygen at extremely high catalytic rates ($k_{cat} \approx 4 \times 10^7 \text{ s}^{-1}$), representing the primary mechanism of H_2O_2 clearance in hepatocytes and erythrocytes (Aebi, 1984). Glutathione peroxidases (GPx, EC 1.11.1.9), a family of selenocysteine-containing enzymes with eight isoforms in humans, catalyse the reduction of H_2O_2 and lipid hydroperoxides using reduced glutathione (GSH) as the electron donor, producing oxidised glutathione (GSSG) that is subsequently reduced by glutathione reductase using NADPH—a reaction that links the antioxidant defence network to pentose phosphate pathway activity (Brigelius-Flohé & Maiorino, 2013).

Non-enzymatic antioxidants of endogenous origin include reduced glutathione (GSH), the most abundant intracellular thiol antioxidant (intracellular concentration 1–10 mM), which serves as a co-substrate for GPx reactions, as a direct scavenger of $HO^{\bullet}$ and lO_2 , and as a co-factor in the enzymatic conjugation of electrophilic compounds by glutathione S-transferases (GSTs); uric acid, the major antioxidant in human plasma; bilirubin; α -lipoic acid; and melatonin (Halliwell & Gutteridge, 2015).

Exogenous dietary antioxidants include the fat-soluble vitamins E (α -tocopherol, the principal lipid peroxidation chain-breaking antioxidant in biological membranes) and carotenoids (β -carotene, lycopene, lutein), and the water-soluble vitamin C (ascorbic acid), which also regenerates α -tocopherol from the α -tocopheroxy radical at the membrane–aqueous interface (Sies *et al.*, 2017).

Phytochemical antioxidants—the class most directly relevant to (*Marrubium deserti de Noé*) research—contribute to the dietary antioxidant intake through consumption of plant-based foods and herbal preparations. As discussed in Section 1.3, phenolic compounds and flavonoids exhibit antioxidant activity through multiple concurrent mechanisms: (i) direct hydrogen atom transfer (HAT) or single electron transfer (SET) to radical species; (ii) chelation of redox-active metal ions (Fe^{2+} , Cu^{2+}), preventing Fenton and Haber–Weiss radical generation; (iii) inhibition of pro-oxidant enzyme systems (xanthine oxidase, NADPH oxidase, COX, LOX); and (iv) upregulation of endogenous antioxidant enzyme expression via activation of the Nrf2/ARE signalling axis (Fresco *et al.*, 2006; Sies *et al.*, 2017). The relative contribution of each mechanism varies between individual compounds and between *in vitro* and cellular contexts, necessitating a multi-assay approach to obtain a comprehensive assessment of antioxidant capacity—an approach that informs the experimental design described in the subsequent chapters of this thesis.

Table 5 presents a classification of antioxidants according to their mechanisms of action. Antioxidant defense systems are generally divided into primary (chain-breaking), secondary (preventive), and repair mechanisms. These systems include both enzymatic and non-enzymatic antioxidants that work synergistically to protect cells against oxidative damage caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). The table summarizes the main antioxidant categories, representative examples, and their respective biological functions.

Table (5): Classification of antioxidants by mechanism of action.

Category	Sub-type	Examples	Mechanism
Chain-breaking (primary)	Enzymatic	SOD, CAT, GPx	Intercepts radical chain propagation
Chain-breaking (primary)	Non-enzymatic	Vitamin E (α -tocopherol), Vitamin C, flavonoids	Hydrogen donation to ROS
Preventive (secondary)	Enzymatic	Glutathione reductase, thioredoxin reductase	Regeneration of reduced antioxidants
Preventive (secondary)	Non-enzymatic	Ferritin, albumin, transferrin	Metal chelation; limits Fenton reaction
Repair / de novo	Enzymatic	DNA repair enzymes, proteasomes, lipases	Removal of oxidised biomolecules

Source: Adapted from Halliwell & Gutteridge (2015) and Sies (2015).

Antioxidant protection relies on several complementary mechanisms. Primary antioxidants directly neutralize free radicals and stop oxidative chain reactions, while secondary antioxidants regenerate oxidized antioxidants and chelate metal ions to prevent the formation of highly reactive radicals. Repair systems further protect cells by removing and repairing oxidatively damaged DNA, proteins, and lipids. Together, these enzymatic and non-enzymatic defenses form an effective network that maintains cellular integrity and limits oxidative stress.

Conclusion

This chapter reviewed the botanical, phytochemical, and medicinal aspects of *Marrubium deserti de Noé*. The literature showed that this species is rich in bioactive compounds, particularly phenolics, flavonoids, and terpenoids, which are associated with antioxidant, antimicrobial, and anti-inflammatory activities. The chapter also highlighted the concepts of oxidative stress, free radicals, and antioxidant defense systems, emphasizing the therapeutic importance of natural antioxidants. Overall, the reviewed data support the medicinal potential of *Marrubium deserti de Noé* and provide the scientific basis for the experimental work presented in the subsequent chapters.

CHAPTER II

Wounds and Their Healing — The Case of Cesarean Wounds

Introduction

The integrity of biological tissues is central to the homeostasis and survival of all multicellular organisms. When that integrity is disrupted—whether by mechanical trauma, thermal injury, ischaemia, or deliberate surgical incision—a cascade of precisely orchestrated biological events is set in motion with the singular objective of restoring structural continuity and functional capacity. This process, collectively known as wound healing, is among the most extensively studied phenomena in biomedical science, yet it continues to generate significant clinical and research interest because its failure or dysregulation carries profound consequences for patient outcomes (Guo & DiPietro, 2010).

Among the many wound types encountered in clinical practice, the cesarean section wound warrants particular attention for several reasons. Cesarean delivery is one of the most commonly performed surgical procedures worldwide, accounting for approximately 21% of all live births globally, a figure that has risen steadily over recent decades (Boerma *et al.*, 2018). Although the incision is deliberately created under sterile conditions and classified as a clean surgical wound, it is not exempt from complications. Wound infection, dehiscence, and abnormal scar formation represent significant sources of maternal morbidity, prolonged hospitalisation, and psychological distress (Wloch *et al.*, 2012). Moreover, in the context of toxicology, the pharmacological management of these wounds—particularly through topical antibiotics and antiseptic agents—raises important questions regarding drug efficacy, systemic absorption, cytotoxicity, and the growing global challenge of antimicrobial resistance.

This chapter synthesises the existing theoretical and empirical literature on wounds and their healing, organised around six interrelated themes. We first define the concept of a wound and establish a classification framework; we then examine the specific characteristics of the cesarean wound and its complications; we analyse the systemic and local factors that modulate healing; we describe the molecular and cellular biology of the three healing phases; and we conclude with a critical appraisal of conventional treatment approaches. This theoretical framework is intended to provide the conceptual scaffolding for the experimental work reported in later chapters of this thesis.

2.1 Definition of a Wound

The term 'wound' is used widely in clinical and biological discourse, yet a rigorous definition remains important for scientific precision. In its broadest biological sense, a wound may be defined as any disruption of the continuity of tissues resulting from physical, chemical,

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thermal, or pathological agents that damages the structural integrity of the skin or underlying tissues (Dealey, 2012). This definition encompasses phenomena ranging from a superficial epidermal abrasion to a complex full-thickness surgical incision involving muscle and fascia.

More formally, the World Health Organization and major wound care authorities have characterised a wound as a break in the epithelial integrity of the skin or a deeper disruption affecting the dermis, subcutaneous tissue, muscles, or bones, frequently accompanied by vascular injury, haemorrhage, and the initiation of a systemic reparative response (Lazarus *et al.*, 1994).

The physiological significance of this definition lies in the dual nature of tissue disruption: on one hand, breaching the epithelial barrier removes the body's primary defence against microbial colonisation; on the other, it simultaneously activates the haemostatic and immune responses that initiate repair.

At the cellular and molecular level, tissue continuity is maintained by the extracellular matrix (ECM), a three-dimensional scaffold of structural proteins—predominantly collagens, elastin, fibronectin, and proteoglycans—that supports cell adhesion, differentiation, and communication (Frantz *et al.*, 2010). A wound, by this cellular definition, represents a rupture of this scaffold and of the cellular architecture it supports, triggering an obligatory sequence of haemostasis, inflammation, proliferation, and remodelling. This four-stage model, originally proposed by Robbins and Cotran (2004) and subsequently refined by numerous investigators (Singer & Clark, 1999; Krafts, 2010), serves as the canonical theoretical framework for understanding wound biology and underpins much of the clinical and pharmacological reasoning explored in subsequent sections of this chapter.

It is worth emphasising that a wound is not simply a static defect but a dynamic, evolving biological environment. From the moment of tissue breach, a complex interplay of cellular populations, soluble mediators, mechanical forces, and microbial signals shapes the trajectory of healing. This dynamism means that the same wound may follow vastly different courses depending on the host's physiological state, the wound's aetiology and location, and the nature of any therapeutic interventions applied.

Understanding this complexity is essential to appreciating why some wounds—including many cesarean section incisions—fail to heal in an uncomplicated manner despite apparently favourable initial conditions.

