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THEME

Development of a Burn-Healing Herbal Cream: Exploring the Biological Potentials of *Neurada procumbens*

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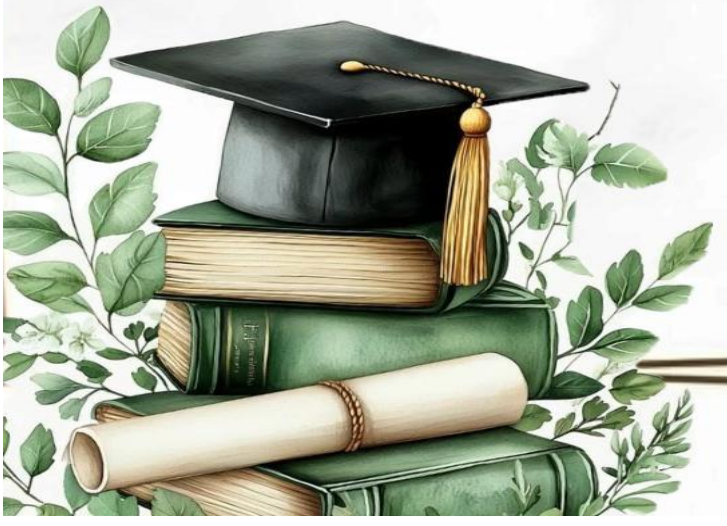

اهداء

" وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ "

بعد سنوات من الاجتهاد والسعي المتواصل، سنوات حملت في طياتها التعب والصبر والتحديات أقف اليوم على عتبة التخرج لأقدم ثمرة جهدي وما وصلت إليه بعد فضل الله وتوفيقه فهذا الانجاز لم يكن خطوة فردية، بل كان حصيلة دعم وحب ومساندة اشخاص كان لوجودهم أعظم الأثر في مسيرتي.

إلى من منحوني القوة لأصل . . . أهدي هذا الإنجاز:
إلى والدتي نبع الحنان وقلب الدعاء الذي لم يغلق بابه يوما ، إلى من كانت عيونها تلاحق خطواتي خوفا علي . يا امن ايامي، ويا هدايا الله في عمري نجاحي هذا امتداد لدعائك، وقطف الثمار حبك، وهدية متواضعة له لها أهدي نجاحي وفخري.

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إلى إخوتي وأخواتي
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إلى رفاق الرحلة.
أصدقائي الذين شاركوني الطريق وساهموا في صنع ذكريات لا تنسى . وجود كم كان مساحة رحبة القلبي، وسندا لتجاوز الصعوبات.

ولكل من وقف إلى جانبي وترك أثراً طيباً في قلبي، أو دعم خطوة من خطواتي، أو قال كلمة صنعت فرق . . .لكم جميعا أجمل الدعاء.

وها أنا اليوم
أرفع مشروعي هذا عربون شكر الله تعالى، الذي وفقني وأعانني، ومنحني القدرة على الوصول إلى هذه المرحلة التي لطالما حلمت بها .

Abstract

This study aimed to evaluate the therapeutic efficacy of *Neurada procumbens L.* (collected from El Oued region, Algeria) in burn healing and the mitigation of oxidative stress and inflammation. Two extracts, aqueous (AENP) and methanolic (MENP), were prepared. LC–ESI–MS/MS analysis revealed their richness in phenolic and bioactive compounds (such as curcumin, oleuropein, and myricetin). The aqueous extract was characterized by a higher yield, stronger antioxidant activity, and better photoprotective capacity (SPF), while both extracts demonstrated hemolytic safety and excellent biocompatibility.

Topical ointments were formulated and evaluated in vivo on rats with second-degree burns. The AENP-based ointment exhibited remarkable superiority in wound healing, achieving a contraction rate of 98.3% within 19 days, outperforming the commercial MEBO ointment. These results were accompanied by a significant improvement in hematological and histopathological parameters, alongside a reduction in oxidative stress. To mechanistically elucidate this efficacy, molecular docking analysis proved that the plant's active compounds act as potent inhibitors of the inflammation-related protein (NF-κB).

These findings confirm the promising pharmaceutical and cosmetic potential of the plant as a natural burn healer, antioxidant, and anti-inflammatory agent, thereby valorizing and supporting its traditional medicinal uses.

Keywords: *Neurada procumbens L.*; burn wound healing; antioxidant; anti-inflammatory; phytochemistry; NF-κB molecular docking; topical cream; Algerian Sahara.

Résumé

Cette étude visait à évaluer l'efficacité thérapeutique de la plante *Neurada procumbens L.* (récoltée dans la région d'El Oued, en Algérie) dans la cicatrisation des brûlures et l'atténuation du stress oxydatif et de l'inflammation. Deux extraits, aqueux (AENP) et méthanolique (MENP), ont été préparés. L'analyse (LC–ESI–MS/MS) a révélé leur richesse en composés phénoliques et bioactifs (tels que la curcumine, l'oleuropéine et la myricétine). L'extrait aqueux s'est distingué par un rendement plus élevé, une plus forte activité antioxydante et une meilleure capacité photoprotectrice (SPF), tout en démontrant l'innocuité hémolytique et l'excellente biocompatibilité des deux extraits.

Des pommades topiques ont été formulées et évaluées in vivo sur des rats souffrant de brûlures du deuxieme degré. La pommade à base de l'extrait aqueux (AENP) a montré une supériorité remarquable dans la cicatrisation des plaies, atteignant un taux de contraction de 98,3 % en 19 jours, surpassant ainsi la pommade commerciale (MEBO). Ces résultats ont été accompagnés d'une amélioration significative des paramètres hématologiques et histopathologiques, ainsi que d'une diminution du stress oxydatif. Afin d'élucider mécanistiquement cette efficacité, l'analyse d'amarrage moléculaire (molecular docking) a prouvé que les composés actifs de la plante agissent comme de puissants inhibiteurs de la protéine liée à l'inflammation (NF-κB).

Ces résultats confirment le potentiel pharmaceutique et cosmétique prometteur de la plante en tant que cicatrisant naturel des brûlures, agent anti-inflammatoire et antioxydant, valorisant et soutenant ainsi ses utilisations médicinales traditionnelles

.Mots-clés : *Neurada procumbens L.* ; cicatrisation des brûlures ; antioxydant ; anti-inflammatoire ; phytochimie ; amarrage moléculaire de NF-κB ; crème topique ; Sahara algérien

الملخص

هدفت هذه الدراســـة إلى تقييم الفعالية العلاجية لنبات *Neurada procumbens L.* (الذي جُمع من منطقة الوادي، الجزائر) في التئام الحروق وتخفيف الإجهاد التأكسدي والالتهاب. تم تحضير مستخلصين، مائي (AENP) وميثانولي(MENP) ، حيث أظهر تحليل (LC–ESI–MS/MS) غناهما بالمركبات الفينولية والنشطة (مثل الكركمين، الأوليوروبين، والميريسيتين). وقد تميز المستخلص المائي بمردود أعلى، وفعالية أقوى كمضـاد للأكسدة، وقدرة أفضـل على الوقاية الضـوئية(SPF) ، مع إثبات السـلامة الانحلالية الدموية والتوافق الحيوي لكلا المستخلصين.

تم تحضــير مراهم موضــعية وتقييمها حيوياً على جرذان مصــابة بحروق من الدرجة الثانية. أظهر المرهم المعتمد على المســتخلص المائي (AENP) تفوقاً ملحوظاً في التئام الجروح، محققاً نســبة انكماش بلغت 98.3% خلال 19 يوماً، متجاوزاً مرهم (MEBO) التجاري. وترافقت هذه النتائج مع تحســن كبير في المؤشرات الدموية والنسيجية وانخفاض الإجهاد التأكسدي. ولتفسير هذه الفعالية ميكانيكياً، أثبتت دراسة الالتحـام الجزيئي أن المركبـات الفعـالـة في النبـات تعمـل كمثبطـات قويـة لبروتين (NF–κB) المرتبط بالالتهاب.

تؤكد هذه النتائج الإمكانيات الصـيدلانية والتجميلية الواعدة للنبات كمعالج طبيعي للحروق، ومضـاد للالتهاب والأكسدة، مما يثمن ويدعم استخداماته الطبية التقليدية.

الكلمات المفتاحية: *Neurada procumbens L.*؛ التئام جروح الحروق؛ مضاد للأكسدة؛ مضاد للالتهاب؛ الكيمياء النباتية؛ الالتحام الجزيئي لـ NF–κB؛ كريم موضعي؛ الصحراء الجزائرية.

List of abbreviations

AENP	Aqueous Extract of *Neurada procumbens*
MENP	Methanolic Extract of *Neurada procumbens*
DPPH	2,2-Diphenyl-1-picrylhydrazyl
RP	Reducing Power
TAC	Total Antioxidant Capacity
SPF	Sun Protection Factor
PPF	Photoprotection Factor
ROS	Reactive Oxygen Species
LC–MS	Liquid Chromatography–Tandem Mass Spectrometry
LC–ESI–MS	Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry
ESI	Electrospray Ionization
CE	Collision Energy
UV	Ultraviolet
UV-A	Ultraviolet A
UV-B	Ultraviolet B
HRBC	Human Red Blood Cell
DCM	Dichloromethane
SDS	Sodium Dodecyl Sulfate
PI	Percent Inhibition
H&E	Hematoxylin and Eosin
MDA	Malondialdehyde
GSH	Reduced Glutathione
SOD	Superoxide Dismutase
CAT	Catalase
EDTA	Ethylenediaminetetraacetic Acid
NF-κB	Nuclear Factor Kappa B
ADT	AutoDock Tools
LGA	Lamarckian Genetic Algorithm
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial Charge (Q), and Atom Type (T) Format
MMFF94	Merck Molecular Force Field 94
BAP	Benzylaminopurine
NAA	Naphthaleneacetic Acid
MS	Murashige and Skoog Medium
O/W	Oil-in-Water
THC	Tetrahydrocannabinol
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
SD	Standard Deviation

Table of Content

List of figure

List of table

Introduction

Introduction

Medicinal plants are among the most important natural sources of bioactive compounds, and have been used for centuries in traditional medicine to treat various diseases. With the significant advancements in biological and chemical sciences, scientific interest in studying medicinal plants and extracting their active compounds has grown considerably, owing to their diverse pharmacological properties and important therapeutic effects. Furthermore, the global trend toward seeking natural alternatives to synthetic drugs — due to the latter's potential side effects and increasing resistance — has reinforced research focusing on plant sources rich in phenolic compounds, antioxidants, and antimicrobial agents (Cock, 2015; Newman & Cragg, 2020).

Among the most severe and debilitating forms of traumatic injury, burn wounds represent a persistent global public health challenge. According to the World Health Organization (2018), burns cause approximately 180,000 deaths per year worldwide, with the majority occurring in low- and middle-income countries. Beyond mortality, non-fatal burns are a major cause of long-term disability, disfigurement, and psychological sequelae. The healing of burn wounds is a complex biological process involving three overlapping phases — inflammatory, proliferative, and remodeling — each requiring precise coordination of cellular and molecular events including inflammation control, oxidative stress regulation, collagen synthesis, angiogenesis, and re-epithelialization (Evers et al., 2010; Pham et al., 2008).

Conventional burn management relies on antiseptic agents, synthetic wound dressings, skin grafting, and pharmacological interventions. While such approaches have improved clinical outcomes, they remain associated with high costs, limited accessibility in resource-poor settings, risk of microbial resistance, and adverse effects. These limitations have intensified scientific interest in natural therapeutic alternatives, particularly plant-derived preparations, which offer a broad spectrum of bioactive compounds capable of simultaneously targeting multiple pathways involved in wound healing (Atiyeh et al., 2007; Dhivya et al., 2015).

Oxidative stress is one of the main factors associated with the onset and aggravation of burn injuries. Following thermal injury, a massive generation of reactive oxygen species (ROS) overwhelms endogenous antioxidant defenses, leading to lipid peroxidation, protein oxidation, and cellular damage that impair the healing process. Therefore, the search for natural antioxidants capable of neutralizing free radicals and restoring redox homeostasis has become a highly significant topic in wound care research (Khurshid et al., 2022; Oryan et al., 2018).

Additionally, the increasing resistance of bacteria to conventional antibiotics represents a critical global health challenge, driving researchers to explore new natural compounds with antibacterial activity. The severity of burn wound infections is further compounded by the ability of bacteria to form biofilms — complex microbial communities embedded in an extracellular matrix — which significantly enhance their resistance to antimicrobial agents and the host immune response. Searching for natural substances capable of inhibiting biofilm formation has therefore become a key focus in modern microbiological studies related to wound management (Aslam et al., 2023).

