NATURAL PRODUCTS: *Research Reviews*

— *Volume 3* —

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The Editor

Dr. Vijay Kumar Gupta, Ph.D., FLS, London (born 1953-) Ex. Chief Scientist, CSIR- Indian Institute of Integrative Medicine, Jammu, India. He did his M.Sc. (1975) and Ph.D. (1979) in Zoology both from University of Jammu, Jammu-India. His research capabilities are substantiated by his excellent work on histopathology, ecology and reproductive biology of fishes, turtles, birds and mammals, which has already got recognition in India and abroad.

Dr. Gupta has to his credit more than 150 scientific publications and review articles which have appeared in internationally recognized Indian and foreign journals. Founder fellow, life member and office bearer of many national societies, academies and associations. He has successfully completed a number of research/consultancy projects funded by government, private and multinational agencies. His current areas of interest are histopathology, toxicology, pre-clinical safety pharmacology, reproductive efficacy studies of laboratory animals and biodiversity.

He is the Series Editor of the recently published multi-volume set of books, **"Comprehensive Bioactive Natural Products (Vols. 1-8)"**, published by M/S Studium Press, LLC, USA. He is also Editor-in-Chief of the books, **"Utilisation and Management of Medicinal Plants (Vols. 1-3)", "Medicinal Plants: Phytochemistry, Pharmacology and Therapeutics (Vols. 1-4)", "Traditional and Folk Herbal Medicine (Vols. 1-3)", "Natural Products: Research Reviews (Vols. 1-4)", "Bioactive Phytochemicals: Perspectives for Modern Medicine (Vols. 1-3)", "Perspectives in Animal Ecology and Reproduction (Vols. 1-10)"** and **"Animal Diversity, Natural History & Conservation (Vols. 1-5)"**. The Editor-in-chief of the American Biographical Institute, USA, has appointed him as *Consulting Editor* of *The Contemporary Who's Who*. Dr. Gupta also appointed as Nominee for the *Committee for the Purpose of Control and Supervision of Experiments on Animals* (CPCSEA, Govt. of India). The *Linnaean Society of London, U.K.* has awarded fellowship to him in November 2009 in recognition of his contribution towards the cultivation of knowledge in Science of Natural History. Recently, Modern Scientific Press, USA has nominated Dr. Gupta as the Editor of the *International Journal of Traditional and Natural Medicine*.

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Foreword

Natural products, particularly from microbial and plant origin have been used for centuries by people as a source of an unlimited range of chemically diverse structures. The interest for new, natural compounds is renewed as a result of the growing resistance of microorganisms to commonly used drugs. Thus, nosocomial infections have become great public health problems. The selection of plants is a first research step based on certain criteria such as ethnobotanical and ethnopharmacological approaches, including information about the traditional use of plants to treat specific diseases. Also, many studies explore chemotaxonomic approach, which relay on the study of plants from the same family or genus of a species from which active compounds have been already isolated.

The volume 3 of **Natural Products: Research Reviews** edited by Dr. V. K. Gupta includes twenty two chapters devoted to the identification and isolation of bioactive components with antimicrobial and antioxidant properties and also chapters related to the potential application of natural products in cardiovascular, cancer and neurodegenerative disorders. Drawing upon the collective wisdom of traditional medical systems and modern science, this book identifies the revered herbs from around the world that can comprehensively inhibit pathologic conditions. It discusses the challenges of using traditional medicines for diseases where access to modern medicine is limited, and the research areas needed to improve quality, safety, and efficacy for enhancing healthcare. It is a pleasure for me to recommend this volume to all researchers in the field of natural products and I believe that the book will attract the attention of great number of readers.

Prof. Nina Ivanovska

Preface

Natural products have been investigated and utilized to alleviate disease since early human history. Medicine among the ancient civilizations was a blend of religion and science. Nature produces a large number of chemical compounds whose structure and properties are of great interest and fascination for the majority. These are organic compounds formed by living system and are emerging from the cocoon of organic chemistry. The term 'natural products' is applied to materials derived from plants, microorganisms, invertebrates and vertebrates, which are the fine biochemical factories for biosynthesis of these metabolites and over 40 per cent of medicines, have origin from these compounds. Natural products are the cornerstone of modern therapeutics. Building on the discovery of morphine from poppy seeds in the 1800s and penicillin from mold in the 1920s, natural products have been a key source of new therapeutics. Natural products are currently used to treat cancer, high levels of cholesterol, fungal infections, bacterial infections, and parasitic diseases. As of 1990, nearly 80 per cent of all drugs in use were natural products or natural product derivatives. In developing countries, the search for biologically active compounds obtainable from locally available plants is also important to reduce public health.