2.2 Classification of Wounds

Wounds may be classified according to multiple intersecting criteria, including their temporal trajectory (acute versus chronic), their aetiology (traumatic, surgical, pressure-related, vascular), their depth, and their microbial status. Each classification system serves a distinct clinical and scientific purpose, and we discuss the most relevant frameworks here, as these distinctions directly inform the management strategies reviewed later in this chapter.

2.2.1 Acute Wounds

Acute wounds are defined by their expected progression through the normal sequence of haemostasis, inflammation, proliferation, and remodelling, reaching complete re-epithelialisation within a predictable timeframe—typically two to four weeks in otherwise healthy individuals (Harding *et al.*, 2002). This category encompasses traumatic lacerations, burns, donor-site wounds, and, critically, planned surgical incisions.

The key distinguishing feature of an acute wound is that its healing trajectory is not inherently compromised: provided the wound is managed appropriately and the host's physiology is intact, closure and restoration of tissue integrity are the expected outcomes.

From a molecular standpoint, acute wounds are characterised by an orderly, time-limited inflammatory response driven by the early release of platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), and epidermal growth factor (EGF) from degranulating platelets at the wound site (Werner & Grose, 2003). These mediators orchestrate the recruitment of neutrophils and macrophages, the subsequent activation of fibroblasts, and the eventual maturation of the reparative connective tissue. The temporal predictability of acute wound healing is largely a function of these well-regulated molecular cascades.

2.2.2 Chronic Wounds

Chronic wounds are broadly defined as wounds that fail to progress through the normal healing sequence within the expected timeframe, typically operationalised as healing that has not advanced substantially after four to twelve weeks of standard care (Mustoe *et al.*, 2006). This category includes pressure injuries (decubitus ulcers), venous leg ulcers, arterial ulcers, and diabetic foot ulcers. The pathophysiology of chronic wounds is multifactorial and differs fundamentally from acute healing failure; chronic wounds are characterised by a persistently elevated and dysregulated inflammatory environment, elevated levels of matrix

metalloproteinases (MMPs) that degrade the ECM and growth factors, reduced cellular proliferative capacity, and often, polymicrobial biofilm colonisation (Bjarnsholt *et al.*, 2008).

The distinction between acute and chronic wounds is not merely temporal; it reflects qualitatively different molecular environments. Research by Trengove *et al.* (1999) demonstrated that chronic wound fluid, compared with acute wound fluid, contains significantly higher concentrations of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumour necrosis factor-alpha (TNF- α), alongside markedly elevated MMP activity and reduced concentrations of the tissue inhibitors of metalloproteinases (TIMPs) that would normally regulate ECM turnover.

This biochemical profile creates a self-perpetuating cycle of tissue degradation that resists conventional treatment and is of direct relevance to the management of complicated post-surgical wounds in patients with metabolic comorbidities.

2.2.3 Surgical Wounds

Surgical wounds constitute a distinct subcategory within the broader classification of acute wounds. They are defined as wounds created intentionally during operative procedures under controlled conditions, with the explicit aim of gaining access to deeper structures for diagnostic or therapeutic purposes. By virtue of their controlled creation, surgical wounds are generally expected to heal by primary intention—that is, the wound edges are approximated and secured by sutures, staples, or adhesive strips, allowing the healing response to proceed across a minimal tissue gap (Leaper & Gottrup, 1998).

Healing by primary intention is distinguished from secondary intention, in which the wound is left open and heals through granulation tissue formation and wound contraction across a larger tissue defect. A third mechanism, tertiary intention (also called delayed primary closure), involves the deliberate delay of wound closure to allow initial contamination to resolve before formal approximation of the wound edges.

The choice between these strategies is directly influenced by the wound's microbial status, as discussed in the following section, and has significant implications for the cesarean wound specifically when complications arise.

2.2.4 Clean Versus Infected Wounds

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The classification of wounds by their microbial status is among the most clinically consequential frameworks in wound care and surgical practice. The widely used Centers for Disease Control and Prevention (CDC) wound classification system distinguishes four categories: Class I (clean), Class II (clean-contaminated), Class III (contaminated), and Class IV (dirty-infected) (Mangram *et al.*, 1999).

This system was originally developed in the context of surgical site infections (SSIs) and provides a structured framework for predicting infection risk and guiding prophylactic antibiotic therapy.

Clean wounds (Class I) are those created electively under sterile conditions, with no entry into the respiratory, gastrointestinal, or genitourinary tracts and no break in aseptic technique. Clean-contaminated wounds (Class II) involve controlled entry into a hollow viscus.

Contaminated wounds (Class III) involve gross spillage or wounds sustained in non-sterile environments, while dirty wounds (Class IV) are associated with established infection or perforated viscera.

As will be discussed in Section 2.3, the cesarean incision is classified as Class II—clean-contaminated—due to the controlled entry into the uterine cavity and the unavoidable, if transient, exposure to the vaginal microbiome during delivery. This classification has important implications for SSI risk and antibiotic prophylaxis protocols.

Table (6): Classification of wound types by healing trajectory and clinical characteristics.

Category	Healing Trajectory	Key Features	Clinical Examples
Acute	2–4 weeks; orderly progression	Time-limited inflammation; ordered molecular cascade	Laceration, burn, surgical incision

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Chronic	> 4–12 weeks; stalled healing	Elevated MMPs; dysregulated inflammation; biofilm	Diabetic foot ulcer, venous ulcer, pressure injury
Surgical	Primary intention expected	Controlled creation; edges approximated	Cesarean incision, laparotomy, appendectomy
Clean (Class I)	Low infection risk (< 2%)	No hollow viscus entry; strict asepsis maintained	Elective hernia repair, thyroidectomy
Infected (Class IV)	Variable; may require reoperation	Established infection; necrotic tissue present	Perforated bowel, contaminated trauma wound

Note. Adapted from Dealey (2012), Mustoe *et al.* (2006), and Mangram *et al.* (1999). CDC = Centers for Disease Control and Prevention; MMP = matrix metalloproteinase. Table 2. Surgical site infection (SSI) classification according to the CDC criteria.

Table(7): Surgical site infection (SSI) classification according to the CDC criteria.

SSI Class	Definition	Clinical Relevance to Cesarean
Superficial incisional	Involves skin and subcutaneous tissue only; within 30 days of surgery	Most common post-CS SSI; erythema, purulent discharge
Deep incisional	Involves fascia and/or muscle layer; within 30–90 days	Risk associated with obesity; fascial dehiscence possible
Organ/space	Involves any organ or space opened or manipulated during surgery	Endometritis, pelvic abscess; associated with prolonged labour

Note. Based on Mangram *et al.* (1999) and Wloch *et al.* (2012). CS = cesarean section; SSI = surgical site infection.

2.3 Cesarean Wounds

Cesarean section (CS) wounds represent a clinically important and increasingly prevalent wound type that occupies a unique position within the spectrum of surgical wounds. Their management raises specific toxicological, microbiological, and surgical considerations that justify their treatment as a distinct topic within this chapter.

2.3.1 Definition and Surgical Anatomy of Cesarean Section

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Cesarean section is defined as the delivery of a fetus through a surgical incision in the abdominal wall (laparotomy) and the uterine wall (hysterotomy), bypassing the normal vaginal route of delivery (Cunningham *et al.*, 2018).

The procedure may be performed as a planned (elective) operation or as an emergency intervention in response to maternal or foetal compromise. Globally, CS rates have increased dramatically over the past four decades, rising from approximately 7% in 1990 to over 21% by 2015, with projections suggesting rates may reach 29% by 2030 in the absence of effective policy intervention (Boerma *et al.*, 2018; Betran *et al.*, 2016).

The surgical anatomy of the CS incision is of direct relevance to wound healing. The most commonly employed approach is the Pfannenstiel incision, a transverse, slightly curved incision made approximately 3 cm above the pubic symphysis, through the skin, subcutaneous fat, and the anterior rectus sheath, followed by blunt separation of the rectus abdominis muscles and entry into the peritoneal cavity.

The uterine incision is then typically made in the lower uterine segment. This approach offers superior cosmetic outcomes and lower rates of postoperative hernia compared with the midline vertical incision, which remains the preferred approach for emergency CS or when rapid access to the peritoneal cavity is required (Mathai *et al.*, 2013). The tissue layers traversed—skin, Camper's fascia, Scarpa's fascia, anterior rectus sheath, rectus abdominis muscle, posterior rectus sheath, peritoneum—each possess distinct vascular supplies, structural properties, and healing capacities, and their disruption contributes to the specific wound healing challenges discussed subsequently.