Among the medicinal plants that have received growing scientific attention is *Neurada procumbens L.* (family Neuradaceae), commonly known as "Sa'adan" or "Saadan" in North Africa. This prostrate annual desert herb is widely distributed across the Saharo-Arabian phytogeographic region, particularly in the Algerian Sahara, Egypt, Saudi Arabia, and Pakistan, where it has been used for centuries in traditional folk medicine for the topical treatment of wounds, skin infections, and burns, as well as for inflammatory and gastrointestinal disorders (Albrecht et al., 2002; Sareen et al., 2017; Bahri & Mansouri, 2020). The biological activity of this plant is mainly attributed to its rich content of bioactive compounds, including polyphenols, flavonoids, acidic mucilaginous polysaccharides, dihydroflavonol glycosides, terpenoids, and carotenoids (Afifi et al., 1991, 2020; Khurshid et al., 2022).

In addition to its bioactive compounds, the aerial parts of *Neurada procumbens* represent an important source of pharmacologically relevant molecules that can be incorporated into topical formulations. The preparation of oil-in-water herbal creams from plant extracts is an established Galenic approach for delivering bioactive phytoconstituents to wound sites, facilitating sustained release, improved skin penetration, and enhanced therapeutic efficacy. These formulations may also possess photoprotective properties against ultraviolet (UV) radiation and the ability to inhibit certain enzymes associated with skin pigmentation processes, such as tyrosinase, making them increasingly interesting for applications in both pharmaceutical and cosmetic dermatology (Chelalba et al., 2020)

This study aims to evaluate the biological properties of *Neurada procumbens L.* extracts and their topical formulations for burn wound healing. To achieve this, the phytochemical composition of aqueous and methanolic extracts was characterized by LC–ESI–MS/MS analysis; antioxidant activity was assessed using DPPH radical scavenging, reducing power, and total antioxidant capacity assays; anti-inflammatory activity was evaluated by albumin denaturation inhibition; the sun protection factor (SPF) was determined; and antibacterial activity was investigated through minimum inhibitory concentration (MIC) and hemolytic

safety evaluation. Furthermore, the in vivo burn-healing efficacy of formulated topical creams was assessed in an experimental rat model, and an in silico molecular docking study was conducted against the NF-κB transcription factor to elucidate the molecular mechanisms of anti-inflammatory action.

This study seeks to answer the following main research questions:

To what extent do the aqueous and methanolic extracts of *Neurada procumbens L.* contain bioactive phytochemical compounds that reflect their pharmacological potential, as characterized by LC–ESI–MS/MS analysis?

Do the extracts show significant antioxidant activity, particularly when evaluated using the DPPH radical scavenging assay, reducing power assay, and total antioxidant capacity?

Does *Neurada procumbens L.* possess anti-inflammatory activity against protein denaturation, and does it exhibit antibacterial activity against certain pathogenic bacterial strains relevant to burn wound infections?

Does the plant extract possess the ability to provide protection against ultraviolet radiation through evaluation of the Sun Protection Factor (SPF), and to inhibit hemolytic activity, confirming its biocompatibility for topical use?

Can topical cream formulations prepared from *Neurada procumbens L.* aqueous and methanolic extracts effectively promote burn wound healing in an experimental rat model, as assessed by wound contraction rate, epithelialization time, hematological, biochemical, and oxidative stress parameters?

At the molecular level, which specific phytoconstituents of *Neurada procumbens L.* are responsible for the anti-inflammatory activity, and how do they interact with the NF-κB transcription factor as revealed by molecular docking simulations?

The present work is structured into two main parts: a theoretical part and an experimental part. The theoretical part includes two chapters. Chapter I presents a general background and literature review on *Neurada procumbens L.*, covering its botanical description, morphological and structural adaptations to arid environments, taxonomic classification, geographical distribution, vernacular names, and documented traditional and medicinal uses. Chapter II deals with the biologically active compounds present in *Neurada procumbens L.* aerial parts and their extracts, with a focus on phenolic compounds, flavonoids, mucilaginous polysaccharides, terpenoids, and carotenoids. It also reviews the pharmacological properties of the plant, including anti-inflammatory, antioxidant, antibacterial, and burn-healing activities documented in the literature.

The experimental part also includes two chapters. Chapter I describes the plant material, animals, chemicals, and experimental methods and protocols used in the study, including

extraction procedures, phytochemical screening, in vitro biological assays, topical cream formulation, in vivo burn wound healing design in Wistar albino rats, and in silico molecular docking methodology. Chapter II presents and discusses the obtained results, including LC–ESI–MS/MS phytochemical profiling, antioxidant assays, evaluation of the sun protection factor (SPF) and hemolytic safety, anti-inflammatory and antibacterial activity tests, physicochemical characterization of formulated creams, in vivo burn healing outcomes (wound contraction, epithelialization, hematological, biochemical, and oxidative stress parameters), and molecular docking analysis against NF-κB.

First part

Bibliographic

CHAPTER I

Neurada procumbens L.

1. Generality:

Neurada procumbens is a prostrate, annual desert plant belonging to the monotypic genus Neurada and is primarily recognized for its high adaptation to arid environments. It thrives in high temperatures and hyper-arid situations, for instance, in deep sands, sand dunes, and sandy plains of desert ecosystems (Albrecht et al., 2002).

The Saharo-Arabian and North African regions exhibit a significant presence of this plant. The genus Neurada traditionally comprises specific variations, and recent taxonomic studies recognize some three distinct varieties (including var. procumbens, var. al-eisawii, and var. stellata), many of which possess unique woolly stems and characteristic spiny, disc-shaped fruits (Turki, 2007).

In fact, *Neurada procumbens* is also noted for its ecological and ethnobotanical value, as its distinctive spiny fruits are easily dispersed over large distances by animals, humans, and desert vehicles, allowing it to colonize new sandy habitats effectively (Albrecht et al., 2002).

It is also documented in local regional floras, mainly in desert sandy habitats, and is widely studied for its pharmacological potential. Under specific desert conditions, the plant plays a dual role as both an indicator of sand dune stability and a source of natural bioactive compounds used in traditional remedies (Albrecht et al., 2002; Turki, 2007).

2. Botanical description:

Plants belong to the botanical family Neuradaceae, which comprises a small group of genera distributed across arid and desert regions. The genus Neurada L. is traditionally recognized as a monotypic genus, with *Neurada procumbens L.* serving as its primary type species originally described from Aegypto-Arabia.

Morphological variations within the species have led to the classification of distinct races treated as varieties, including N. procumbens var. procumbens, N. procumbens var. stellata, and N. (Albrecht et al., 2002; Cronquist, 1981).

procumbens var. al-eisawii. Historically, the taxonomic placement of the family Neuradaceae has been a subject of intensive debate; earlier authors treated it as closely allied to or a subfamily (Neuradoideae) of the Rosaceae. However, modern classification systems and

phylogenetic insights place Neuradaceae as an aberrant family within the order Malvales due to its distinctive floral and pollen morphology. The plant's regional common names include sa'adan or anfel in Africa, pata camello in Spanish-speaking regions, and chapari in India.

Due to its high evolutionary adaptation to hyper-arid conditions, *Neurada procumbens* exhibits significant phenotypic variation governed by environmental constraints such as water availability, salinity, and temperature. The species possesses a unique biological phenomenon where the seeds germinate directly within the hard, persistent fruit pericarp. Upon germination, both the radicle and plumule perforate the woody structure, growing downwards and upwards respectively, leaving the old spiny fruit attached as a protective collar at the base of the stem.

Morphological and Structural Adaptations

Neurada procumbens is a prostrate, non-succulent annual herb capable of forming a dense leafy mat up to approximately 1 meter in diameter.

Floral Morphology: Flowers arise as small, solitary, and regular structures situated in the leaf axils. The perianth is pentacyclic with a distinct calyx and corolla. The corolla consists of 5 petals (measuring 2.5 to 3.0 mm long and 1.5 to 2.0 mm wide), ranging in color from white and whitish-yellow to having pale purple margins. The androecium features 10 free diplostemonous stamens arranged in two whorls (alternating short and long filaments) inserted at the constriction of the calyx tube. The gynoecium is characterized by a syncarpous, partly inferior ovary containing 10 united carpels—each holding a single ovule—surmounted by 10 free styles ending in capitate stigmas (Albrecht et al., 2002).

Root System Adaptations: The root system of N. procumbens is highly specialized to cope with extreme desert droughts. Seedlings rapidly project a deep, narrow vertical taproot that subsequently establishes lateral branches. Once well-established, this deep root system allows the plant to withstand high-temperature stress and thrive for extended periods in mobile sand plains, dunes, and sandy desert swales (Albrecht et al., 2002; Cronquist, 1981).

Morphology and Anatomical Characteristics of *Neurada procumbens L.*

1 .Vegetative Architecture and Stem Anatomy In botanical literature, the vegetative architecture of *Neurada procumbens* is characterized by prostrate, alternately branched main stems that radiate outwardly from a persistent basal fruit coat. Structurally, the stems are terete in outline. Anatomical analysis reveals that the stem cross-section consists of a uni-layered epidermis composed of cubic to barrel-shaped cells, followed by a continuous collenchyma band intermingled with specialized tannin-containing cells. Furthermore, the secondary thickening of the stem develops from a conventional cambial ring, which notably lacks both cortical and medullary bundles (Huber, 1993).

2. Epidermal Indumentum and Adaptive Strategies The surfaces of both the stems and leaves are equipped with a very dense, woolly grey indumentum composed of short and long hairs, granting the plant a characteristic greyish-blue or whitish-green appearance. This dense trichome matrix serves as a vital defensive and adaptive strategy in hyper-arid environments. It functions primarily to maximize light reflection, suppress excessive transpiration, and mitigate heat stress across exposed desert dunefields (Cronquist, 1981; Huber, 1993).

3. Foliar Morphology The leaves of N. procumbens are alternate, petiolate, and frequently fasciculate. Morphologically, the leaf lamina is dorsiventral, relatively non-succulent, and deeply dissected into an ovate to ovate-oblong shape, featuring toothed or pinnatifid margins and obtuse apices. Leaf dimensions generally range from 6 to 23 mm in length and 4 to 17 mm in width, supported by petioles measuring 3 to 15 mm long and flanked by minute, linear-subulate stipules. Additionally, the stomatal complexes present on the leaf epidermis are anatomically classified as anomocytic (Cronquist, 1981; Huber, 1993).

2.2. Distribution of mucilage

Mucilage is widely distributed in different plant organs including seeds, leaves, stems, roots, bark, flowers, and fruits. It is commonly produced by specialized mucilage-secreting cells and accumulates mainly in epidermal tissues and seed coats. In desert and medicinal plants, mucilage plays an important physiological role in water retention, seed germination, protection against drought stress, and tissue hydration (Tosif et al., 2021). In *Neurada procumbens L.*, the presence of mucilage polysaccharides has been previously reported together with flavonoid compounds, supporting the medicinal importance of the plant (Afifi et al., 1991). Plant mucilage forms a gelatinous layer upon hydration and contributes to wound protection and tissue regeneration because of its moisturizing and soothing properties.

Figure 1:: Morphological characteristics of *Neurada procumbens* L leaves and stems (POWO,2026).

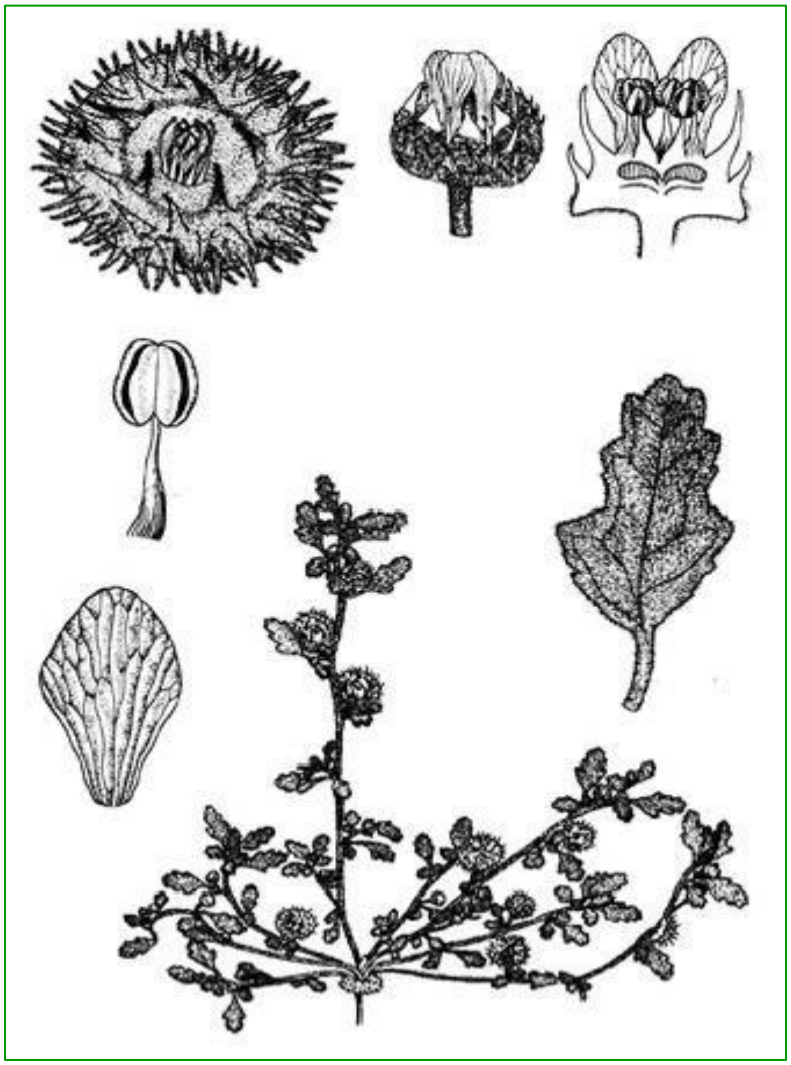

Figure 2: Diagram illustrating the different parts of *Neurada procumbens*. (Flora Canaria, 2026).