Despite the great successes already achieved, we have barely begun to tap the potential of our molecular diversity and just 5-15 per cent of the 2,50,000 species of higher terrestrial plants in existence have been pharmacologically investigated in systematic fashion. The percentage of insects, marine organisms and microbes investigated is far lower still. In the case of microbes, it is estimated that 95 to 99 per cent of existing species are currently not even known, never mind analyzed. Even with all the challenges facing drug discovery, natural products isolated from natural sources can be predicted to remain an essential component in the research for new medicines.

It was with this approach that this volume "***Natural Products: Research Review Vol. 3***" has been compiled. The contributions made by some of the worlds leading scientists in the field should prove of wide interest to the readers. This is a timely and interesting collection of review articles covering a very wide range of biologically active natural products. The book is produced to the high standard that we have come to expect of this volume. It contains a good subject index. There is a great deal of information in these chapters which should be of interest not just to the natural product chemist but also to those in the pharmaceutical and agrochemical industries who are interested in potential leads for structure activity studies. The book can be recommended to all who are interested in biologically active natural products.

V.K. Gupta

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Essential Oils and their Use in Skin Wound Healing

L.F.S. Gushiken[1], A.L. Rozza[1], A.J. Vieira[1], F.P. Beserra[2] and C.H. Pellizzon[1*]

ABSTRACT

Skin is a barrier that protects organisms against physical, chemical and biological agents as well as playing a role in thermoregulation and impermeability. If the skin integrity is disturbed, the process of wound healing is initiated, which involves molecular and cellular mechanisms in the inflammatory, proliferative and remodeling phases. Medicinal plants and other natural products are widely used in wound healing, especially in the pharmaceutical industry. Essential oils are secondary metabolites of aromatic plants, which have produced promising results in preclinical studies of wound healing. This manuscript reviews the use of essential oils in the skin wound healing process, with the aim of identifying the main mechanisms from which essential oils operate and promoting novel studies for plant-derived natural products that can be successfully used in the treatment of skin wounds.

Keywords: Essential oil, Inflammation, Medicinal plants, Natural products, Re-epithelization, Skin wound healing, Terpenes.

Introduction

Skin is a barrier that covers the body surface and is formed by the epidermis, which is an epithelial barrier of ectodermal origin, and the dermis, which is the connective portion of mesoderm. Separating these two layers is a network of

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extracellular matrix proteins known as the basal membrane. The epidermis has many layers comprised primarily of keratinocytes, melanocytes, Langerhans cells and Merkel cells. The dermis is formed by the reticular and papillary layers, with the predominance of extracellular matrix compounds such as collagen I, elastin (which promotes tissue tensile strength) and fibroblasts, which play a role in the organization of the dermis matrix (Evans *et al.*, 2013). Skin is the largest organ of human body and represents approximately 16 per cent of the total body weight. Skins serves as a vital barrier for vertebrates to protect the organism in many environments, including exposure to antigens, solvents, ultraviolet light, detergents, microorganisms, toxins, nanoparticles and local physical variations (Guo and Dipietro, 2010; Benedetto *et al.*, 2012). Moreover, the skin is impermeable to water and electrolytes because of the lipid covering produced by epidermal cells (Benedetto *et al.*, 2012).

Skin integrity is lost when an injury occurs that results in the opening or rupture of the skin, which is commonly referred to as an ulcer or lesion (Fikru *et al.*, 2012). This type of open wound can be caused by trauma, extreme heat or cold, radiation, corrosive materials or circulatory problems (Morin *et al.*, 2012). In response to this damage, the organism initiates the wound healing process, which is complex and involves a sequence of interdependent and overlapping stages: the inflammatory or exudative phase, the proliferative or reconstruction phase and the remodeling or epithelization phase. All of these stages depend on cellular, molecular and physiological processes for the structural and physiological restoration of the injured area (Martins *et al.*, 2006; Araújo, *et al.*, 2010; Agha *et al.*, 2011).