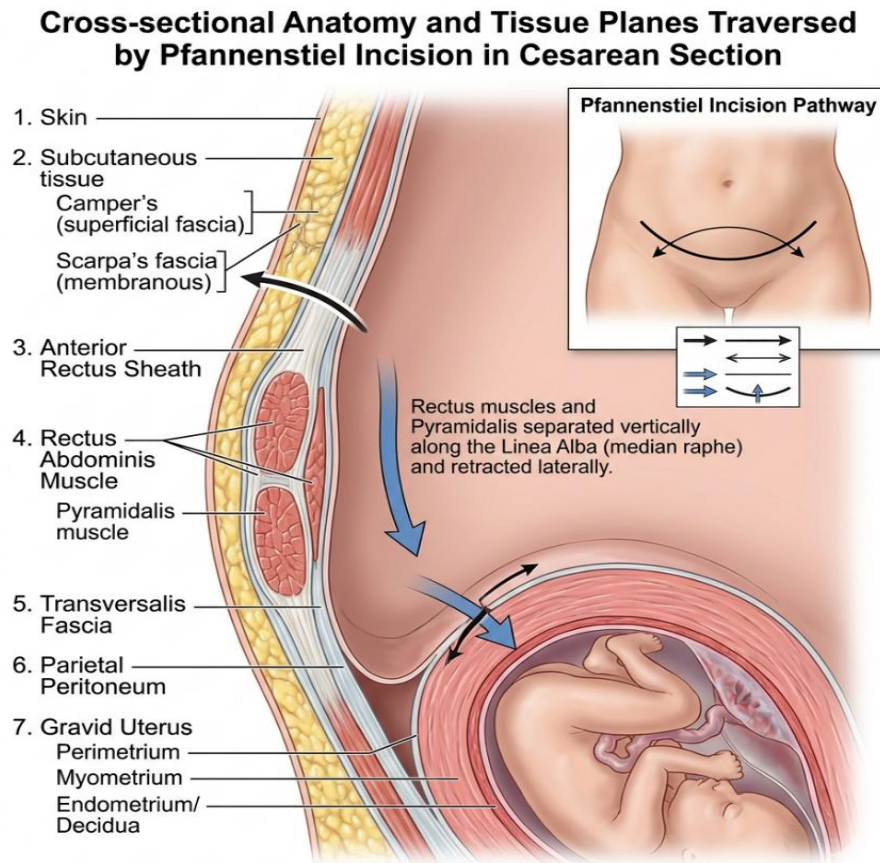


Figure (3): Cross-sectional anatomy of abdominal wall layers incised during cesarean section, illustrating the tissue planes traversed by the Pfannenstiel incision. Source: Cunningham *et al.* (2018).

Figure (3) illustrates the cross-sectional anatomy of the abdominal wall layers traversed during a cesarean section performed through a Pfannenstiel incision. The incision passes sequentially through the skin, subcutaneous tissues, fascial layers, abdominal muscles, and peritoneum before reaching the uterus. Each of these tissue layers possesses distinct anatomical characteristics, vascularization patterns, and regenerative capacities that influence the wound-healing process. Consequently, damage to these structures during surgery may contribute to postoperative complications such as infection, wound dehiscence, delayed healing, or excessive scar formation. Understanding the anatomical organization of these layers is therefore essential for evaluating the mechanisms of cesarean wound healing and the effectiveness of therapeutic interventions aimed at improving tissue repair.

Comparison of Pfannenstiel and Midline Vertical Incisions for Cesarean Section

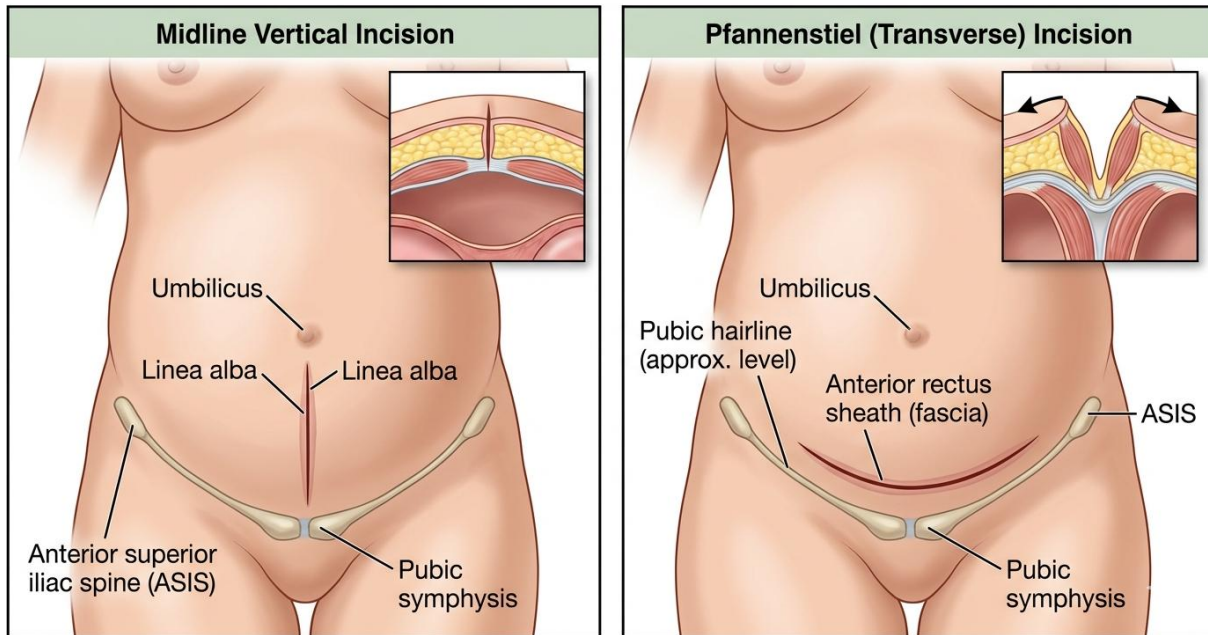


Figure (4): Comparison of Pfannenstiel (transverse) and midline vertical incisions for cesarean section, with anatomical landmarks indicated. Source: Mathai *et al.* (2013).

Figure (4) compares the two principal abdominal incisions used in cesarean section: the Pfannenstiel transverse incision and the midline vertical incision. The Pfannenstiel incision, located in the lower abdomen above the pubic symphysis, is generally preferred because it provides better cosmetic results, reduced postoperative pain, and a lower risk of incisional hernia. In contrast, the midline vertical incision allows more rapid access to the abdominal cavity and is therefore often reserved for emergency situations or cases requiring extensive surgical exposure. The anatomical landmarks illustrated in the figure highlight the differences in incision placement and emphasize how the choice of surgical approach may influence postoperative recovery and wound-healing outcomes.

2.3.2 The Nature of the Cesarean Wound: A Clean Surgical Wound

Notwithstanding its CDC Class II designation due to controlled entry into the uterine cavity, the cesarean incision is broadly regarded in the obstetric surgical literature as a clean surgical wound under standard operating conditions. This designation reflects the planned, elective creation of the wound in a sterile environment, the absence of gross faecal or purulent contamination, and the use of prophylactic antibiotic therapy, which has substantially reduced SSI rates (Smail & Gyte, 2010).

Pre-operative skin preparation with chlorhexidine or povidone-iodine, standardised surgical draping, and the widespread adoption of antibiotic prophylaxis—typically a first-generation cephalosporin administered within 60 minutes of skin incision—are now standard elements of CS care in high-income settings (WHO, 2018).

Nevertheless, the clean designation should not be interpreted as implying that the wound is free of infection risk. The uterine cavity communicates with the vaginal flora during delivery, and ascending microbial contamination, even in the context of an otherwise well-conducted procedure, is a documented mechanism of post-CS SSI. Research by Tita *et al.* (2009) demonstrated that extending antibiotic prophylaxis beyond the single-dose cephalosporin regimen to include azithromycin

significantly reduced post-CS infection rates, suggesting that vaginal microbiome contamination contributes meaningfully to wound infection risk even in ostensibly clean procedures.

2.3.3 Possible Complications of Cesarean Wounds

Despite the controlled conditions under which cesarean wounds are created and managed, they are associated with a range of complications that reflect both the specific anatomical and physiological demands of the procedure and the broader systemic vulnerabilities of the postpartum patient. The three most clinically significant complications are infection, dehiscence, and hypertrophic scar formation.

2.3.3.1 Wound Infection

Surgical site infection following cesarean delivery represents the single most common infectious complication of the procedure and a leading cause of postpartum maternal morbidity. Published estimates of post-CS SSI rates vary considerably depending on the definition employed, the surveillance methodology, and the population studied, but typically range from 3% to 15% in the absence of prophylactic antibiotics, falling to 2–5% with appropriate prophylaxis (Wloch *et al.*, 2012; Allegranzi *et al.*, 2016). In low- and middle-income countries, where prophylaxis protocols may be less consistently applied and patient-level risk factors such as malnutrition and anaemia are more prevalent, SSI rates may exceed 20% (WHO, 2018).

The predominant causative organisms of post-CS SSI are skin commensals, particularly *Staphylococcus aureus* (including methicillin-resistant *S. aureus*, or MRSA), coagulase-

negative staphylococci, and gram-negative enteric organisms such as *Escherichia coli* and *Klebsiella pneumoniae*, the latter two reflecting contamination from the vaginal and uterine microenvironments (Kabiri *et al.*, 2019).

Polymicrobial infection, involving multiple co-colonising species, is documented in a significant proportion of deep incisional and organ-space SSIs and is associated with more severe clinical presentations and greater therapeutic complexity.