3. Taxonomy and Classification of *Neurada procumbens L.*

Neurada procumbens L. belongs to the family Neuradaceae, a small family native to desert and semi-desert regions of Africa, the Middle East, and India. According to Cronquist (1981), the family Neuradaceae includes three genera: Neurada, Grielum, and Neuradopsis. Historically, the taxonomic placement of Neuradaceae has been controversial. Earlier authors considered the family closely related to Rosaceae because of certain floral characteristics and pollen morphology, while later studies investigated its affinities within the order Malvales (Huber, 1993; Angiosperm Phylogeny Group, 1998). However, current taxonomic systems classify *Neurada procumbens L.* under the family Neuradaceae within the order Rosales.

Table 1: Taxonomic classification of *Neurada procumbens L.* (ALBRECHT et al., 2002)

Rank	Classification
Kingdom	Plants
Division	Spermatophyta
Subdivision	Angiosperms
Class	Dicotylédons
Order	Rosales
Family	Neuradaceae
Genus	Neurada
Species	*Neurada procumbens L*
Common name	S'aadan

4. Cultivation System

- *Structural and Physiological Adaptations*

Neurada procumbens is a prostrate, non-succulent, and densely branched annual herb that forms a distinct vegetative mat spreading up to approximately 1 meter in diameter over sand dunes. The plant is characterized by a prominent and unique biological phenomenon regarding its establishment: the seeds are physically retained and germinate directly within the hard, persistent, woody, and spiny discoid fruit pericarp (Albrecht et al., 2002; Turki, 2007).

Upon natural germination under arid conditions, both the radicle and plumule forcefully perforate this rigid maternal hypanthium, growing downwards into the soil and upwards into the atmosphere respectively, leaving the old spiny fruit structure permanently attached as a protective collar at the collar zone of the new primary stem . The vegetative architecture features terete, alternately branched stems and alternate, petiolate, deeply dissected (pinnatifid) leaves (Turki, 2007).

Both the stems and leaves are structurally insulated by a very dense, woolly grey indumentum composed of a complex matrix of short and long trichomes. This dense trichome covering functions as a vital adaptive strategy that suppresses excessive transpiration, reflects solar radiation, and prevents cellular heat stress within severe desert swales (Albrecht et al., 2002). The small, solitary flowers emerge directly from the leaf axils, featuring a pentacyclic perianth with 5 small petals (white, whitish-yellow, or pale purple) and 10 free diplostamenous stamens, which eventually develop into the heavily armed, persistent spiny fruits responsible for zoochorous dispersal via animals and vehicles (Albrecht et al., 2002; Turki, 2007).

- *Root System and Drought Tolerance*

The root system of N. procumbens is deeply penetrating and highly specialized, designed to exploit scarce moisture in hyper-arid mobile sands and dune fields.

Immediately following germination, the seedling projects a rapid, narrow, and vertically elongated taproot that anchors deep into the sand substrate before the extensive development of lateral branches takes place. This deep rooting mechanism, paired with an advanced regulation of stomatal complexes and physiological transpiration control via its trichome matrix, allows the plant to withstand high temperature stress and complete its reproductive life cycle in extremely dry environments (Albrecht et al., 2002; Turki, 2007). Furthermore, due to the natural challenges of seed dormancy imposed by the hard woody pericarp and its status as an endangered medicinal species in certain overexploited regions like the Cholistan and Algerian deserts, modern cultivation systems have successfully integrated in vitro micropropagation protocols (Sareen et al., 2017; Bahri & Mansouri, 2020). These advanced propagation systems utilize MS (Murashige and Skoog) media supplemented with specialized plant growth regulators, such as Benzylaminopurine (BAP) and Naphthaleneacetic acid (NAA), to overcome structural germination barriers, achieve rapid shoot multiplication, and optimize the systematic cultivation of this robust desert specialist (Sareen et al., 2017).

5.Origin and geographical diffusion:

The genus Neurada originated in the hyper-arid regions of Aegypto-Arabia, from which it naturally diffused across a vast geographic belt spanning North Africa, the Mediterranean region, the Middle East, and Northwest India (Turki, 2007; Sareen et al., 2017). Due to its high evolutionary adaptation to sand dunes, the species has established a continuous native distribution throughout the Saharo-Arabian phytogeographical region, with notable extensions into the Sudanian zone (Turki, 2007). In recent years, historical records and botanical surveys documented its intercontinental diffusion, confirming *Neurada procumbens* as a newly naturalized alien record in the southern hemisphere, specifically invading the highly arid sandy situations on the northwestern edge of the Simpson Desert in Australia (Albrecht et al., 2002).

Historical records and floristic treatments show that the plant is widely distributed across more than twenty countries in the arid world, including Egypt, Libya, Saudi Arabia, Jordan, Qatar, and Pakistan (Jafri, 1977; El-Ghazaly, 1991; Borzatti de Loewenstern & Garbari, 2000; Turki, 2007; Sareen et al., 2017).

In Egypt, for instance, the species thrives across diverse ecological phytogeographical sub-regions, including the Mediterranean coastal strip, the Oases of the Western Desert, the Eastern Desert, and the Sinai Peninsula (Boulos, 1999; Turki, 2007).

In Algeria, the natural populations of *Neurada procumbens* are subjected to ongoing horizontal expansion across the northern and eastern parts of the Algerian Sahara, with a dense concentration in the El-Oued region (Souf), where the surface area of mobile sand dunes provides an ideal ecosystem for its survival (Bahri & Mansouri, 2020).

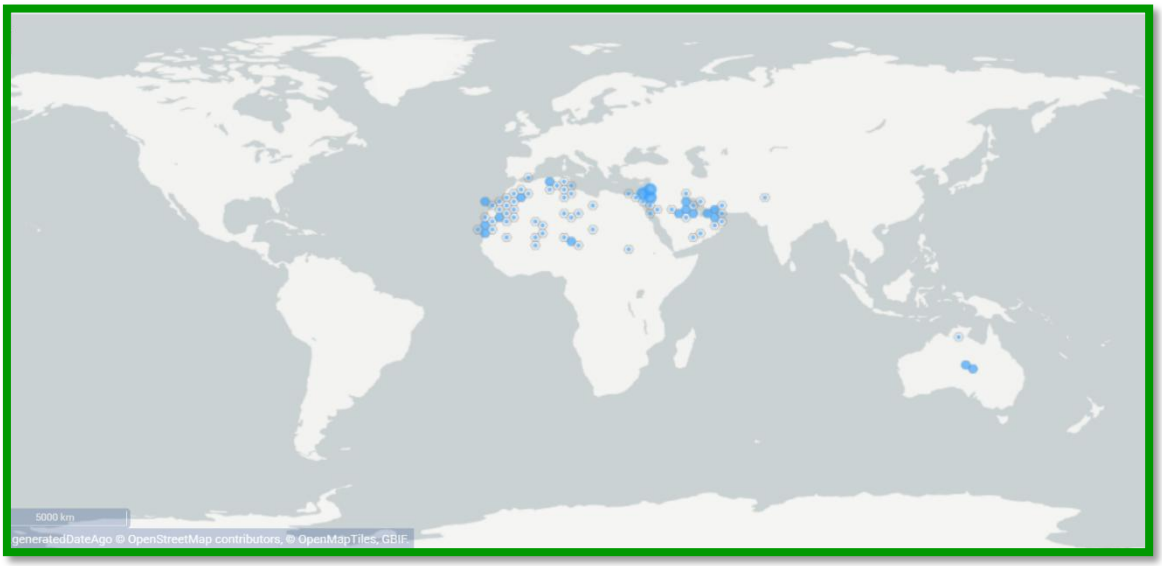

Figure 3 : Geographic distribution map of *Neurada procumbens* (Source: GBIF Secretariat, 2023).

6. Local name of *Neurada procumbens* L

In the arid and desert regions where *Neurada procumbens* thrives, local communities have developed various vernacular names that reflect the plant's morphology and cultural presence. In Saudi Arabia, the species is widely known by the traditional Arabic names "Al-Sadan" and "Sa'dan", names deeply rooted in ancient Arabic botanical lexicon (Al-Yemeni et al., 2013). In North Africa, particularly in the desert areas of Algeria, the plant is similarly recognized by its vernacular name "Saadan" (Bahri & Mansouri, 2020). Beyond the Arab world, in the Cholistan Desert of Pakistan, the species is commonly referred to as "Chipri Booti" (Sareen et al., 2017). This diverse vernacular nomenclature highlights the widespread geographical recognition and ethnobotanical relevance of N. procumbens across different desert cultures.

7. Bioactive compound

Neurada procumbens is recognized as a rich source of bioactive and nutritional phytochemicals, including phenolic acids, flavonoids, acidic mucilages, sterols, and triterpenes (Afifi et al., 2020; Khurshid et al., 2022). Seeds and the surrounding persistent spiny fruits are notably dense in fixed oils, specific fatty acids, and essential minerals (Khurshid et al., 2022). Bioactive antioxidants, distinct dihydroflavonol glycosides, and water-soluble polysaccharides are abundant throughout the vegetative aerial parts, including the stems and leaves (Afifi et al., 2020), while the whole plant extracts exhibit remarkable concentrations of total phenolics and total flavonoids responsible for high radical scavenging capacities (Khurshid et al., 2022).

7.1.Polyphenols:

Polyphenols in *Neurada procumbens* are primarily composed of flavonoids (specifically flavonols, dihydroflavonols, and flavanones) and phenolic acids. These compounds are well-known for their strong antioxidant, antibacterial, and enzyme-inhibitory activities (Khurshid et al., 2022).

Flavonoids protect against oxidative damage by directly scavenging free radicals (Jakobek, 2015), inhibiting vital enzymes such as xanthine oxidase, α-glucosidase, and tyrosinase (Khurshid et al., 2022), and preventing oxidative cellular stress.

7.2. Mucilages and Polysaccharides:

Polysaccharides of plant origin in N. procumbens include unique water-soluble acidic

mucilages structured with core monosaccharide units such as D-galacturonic acid, D-galactose, L-arabinose, and L-rhamnose (Afifi et al., 2020). These macromolecules act as efficient protective agents and physiological texturizers in arid systems (Tomoda & Ishikawa, 1987). Biochemically, the yield and physical properties of these mucilages vary across different plant organs and varieties, with the vegetative mats exhibiting highly viscous polymeric systems that enhance moisture retention and display significant anti-inflammatory and cytoprotective properties (Afifi et al., 2020).

8. **Traditional and Medicinal Uses of *Neurada procumbens*:**

Local populations traditionally use the plant as an effective remedy for respiratory conditions such as coughs and asthma, as well as for treating stomach aches, diarrhea, and fever (Sareen et al., 2017). Moreover, the fresh juice or crushed leaves of the plant are applied topically to treat eye infections and skin wounds, while a decoction of its roots is traditionally administered to relieve kidney pain and urinary tract infections (Sareen et al., 2017). Additionally, the spiny fruits of the plant have been reported to serve as a nutritious food source for camels and livestock in desert environments, contributing to their physical strength (Albrecht et al., 2002).

In North African and Middle Eastern traditional practices, parts of *Neurada procumbens* are widely employed as a general tonic, an aphrodisiac, and for strengthening the nervous system, as well as for treating diabetes and gastrointestinal disorders (Afifi et al., 2005). Modern pharmacological evaluations have validated many of these traditional applications, demonstrating that the plant extracts possess significant biological activities. Phytochemical studies have revealed the presence of potent secondary metabolites, such as acidic mucilages and dihydroflavonol glycosides (including astilbin), which are responsible for its therapeutic effects (Afifi et al., 2005). Recent laboratory investigations have confirmed that its aqueous and alcoholic extracts exhibit robust anti-inflammatory, antioxidant, and antibacterial properties, particularly against pathogenic bacterial strains, alongside a notable analgesic and protective effect on gastric and liver tissues (Bahri and Mansouri, 2020).