The inflammatory phase is the initial process of response after the formation of a lesion (0 to 3 days) in which the priority is the restoration of local hemostasis, with subsequent clearance of dead tissue and prevention of infection. At the edges of the wound, a hemostatic clot forms upon fibrin deposit to block blood efflux and promote platelet retention, which activates local vascular alterations (Gurtner, 2007; Profyris *et al.*, 2011). In this stage, the dilation of vessels occurs near the injured area due to the release of vasoactive amines (histamine and serotonin). There is also an increase in vessel permeability, which facilitates leukocyte migration to the injured region – a primary hallmark of the inflammatory process. Upon tissue destruction, keratinocytes in the region release interleukin 1 (IL-1) and tumor necrosis factor (TNF-α), which are essential cytokines to the inflammatory process that recruit and activate local leukocytes. Platelets release vesicles containing growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming-β growth factor (TGF-β) and chemokines (Kondo and Ishida, 2010). During the first five days, neutrophils invade the lesion area, which contains abundant levels of fibrin, and promote the elimination of bacteria and degradation of dead tissue via phagocytosis and secretion of proteases. Three days after the lesion first forms, macrophages migrate to the region as part of the increasing inflammatory response, which is maintained by the presence of cellular debris and release of growth factors (FGF – fibroblast growth factor, EGF, TGF-β and PDGF), chemokines and pro-inflammatory cytokines (IL-1 and IL-6). These mitigating factors coordinate events that will manifest into the proliferative phase of wound healing (Profyris *et al.*, 2011).

The proliferative phase is characterized by the deposition of granulation tissue, which contains macrophages, fibroblasts and newly formed vessels. This phase lasts between 4 and 21 days and can be observed by the decrease in clot size (formed upon initial skin breakage). The primary mediators during this phase are PDGF, TGF-β1 and FGF, which determine fibroblast proliferation in the basal layer and initiate the synthesis of collagen III and other extracellular matrix proteins such as fibronectin and proteoglycans. ECM protein provides support for endothelial cell proliferation, which is mediated by release of VEGF, and aids in the formation of new vessels and organization of the granulation tissue (Kondo and Ishida, 2010; Profyris *et al.*, 2011). This re-epithelization process is important to establishment of the tissue integrity. The keratinocytes at the wound edges migrate towards the surface because of the cellular dedifferentiation and reorganization of adhesion molecules. The loss of adhesion with other keratinocytes and the basal membrane are critical in promoting wound closure (Profyris *et al.*, 2011).

The remodeling phase is an important process to avoid aberrations left by scarring, such as keloids. Granulation tissue formation is interrupted by cellular apoptosis, and collagen III is degraded by metalloproteinases released from macrophages, fibroblasts and endothelial cells to form collagen I, which has higher tensile strength and can better support new epithelium growth (Kondo and Ishida, 2010). Furthermore, the tissue receives a contractile response from myofibroblast activity because these cells connect to the collagen at multiple points, which facilitates the retraction of the wound area (Profyris *et al.*, 2011).

Due to the possibility of physical disability, psychosocial consequences or even death, skin injuries require significant attention in public health and would benefit from increased investments from public resources (Bayat *et al.*, 2003; Guo and Dipietro, 2010). An important aspect of wound healing by secondary intention is the decrease of the cellular response time to reconstruction of the tegmental system. For this, identifying elements that promote wound healing, such as the use of biological agents, is one of the oldest medicinal practices of humanity. Plants have been used for the treatment of diseases for thousands of years,and the utilization of natural products for the treatment of disorders has increased over the past few decades (Fabricant and Farnsworth, 2001; Veiga Jr *et al.*, 2005; Gurib-Fakim, 2006; Ganeshkumar *et al.*, 2012).