From a toxicological perspective, the management of post-CS SSI raises important questions about the appropriate selection, dosing, and monitoring of antimicrobial agents in postpartum women, many of whom are breastfeeding. The systemic absorption of topically applied agents and the potential for both maternal toxicity and infant exposure via breast milk are considerations that inform the pharmacological discussions in Section 2.6.

2.3.3.2 Wound Dehiscence

Wound dehiscence—the partial or complete breakdown of a previously closed surgical wound—is a serious complication that may occur at any tissue layer and at any point in the postoperative period. Superficial dehiscence involves separation of the skin and subcutaneous layers only, while fascial dehiscence—the more dangerous form—involves disruption of the rectus sheath, potentially leading to evisceration or hernia formation (Horan *et al.*, 2008).

In the context of CS wounds, the reported incidence of dehiscence ranges from 0.2% to 3.5%, with higher rates observed in obese patients, those with poorly controlled diabetes, and those in whom SSI has occurred (Tran *et al.*, 2014).

The pathophysiology of wound dehiscence involves a failure of the mechanical tensile strength that should be provided by the nascent scar tissue during the first two to three weeks following closure. Suture cut-through, inadequate suture technique, premature collagen lysis driven by elevated MMP activity in infected wounds, and the mechanical stress of a distended or heavily loaded abdominal wall all contribute to dehiscence risk.

From a healing biology standpoint, dehiscence effectively converts a primary-intention wound into a secondary-intention wound, substantially increasing the duration of healing, the risk of further infection, and the probability of a functionally and cosmetically inferior scar.

2.3.3.3 Hypertrophic Scar Formation

Hypertrophic scarring is a pathological outcome of wound healing characterised by an excessive deposition of collagen within the boundaries of the original wound, resulting in a raised, firm, often pruritic scar that may cause significant physical discomfort and psychological distress (Gauglitz *et al.*, 2011). In contrast to keloids, which extend beyond the wound margins, hypertrophic scars are confined to the wound site and have a greater capacity for partial spontaneous resolution over time.

The Pfannenstiel incision is particularly prone to hypertrophic scar formation because it lies along a naturally high-tension line in the lower abdomen, where mechanical forces during ambulation and respiration may continuously stress the healing tissue.

At the molecular level, hypertrophic scar formation appears to result from an imbalance in the TGF- β signalling pathway, specifically an overactivation of TGF- β 1 (which promotes fibrosis) relative to TGF- β 3 (which promotes scarless healing), leading to abnormal fibroblast activity, excessive collagen type I synthesis, and impaired remodelling (Bayat *et al.*, 2003; Shah *et al.*, 1995). This molecular understanding has informed the development of potential anti-fibrotic treatments, including intralesional corticosteroid injection and TGF- β 3-based topical preparations, though evidence for the efficacy of the latter in the CS wound context remains limited.

2.4 Factors Influencing Wound Healing

Wound healing is not an isolated cellular event but a systemic biological process profoundly influenced by the host's physiological state. A large and converging body of evidence has identified both systemic and local factors that impair or dysregulate the healing cascade, and their understanding is essential for the clinical management of complex wounds such as those encountered following CS in high-risk patients. Table 3 provides a summary of the principal risk factors and their mechanistic effects.

To facilitate the understanding of the multifactorial nature of wound healing impairment, the principal systemic and local risk factors identified in the literature are summarized in Table (8). These factors interfere with one or more phases of the healing process through distinct pathophysiological mechanisms, including alterations in inflammatory responses, cellular proliferation, angiogenesis, extracellular matrix remodeling, and tissue oxygenation. The table below presents the major risk factors associated with delayed or impaired wound healing, together with their primary mechanisms of action and supporting references.

Table (8): Key risk factors for impaired wound healing and their pathophysiological mechanisms.

Risk Factor	Primary Mechanism(s) of Impairment	Key References
Diabetes mellitus	Hyperglycaemia impairs neutrophil chemotaxis; reduces VEGF expression; promotes AGE accumulation; impairs fibroblast proliferation	Guo & DiPietro (2010); Falanga (2005)
Obesity	Adipose tissue ischaemia; chronic low-grade inflammation; increased mechanical tension; elevated adipokine levels disrupting cytokine balance	Pierpont <i>et al.</i> (2014); Lumio <i>et al.</i> (2006)
Bacterial infection	Sustained neutrophil recruitment; elevated MMP activity degrading ECM; biofilm formation impairs antibiotic penetration	Bjarnsholt <i>et al.</i> (2008); Percival <i>et al.</i> (2012)
Poor vascularization	Hypoxia reduces HIF-1 α -driven VEGF synthesis; impairs fibroblast and keratinocyte migration; reduces nutrient delivery	Sen (2009); Thackham <i>et al.</i> (2008)
Malnutrition	Deficiency of amino acids (esp. arginine), zinc, and vitamins C and A impairs collagen synthesis and immune function	Wild <i>et al.</i> (2010); Stechmiller (2010)
Advancing age	Decreased fibroblast proliferation; attenuated growth factor response; impaired re-epithelialisation	Sgonc & Gruber (2013); Guo & DiPietro (2010)

Note. AGE = advanced glycation end-products; ECM = extracellular matrix; HIF-1 α = hypoxia-inducible factor 1-alpha; MMP = matrix metalloproteinase; VEGF = vascular endothelial growth factor.

2.4.1 Diabetes Mellitus

Diabetes mellitus represents one of the most well-characterised and clinically significant impediments to wound healing, and its interaction with surgical wound biology has been extensively studied over the past three decades. The pathophysiology is multifactorial. At the cellular level, sustained hyperglycaemia impairs the chemotaxis, phagocytic capacity, and bactericidal activity of neutrophils, thereby attenuating the first-line antimicrobial defence at

the wound site (Guo & DiPietro, 2010). Concurrently, hyperglycaemia promotes the non-enzymatic glycation of structural proteins, leading to the accumulation of advanced glycation end-products (AGEs) within the ECM, which crosslink collagen fibres, reduce tissue compliance, and impair normal matrix remodelling (Falanga, 2005).

The effects of diabetes on the angiogenic response are equally significant. Under normoglycaemic conditions, hypoxia at the wound site induces the expression of hypoxia-inducible factor 1-alpha (HIF-1 α), which in turn drives the synthesis and release of vascular endothelial growth factor (VEGF), promoting new vessel formation. In diabetic individuals, this HIF-1 α -VEGF axis is impaired, partly due to elevated prolyl hydroxylase activity that accelerates HIF-1 α degradation under hyperglycaemic conditions (Catrina *et al.*, 2004). The result is a blunted angiogenic response, reduced wound perfusion, and sustained local hypoxia that perpetuates the cycle of impaired healing. These considerations are particularly relevant in the obstetric context, where gestational diabetes—affecting approximately 14% of pregnancies worldwide (IDF, 2019)—represents an increasingly prevalent risk factor for post-CS wound complications.

2.4.2 Obesity

Obesity, defined as a body mass index (BMI) of 30 kg/m² or above, is a well-established independent risk factor for post-surgical wound complications, and its impact on CS wound healing is particularly pronounced because the surgical site traverses multiple layers of subcutaneous adipose tissue with a relatively poor intrinsic blood supply compared with well-vascularised muscular tissue (Pierpont *et al.*, 2014).

The subcutaneous fat of the obese abdomen may be poorly perfused, predisposing the wound to hypoxia-mediated healing failure and creating an environment that is conducive to bacterial proliferation.

Beyond the mechanical and vascular effects, obesity is associated with a state of chronic low-grade systemic inflammation, characterised by elevated circulating concentrations of pro-inflammatory adipokines—notably leptin, resistin, and interleukin-6 (IL-6)—alongside reduced levels of the anti-inflammatory adiponectin (Lumio *et al.*, 2006). This cytokine milieu overlaps substantially with the inflammatory environment of a wound, potentially creating a dysregulated and prolonged inflammatory phase that delays transition to the proliferative stage.

Furthermore, obese patients undergoing CS may be subjected to greater mechanical tension at the wound edges due to the weight of the abdominal panniculus, increasing the risk of both dehiscence and hypertrophic scar formation.

2.4.3 Bacterial Infection

The introduction of bacteria into a wound creates a competing biological dynamic in which the host's healing response must simultaneously manage tissue repair and antimicrobial defence. Bacterial contamination of a wound triggers a sustained and often exaggerated inflammatory response, characterised by the continuous recruitment of neutrophils and the elevated secretion of pro-inflammatory cytokines and MMPs. While this response is initially protective, its prolongation at the expense of the proliferative phase substantially delays healing. Of particular significance is the phenomenon of bacterial biofilm formation, in which polymicrobial communities of bacteria encase themselves within a self-produced extracellular polymeric matrix that confers substantial resistance to both host immune effectors and topical antimicrobial agents (Bjarnsholt *et al.*, 2008; Percival *et al.*, 2012).

Biofilm-associated infections are now recognised as a major obstacle in the management of both acute and chronic wounds, and there is growing evidence that biofilm formation may occur on surgical wounds, including CS incisions, within the first 24 to 48 hours of contamination (James *et al.*, 2008).