Chapter II
Biological Activities of
(Neurada procumbens L.)

1. Anti-inflammatory activity:

Neurada procumbens, commonly known as prickly spike-headed pod, has garnered significant attention for its potential anti-inflammatory properties, which have been extensively studied through various extracts, including those from its aerial parts, lyophilized flowers, and stems. Numerous studies have demonstrated the anti-inflammatory and antioxidant activities of Neurada, thanks to its rich content of bioactive compounds such as alkaloids, flavonoids, polyphenols, and tannins, notably phenolic acids, found in both the aerial and floral extracts (Aslam et al., 2023; Hegazi et al., 2019; Salama et al., 2021). These compounds are known to play crucial roles in modulating inflammatory pathways.

One of the primary mechanisms through which Neurada exerts its anti-inflammatory effects is by inhibiting the release of lysosomal enzymes and stabilizing the cellular membrane (Chou, 1997). The suppression of these enzymes is crucial, as they are involved in the initiation and perpetuation of the inflammatory process and tissue damage in various chronic diseases.

In an animal model, Aslam et al. (2023) observed that *Neurada procumbens* floral extract effectively reduced inflammatory markers and inhibited the carrageenan-induced hind paw edema, a key experimental model that measures the acute phase of inflammation. Furthermore, Neurada's ability to suppress membrane lysis—an event responsible for the production of inflammatory mediators—further corroborates its role as a natural anti-inflammatory agent (Amresh et al., 2007).

Neurada procumbens's anti-inflammatory properties have been linked to its potential benefits in treating various inflammatory conditions. For instance, it has been traditionally used to alleviate symptoms associated with fever, inflammatory disorders, and gastrointestinal disturbances such as dysentery and internal ulcers (Said et al., 2002).

Its ability to reduce inflammation in these contexts positions Neurada as a promising therapeutic agent for conditions characterized by chronic inflammation.

Additionally, specific phenolic monomers, including p-coumaric acid and chlorogenic acid found in the plant, are recognized for their own anti-inflammatory properties. While its effects have been demonstrated in several studies (Hegazi et al., 2019), it is worth noting that the anti-inflammatory activity of the dichloromethane (DCM) floral extract of *Neurada procumbens* through human red blood cell (HRBC) membrane stabilization is being reported for the first time, adding a novel dimension to the plant's therapeutic potentia.

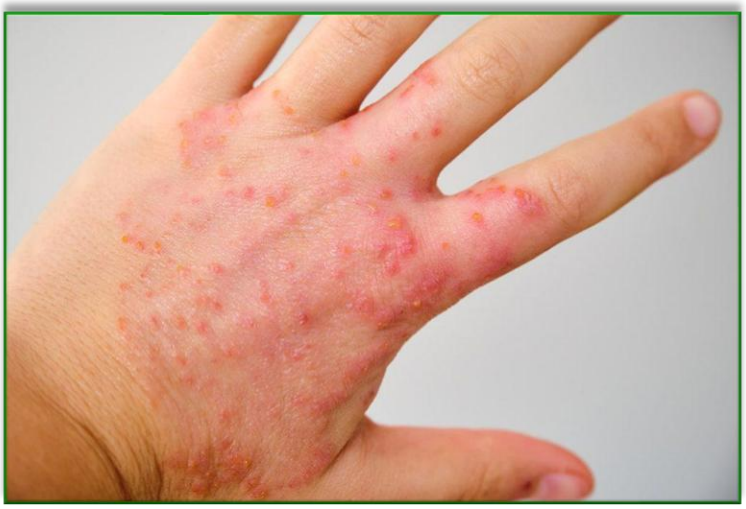

Figure 4: Skin infections (Basirclinc .2019)

2. Antioxidant activity:

Neurada procumbens L., commonly known as Al-Sa'adan, has been studied for its antioxidant properties, which are primarily attributed to its composition of polyphenols, flavonoids, and condensed tannins. These bioactive compounds work to combat oxidative stress, protect against cellular damage, and mitigate harmful free radicals.

2.1. Mechanism of Antioxidant Action

Research on the extracts of *Neurada procumbens L.* has demonstrated its significant ability to inhibit oxidative processes in vitro. Studies evaluating the plant's biological profile have revealed a potent concentration-dependent free radical scavenging activity using the stable free radical DPPH assay, highlighting the capacity of the plant's secondary metabolites to neutralize oxidative damage (Khurshid et al., 2022). This strong antioxidant capability is deeply linked to the structural and chemical diversity of its natural constituents, which effectively counteract the toxic accumulation of reactive oxygen species that induce tissue destruction and molecular transformation (Khurshid et al., 2022).

These protective effects are primarily driven by the rich presence of total phenolic and flavonoid compounds distributed within the plant's polar extracts (Khurshid et al., 2019). The bioactive fractions act as natural free radical scavengers and reducing agents, directly correlating with maximum antioxidant capacities and showcasing prominent ferric reducing antioxidant power (Khurshid et al., 2019). Furthermore, the complex composition of specialized flavonoids—including taxifolin, vitexin, and orientin derivatives—underpins the plant’s robust potential to mitigate oxidative stress, prevent cellular degradation, and

support overall cellular homeostasis under pathological conditions (Khurshid et al., 2019; Khurshid et al., 2022).

2.2. Supporting Evidence from In Vitro and In Vivo Studies

In vitro studies have shown that Al-Sa'adan (*Neurada procumbens L.*) extracts exhibit notable free radical scavenging activity. For instance, Chelalba et al. (2020) demonstrated that the plant extracts possess strong radical scavenging effects, particularly when evaluated using the stable free radical DPPH assay system. This study highlighted that the polar fractions of the plant are highly efficient in neutralizing reactive oxygen species (ROS) and scavenging free radicals, which are the main driving forces behind lipid peroxidation and severe biochemical tissue destruction under elevated oxidative stress conditions.

In vivo and cellular-based models also support the protective and physiological benefits of *Neurada procumbens L.* extracts. In experimental setups evaluating cellular damage, Bahri et al. (2020) observed that the plant's leaf and fruit extracts significantly minimized cellular degradation and enhanced membrane stability within red blood cell hemolysis assays. This protective property prevents cell lysis and lipid breakdown under chemical induction, thereby validating the plant's capacity to maintain cellular homeostasis.

2.3. Health Benefits and Therapeutic Potential

The phytochemical richness of *Neurada procumbens L.* provides important therapeutic potential against several oxidative stress-related disorders. Traditionally, the plant has been used for the treatment of diabetes, fever, inflammation, jaundice, wounds, and rheumatic disorders in desert regions and folk medicine systems (Aslam et al., 2023). Recent pharmacological investigations confirmed the antioxidant, anti-inflammatory, neuroprotective, and antibacterial activities of the plant extracts, supporting its ethnomedicinal importance.

The presence of bioactive metabolites such as flavonoids, phenols, tannins, coumarins, and terpenoids may contribute to tissue protection, enhancement of wound healing, and prevention of cellular damage associated with chronic inflammatory and degenerative diseases. The low toxicity profile reported for floral extracts further supports the potential pharmaceutical and cosmetic applications of N. procumbens L.

2.4. Sun protection factor (SPF)

Different photoprotection factors (PPFs) of the leaf and fruit extracts of *Neurada procumbens L.* were analyzed using a UV-visible spectrophotometric method across the

ultraviolet radiation spectrum. Sun protection factor (SPF) was determined in vitro to evaluate the plant's efficacy against erythematic action and UV-B induced cellular damage. Additionally, the UV-A radiation protection capacity was assessed relative to the persistence of pigmentation and biological protective benchmarks related to overall ultraviolet exposure, referencing the established parameters of solar radiation absorption (Chelalba et al., 2020).

The critical wavelength and spectrophotometric absorbance data were utilized to calculate the definitive SPF values across different extract concentrations. To evaluate these biological protection effects, the dissolved plant extracts were evaluated via transmittance measurements across specific wavelength ranges mimicking human skin exposure thresholds. The sample preparation was evaluated using quartz cells or adapted plates to measure transmittance via a specialized spectrophotometer equipped with an integrating sphere to capture total diffuse radiation (Khurshid et al., 2019). Multiple readings per sample extract were systematically recorded to ensure mathematical replication and precisely establish the screening capabilities of *Neurada procumbens L.* formulations against light-induced structural degradation (Chelalba et al., 2020; Khurshid et al., 2019).

Figure 5: Skin pigmentation due to sun rays (Dermedica, n.g.)

3. Anti-burn effect:

3.1. Mechanism of Action in Burn Treatment

3.1.1. Inflammatory Phase:

During the inflammatory phase of burn wound healing, *Neurada procumbens L.* contributes to reducing inflammation and protecting damaged tissues against microbial invasion. The plant is rich in flavonoids, phenolic acids, tannins, coumarins, and terpenoids, which possess significant antioxidant and anti-inflammatory activities (Khalid et al., 2023; Locatelli et al., 2022). These bioactive compounds help neutralize reactive oxygen species (ROS) generated after burn injury, thereby limiting oxidative stress and preventing further cellular damage.

3.1.2. Proliferative Phase:

During the proliferative phase, *Neurada procumbens L.* promotes tissue regeneration and accelerates wound closure through several biological mechanisms. The mucilage polysaccharides present in the plant form a moist gelatinous protective layer over the wound surface, helping maintain hydration and preventing excessive water loss from injured tissues (Afifi et al., 1991). This hydrated environment is essential for optimal cellular migration and re-epithelialization.

Additionally, the antioxidant compounds of N. procumbens L. stimulate fibroblast activity and support granulation tissue formation. Polyphenols and flavonoids may enhance angiogenesis by protecting endothelial cells against oxidative injury and improving microcirculation at the wound site. Improved vascularization facilitates oxygen and nutrient delivery necessary for tissue repair and regeneration.

The plant also contains vitamins, amino acids, and terpenoid compounds that may contribute to collagen biosynthesis and extracellular matrix formation. Phenolic antioxidants help preserve newly formed tissues from oxidative degradation, thereby accelerating wound contraction and reducing healing time. Experimental studies on medicinal plants rich in similar phytochemicals demonstrated enhanced epithelial regeneration and increased collagen deposition during burn healing processes (Shahzad et al., 2023).

3.1.3. Remodeling Phase:

In the remodeling phase, *Neurada procumbens L.* contributes to tissue maturation and restoration of skin integrity. The antioxidant and anti-inflammatory phytochemicals present in the plant help regulate collagen remodeling and extracellular matrix organization, which are essential for restoring the tensile strength and elasticity of healed skin.

Flavonoids and polyphenolic compounds may influence fibroblast proliferation and collagen fiber alignment while reducing excessive scar formation. The reduction of oxidative stress during this phase protects the extracellular matrix from degradation and supports balanced tissue remodeling. In addition, terpenoids and sterol compounds identified in N. procumbens L. may participate in maintaining membrane stability and improving tissue resilience.

The combined antioxidant, moisturizing, anti-inflammatory, and regenerative properties of *Neurada procumbens L.* therefore contribute to faster wound healing, improved tissue organization, and reduced scar formation in burn injuries

Figure 6: First degree burn hand (altibi.2024)

Second part

EXPERIMENTAL STUDY

CHAPTER I:

MATERIALS AND METHODS

Experimental:

I. Materials:

1. Collection and Preparation of *Neurada procumbens L.:*

The *Neurada procumbens* (Sa'dan) plant was collected during the autumn season on September 25, 2025, from the northeastern part of the Algerian Sahara, specifically from the southeastern region in El Oued Province, Reguiba District (Dhabaia area). A second collection was carried out on Friday, February 21, 2026, at 8:00 AM, from the Hiba area in the same district (Reguiba). To remove dust and impurities, the plant samples were thoroughly washed under running tap water. The thorny flowers were carefully removed, keeping only the green leaves, which were subsequently dried and stored for further use.

Figure 7: Images of *Neurada procumbens* in its natural habitat (Original photos)

2.Animals:

A total of thirty (30) adult male albino rats, with an initial body weight ranging between 120 and 140 g, were procured from the animal house of the Pasteur Institute (Algeria). The animals were housed in the animal facility of the Department of Molecular and Cellular Biology, Faculty of Natural and Life Sciences, University of El Oued. They were maintained under strictly controlled laboratory conditions and subjected to a two-week acclimatization period prior to the commencement of the experiments. Throughout both the acclimatization and experimental phases, the rats were provided with ad libitum access to a standard pellet diet and fresh drinking

water.