Essential oils are volatile, lipid-soluble secondary metabolites of aromatic plants that can be synthetized from all parts of the plant and are characterized by their strong odor. In nature, essential oils have important antibacterial, antifungal and antiviral activities, as well as insecticide activity, which can repel herbivores and attract insects for the dispersion of pollen and seeds. In addition to their use in the perfume industry for fragrances, essential oils have medicinal properties, including their ability to function as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic agents. They are comprised of a mixture of components and characterized by the major compounds that, generally, determine the biological properties of the essential oil. These compounds include two groups: terpenes and aromatic compounds. Terpenes are molecules that are formed by various combinations of isoprene $(C_5H_8)_n$ and are classified according to the number of carbons, including monoterpenes (10 carbons), sesquiterpenes (15 carbons) and diterpenes (20 carbons) among others. Monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$) are the primary terpenes present in essential oils and are formed by two and three isoprene units, respectively. The aromatic compounds are molecules derived from phenylpropane and exist at a lower percentage in essential oils compared to terpenes (Bakkali *et al.*, 2008).

This review aims to highlight the role of essential oils in skin wound healing with the intention of promoting novel and extensive studies to identify improved treatments.

Materials and Methods

Searches for this review were performed using primary literature accessible from PubMed through April 2014. The words "essential oil", "wound healing" and "medicinal plants" were used in PubMed and Scopus searches. Moreover, we selected original articles written in English from the previous ten years without Ayurveda research. The results are expressed in Table 21.1 and discussed below.

Results and Discussion

Table 21.1 shows all of the plant species and their respective primary compounds used in skin wound healing studies described in the literature. Twenty-five species of plants belonging to eight different families were studied, and the parts of the plants used to obtain the essential oils were the leaves, trunk, cones and aerial parts.

Skin wound healing is a highly complex process that starts the moment a lesion manifests, which determines the partial or complete tissue repair and involves a series of interdependent and overlapping stages classified in three phases: inflammatory, proliferative and remodeling (Martins *et al.*, 2006).

Selection of the proper experimental model for skin wound healing studies is essential to analyze the biological mechanisms involved in the process. In the analyzed manuscripts, the most commonly implemented experimental models to quantitatively and qualitatively evaluate the healing process were excision, linear incision and wound dead space. The excision model is employed to determine lesion retraction in wound healing by secondary intention. A surgical "punch" is used to induce a lesion on the back of the animal, which allows for the measurement of daily wound retraction as well as molecular and histopathological analysis to determine the healing mechanisms during the different phases. The second model mentioned is performed by introducing paravertebral linear incisions equidistant of spinal column, which are subsequently sutured, to evaluate the primary closure. Incisions are analyzed with a densitometer to evaluate the tissue tensile strength. The wound dead space model is used to analyze the granulation tissue synthesis during the proliferative phase of wound healing. A cellulose sponge pellet is implanted under the skin in the back of animals and, after apre-determined number of days corresponding to the different phases of the healing process, the granulation tissue formed in the pellet is removed and weighed. Therefore, the skin excision and linear incision models are used to directly evaluate the three phases of skin wound healing, but the wound dead space model has emerged as an alternative to analyze the parameters of granulation tissue formation during the proliferative stage.

Table 20.1: Essential Oils Used in Skin Wound Healing Treatment

Plant Species	*Family*	*Part of the Plant*	*Majority Compound*	*Model*	*Mechanism of Action*	*Reference*
Cinnamomum zeylanicum	Lauraceae	leaves	Eugenol/trans-cinnamaldehyde	Excision	Antimicrobial/ re-epithelization	Ghosh *et al.*, 2013
Croton adamantinus	Euphorbiaceae	leaves	methyl-eugenol/ 1,8-cineole	Excision/wound dead space	Antimicrobial/ re-epithelization/collagen deposition/increase in fibroblast population	Ximenes *et al.*, 2013
Croton zehntneri	Euphorbiaceae	leaves	Trans-anethole	Excision	Increase in fibroblasts population and collagen fibers	Cavalcanti *et al.*, 2012
Eucalyptus globulus	Myrtaceae	n.i.	1,8-cineole (eucalyptol)	Excision	Anti-inflammatory/ antimicrobial	Sugumar *et al.*, 2014
Origanum majorana	Lamiaceae	n.i	Carvacrol	Linear incision/excision	Antimicrobial/anti-inflammatory/increase in connective tissue formation	Suntar *et al.*, 2011
Origanum minutiflorum	Lamiaceae	n.i	Carvacrol	Linear incision/excision	Antimicrobial/anti-inflammatory/increase in connective tissue formation	Suntar *et al.*, 2011
Salvia triloba	Lamiaceae	n.i	1,8-cineole	Linear incision/ excision	Antimicrobial/anti-inflammatory/increase in connective tissue formation	Suntar *et al.*, 2011
Juniperus virginiana	Cupressaceae	trunk	cedrol	Linear incision/excision	Ineffective	Tumen *et al.*, 2013

Contd...