The toxicological relevance of this phenomenon is considerable: the concentrations of topical antibiotics required to penetrate and disrupt a mature biofilm may substantially exceed the minimum inhibitory concentrations (MICs) established in standard in vitro susceptibility testing, raising concerns about local tissue toxicity and systemic absorption.

2.4.4 Poor Vascularisation

Adequate blood supply is a foundational requirement for normal wound healing. Oxygen, glucose, amino acids, growth factors, and immune cells are all delivered to the wound microenvironment via the vasculature, while metabolic waste products and carbon dioxide are cleared by the same route. Local or regional ischaemia—whether due to peripheral arterial disease, excessive wound tension, haematoma formation, or pressure necrosis—creates a

hypoxic wound environment that profoundly impairs all phases of the healing response (Sen, 2009).

Under hypoxic conditions, fibroblast and keratinocyte migration and proliferation are substantially reduced, the production of collagen and other ECM components is impaired, and the bactericidal capacity of neutrophils—which relies on the oxygen-dependent respiratory burst—is attenuated, predisposing the wound to infection.

While short-lived, moderate hypoxia at the wound edge may actually stimulate the angiogenic response through the HIF-1 α pathway, sustained or severe hypoxia typically leads to cell death and tissue necrosis that can perpetuate and expand the wound defect (Thackham *et al.*, 2008). In the CS wound, poor vascularisation may result from previous abdominal surgery (including prior CS), aberrant scar tissue formation around the incision, or excessive use of electrocautery during haemostasis, all of which may devitalise the wound edges.

2.5 Phases of Wound Healing

The process of wound healing is conventionally described as comprising three overlapping phases: inflammatory, proliferative, and remodelling. These phases are not rigidly sequential but rather temporally overlapping and interdependent processes orchestrated by a complex network of cellular communication and molecular signalling. A foundational understanding of this biology is essential for appreciating both the normal trajectory of cesarean wound healing and the mechanistic basis of complications and pharmacological interventions. Table 4 provides an integrated summary of these phases.

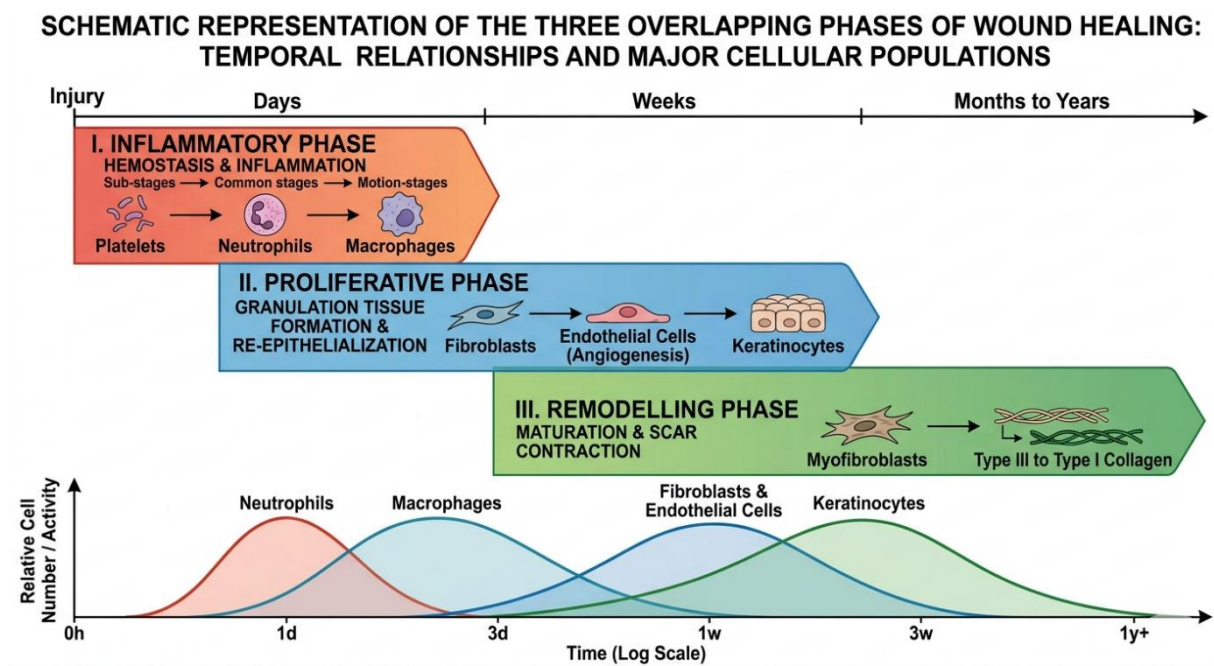


Figure (5): Schematic representation of the three overlapping phases of wound healing, illustrating the temporal relationships between the inflammatory, proliferative, and remodelling phases and the major cellular populations active at each stage. Source: Guo & DiPietro (2010).

As shown in Figure 5, wound healing is a highly regulated process involving a succession of overlapping phases rather than distinct and isolated events. The inflammatory phase occurs immediately after injury and is characterized by the recruitment of neutrophils and macrophages to eliminate pathogens and cellular debris. This phase is followed by the proliferative phase, during which fibroblasts, endothelial cells, and keratinocytes promote granulation tissue formation, angiogenesis, collagen synthesis, and re-epithelialization. Finally, the remodelling phase involves the reorganization and maturation of collagen fibers, leading to increased tissue strength and functional recovery. The overlap between these phases demonstrates the complexity of wound healing and emphasizes the importance of coordinated cellular interactions for efficient tissue repair and successful healing outcomes.

Table (9): Summary of the three phases of wound healing: cellular actors, molecular mediators, and clinical correlates.

Phase	Timing	Key Cells & Mediators	Clinical Correlate
Inflammatory	0–5 days	Platelets, neutrophils, macrophages ; IL-1 β , TNF- α , PDGF, TGF- β , IL-6, IL-10	Erythema, oedema, warmth, pain; serous or serosanguinous exudate
Proliferative	3–21 days	Fibroblasts, keratinocytes, endothelial cells; VEGF, FGF, EGF, collagen I/III, fibronectin	Granulation tissue formation; re- epithelialisation; wound contraction
Remodelling	21 days–2 years	Fibroblasts→myofibroblasts; MMP-1/3/13, TIMP-1/2; collagen I dominant	Scar maturation; tensile strength recovery to ~80% of normal

Note. EGF = epidermal growth factor; FGF = fibroblast growth factor; IL = interleukin; MMP = matrix metalloproteinase; PDGF = platelet-derived growth factor; TGF- β = transforming growth factor-beta; TIMP = tissue inhibitor of metalloproteinases; TNF- α = tumour necrosis factor-alpha; VEGF = vascular endothelial growth factor. Adapted from Singer & Clark (1999) and Werner & Grose (2003).

2.5.1 The Inflammatory Phase

The inflammatory phase commences within seconds of tissue injury and serves three essential functions: haemostasis, antimicrobial defence, and the initiation of the repair signal cascade. Immediately upon wounding, the disruption of blood vessels triggers the activation of the coagulation cascade and platelet aggregation at the wound site. Activated platelets release dense granule contents—including adenosine diphosphate (ADP), serotonin, and thromboxane A₂—that amplify the platelet aggregation response and initiate vasoconstriction to limit blood loss (Li *et al.*, 2007).

Simultaneously, the coagulation cascade generates thrombin, which cleaves fibrinogen into fibrin monomers that polymerise to form the fibrin clot—a provisional ECM that serves as both a physical barrier and a scaffold for subsequent cell migration.

The fibrin clot is not merely haemostatic; it is also a potent reservoir of signalling molecules. Platelets degranulate and release alpha-granule contents rich in PDGF, TGF- β 1, TGF- β 2, fibroblast growth factor (FGF), and EGF, which diffuse into the wound environment to recruit inflammatory cells and initiate tissue repair (Werner & Grose, 2003). These growth

factors bind to receptors on neutrophils, monocytes, and later fibroblasts, initiating the chemotactic gradients that guide cellular responses over the following days.

2.5.1.1 Neutrophil Recruitment

Neutrophils are the first leukocytes to arrive at the wound site, typically within the first hour following injury. Their recruitment is driven by chemotactic agents including complement fragments (C3a, C5a), leukotriene B₄ (LTB₄), IL-8, and formylated bacterial peptides (Singer & Clark, 1999). Once at the wound, neutrophils perform phagocytosis of bacteria and cellular debris, release proteolytic enzymes (elastase, cathepsin G, gelatinase) to degrade damaged matrix components, and generate reactive oxygen species (ROS) through the NADPH oxidase pathway to kill pathogens. The neutrophilic infiltrate is typically maximal within 24 to 48 hours and, in an uncomplicated wound, largely resolved by 72 hours.

