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„HEALING IS A MATTER OF TIME, BUT IT IS
SOMETIMES ALSO A MATTER OF OPPORTUNITY“

- **HIPPOKRATES** -

ABSTRACT

Wound healing is a complex biological process, in which many problems, e.g. infection and inflammation can occur. Especially infections with multi-resistant bacteria are concerning problems. Because wounds are common health problems and the treatment options which are available by now are not satisfying and cannot deal with these issues, save and efficient alternatives are desperately required. The aim of this diploma thesis is to give a résumé of different commonly used essential oils with relevance or potential in wound treatment. Therefore, several trials, which were obtained with the aid of database like PubMed, Medline, SciFinder, Scopus, Biosis and Toxline are summarized. Furthermore, studies which were found by cross-checking the references are included.

ZUSAMMENFASSUNG

Wundheilung ist ein komplexer biologischer Prozess, bei dem zahlreiche Probleme wie zum Beispiel Infektionen oder Entzündungen auftreten können. Besonders Infektionen mit multiresistenten Bakterien stellen ernstzunehmende Probleme dar. Weil Wunden zu den häufigsten Gesundheitsproblemen zählen und es nach wie vor noch keine zufriedenstellenden Therapieoptionen gibt, die mit diesen Problemen fertig werden können, werden sichere und effektive Alternativen dringend benötigt. Das Ziel dieser Diplomarbeit ist es eine Zusammenfassung von verschiedenen häufig verwendeten ätherischen Ölen mit Relevanz oder Potential in der Wundbehandlung zu geben. Dazu wurden Studien, die mithilfe von Datenbanken wie PubMed, Medline, SciFinder, Scopus, Biosis und Toxline gefunden wurden, zusammengefasst. Weiters wurden Studien, die mithilfe der Literaturverzeichnisse gefunden wurden, inkludiert.

ABBREVIATIONS

A.	<i>Acinetobacter</i>
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
B.	<i>Bacillus</i>
bFGF	basic fibroblast growth factor
C.	<i>Candida</i>
COX	cyclooxygenase
DPPH	2,2-diphenyl-1-picrylhydrazyl
EGF	epidermal growth factor
ERK	extracellular signal-regulated kinase
ESBL	extended spectrum β-lactamase
fMLP	N-formyl-methionyl-leucyl-phenylalanine
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GM-CSF	granulocyte macrophage-colony stimulating factor
GRAS	generally recognized as safe
HbA _{1c}	hemoglobin A_{1c}
HSV-1	<i>Herpes simplex virus type 1</i>
IL	interleukin
ILGF	insulin like growth factor
iNOS	inducible nitric oxide synthase
IUCN	International Union for Conservation of Nature and Natural Resources
K.	<i>Klebsiella</i>
LPS	lipopolysaccharide
MAP	mitogen-activated protein kinase

MIC	m inimum i nhibitory c oncentration
MRSA	m ethicillin- r esistant <i>Staphylococcus aureus</i>
NF- κ B	n uclear f actor k appa-light-chain-enhancer of activated b -cells
NO	n itric o xide
OR	o lfactory r eceptor
PDGF	p latelet- d erived g rowth f actor
PG	p rosta g landin
<i>P.</i>	<i>Pseudomonas</i>
RAS	r ecurrent a phthous s tomatitis
SDS-PAGE	s odium d odecyl s ulfate- p olyacrylamide g el electrophoresis
<i>St.</i>	<i>Staphylococcus</i>
<i>Str.</i>	<i>Streptococcus</i>
TCM	T raditional C hinese M edicine
TGF	t ransforming g rowth f actor
TNF	t umor n ecrosis f actor
TTO	t ea t ree o il
VEGF	v ascular e ndothelial g rowth f actor

TABLE OF CONTENTS

<u>A) WOUND HEALING PROCESS</u>	13
<u>B) PROBLEMS ASSOCIATED WITH WOUND HEALING</u>	16
<u>C) ROLE OF ESSENTIAL OILS IN WOUND TREATMENT</u>	20
<u>D) ESSENTIAL OILS WITH RELEVANCE IN WOUND TREATMENT</u>	
Blumea balsamifera essential oil	21
Calamus essential oil	23
Chamomile essential oil	25
Cinnamon essential oil	29
Eucalyptus essential oil	32
Lavender essential oil	36
Myrtle essential oil	39
Oregano essential oil	43
Rosemary essential oil	46
Sandalwood oil	48
Tea tree oil	50
Thyme essential oil	54
<u>E) REFERENCES</u>	57

WOUND HEALING PROCESS

A wound is a condition which comes along with the loss of skin integrity. Wound degrees as well as wound causes are extremely various. Common causes for wounds are, e.g. abrasion, bite, burn, contusion, laceration, surgical excision or trauma. Because numerous cell types, cytokines, growth factors and other mediators are involved in the process of wound healing, it is a very complex biological cascade. Wound healing can be divided into four phases - hemostasis, inflammation, proliferation and remodeling and scar maturation. Because these four phases overlap each other, the separating lines are blurred.

HEMOSTASIS

Hemostasis is the first phase of the wound healing process and starts immediately after the injury was happened. If vessels are destroyed it comes to vasoconstriction to reduce the blood loss. Thrombocyte adhesion and thrombocyte aggregation results in a clot, which is mainly comprised of platelets and a meshwork of fibrin, fibronectin and von Willebrand factor. The purpose of the fibrin platelet clot is to seal the wound temporary. When the platelets degranulate, they release several growth factors like epidermal growth factor (EGF), insulin like growth factor (ILGF), platelet-derived growth factor (PDGF) and transforming growth factor (TGF). The growth factors activate endothelial cells, fibroblasts and macrophages [1, 2].