Table 20.1–*Contd...*

Plant Species	*Family*	*Part of the Plant*	*Majority Compound*	*Model*	*Mechanism of Action*	*Reference*
Juniperus occidentalis	Cupressaceae	trunk	cedrol	Linear incision/excision	Re-epithelization/anti-inflammatory/increase in tensile strength/high hydroxyproline content	Tumen *et al.*, 2013
Juniperus ashei	Cupressaceae	trunk	thujopsene	Linear incision/excision	Ineffective	Tumen *et al.*, 2013
Lavandula x allardii	Lamiaceae	n.i	1,8-cineole	Excision	Ineffective	Lusby *et al.*, 2006
Lippia sidoides	Verbenaceae	leaves	thymol	Excision	Antimicrobial/amplify inflammatory response	Oliveira *et al.*, 2014
Murraya koenigii	Rutaceae	leaves	β-Caryophyllene	Excision	Ineffective	Nagappan *et al.*, 2012
Abies cilicica subsp. *Cilicica*	Pinaceae	Coniferous cones	n.i	Linear incision/excision	Increase in tensile strength/inhibition of capillary permeability	Tumen *et al.*, 2011
Abies nordmanniana subsp. equi-trojani	Pinaceae	Coniferous cones	n.i	Linear incision/excision	Re-epithelization	Tumen *et al.*, 2011
Abies nordmanniana subsp. *bornmulleriana*	Pinaceae	Coniferous cones	n.i	Linear incision/excision	Increase in tensile strength/re-epithelization	Tumen *et al.*, 2011
Abies nordmanniana subsp. *nordmanniana*	Pinaceae	Coniferous cones	n.i	Linear incision/excision	Re-epithelization	Tumen *et al.*, 2011
Cedrus libani	Pinaceae	Coniferous cones	n.i	Linear incision/excision	Increase in tensile strength/inhibition of capillary permeability/re-epithelization	Tumen *et al.*, 2011
Picea orientalis	Pinaceae	Coniferous cones	n.i	Linear incision/excision	Ineffective	Tumen *et al.*, 2011
Pinus brutia	Pinaceae	Cones	n.i	Linear incision/excision	Ineffective	Süntar *et al.*, 2012

Contd...

Table 20.1–*Contd...*

Plant Species	*Family*	*Part of the Plant*	*Majority Compound*	*Model*	*Mechanism of Action*	*Reference*
Pinus halepensis	Pinaceae	Cones	α-Pinene	Linear incision/excision	Increase in tensile strength/ re-epithelization/ anti-hyaluronidase activity/high hydroxyproline content	Süntar *et al.*, 2012
Pinus nigra	Pinaceae	Cones	α-Pinene	Linear incision/excision	Inhibition of capillary permeability/ re-epithelization	Süntar *et al.*, 2012
Pinus pinea	Pinaceae	Cones	Limonene and β-pinene	Linear incision/excision	Increase in tensile strength/inhibition of capillary permeability/ re-epithelization/ anti-hyaluronidase activity/high hydroxyproline content	Süntar *et al.*, 2012
Pinus sylvestris	Pinaceae	Cones	α-Pinene	Linear incision/excision	Ineffective	Süntar *et al.*, 2012
Rosmarinus officinalis	Lamiaceae	Aerial parts	n.i	Excision	Increase in granulation tissue weight/increase in wound contraction	Abu-Al-Basal, M.A., 2010

n.i: not identified.