However, if bacterial contamination persists—as occurs in SSI—neutrophil recruitment continues indefinitely, driven by sustained chemotactic signalling. This perpetuated neutrophilic inflammation is both diagnostically useful (as a clinical sign of infection) and mechanistically harmful, as the continued proteolytic and oxidative activity of neutrophils damages the nascent granulation tissue and delays the transition to the proliferative phase. The toxicological dimension of this process is relevant when considering topical antimicrobial agents: excessive or prolonged exposure of the wound to bactericidal agents with cellular toxicity may also impair host neutrophil function, paradoxically worsening the antimicrobial response.

2.5.1.2 Macrophage Activation and Cytokine Release

Macrophages are the central orchestrators of the wound healing response, and their role extends far beyond simple phagocytosis of debris and microorganisms. Monocytes recruited from the circulation by MCP-1 (monocyte chemoattractant protein-1) and CCL2 differentiate within the wound tissue into wound macrophages, which adopt functionally distinct polarisation states depending on the local cytokine environment (Mosser & Edwards, 2008). The classically activated M1 macrophage phenotype, induced by IFN- γ and bacterial lipopolysaccharide (LPS), is characterised by high production of pro-inflammatory mediators including TNF- α , IL-1 β , IL-6, IL-12, and nitric oxide, and plays a critical role in antimicrobial defence during the early inflammatory phase.

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As the inflammatory phase resolves, macrophages transition towards the alternatively activated M2 phenotype under the influence of IL-4, IL-13, and IL-10. M2 macrophages produce anti-inflammatory cytokines (IL-10, TGF- β), express scavenger receptors for efferocytosis of apoptotic neutrophils, and release VEGF, TGF- β 1, and PDGF, thereby providing the angiogenic and fibrogenic signals that initiate the proliferative phase (Novak & Koh, 2013). This M1-to-M2 transition is now considered a critical checkpoint in normal healing, and its dysregulation—as may occur in diabetic wounds where macrophages demonstrate impaired M2 polarisation—is increasingly recognised as a central mechanism of chronic wound pathology (Mirza & Koh, 2015).

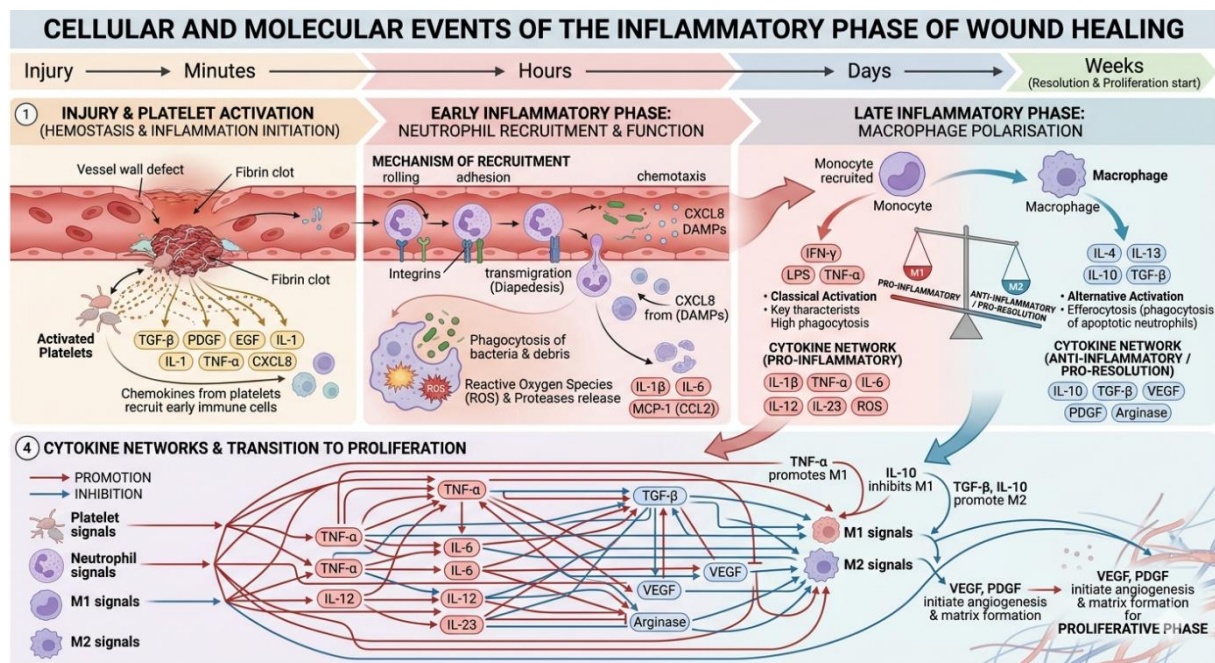


Figure (6): Cellular and molecular events of the inflammatory phase of wound healing, depicting platelet activation, neutrophil recruitment, and macrophage polarisation from M1 to M2 phenotype, with associated cytokine networks. Source: Novak & Koh (2013).

2.5.2 The Proliferative Phase

The proliferative phase, spanning approximately days 3 to 21 following wounding, is characterised by the coordinated activities of fibroblasts, keratinocytes, and endothelial cells, whose collective efforts produce granulation tissue, re-epithelialise the wound surface, and contract the wound margins. This phase represents the constructive counterpart to the

destructive early inflammation, and its efficiency and quality directly determine the structural integrity and aesthetic appearance of the eventual scar.

2.5.2.1 Fibroblast Activation and Collagen Synthesis

Fibroblasts are the principal effector cells of the proliferative phase. Quiescent fibroblasts in the wound periphery are activated by growth factor signals—principally TGF- β 1, PDGF, and FGF—released from degranulating platelets and M2 macrophages, causing them to proliferate and migrate into the fibrin scaffold of the wound (Barrientos *et al.*, 2008). Activated fibroblasts adopt a biosynthetically active phenotype characterised by abundant rough endoplasmic reticulum and Golgi apparatus, and begin synthesising the components of a new ECM, most notably collagen.

Collagen synthesis by fibroblasts involves a multi-step intracellular and extracellular process. Intracellularly, the procollagen alpha chains are synthesised on ribosomes, hydroxylated at proline and lysine residues by prolyl and lysyl hydroxylases (reactions that require vitamin C as a cofactor), and glycosylated before trimerisation into procollagen triple helices.

Procollagen is secreted into the ECM, where proteolytic cleavage of propeptides generates tropocollagen, which spontaneously self-assembles into collagen fibrils and fibres (Myllyharju & Kivirikko, 2004). The initial scar is dominated by type III collagen (foetal collagen), which is thinner and less well-organised than the type I collagen that characterises mature scar tissue and normal dermis. Over time, type III collagen is progressively replaced by type I collagen during the remodelling phase.

A proportion of activated wound fibroblasts differentiate into myofibroblasts, a specialised contractile cell type characterised by the expression of alpha-smooth muscle actin (α -SMA) and the development of stress fibres that exert contractile forces on the wound margins (Tomasek *et al.*, 2002). Wound contraction—mediated by myofibroblast-generated force transmitted through the fibronectin-rich provisional matrix—serves to reduce the size of the tissue defect and thereby decrease the extent of scar required for wound closure. In small wounds, contraction alone may achieve substantial closure, but in large or tension-prone wounds such as the lower abdominal CS incision, excessive myofibroblast activity may contribute to hypertrophic scar formation.

2.5.2.2 Angiogenesis

Angiogenesis—the sprouting of new capillaries from pre-existing vessels—is an essential component of the proliferative phase that provides the nascent granulation tissue with oxygen and nutrients and supports the metabolically active wound microenvironment. The angiogenic cascade is initiated by the hypoxic conditions that prevail in the wound centre following injury, which stabilise HIF-1 α and drive the transcriptional upregulation of VEGF-A, the principal angiogenic growth factor (Semenza, 2011). VEGF-A signals through endothelial cell receptors (VEGFR1 and VEGFR2) to promote endothelial cell proliferation, migration, and tube formation, processes that are further supported by angiopoietins, FGF-2, and PDGF, which contribute to vessel stabilisation and maturation (Tonnesen *et al.*, 2000).

The formation of a well-vascularised granulation tissue is visually characterised by the pink or red, moist, granular appearance that gives this tissue its name and serves as a macroscopic indicator of normal healing progression. Histologically, granulation tissue comprises a dense network of newly formed capillaries embedded within a loose matrix of fibronectin, hyaluronan, and type III collagen, infiltrated by macrophages and fibroblasts. The metabolic demands of this tissue are high, and any factor that limits local oxygen delivery—including haematoma, wound infection, or systemic cardiovascular compromise—may substantially impair granulation tissue formation and delay wound closure.