II. Methods

1. Phytochemical analysis of *Neurada procumbens L*

1.1. Preparation of the Aqueous and Hydroethanolic Extract of *Neurada procumbens L.*:

The aerial parts of *Neurada procumbens*, collected from the southeastern region of Algeria (El Oued) during the flowering stage, were air-dried at room temperature and then ground into a fine powder using a blender.

To prepare the hydroethanolic extract, 100 g of the obtained powder was macerated in 1 L of 70% ethanol solution (700 mL ethanol and 300 mL distilled water). The maceration process was carried out for 48 hours at room temperature.

The aqueous extract was prepared by infusion of 100 g of plant powder in 1 L of distilled water.

Both extracts were filtered using Whatman filter paper and then concentrated under reduced pressure using a rotary evaporator.

Finally, the obtained extracts were stored at 4 °C until further use.(Figure 10)

1.2. Extraction yield

Extraction yield was calculated by the following equation (1):

$$\text{Yield (\%)} = W1/W2 \times 100 \qquad (1)$$

Where W1 = weight of the extract and W2 = weight of plant material dried powder *(Boutennoun et al., 2017)*

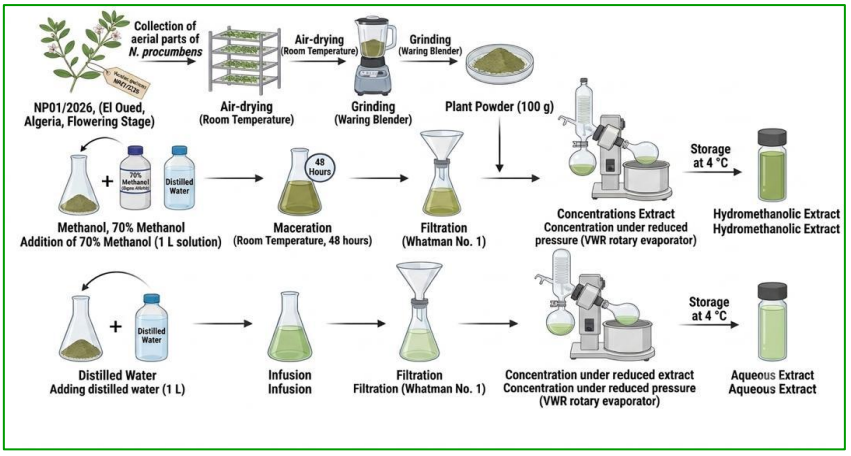

Figure 8: Schematic diagram for the preparation of the aqueous and hydroehanolic extracts of *Neurada procumbens*.

2. In Vitro Activity of the aqueous extract of *Neurada procumbens L.*

2.1. Antioxidant activity:

2.1.1. DPPH free-radical scavenging activity (DPPH):

The antioxidant activity of the extracts was evaluated using the DPPH free radical scavenging assay. Briefly, 1 mL of DPPH• solution was mixed with 1 mL of each extract, while ascorbic acid was used as a reference standard. The reaction mixture was thoroughly shaken and incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance of the reaction medium was measured at 517 nm (Equation 04) (Mansouri et al., 2005).

Percent inhibition (PI) = [(Abs control - Abs sample) / Abs control) times *100] (4)

Where Abs control represents the absorbance of the control solution containing all reagents except the sample, and Abs sample corresponds to the absorbance of the tested extract (Gulcin & Alwasel, 2023).

2.1.2. Reducing Power (RP):

The reducing power of the extracts was determined according to the method described by Oyaizu. Different concentrations of the extract (mg/mL) were prepared in distilled water and mixed with phosphate buffer (1.25 mL, 0.2 M, pH 6.6) and potassium ferricyanide solution (1.25 mL, 1% $K_3[Fe(CN)_6]$). The resulting mixture was incubated at 50°C for 20 minutes. Afterwards, 1.25 mL of trichloroacetic acid solution (10%) was added, followed by centrifugation at 3000 rpm for 10 minutes.

An aliquot of the supernatant (1.25 mL) was then combined with distilled water and freshly prepared ferric chloride solution (0.25 mL, 0.1% $FeCl_3$). The absorbance was measured at 700 nm. Ascorbic acid was used as a positive control (Oyaizu, 1986).

2.2. Anti-inflammatory activity:

The anti-inflammatory activity of the aqueous extract of *Neurada procumbens L.* was assessed using the egg albumin denaturation inhibition method. The reaction mixture (5 mL) consisted of 100 µL of fresh hen egg albumin, 1.4 mL of phosphate buffer solution (pH 6.4), and 1 mL of different concentrations of the tested extract or the standard drug diclofenac sodium. For the control, 1 mL of distilled water was used instead of the extract or diclofenac sodium.

The prepared mixtures were incubated in a water bath at 37°C for 15 minutes, followed by heating at 70°C for 5 minutes. After cooling, the absorbance was measured at 660 nm using a

UV–visible spectrophotometer, with phosphate buffer serving as the blank (Dharmadeva et al., 2018).

2.3. Hemolytic activity

The hemolytic activity of the extracts was evaluated using the erythrocyte hemolysis assay. Fresh blood was collected in anticoagulant tubes and centrifuged to obtain red blood cells (RBCs). The cells were washed three times with phosphate-buffered saline (PBS) and re-suspended to prepare an erythrocyte suspension. Different concentrations of the extracts were incubated with the RBC suspension at 37°C for 1 h. After centrifugation, the absorbance of the released hemoglobin in the supernatant was measured at 540 nm using a UV–Visible spectrophotometer. Sodium dodecyl sulfate (SDS) was used as the positive control, while PBS served as the negative control. The percentage of hemolysis was calculated relative to the controls.

2.4. Photoprotective activity

The photoprotective activity of the extracts (AENP and MENP) was evaluated in vitro using the UV-Vis spectrophotometric method, as described by Mansur et al. (1986). The Sun Protection Factor (SPF) was determined by measuring the absorbance of the extracts in the UVB wavelength range (290–320 nm).

2.5. characteristic LC-MC

The plant extracts were comprehensively characterized using an ultra-performance liquid chromatography–electrospray ionization tandem mass spectrometry (UPLC–ESI–MS/MS) system (Shimadzu LCMS-8040, Kyoto, Japan), which was equipped with Ultra-Fast Mass Spectrometry (UFMS) technology to ensure high sensitivity, rapid analysis, and precise compound detection. The system was coupled to a Nexera XR LC-20AD binary pump, enabling efficient solvent delivery and gradient control throughout the chromatographic run. Separation of the phytochemical constituents was carried out on a Restek Ultra C18 analytical column (150 × 4.6 mm internal diameter, 3 μm particle size), which was maintained at a constant temperature of 40 °C to optimize peak resolution and reproducibility.

The chromatographic analysis employed a binary mobile phase system consisting of solvent A (water supplemented with 0.1% formic acid) and solvent B (methanol). Elution was performed using a programmed linear gradient designed to facilitate effective separation of compounds with varying polarities. The gradient profile was established as follows: from 0 to 0.2 min, 98% solvent A; from 0.2 to 2.5 min, reduced to 25% solvent A; from 2.5 to 4.0 min, decreased to 0% solvent A; maintained at 0% solvent A from 4.0 to 7.0 min; then rapidly returned to 98% solvent A between 7.0 and 7.1 min; and finally held at 98% solvent A from

7.1 to 12.0 min to allow proper column re-equilibration before the next injection. The flow rate was consistently maintained at 0.2 mL/min, while the injection volume for each sample was set at 5 µL.

Mass spectrometric detection was conducted using electrospray ionization (ESI) in tandem MS mode under optimized operating conditions to maximize ion generation and fragmentation efficiency. The collision-induced dissociation (CID) gas pressure was set at 230 kPa, while the conversion dynode voltage was maintained at −6.0 kV. The desolvation line temperature was adjusted to 250 °C, and the heat block temperature was set at 400 °C to ensure efficient solvent evaporation and ion transfer. Additionally, the nebulizing gas flow was maintained at 3.0 L/min, and the drying gas flow was set at 10 L/min to promote stable ionization and enhance analytical performance.

3. In vivo activity of aqueous extract and methanolic of *Neurada procumbens L.*

3.1. Preparation of Formulation creams

The topical formulations were developed using classical Galenic pharmaceutical techniques to create an Oil-in-Water (O/W) emulsion. Two distinct ointments were prepared: one incorporating the aqueous extract and the other incorporating the methanolic extract of Haplophyllum tuberculatum.

The formulation process followed a three-phase protocol to ensure stability and homogeneity:

1- Phase A (Aqueous Phase): Consisted of distilled water (64.6%) and glycerin (3%), heated to 75°C.

2- Phase B (Oil Phase): Comprised of Shea butter (1%), vegetable oils (10.5%), and emulsifying agents including Lanette O (4%), Lanette 16 (2%), and Eumulgin B2 (2%), melted at the same temperature. Phase A was then gradually incorporated into Phase B under continuous and vigorous mechanical stirring until a uniform emulsion was formed.

3- Phase C (Active Ingredients): Once the mixture cooled to approximately 40°C, the plant extract (8%) was added along with essential oils (0.5%), Vitamin E (0.2%) as an antioxidant, and a preservative (0.2%).

The pH of the resulting creams was measured to ensure compatibility with the skin. The final formulations were stored in sterile, airtight containers at 4°C for subsequent biological evaluation.

3.2. Characterization of cream:

. Moisture (H%):

Using the oven-drying method, the process was carried out on two samples (H_1 and H_2):

Step 1: Weighing the container completely empty and clean, and recording the mass as (m_0).

Step 2: Adding an amount of the sample into the container, and weighing the container with the wet sample before drying, and recording the mass as (m_1).

Step 3: Placing the container in the dryer/oven to completely remove moisture (water).

Step 4: Weighing the container after complete drying with the remaining dry matter, and recording the mass as (m_2).

- Moisture Content Calculation Formula (H%) :

$$H\% = \frac{m1-m2}{m1-m0} \times 100$$

- Dry Matter Calculation Formula (E.S%):

$$E.S\% = 100\% - H\%$$

- Calculation of the Final Average Moisture Content*:*

$$\mathbf{H}moy\% = \frac{\mathbf{H1}\% + \mathbf{H2}\%}{\mathbf{2}}$$

Figure 9: Experimental steps and equipment used for moisture determination (Original photos).

. Density Determination:

This method is based on the precise weighing of identical volumes of both water and the

sample at a specific temperature (usually 20°C).

- {Pv} (Empty Weight): The weight of the completely empty pycnometer
- {PH2O} (Water-filled Weight): The weight of the pycnometer filled with distilled water.
- PEch (Sample Weight): The weight of the pycnometer filled with the sample to be tested.
- Formula for calculating P {20}:

$$P20 = 997 \times \frac{\boldsymbol{P}Ech - Pv}{PH2O - Pv} + F$$

Figure 10: Experimental steps and equipment used for density determination (Original photos).

pH Measurement:

The measurement was conducted on samples of a specific weight and under different temperatures to ensure accuracy and to document the effect of temperature on pH:

- Weighing a specific amount of the sample.
- Submerging the pH meter electrode into the sample, reading the value, and accurately recording the accompanying temperature.

3.3.Experimental Design and Burn induction

Adult Wistar albino rats were acclimatized for two weeks under standard laboratory conditions. The animals were then randomly divided into six groups, each consisting of five rats (n = 5), as follows:

Detailed Experimental Group Descriptions

Group NC (Negative Control): This group consists of rats subjected to the standard burn injury protocol but left completely untreated. They serve as a baseline to observe the natural pathological progression, inflammatory response, and spontaneous healing rate of the

burn wound without any pharmacological intervention.

Group BASE (Vehicle Control): Rats in this group are subjected to the burn injury and subsequently treated solely with the empty cream base (the excipients/vehicle without any active plant extracts). This group is methodologically crucial for excluding any potential healing or adverse physiological effects caused by the inert delivery medium itself.

Group MEBO (Positive Control): Following the burn injury, this group receives topical application of MEBO (Moist Exposed Burn Ointment), a globally recognized standard commercial burn therapy. This group acts as a standard pharmacological benchmark to quantitatively and qualitatively compare the relative therapeutic efficacy of the novel plant extracts.

Group MENB (Experimental Group - Methanolic Extract): Burned rats in this experimental group are treated topically with the formulated cream containing the methanolic extract. The objective is to evaluate the specific wound healing properties—such as the rate of wound contraction, re-epithelialization, and collagen synthesis—mediated by the less polar bioactive compounds extracted via methanol.

Group AENP (Experimental Group - Aqueous Extract): This experimental group comprises burned rats receiving topical treatment with the formulation containing the aqueous extract. This allows for the assessment of the therapeutic efficacy of highly polar, water-soluble phytochemical constituents on the burn healing process, providing a comparative analysis against the methanolic extract and control groups.

Group N (Normal / Intact Control): This group consists of healthy, intact rats that are neither subjected to the burn injury nor administered any topical treatment. They provide the absolute baseline for normal physiological, biochemical, and histological parameters to be compared against both the injured and treated states.