Essential oils are substances comprised of volatile and fat-soluble secondary metabolites, which can be extracted from different parts of the plant. Secondary metabolites present in essential oils can have biological activity on the healing mechanism by decreasing the retraction time and helping in wound healing. Many essential oils with reported healing activities have terpenes as their primary compounds, which are hydrocarbons whose chemical formula is derived from one or more isoprene molecules $(C_5H_8)_n$. Regarding the plant species identified in our search, the majority have either monoterpenes (a molecule derived from two isoprenes with chemical formula $C_{10}H_{16}$) or sesquiterpenes (a hydrocarbon with formula $C_{15}H_{24}$) as their primary compound, which suggests a possible relationship between this group of molecules and the mechanisms involved in skin wound healing. However, some species such as *Juniperus virginiana*, *Juniperus ashei* (Tumen *et al.*, 2013), *Lavandula allardii* (Lusby *et al.*, 2006), *Murraya koenigii* (Nagappan *et al.*, 2012) and *Pinus sylvestris* (Süntar *et al.*, 2011), which have the terpenes cedrol, thujopsene, 1,8-cineole, β-caryophyllene, and α-pinene, respectively, as their major compounds, had no healing activity. Therefore, it is possible that the terpene concentrations in the studied samples are a determining factor for its ability to promote healing. There is also the possibility of a synergistic effect between the different compounds in essential oils instead of only one major compound or isolated substances acting on the healing pathways. Furthermore, skin lesions treated with essential oils from *Cinnamomum zeylanicum* (Ghosh *et al.*, 2013) and *Croton adamantinus* (Ximenes *et al.*, 2013), which have the phenylpropanoids eugenol and methyl-eugenol, respectively, as their primary compounds, demonstrated wound healing activity. Phenylpropanoids are molecules consisting of phenolic compounds and derived from the aromatic amino acids phenylalanine and tyrosine; these organic compounds have been shown to exert antimicrobial and antioxidant activities and may help in the healing process (Korkina, 2007). Cavalcanti *et al.*(2012) showed that the animals treated with *Croton zehntneri* and trans-anethole essential oils presented similar results in wound healing, demonstrating a likely relationship between the primary compound present and the overall essential oil.

Skin wound healing involves molecular, cellular and physiological mechanisms that play a role in either partial or complete tissue repair (Agha *et al.*, 2011). The process may be divided into three interdependent and overlapping phases: the inflammatory, proliferative and remodeling phases. The inflammatory phase begins with the coagulation cascade to maintain hemostasis and promote of local inflammation; the proliferative phase is characterized by the deposition of granulation tissue (macrophages, fibroblasts and newly formed vessels), lesion contraction and re-epithelization; and the remodeling phase is marked by the replacement of collagen III for collagen I, which provides increased tensile strength of the tissue, complete epithelization and synthesis of the new dermis connective tissue (Rodero and Khosrotehrani, 2010; Kondo and Ishida, 2010). During the inflammatory phase after the lesion initially forms, the injured keratinocytes release IL-1 and TNF-α, which are cytokines responsible for the recruitment of neutrophils and macrophages to promote bacterial degradation and dead tissue clearance. The antimicrobial activity observed in essential oils from *Cinnamomum zeylanicum* (Ghosh *et al.*, 2013), *Croton adamantinus*

(Ximenes *et al.*, 2013), *Eucalyptus globulus* (Sugumar *et al.*, 2014), *Origanum majorana*, *Origanum minutiflorum*, *Salvia triloba* (Suntar *et al.*, 2011) and *Lippia sidoides* (Oliveira *et al.*, 2014) play an important role in wound healing, preventing infections and the consequent delay in the process. Inflammatory mediators such as IL-1 and TNF-α play a vital function in tissue reconstruction (Midwood *et al.*, 2004). Inflammation could cause destruction of the extracellular matrix, cell senescence and delays in wound healing (Oliveira *et al.*, 2014). Ahanger *et al.* (2010) showed that a decrease in TNF-α synthesis accelerated skin wound repair. Therefore, the anti-inflammatory activity of the essential oils from *Eucalyptus globulus* (Sugumar *et al.*, 2014), *Origanum majorana*, *Origanum minutiflorum*, *Salvia triloba* (Suntar *et al.*, 2011) and *Juniperus occidentalis* (Tumen *et al.*, 2013) enabled a decrease in the local edema and prevented loss of tissue function normally observed upon exacerbated inflammation. However, in cases of an attenuated inflammatory response after injury, there is a risk of infection risk due to the delayed recovery of damaged skin. There are studies demonstrating the positive modulatory effects of TNF-α and other inflammatory cytokines in healing as this pro-inflammatory cytokine synthesis influences the expression of other growth factors. Therefore, a decrease or complete abolishment of this cytokine synthesis process could impair the mechanisms involved in the proliferative and remodeling phases (Lim *et al.*, 2006). Oliveira *et al.*(2014) showed the beneficial effects amplifying inflammation in skin wounds treated with the essential oil from *Lippia sidoides* at 6 per cent and 12 per cent concentrations, suggesting the use of this oil as an alternative treatment for skin wound.