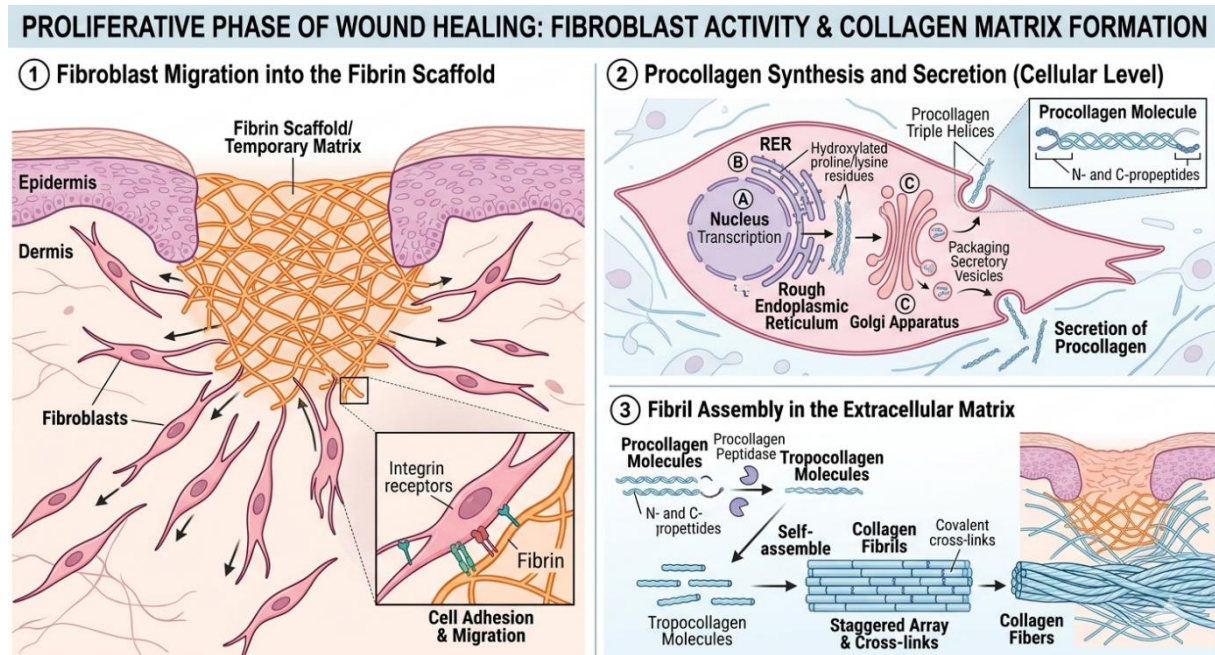


Figure (7): Fibroblast activity and collagen matrix formation during the proliferative phase of wound healing, illustrating fibroblast migration into the fibrin scaffold, procollagen synthesis and secretion, and fibril assembly in the extracellular matrix. Source: Myllyharju & Kivirikko (2004).

2.5.3 The Remodelling Phase

The remodelling phase begins approximately three weeks after wounding and may continue for up to two years in large or surgically complex wounds. It is characterised by the progressive reorganisation and strengthening of the collagen matrix, the reduction of wound cellularity through apoptosis of excess fibroblasts and vascular structures, and the gradual increase in tensile strength that characterises the maturing scar. While the scar never fully recovers the mechanical properties of the original unwounded tissue—mature scar reaches approximately 70–80% of the tensile strength of unwounded skin—the remodelling phase represents the closest biological approximation to tissue restoration (Levenson *et al.*, 1965; Robson *et al.*, 2001).

2.5.3.1 Collagen Reorganisation

The key biological event of the remodelling phase is the replacement of type III collagen with type I collagen and the progressive reorganisation of collagen fibres from a disorganised, woven arrangement into the more structured, cross-linked bundles characteristic of mature

connective tissue. This process is mediated by the coordinated activities of MMPs—particularly collagenases (MMP-1, MMP-13) and stromelysins (MMP-3)—which degrade existing collagen, alongside lysyl oxidase-mediated crosslinking of newly synthesised collagen fibres (Robson *et al.*, 2001). The ratio of MMP activity to TIMP activity is a critical determinant of net matrix turnover during this phase, and aberrations in this balance—whether excessive MMP activity (as in chronic wounds) or insufficient MMP activity (contributing to excessive fibrosis)—will produce pathological scar outcomes.

It should be noted that the collagen remodelling process is not merely mechanical but is also guided by the physical stress environment of the wound. Mechanotransduction signals, transmitted through integrins at the fibroblast-ECM interface, direct collagen fibre orientation along lines of mechanical stress, producing the characteristic parallel fibre bundles of mature scar tissue (Wang *et al.*, 2007). This has practical implications for CS wound management: post-operative strategies that minimise excessive mechanical loading of the wound—including appropriate wound dressing selection, activity restriction, and management of obesity-related abdominal panniculus weight—may contribute to more organised and functionally superior scar formation.

2.5.3.2 Tissue Strengthening

The progressive increase in wound tensile strength during the remodelling phase follows a well-characterised curve. At one-week post-wounding, a sutured wound has recovered only approximately 3% of the tensile strength of unwounded tissue; by three weeks, this rises to approximately 20%; by three months, it reaches 80%; and by one year, the scar stabilises at approximately 70–80% of normal tissue strength (Levenson *et al.*, 1965). The rate and extent of tensile strength recovery is directly dependent on the rate and quality of collagen synthesis and crosslinking, and may be substantially impaired by the complicating factors discussed in Section 2.4.

The clinical relevance of these strength recovery curves to the CS wound is significant. The fascial layer of the abdominal wall, which bears the principal mechanical loads of the healing wound, is particularly vulnerable in the early post-operative weeks. Activities that substantially increase intra-abdominal pressure—including coughing, straining, and lifting—may exceed the tensile capacity of the healing fascia and contribute to wound dehiscence or

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hernia formation. Understanding this temporal vulnerability is essential for the design of appropriate post-operative activity guidelines and wound care protocols.

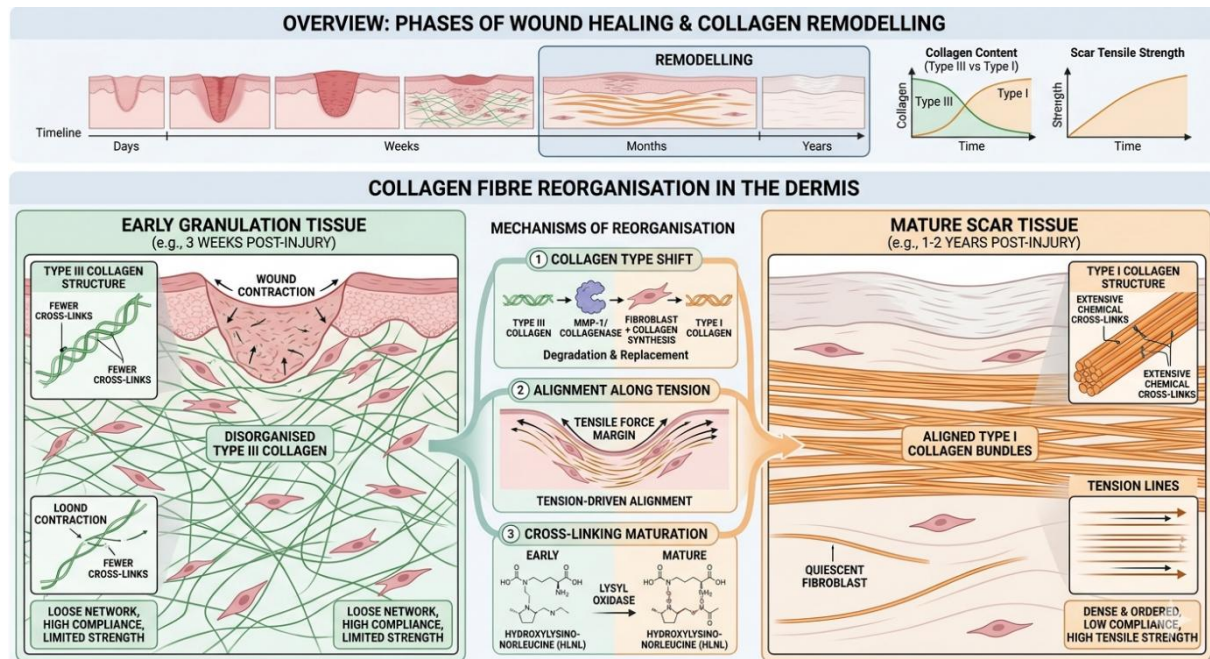


Figure (8): Collagen fibre reorganisation during the remodelling phase, showing the transition from disorganised type III collagen of early granulation tissue to the aligned, crosslinked type I collagen bundles of mature scar tissue. Source: Robson *et al.* (2001).

2.6 Conventional Treatments for Wound Management

The pharmacological management of surgical wounds, including CS incisions and their complications, relies on a range of topical agents broadly categorised as antibiotics, antiseptics, and wound-conditioning preparations. These agents aim to reduce the microbial burden at the wound surface, create conditions conducive to healing, and manage exudate. However, each category carries specific limitations from both a toxicological and a microbiological standpoint, and a critical appraisal of these limitations is essential context for the experimental work that follows in subsequent chapters.

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Table (9): Conventional topical treatments for wound management: agents, mechanisms, and limitations.