For burn induction, the dorsal region of the animals was shaved 24 hours prior to the experiment. Under general anesthesia induced by chloroform inhalation in a closed chamber, a standardized burn wound was created on the dorsal surface using an aluminum plate (2 cm in diameter) heated to 120°C and applied to the skin for 6 seconds, producing a second-degree burn.

After burn induction, the animals were returned to their respective groups and housed collectively (five rats per cage). No specific treatment was administered immediately after injury.

Treatment protocols were initiated 24 hours post-burn and applied topically once daily

for 19 consecutive days. The respective extracts were applied directly to the wound area in appropriate doses.

At the end of the experimental period, the animals were sacrificed under anesthesia for sample collection and further analysis.

3.4.Parameters for Evaluating Burn Healing in an Excision Wound Model

Morphological evaluation: Following the method described by *(Fikru et al.,2012)* gradual changes in wound area throughout the treatment period were documented using a iPhone 8 Plus camera on days 1,3,7,10,14,19 and upon complete healing of each wound.

Measurement of Burn contraction: The size of the wounds (cm2) was monitor according to *(Arunachalam and Parimelazhagan ,2013),* as detailed by *(Fikru et al.,2012),* on days 1, 3, 7,10, 19, and 17. Wound contraction was calculated as the percentage reduction in the original wound area, using the formula described by *(Subramanian et al.,2023***).**

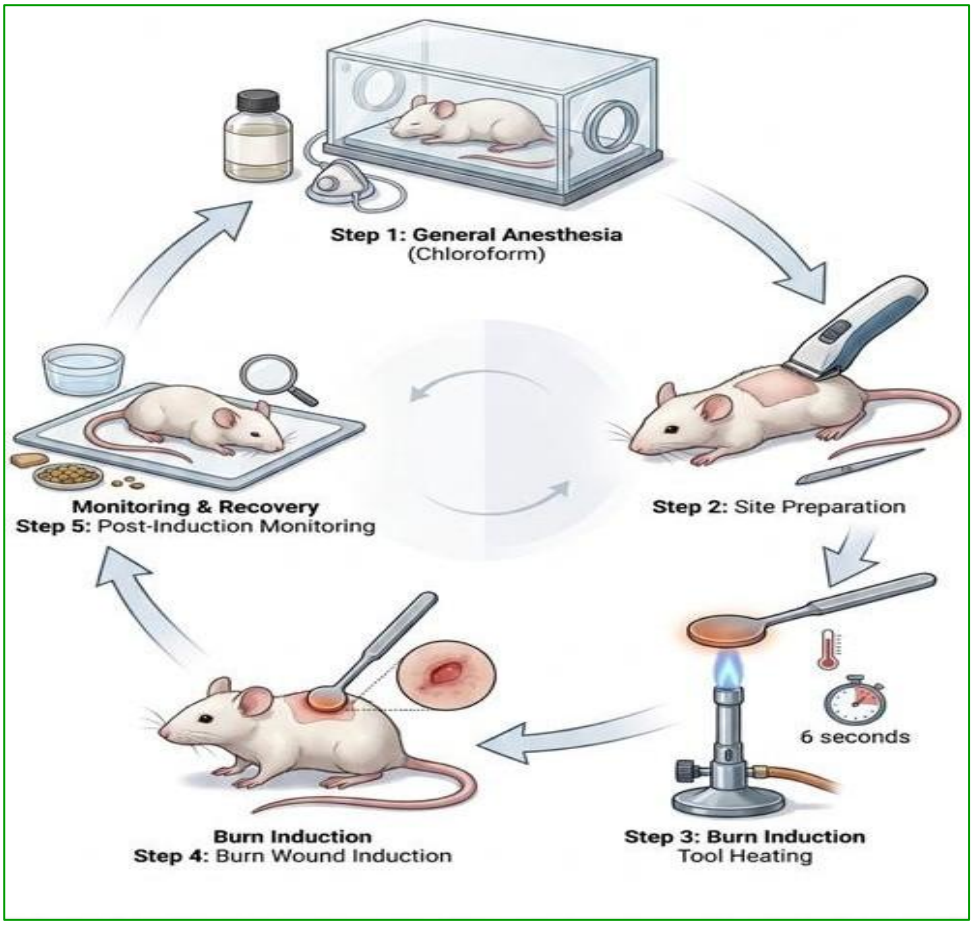

Figure 11: Diagram explaining experimental Design and Burn Wound Induction.

3.5.Samples preparation:

3.5.1.Sacrifice of rats and collection of blood and organs

After a fasting period of 16 hours, the rats were anesthetized using chloroform (94%) and then sacrificed. Blood samples were collected at the time of sacrifice into EDTA tubes

for hematological analysis and into dry tubes (sec) for biochemical analysis.

Following dissection, the liver, kidneys, spleen, thymus gland, and a sample from the burned skin area were carefully excised. The organs were rinsed first with distilled water and then with 0.9% NaCl solution, after which they were weighed. Subsequently, the collected organs were stored in ECBE containers.

A portion of these organs was used for the preparation of tissue homogenates, while the remaining samples were preserved for histological examination. The homogenates were utilized to evaluate oxidative stress parameters, including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT).

3.6. Methods of blood analysis

3.6.1. Hematological parameters assay

Hematological parameters—including white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA), red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), and platelets (PLT)—were quantified via the Coulter principle, utilizing a Medonic automated hematology analyzer designed for comprehensive Complete Blood Count (CBC) analysis.

3.6.2. Biochemical parameters assay

The biochemical profile of the serum samples was determined using various analytical techniques, ranging from enzymatic kinetic assays to automated immunoassay protocols. The specific methodologies employed for each parameter are detailed below:

- Blood Glucose: Plasma glucose levels were determined using the glucose oxidase method. Beta-D-glucose is oxidized to D-glucono-1,5-lactone, producing hydrogen peroxide, which is subsequently converted by peroxidase to a colored complex detectable colorimetrically (Trinder,1969).
- Calcium: Calcium concentrations were measured using the Arsenazo III colorimetric method. In an alkaline medium, calcium forms a colored complex with Arsenazo III, the intensity of which is directly proportional to the calcium concentration (Michaylova and Ilkova,1971).
- Phosphor: Inorganic phosphorus was analyzed via the photometric molybdenum blue method. Following the liberation of phosphate using acid reagents, the complex is reduced to molybdenum blue, which is quantified photometrically (Leiboff.1928).
- Testosterone and Thyroid Hormones: Serum concentrations of testosterone and thyroid

hormones (FT3, FT4, and TSH) were quantified using the Cobas e411 analyzer. This system utilizes the electrochemiluminescence (ECL) technique, which relies on electron valence changes to generate measurable photons proportional to the analyte concentration (Wiwanitkit,2007).

- C-Reactive Protein (CRP): Serum CRP levels were determined using a turbidimetric immunoassay on the COBAS INTEGRA 400 analyzer. This method employs specific antibodies, with Polyethylene glycol-6000 and Tween-20 to enhance the immunoprecipitation reaction and minimize sample interference (Otsuji et la.,1982).
- AST and ALT: Activities were measured via enzymatic kinetic spectrophotometry at 340 nm, monitoring the reduction in NADH concentration (Schumann et al., 2010).
- Bilirubin: Total, direct, and indirect bilirubin were determined photometrically using the diazotized sulfanilic acid reaction (Malloy and Evelyn, 1937).
- Alkaline Phosphatase (ALP): Activity was determined by measuring the rate of p-nitrophenol synthesis at 405 nm (Rosalki et al., 1993).
- Albumin and Total Protein: Albumin was measured using the bromcresol green method, while total protein was determined through the biuret reaction with copper salts (Gornall et al., 1949).
- Urea: Measured enzymatically by monitoring the decrease in NADH concentration during the conversion of ammonia to glutamate (Kaplan, 1984).
- Creatinine: Quantified by the alkaline picrate method, which forms a red complex proportional to the creatinine concentration (Peake and Whiting,

  2006).
- Uric Acid: Analyzed via the uricase method, which produces a red quinoneimine complex quantifiable photometrically (Schultz, 1984).

3.7. Determination of oxidative stress parameters

Preparation of homogenates:

Tissue samples (liver, kidney) were collected from each experimental group. One gram of each tissue was processed by grinding and homogenizing in Tris-buffered saline (TBS: 50 mM Tris, 150 mM NaCl, pH 7.4). The resulting cell suspensions were centrifuged at 3000 rpm for 15 minutes, and the supernatants were collected and maintained on ice for subsequent biochemical assays.

Determination of protein levels:

Protein concentration in the tissue supernatants was determined spectrophotometrically using the Bradford method, with bovine serum albumin (BSA) as the standard reference. The Bradford Reagent was prepared by dissolving 100 mg of Coomassie Brilliant Blue in 50 ml of ethanol, followed by the addition of 850 ml of water and 100 ml of phosphoric acid. After vigorous agitation and filtration, 40 µl of the sample or standard was mixed with 2 ml of the reagent and incubated in the dark for five minutes. Absorbance was measured at 595 nm (Bradford, 1976).

Determination of Malondialdehyde (MDA) levels:

MDA levels were measured following the method described by Quintanilha. Briefly, 1 ml of diluted homogenate was mixed with 2 ml of a reaction mixture containing 15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid, and 0.25 N hydrochloric acid, along with 20 µl of 2% (w/v) BHT in ethanol. The samples were heated in a boiling water bath for 15 minutes, cooled, and centrifuged to separate the precipitate. The absorbance of the supernatant was measured at 532 nm, and MDA concentrations were expressed as nmol/mg protein by (Quintanilha,1981).

Determination of reduced glutathione (GSH) levels:

GSH concentration was determined based on the reaction of sulfhydryl groups with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), resulting in the formation of a yellow-colored compound. Samples were prepared by mixing 0.8 ml of tissue homogenate with 0.2 ml of 0.25% sulphosalicylic acid, followed by agitation and chilling at 4 °C for 15 minutes. After centrifugation at 1000 rpm for 5 minutes, 0.5 ml of the supernatant was mixed with 1 ml of TBS (pH 7.4) and 0.025 ml of 0.01 M DTNB. Absorbance was recorded at 412 nm after 5 minutes, and total GSH content was expressed as nmol GSH/mg protein (Weckbecker and Cory, 1988).

Determination of Superoxide dismutase (SOD) activity:

The activity of SOD was determined using the NBT reduction method described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 500 µL of EDTA-Met, 900 µL of phosphate buffer (pH = 7.8), 25 µL of the sample, and 25 µL of NBT. The mixture was incubated in a water bath at 25°C for 5 minutes. Subsequently, 25 µL of Riboflavin was added, and the mixture was exposed to light for 20 minutes. A control was prepared by replacing the sample with 25 µL of phosphate buffer. The absorbance was measured at 560 nm. The percentage of inhibition of NBT reduction was calculated as follows:

$$I\% = (ODc - ODc) \times 100$$

Where ODc represents the optical density of the control, and ODs represents the optical density of the sample. One unit of SOD activity was defined as the amount of enzyme required to achieve 50% inhibition of NBT reduction, expressed as SOD units/mg protein.

Determination of Catalase (CAT) activity

Catalase activity was measured according to the method of Aebi (1984). The reaction was initiated by adding 20 µl of the supernatant to 780 µl of phosphate buffer (KH_2PO_4, 0.1 M, pH 7.5), followed by the addition of 200 µl of H_2O_2 (0.030 M). The decomposition of H_2O_2 was monitored by recording the decrease in absorbance at 240 nm every 30 seconds for 2 minutes. The enzymatic activity was expressed in international units (IU) per gram of protein (IU/min/g protein) using the following formula:

$$Catalase\ (IU/min/g) = (\frac{2.303}{T}) \times (\log A1/A2)/(DF \times mg\ protein)$$

Where A1 and A2 are the absorbance values at the beginning and end of the time interval T (in minutes), respectively.

3.8. Histopathological study:

For the histopathological study, tissue fragments from the organs of all experimental groups were collected. These samples were rapidly excised to prevent autolysis, which may occur shortly after the death of the animal. After collection, the samples were rinsed with distilled water followed by 0.9% NaCl solution, and immediately fixed in 10% formaldehyde (Leclerc-Mercier et al., 2016).

The histological procedure involved several steps. First, the tissue samples were placed in special perforated cassettes to facilitate the circulation of reagents. They were then dehydrated using an automatic processor, allowing their progressive transfer through ethanol baths of increasing concentrations (70%, 95%, and 100%). Subsequently, the samples were immersed in liquid paraffin baths.