After initiation of fibrin clot synthesis and the inflammatory process, chemical mediators and growth factors such as PDGF, EGF, TGF-β1, FGF and VEGF determine specific alterations of the proliferative phase, including re-epithelization and granulation tissue synthesis (Kondo and Ishida, 2010). In the analyzed papers, the essential oils of some species from the*Pinaceae* family described by Süntar *et al.* (2011) and Tumen *et al.*(2011), as well as the *Cinnamomum zeylanicum* (Ghosh *et al.*, 2013), *Croton adamantinus* (Ximenes *et al.*, 2013) and *Juniperus occidentalis* (Tumen *et al.*, 2013) plant species, showed increased re-epithelization of the injured tissue, which occurs by the restructuring of the cell adhesion molecules near the wound edge keratinocytes. The loss of adhesion with other keratinocytes and basement membrane proteins allows for migration of these cells to aid in closing the lesion (Tziotzios *et al.*, 2012). Granulation tissue, which is comprised of macrophages, fibroblasts and newly formed vessels, plays an important role in skin wound healing because it initiates the synthesis of new extracellular matrix compounds (collagen III, proteoglycans and fibronectina) and provides support for the new tegument development (Kondo and Ishida, 2010; Tziotzios *et al.*, 2012). Subsequently, fibroblasts differentiate into myofibroblast, which are involved in the wound edge contractile response and consequently decrease the size of the injured area (Rodero and Khosrotehrani, 2010). Thus, substances that induced an increase in the number of cells in the granulation tissue could result in decreasing the healing time by promoting extracellular matrix compound synthesis, deposition of collagen fibers and retraction of the injured surface by myofibroblasts, as demonstrated by the essential oils from *Origanum majorana* (Süntar *et al.*, 2011), *Croton adamantinus* (Ximenes *et al.*, 2013) and *Croton zehntneri* (Cavalcanti *et al.*, 2012). Migration and cell proliferation are important during the proliferative phase of wound healing. Hyaluronic acid is one of the primary extracellular matrix constituents and helps in proliferation and cellular migration 6-mechanisms (Stern, 2004). Thus, compounds that have anti-hyaluronidase activity may contribute to wound healing process, such as the *Pinus pinea* and *Pinus halepensis* (Süntar *et al.*, 2012) and the *Juniperus occidentalis* essential oil (Tumen *et al.*, 2013).

During the remodeling phase, collagen III synthetized by fibroblasts is degraded by metalloproteinase and replaced with collagen I, which has greater tensile strength and can support the new epithelium (Kondo and Ishida, 2010). Collagen is the major component of the extracellular matrix, which contains the amino acid hydroxyproline–this component is critical in providing the tensile strength of collagen as well as aiding in the remodeling of injured tissue (Ganeshkumar *et al.*, 2012). Thus, hydroxyproline is used as biochemical marker for measuring collagen levels in a sample (Kumar *et al.*, 2006). Some essential oils from the *Pinaceae* family species (Süntar *et al.*, 2012) and *Juniperus occidentalis* (Tumen *et al.*, 2013) showed high hydroxyproline content, indicating higher collagen levels in the tissue samples. Increased levels of collagen, especially collagen I, correlate to improved tensile strength in the linear incision models as shown by essential oils of aforementioned species.

Conclusions and Future Directions

Most of the essential oils from the species studied in this review presented promising results in skin wound healing, by influencing the mechanisms involved in the inflammatory, proliferative and remodeling phases. Furthermore, among the studied species, the primary compounds classes identified were monoterpenes and sesquiterpenes, suggesting a possible healing effect of either these isolated compounds or a synergic effect among the secondary metabolites within the essential oils. These observations reinforce the importance of ethnopharmacological studies in skin wound healing and encourage the development of future studies in this field.

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