Agent	Mechanism of Action	Clinical Use	Key Limitations
Fusidic acid	Inhibits bacterial protein synthesis by blocking elongation factor G (EF-G); bacteriostatic at low concentrations	Superficial skin infections; SSTI due to <i>S. aureus</i> ; post-CS wound infections	Rapid resistance development; cross-resistance with mupirocin; limited gram-negative activity
Mupirocin	Inhibits isoleucyl-tRNA synthetase; highly selective for gram-positive organisms	<i>S. aureus</i> decolonisation; infected wounds	Resistance; limited depth of penetration; inactive in wounds with high exudate
Silver O sulfadiazine	Ag ⁺ ions disrupt bacterial DNA, cell wall, and respiratory chain; broad spectrum	Burns; chronic wounds; infected surgical wounds	Cytotoxic to keratinocytes and fibroblasts at therapeutic concentrations; argyria risk with prolonged use
Povidone-iodine	Releases free iodine; non-specific oxidative damage to proteins and DNA; broad spectrum	Pre-operative skin antisepsis; wound irrigation	Cytotoxic to all wound cells; impairs angiogenesis; iodine absorption risk in neonates and thyroid patients
Chlorhexidine	Disrupts bacterial cell membranes; binds to skin; broad spectrum; residual activity	Pre-operative skin preparation; wound cleansing	Cytotoxic at concentrations > 0.05%; neurotoxicity if enters middle ear; rare anaphylaxis
Hydrogen peroxide	ROS-mediated non-specific oxidative bactericidal action	Historical wound cleansing	Highly cytotoxic; impairs all healing phases; largely superseded in current guidelines

Note. EF-G = elongation factor G; ROS = reactive oxygen species; SSTI = skin and soft tissue infection; tRNA = transfer RNA. Adapted from Lipsky & Hoey (2009), Leaper (2006), and Templeton (2005).

2.6.1 Topical Antibiotics: Fusidic Acid as a Case Study

Fusidic acid is a steroidal antibiotic derived from the fungus *Fusidium coccineum* that has been used in clinical practice since the 1960s primarily for the treatment of skin and soft-tissue infections caused by *S. aureus* (Turnidge, 1999). Its mechanism of action is distinctive: fusidic acid binds to the elongation factor G (EF-G)–GDP complex on the bacterial ribosome, preventing the translocation step of protein synthesis and thereby exerting a bacteriostatic effect at clinical concentrations. At higher concentrations, it may exhibit bactericidal activity against susceptible organisms. Its lipophilicity facilitates reasonable penetration of intact and inflamed skin, making topical formulations pharmacologically plausible for superficial wound infections.

In the context of post-CS wound infections, fusidic acid cream or ointment (typically 2% w/w formulations) has been used for the management of superficial incisional SSI attributable to *S. aureus*, and its clinical efficacy in this setting has been reported in several observational series (Wilkinson, 1998). However, the application of fusidic acid to open wounds is associated with several toxicological concerns. At the wound level, the evidence regarding its impact on healing cells is mixed: some *in vitro* studies have demonstrated dose-dependent inhibitory effects on fibroblast proliferation and keratinocyte migration at concentrations achievable in topical formulations, raising the possibility that the antibiotic may, paradoxically, delay healing in the very wounds it is intended to treat (Hansson *et al.*, 1994).

Perhaps the most significant concern associated with topical fusidic acid use is the well-documented propensity for resistance development. Fusidic acid resistance in *S. aureus* is primarily mediated by point mutations in the *fusA* gene encoding EF-G, by the acquisition of the plasmid-encoded *fusB*, *fusC*, or *fusD* genes (which encode fusidic acid-binding protection proteins), or by upregulation of efflux pump systems (Farrell *et al.*, 2011). Because fusidic acid is used extensively and often without microbiological guidance in the community setting, the selective pressure it exerts on *S. aureus* populations has contributed to rising rates of resistance, particularly in countries where topical antibiotics are available without prescription. Cross-resistance with other antibiotics is not well established, but the emergence of fusidic acid-resistant MRSA is a particularly concerning development given the limited therapeutic options available for this pathogen (McLaws *et al.*, 2011).

2.6.2 Antiseptic Agents and Their Limitations

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Antiseptics—agents that reduce or eliminate microbial viability on living tissue—have been employed in wound management for well over a century, and their role in the pre-operative preparation of the CS surgical field is well established and evidence-based (WHO, 2018). However, their application to open wounds in the post-operative period is considerably more controversial, and contemporary wound care guidelines increasingly caution against the routine use of several traditional antiseptic preparations.

The central toxicological concern with antiseptic agents applied to open wounds is their non-selective cytotoxicity. Unlike antibiotics, which are designed to exploit structural or biochemical differences between bacterial and mammalian cells, most antiseptics act through broad mechanisms—membrane disruption, oxidative damage, or protein denaturation—that can harm host cells as readily as microbial cells.

Povidone-iodine, historically one of the most widely used wound antiseptics, has been demonstrated in multiple *in vitro* and *in vivo* studies to be toxic to fibroblasts, keratinocytes, and endothelial cells at concentrations that retain antimicrobial efficacy (Balin & Pratt, 2002). The net clinical effect of these observations has led to a substantial reconceptualisation of its role in wound care, with current evidence supporting its use as a pre-operative skin antiseptic but not as a routine wound irrigation agent for surgical wounds in the post-operative period.

Chlorhexidine gluconate presents a somewhat more nuanced picture. Its broad-spectrum antimicrobial activity, residual skin binding, and favourable toxicological profile at low concentrations ($\leq 0.05\%$) have made it the antiseptic of choice for pre-operative skin preparation in many guidelines. However, at concentrations typically formulated in wound cleansing products (0.5–4%), chlorhexidine demonstrates significant *in vitro* cytotoxicity against fibroblasts and keratinocytes, as well as potential neurotoxic effects if inadvertently introduced into wound tracts communicating with neural structures (Denton, 2001).

These findings underscore the need for a critical, evidence-based approach to antiseptic selection in wound management, guided by consideration of wound type, depth, and the individual patient's systemic risk factors.

The limitations of conventional pharmacological approaches to wound management—resistance, cytotoxicity, restricted spectrum, and the challenges of biofilm penetration—have generated significant interest in the development of alternative or complementary therapeutic strategies. Among these, plant-derived compounds with anti-infective and wound-healing-promoting properties have attracted growing attention from both basic science and clinical

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research perspectives. This interest provides the intellectual motivation for the experimental investigation that forms the core of the subsequent chapters of this thesis.

Conclusion

Wound healing is a complex biological process involving inflammatory, proliferative, and remodeling phases that work together to restore tissue integrity after injury. Cesarean wounds, despite being created under sterile surgical conditions, remain susceptible to complications such as infection, dehiscence, delayed healing, and abnormal scar formation. Several factors, including diabetes, obesity, bacterial infection, poor vascularization, malnutrition, and aging, can negatively affect the healing process. Although conventional treatments such as antibiotics and antiseptics are widely used, their effectiveness may be limited by antimicrobial resistance and cytotoxic effects. Consequently, increasing attention is being directed toward natural therapeutic alternatives, particularly medicinal plants with antioxidant, anti-inflammatory, and antimicrobial properties. This chapter therefore provides the theoretical foundation for evaluating the wound-healing potential of *Marrubium deserti de Noé* in the experimental part of this study.

Conclusion

Conclusion

The present study aimed to evaluate the therapeutic potential of (*Marrubium deserti de Noé*) in accelerating wound healing and reducing complications associated with cesarean section surgery through the investigation of the chemical and biological properties of its methanolic extract, as well as the assessment of its therapeutic efficacy in an animal model using female Wistar rats. The results of the qualitative and quantitative phytochemical analyses revealed that the methanolic extract contained a wide range of biologically active compounds, including phenolic compounds, flavonoids. High levels of phenolic and flavonoid compounds, known for their antioxidant and anti-inflammatory properties, were particularly detected. Furthermore, the in vitro assays demonstrated significant antioxidant, anti-inflammatory, and antibacterial activities against several bacterial strains.

These findings confirm the therapeutic value of this plant and support its traditional use in the treatment of wounds and inflammatory conditions. The in vivo study also showed a remarkable improvement in various biochemical, hematological, and enzymatic parameters in the extract-treated animals compared with the untreated injured group. In addition, treated groups exhibited reduced inflammatory markers and a significant enhancement of the wound-healing process. These observations were further confirmed by histopathological examinations, which revealed restoration of the normal skin architecture, stimulation of tissue regeneration and repair processes, as well as a reduction in scar formation and other surgery-related adverse effects. The therapeutic efficacy of (*Marrubium deserti de Noé*) appears to be mainly attributed to its richness in phenolic compounds and flavonoids, which possess antioxidant, anti-inflammatory, and antimicrobial activities.

These bioactive constituents act synergistically to accelerate tissue repair and limit the progression of inflammatory complications following surgical procedures. In light of the obtained results, it can be concluded that the methanolic extract of (*Marrubium deserti de Noé*) represents a promising natural source for the development of effective and safe topical therapeutic formulations intended to promote skin wound healing, particularly wounds resulting from cesarean section surgery.

Moreover, this study opens new perspectives for future research aimed at isolating and identifying the bioactive compounds responsible for these biological effects, elucidating their

Conclusion

molecular mechanisms of action, and conducting extensive clinical trials to evaluate their efficacy and safety in humans.

Such investigations may contribute to the development of natural, cost-effective, and safer therapeutic alternatives for medical and pharmaceutical applications.

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