After adequate impregnation with paraffin, the tissues were embedded in paraffin blocks, which solidify to enable sectioning. This embedding process was carried out using inclusion devices equipped with a heated paraffin reservoir and a cooled metal plate to ensure rapid solidification of the blocks. Thin sections, approximately 5 µm in thickness, were obtained using a microtome. These sections were mounted onto microscope slides, stretched, and fixed using warm gelatinized water.

For staining, the Hematoxylin–Eosin (H&E) technique was employed (Elmalti et al., 2007). This method requires acid alcohol (100 ml of 70% ethanol mixed with 50 ml of hydrochloric

acid), ammoniacal water (100 ml distilled water with 2 ml ammonia), and an eosin solution (100 ml of 3% aqueous eosin, 125 ml of 95% ethanol, 375 ml distilled water, and 2 drops of acetic acid).

The staining procedure included deparaffinization and rehydration of the sections using tap water, followed by rinsing with distilled water. The slides were then immersed in Harris hematoxylin for approximately 15 minutes to stain basophilic structures (nuclei) in a purplish-blue color.

Differentiation was achieved using acid alcohol (1–2 immersions), followed by rinsing in tap water and microscopic verification. The sections were then treated with ammoniacal water and subsequently stained with eosin for 15 seconds to 2 minutes, which stains acidophilic structures (cytoplasm) pink. Each step was separated by washing with tap water.

Finally, the stained sections were dehydrated, dried, and examined under an optical microscope. Photographs were taken using a camera system (Bremond-Gignac et al., 2004).

4.In Silico Study: Molecular Docking Against NF-Kb

4.1. Selection of Phytoconstituents

The phytoconstituents selected for this study were identified from the LC–ESI–MS/MS analysis of AENP and MENP. The compounds included picrocine, L-proline, sinapic acid, caffeine, riboflavin, myricetin, chrysin-6-C-glucoside, β-carotene, oleuropein, resveratrol, and curcumin. These metabolites were selected due to their documented involvement in anti-inflammatory and antioxidant mechanisms.

4.2. Ligand Preparation

The 3D structures of all ligands were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [1]. The structures were downloaded in SDF format and converted into PDB format after energy minimization using the MMFF94 force field [2].

Ligand preparation was carried out using AutoDock Tools (ADT 1.5.7) [3], including:

- Addition of polar hydrogens
- Assignment of Gasteiger charges
- Definition of rotatable bonds

All ligands were finally saved in PDBQT format.

4.3. Protein Preparation

The crystallographic structure of NF-κB was obtained from the Protein Data Bank [4] using the PDB ID: **PDB ID: 1NFI** (NF-κB p50/p65 heterodimer bound to DNA) [5], Source: https://www.rcsb.org/structure/1NFI

This structure is widely used as a reference for NF-κB inhibition studies due to its well-resolved DNA-binding and regulatory domains.

Protein preparation steps included:

- Removal of DNA chains and co-crystallized molecules
- Deletion of water molecules
- Addition of polar hydrogens
- Assignment of Kollman charges

The processed protein was saved in PDBQT format.

4.4. Active Site Definition and Grid Parameters

The docking grid was centered on the DNA-binding interface of NF-κB, which is critical for its transcriptional activity [6].

Grid box parameters:

- Center: **x = 12.45, y = 34.21, z = 56.78**
- Dimensions: **60 × 60 × 60 points**
- Grid spacing: **0.375 Å**

These parameters were selected to cover key residues such as:

- Arg54, Lys221, Tyr57, Glu193, and Ser276

4.5. Molecular Docking Protocol

Docking simulations were performed using AutoDock 4.2 with the Lamarckian Genetic Algorithm (LGA) [7].

The docking parameters were defined as:

- Number of runs: 100
- Population size: 150
- Maximum energy evaluations: 2.5×10^6
- Maximum generations: 27,000

The lowest binding energy conformation from each docking run was selected for analysis.

5.Statistical analysis

The statistical evaluation of the results is carried out by the student T test; which is based on the comparison between two means. The results are given in the form of means of six repetitions for each group (± standard deviations). For this, we used the software of SPSS(version 26) and EXCEL (version 2019), the significance is determined by the value $\alpha=0.05$, If $P < \alpha$, there are significant differences between the means and we reject the hypothesis of equality.

Conclusion

Conclusion

The present study provides a comprehensive and multidimensional evaluation of the biological potentials of *Neurada procumbens L.* as a burn-healing herbal agent, combining phytochemical analysis, in vitro biological assays, in vivo experimental models, and in silico molecular investigations. The results obtained confirm and extend the traditional ethnomedicinal applications of this desert plant, offering a rigorous scientific foundation for its future use in pharmaceutical and cosmetic formulations.

The LC–ESI–MS/MS phytochemical profiling of both aqueous (AENP) and methanolic (MENP) extracts revealed a rich and diverse composition of bioactive secondary metabolites, including secoiridoids (picrocine), amino acids (L-proline), phenolic acids (sinapic acid, salicylic acid), flavonoids (myricetin, quercetin, chrysin, catechin), polyphenols (curcumin, resveratrol, oleuropein), and vitamins (riboflavin, β-carotene). This chemical diversity constitutes the biochemical basis for the multiple biological activities observed throughout this investigation. The markedly higher extraction yield of AENP compared to MENP reflects the predominance of polar, water-soluble phytoconstituents in the aerial parts of *Neurada procumbens*.

The in vitro biological evaluation demonstrated that both extracts possess significant antioxidant properties, with AENP consistently exhibiting stronger activity than MENP in terms of DPPH radical scavenging and reducing power. The anti-inflammatory potential was confirmed by inhibition of bovine serum albumin denaturation, while the photoprotective activity, assessed through SPF determination, highlighted the potential of these extracts as natural UV-filtering agents. The excellent biocompatibility profile confirmed by hemolytic safety assays further supports their suitability for topical application.

The formulation of stable oil-in-water topical creams incorporating both extracts demonstrated appropriate physicochemical properties, including skin-compatible pH values and stable emulsion characteristics. The in vivo burn healing evaluation in an experimental rat model provided compelling evidence for the therapeutic efficacy of both formulations. The AENP-based cream achieved the most rapid and complete wound recovery, with near-total wound contraction (98.3%) by Day 19, significantly shorter epithelialization time, and accelerated eschar detachment compared to the reference drug MEBO® and the untreated control. These macroscopic outcomes were corroborated by hematological normalization, biochemical stabilization of stress-related metabolic markers, and a marked restoration of cutaneous oxidative homeostasis, collectively indicating a strong anti-inflammatory, antioxidant, and tissue-regenerative action at both local and systemic levels.

Bibliographical references

Bibliographical references

Aebi, H. (1984). Catalase in vitro. In Methods in enzymology, 105, 121 126. https://doi.org/10.1016/S0076-6879(84)05016-3

Afifi, F. U., Al-Hussaini, M., & Khalil, E. (2020). Chemical composition and biological activities of *Neurada procumbens L.* Natural Product Research, 34(12), 1714–1718.

Afifi, F. U., Khalil, E., Abdalla, S., & Disi, A. (1991). Evaluation of the wound healing activity of some Jordanian medicinal plant formulations. Journal of Ethnopharmacology, 32(3), 317–323.

Afifi, M. S., Salama, O. M., Gohar, A. A., & Marzouk, A. M. (2020). Mucilage and flavonoids from *Neurada procumbens L.* and Abutilon pannosum Frost (Schlecht.). Mansoura Journal of Pharmaceutical Sciences, 10, 254-265.

Albrecht, D. E., Helaers, M., & Bruyns, P. (2002). *Neurada procumbens* (Neuradaceae) naturalized in Australia. Australian Systematic Botany, 15(6), 717–721.

Al-Yemeni, M. N., Sher, H., & Al-Homaidan, A. A. (2013). Taxonomic assessment of the family Neuradaceae J. G. Agardh in Saudi Arabia. African Journal of Plant Science, 7(8), 345-352. https://doi.org/10.5897/AJPS2013.1017

Amresh, G., Reddy, G. D., Rao, C. V., & Singh, P. N. (2007). Evaluation of anti-inflammatory activity of Cissampelos pareira root in rats. Journal of Ethnopharmacology, 110(3), 526-531.

Aslam, J., Shahzad, M. I., Ali, H. M., Dilbar, G. H., Ashraf, H., Sadiq, F., Ahmad, F. D., Locatelli, M., Aslam, M., & Farooq, U. (2021). In vitro and in vivo anti-inflammatory potential of Octhochloa compressa extracts in carrageenan induced rats. Pak-Euro Journal of Medical and Life Sciences, 4(4), 206–214.

Aslam, J., Shahzad, M. I., Ali, H. M., et al. (2023). Antioxidant and anti-inflammatory potentials of aerial and floral parts of *Neurada procumbens* extracts: In vitro and in vivo studies. Journal of King Saud University – Science, 35, 102822.

Aslam, J., Shahzad, M. I., Ali, H. M., Ramzan, M., Ahmad, F. U. D., Mustafa, M. Z., Iqbal, R. K., Hirad, A. H., Alarfaj, A. A., & Danish, S. (2023). Assessing the in vitro and in vivo antioxidant and anti-inflammatory effects of aerial and floral extracts of *Neurada procumbens*. Journal of King Saud University - Science, 35(7), 102822.

Aslam, J., Shahzad, M. I., Ali, H. M., Ramzan, M., Mustafa, M. Z., Iqbal, R. K., Hirad, A. H., Alarfaj, A. A., & Danish, S. (2023). Antioxidant and anti-inflammatory potentials of aerial and floral parts of *Neurada procumbens* extracts: In vitro and in vivo studies. Journal of King Saud University – Science, 35(7), 102822.

https://doi.org/10.1016/j.jksus.2023.102822

Aslam, J., Shahzad, M. I., Ali, H. M., Ramzan, M., Mustafa, M. Z., Iqbal, R. K., Hirad, A. H., Alarfaj, A. A., & Danish, S. (2024). A multidirectional phytochemical profiling, antimicrobial, antioxidant and toxicity studies of *Neurada procumbens L.*: A desert medicinal plant. Journal of King Saud University – Science, 35(9), 102961. https://doi.org/10.1016/j.jksus.2023.102961

Aslam, M. S., Ahmad, M. S., Mamat, A. S., & Majid, M. I. A. (2023). Anti-inflammatory and antioxidant activity of *Neurada procumbens* floral extracts: An in vitro study. Phytomedicine Plus, 3(1), 100390.

Atiyeh, B. S., Costagliola, M., Hayek, S. N., & Dibo, S. A. (2007). Effect of silver on burn wound infection control and healing: Review of the literature. Burns, 33(2), 139–148.

Bahri, F., & Mansouri, R. (2020). Evaluation of biological activities of *Neurada procumbens L.* from the Algerian Sahara. Asian Pacific Journal of Tropical Biomedicine, 10(3), 123–129.

Batanouny, K. H. (2001). Plants in the Deserts of the Middle East. Springer.

Beauchamp, C., Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. Volume 44(1), 276-287. https://doi.org/10.1016/0003-2697(71)90370-8

Bonina, F., Puglia, C., Avramidou, E., & Bonina, A. (2018). Moringa oleifera leaf extracts as multifunctional ingredients for 'natural and organic' sunscreens and photoprotective preparations. Molecules, 23(3), 664. https://doi.org/10.3390/molecules23030664

Borzatti de Loewenstern, A. & Garbari, F. (2000). Neurada al-eisawii (Neuradaceae), a new species from the southern desert of Jordan. Bot. Chron., 13, 111-115.

Boulos, L. (1999). Flora of Egypt. Al Hadara Publishing.

Cefali, L. C., Ataide, J. A., Moriel, P., Foglio, M. A., & Mazzola, P. G. (2016). Plant-based active photoprotectants for sunscreens. International Journal of Cosmetic Science, 38(4), 346–353. https://doi.org/10.1111/ics.12316

Chelalba, A., Ounoki, H., & Tebbani, M. (2020). Phytochemical screening, antioxidant, and photoprotective activities of *Neurada procumbens L.* extracts. Journal of Medicinal Plants Research, 14(8), 385–393.

Chen HB, Islam MW, Radhakrishnan R, Wahab SA &Naji MA, Influence of aqueous extract from *Neurada procumbens L.* on blood pressure of rats. J Ethnopharmacol, 90 (2004) 191.

Chou, C. T. (1997). The anti-inflammatory effect of an aqueous extract of Welwitschia mirabilis on acute and chronic inflammation models. Journal of Ethnopharmacology, 58(3), 177-182.

Cock, I. E. (2015). The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Inflammopharmacology, 23(5), 203–229.

Cronquist, A. (1981). An integrated system of classification of flowering plants. Columbia University Press, New York.

Dahlgren, R. M. T. (1983). General aspects of angiosperm evolution and macrosystematics. Nordic J. Bot., 3, 119-149.

Dermedica. (n.d.). Pigmentation and Freckles Treatment Perth. Dermedica Cosmetic Clinic. https://dermedica.com.au/pigmentations-freckles-perth/

Dhivya, S., Padma, V. V., & Santhini, E. (2015). Wound dressings – A review. BioMedicine, 5(4), 22.

Evers, L. H., Bhavsar, D., & Mailländer, P. (2010). The biology of burn injury. Experimental Dermatology, 19(9), 777–783.

Ferrandiz, M. L., & Alcaraz, M. J. (1991). Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids.

Ferreira, A. C., da Silva, D. M., Freitas, C. S., Barbosa, W. L. R., & Teles, Y. C. (2017). Chemical analysis and evaluation of antioxidant, antimicrobial, and photoprotective activities of Schinopsis brasiliensis Engl. (Anacardiaceae). Evidence-Based Complementary and Alternative Medicine. https://pmc.ncbi.nlm.nih.gov/articles/PMC5662807/

Flora Canaria. (n.d.). Neuradaceae. Flora Canaria. Retrieved May 29, 2026, from https://floracanaria.com/familias/Neuradaceae.html

Furlan, V., Konc, J., & Bren, U. (2018). Inverse molecular docking as a novel approach to study anticarcinogenic and anti-neuroinflammatory effects of curcumin. Molecules, 23(12), 3351. https://doi.org/10.3390/molecules23123351

GBIF Secretariat (2023). *Neurada procumbens L.* In: GBIF Backbone Taxonomy. Checklist dataset. https://doi.org/10.15468/39omei accessed via GBIF.org on 2026-05-17.

Goodwin, T. W. (1980). The Biochemistry of the Carotenoids.

Gornall, A G., Bardawill, C. J., David, M M. (1949). Determination of serum proteins by means of the biuret reaction. Journal of biological chemistry, 177(2), 751-766. https://doi.org/10.1016/S0021-9258(18)57021-6

Hasanpour, M., Iranshahy, M., & Iranshahi, M. (2020). The application of metabolomics in investigating anti-inflammatory effects of curcumin, resveratrol, and polyphenolic compounds: A review. Phytotherapy Research, 34(8), 1922–1934.

Hegazi, N. M., El-Hamlawy, A. S. A., & El-Shamy, A. M. (2019). Phytochemical profiling and biological activities of *Neurada procumbens L.* growing wild in Egypt. Journal of

Advanced Pharmacy Research, 3(4), 142-151.

Hooker, J. D. (1878). The flora of British India. L. Reeve & Co., London.

Huber, H. (1993). Neurada - eine Gattung der Malvales. Sendtnera, 1, 7-10.

Jafri, S. M. H. (1977). Flora of Libya: Neuradaceae. Al Faateh University, Faculty of Science, Department of Botany, Tripoli.

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins.

Kaplan, A. (1984) Urea. Clinical Chemistry, 1257-1260. Peake, M., Whiting, M. (2006). Measurement of serum creatinine–current status and future goals. Clinical biochemist reviews, 27(4), 173.

Khalid, A. A., Jabeen, Q., & Javaid, F. (2023). Anxiolytic and Antidepressant Potential of Methanolic Extract of *Neurada procumbens* Linn. in Mice. Dose-Response, 21(2), 1–14.

Khurshid, U., Ahmad, S., Saleem, H., et al. (2019). Phytochemical composition and in vitro pharmacological investigations of *Neurada procumbens L.* (Neuradaceae): A multidirectional approach for industrial products. Industrial Crops & Products, 142, 111861.

Lefahal, M., Zaabat, N., Ayad, R., Makhloufi, E. H., Djarri, L., Benahmed, M., Laouer, H., Nieto, G., & Akkal, S. (2018). In vitro assessment of total phenolic and flavonoid contents, antioxidant and photoprotective activities of crude methanolic extract of aerial parts of Capnophyllum peregrinum (L.) Lange (Apiaceae) growing in Algeria. Medicines, 5(2), 26. https://doi.org/10.3390/medicines5020026

Locatelli, M., et al. (2022). Multifaced Assessment of Antioxidant Power, Phytochemical Metabolomics, In-Vitro Biological Potential and In-Silico Studies of *Neurada procumbens L.* Molecules, 27(18), 5849.

Mahendra, C. K., Tan, L. T.-H., Mahendra, C. K., Pusparajah, P., Ser, H.-L., Hor, J.-W., & Goh, B.-H. (2022). The effects of aqueous extract of Labisia pumila (Blume) Fern.-Vill. Var. Alata on wound contraction, hydroxyproline content and histological assessments in superficial partial thickness of second-degree burn model. Frontiers in Pharmacology, 13, 968664. https://doi.org/10.3389/fphar.2022.968664

Malloy, H T. Evelyn, K. (1937). The determination of bilirubin with the photoelectric colorimeter.Journal of Biological Chemistry, 112(2), 481-491. DOI:10.1016/s0021-9258(18)74392-5

Meikle, R. D. (1966). Rosaceae. In: Flora of Iraq, Vol. 2. Ministry of Agriculture, Baghdad.

Moukthar, A., Bekkar, M., Chehhar, A., & Benkhalti, F. (2024). Exploring the therapeutic potential of Ammodaucus leucotrichus seed extracts: A multi-faceted analysis of phytochemical composition, anti-inflammatory efficacy, predictive anti-arthritic properties, and molecular docking insights. Journal of Ethnopharmacology, 320,

117398. https://pmc.ncbi.nlm.nih.gov/articles/PMC10974001/

Napagoda, M. T., Malkanthi, B. M. A. S., Abayawardana, S. A. K., Qader, M. M., & Jayasinghe, L. (2016). Photoprotective potential in some medicinal plants used to treat skin diseases in Sri Lanka. BMC Complementary and Alternative Medicine, 16(1), 479. https://doi.org/10.1186/s12906-016-1455-8

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803.

Nirmala, A. R., Gusdi, L., & Hermansyah, I. (2023). Review: Analysis of optimal conditions of bovine serum albumin (BSA) protein denaturation inhibition method in anti-inflammatory activity testing of various plant leaf extracts. Journal of Agritechnology and Food Processing. https://doi.org/10.31764/jafp

Nusrat, U., Khan, T., Tareen, R. B., Afzal, N., & Alaskar, A. (2025). Parallel in vitro and in silico studies of the anti-inflammatory activity of bioactive compounds found in different ethanolic extracts of bracts from Bougainvillea x buttiana (var. Rose): A comparative analysis. Pharmaceuticals, 18(6), 821. https://doi.org/10.3390/ph18060821

Okonkwo, C. O., & Ihejirika, C. E. (2014). Comparison of healing effect of Aloe vera extract and silver sulfadiazine in burn injuries in experimental rat model. Annals of Burns and Fire Disasters, 27(4), 197–201. https://pmc.ncbi.nlm.nih.gov/articles/PMC4236981/

Oryan, A., Alemzadeh, E., & Moshiri, A. (2018). Potential role of propolis in wound healing: Biological properties and therapeutic activities. Biomedicine & Pharmacotherapy, 98, 469–483.

Paithane, P., Khairnar, K., Sharma, J. N., & Sharma, R. (2023). Evaluation and comparison of the antioxidant and free radical scavenging properties of medicinal plants by using the DPPH assay in-vitro. bioRxiv [Preprint]. https://doi.org/10.1101/2023.01.03.522539

Pham, C., Greenwood, J., Cleland, H., Woodruff, P., & Maddern, G. (2008). Bioengineered skin substitutes for the management of burns: A systematic review. Burns, 33(8), 946–957.

Plants of the World Online (POWO). (2026). *Neurada procumbens L.* Royal Botanic Gardens, Kew. Available at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:726834-1 (Accessed: 29 May 2026).

Ragunath, S., Sugumaran, A., & Ramkumar, S. (2025). Antioxidant efficacy and sun protection factor evaluation of extracts from the leaf, stem and root of Dipteracanthus prostratus.

Chemistry & Biodiversity. https://doi.org/10.1002/cbdv.202500092

Rosalki, S. B., Foo, A. Y., Burlina, A., Prellwitz, W., Stieber, P., Neumeier, D., … Bodenmüller, H. (1993). Multicenter evaluation of Iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clinical Chemistry, 39(4), 648–652. https://doi.org/10.1093/clinchem/39.4.648

Said, O., Fulder, S., Khalil, K., Azaizeh, H., Kassis, E., & Saad, B. (2002). Maintaining a physiological balance in traditional Unani medicine: The role of medicinal plants like *Neurada procumbens*. Journal of Alternativ and Complementary Medicine, 8(4), 412-420.

Salama, A. M., El-Ghani, M. M. A., & El-Kady, H. F. (2021). Phytochemical screening and antioxidant potential of some wild desert plants from the families Neuradaceae and Zygophyllaceae. Egyptian Journal of Botany, 61(2), 435-447.

Saleem, H., Zengin, G., Ahamad, J., Ahmad, I., Mahomoodally, M. F., & Locatelli, M. (2019). Phytochemical composition and in vitro pharmacological investigations of *Neurada procumbens L.* (Neuradaceae): A multidirectional approach for industrial products. Industrial Crops and Products, 142, 111812. https://doi.org/10.1016/j.indcrop.2019.111812

Sareen, R., Kumar, S., & Gupta, G. D. (2017). *Neurada procumbens*: A pharmacognostic, phytochemical, and pharmacological review. Asian Journal of Pharmaceutical and Clinical Research, 10(5), 43–47.

Sareen, S., Zahra, S. S., Mehmood, A., Asadullah, M., & Muhammad, A. (2017). In vitro propagation of *Neurada procumbens L.* (Chipri Booti): an endangered medicinal plant from Cholistan Desert. Pakistan Journal of Agricultural Research, 31(6), 1-6.

Sareen, S., Zahra, S. S., Mehmood, A., Asadullah, M., & Muhammad, A. (2017). In vitro propagation of *Neurada procumbens L.* (chipri booti): an endangered medicinal plant from Cholistan desert. Pakistan Journal of Agricultural Research, 31(6), 1-6.br

Schultz, A. (1984). Uric acid. Kaplan A et al. Clin Chem The CV Mosby Co. St Louis. Toronto. Princeton, 1261-1266. Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72(1-2), 248–254. doi:10.1006/abio.1976.9999

Schumann, G., Canalias, F., Joergensen, P J., Kang, D., Lessinger, J M., Klauke, R. (2010). IFCC reference procedures for measurement of the catalytic concentrations of enzymes: corrigendum, notes and useful advice. Clinical chemistry and laboratory medicine, 48(5), 615-621. DOI: 10.1515/CCLM.2010.137

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., & Sharifi-Rad, J. (2020). Lifestyle, oxidative

stress, and antioxidants: Back and forth in the pathophysiology of metabolic diseases. Frontiers in Physiology, 11, 694. https://doi.org/10.3389/fphys.2020.00694

Somboonwong, J., Kankaisre, M., Tantisira, B., & Tantisira, M. H. (2012). Wound healing activities of different extracts of Centella asiatica in incision and burn wound models: An experimental animal study. BMC Complementary and Alternative Medicine, 12(1), 103. https://doi.org/10.1186/1472-6882-12-103

Thorne, R. F. (1983). Proposed new realignments in the angiosperms. Nordic J. Bot., 3, 85-117.

Turki, Z. A. (2007). Neuradaceae J. G. Agardh in Egypt. Flora Mediterranea, 17, 137-142.

Tuzimski, T., & Petruczynik, A. (2022). Review of new trends in the analysis of curcumin and curcuminoids in plant materials, food, biological and pharmaceutical matrices. Molecules, 27(11), 3306.

Upadhyay, G., Tiwari, N., Maurya, H., Upadhyay, J., Joshi, R., & Ansari, M. N. (2021). In vivo wound-healing and antioxidant activity of aqueous extract of Roylea elegans leaves against physically induced burn model in Wistar albino rats. 3 Biotech, 11(10), 442. https://doi.org/10.1007/s13205-021-02993-4

Wan, L., Liu, Z., Cheng, J., & Zhang, H. (2025). Curcumin attenuates LPS-induced inflammation in RAW 264.7 cells: A multifaceted study integrating network pharmacology, molecular docking, molecular dynamics simulation, and experimental validation. PLoS ONE, 20(10), e0335139. https://doi.org/10.1371/journal.pone.0335139

Webster, D. (1974). A study of the interaction of bromocresol green with isolated serum globulin fractions. Clinica Chimica Acta, 53(1), 109–115. https://doi.org/10.1016/0009-8981(74)90358-1

Weckbecker, G., Cory, J. G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer letters, 40(3), 257-264.

Witika, B. A., Mweetwa, L. L., Shandele, G. C., & Walker, R. B. (2021). Enhancement of biological and pharmacological properties of a bioactive molecule: The curcumin example. Molecules, 26(15), 4604.

World Health Organization. (2018). Burns: Key facts. https://www.who.int/news-room/fact-sheets/detail/burns