INFLAMMATION

Inflammation is the second phase of the wound healing process and can be divided into early inflammation phase and late inflammation phase. The early inflammation phase starts within one to two days after the injury and is characterized by the infiltration of the wound with neutrophil granulocytes. After infiltration, the neutrophil granulocytes adhere to endothelium (margination) and move through the vascular wall (diapedesis). The main function of the neutrophil granulocytes is to engulf pathogens and kill them with the aid of enzymes and free radicals. So, bacterial contamination and therefore infection risk can be reduced to a minimum. The late inflammation phase starts within two to three days after the injury. In this stage of wound healing mainly macrophages are involved. On the one hand, they act as phagocytic cells and on the other hand they act as producer of growth factors, which are responsible for proliferation. Furthermore, they produce collagenase, which consists of proteolytic enzymes with high importance for wound debridement [1].

PROLIFERATION

Proliferation is the third phase of the wound healing process and starts approximately on the third day after injury. Depending on the wound it lasts up to four weeks. At the beginning of this phase fibroblasts migrate to the wound area, where they proliferate and produce proteins of the extracellular matrix like collagen and elastin. Further parts of the proliferation phase are neovascularization and epithelialization, which is induced by EGF and other growth factors. Neovascularization is induced by growth factors like vascular endothelial growth factor (VEGF) and leads to re-supply of oxygen and nutrients [1].

REMODELING AND SCAR MATURATION

The fourth phase of the wound healing process starts about seven days after wounding and lasts up to several weeks, depending on different factors and on depth, severity, size and type of the scar. Metalloproteinases are enzymes which play an important role during remodeling and scar maturation. They are produced as precursor by fibroblasts, macrophages and neutrophils and can be activated by proteases like plasmin. The main task of metalloproteinases is the degradation of collagen. During the process of scar maturation collagen fibers are tightened and so the tensile strength of the wound increases. Nevertheless, the strength of the collagen fibers can only achieve 80% of the strength of unwounded skin. A wound is classified as a healed wound when the affected tissues have been completely repaired and therefore possess normal anatomical function and structure [1].

PROBLEMS ASSOCIATED WITH WOUND HEALING

AGE

During the aging process the skin underlies numerous changes. Some of these changes are shown to significantly affect the four different phases of the wound healing process. By comparing the wound healing of cutaneous incision wounds in young, middle-old and old mice, it can be shown that age delays the inflammation response as well as re-epithelialization [3]. Furthermore, aged mice showed a significant lower phagocytic capacity of macrophages compared to young mice [4]. Because young mice, which were treated with rabbit antimouse macrophage serum, showed similar delay of wound healing as older mice, Cohen *et al.* came to the conclusion that the age-related reduction of macrophage activity may be the main reason for delayed wound healing of elderly people [5]. Reversely local injections of macrophages received from young mice can promote the wound healing process in aged, wounded skin *in vivo* [6]. Furthermore, macrophages of older individuals produce less VEGF than macrophages of younger individuals. Therefore, it additionally came to a delay in angiogenesis [7]. Besides the fact that the production of growth factors declines with age, the responsiveness of human fibroblasts to growth factors is indirectly proportional to the age [8]. Further factors, which contribute to the dependency of wound healing on the age, are for example delayed epithelialization [9] and comorbidities which are more commonly among elder people such as diabetes mellitus. All the above-mentioned factors are well studied and affect the time, which is necessary to completely heal a wound, in a negative manner. In conclusion, it can be said that the age is directly proportional to wound healing time.

DIABETES

Worldwide there are over 400 million adult diabetics [10]. A lot of them are concerned about severe complications including chronic, non-healing diabetic foot ulcers, which are the principal reasons for amputations, especially lower leg amputations. Furthermore, diabetics have a higher risk to develop postoperative complications. Humphers *et al.* came to the conclusion that the higher the hemoglobin A_{1c} (HbA_{1c}) level, the higher the risk for postoperative complications like infections after foot and ankle surgery [11]. There is no factor which can be defined as the only cause for delayed wound healing in patients who suffer from diabetes mellitus. The wound healing process is per se a complex biological process. However, the wound healing process of diabetic wounds is much more complicated, because diabetic patients show several changes in factors, which are involved in wound healing. Immunohistochemistry showed a reduction in expression of several growth factors like PDGF and VEGF in diabetic foot ulcers compared to normal non-diabetic foot skin. This may lead to disturbance of angiogenesis and re-epithelialization during the wound healing process. Immunohistochemistry additionally showed an enhanced production of pro-inflammatory factors like granulocyte macrophage colony-stimulating factor (GM-CSF) which might also be responsible for the delay of wound healing in diabetics [12]. The up-regulation of interleukin-8 (IL-8), IL-17 and IL-20 which are pro-inflammatory molecules, is considered to prolongate inflammation and therefore impair wound healing [13, 14]. The delay of diabetic wound healing is furthermore associated with down-regulation of the expression of heat shock proteins, which are induced by physiological and pathological stress. Because heat shock proteins attract fibroblasts to the wound and therefore play an important role during the wound healing process, the down-regulation of the expression in diabetic foot ulcers affect wound healing in a negative manner [15].

INFECTION

Because human beings are constantly in contact with numerous microorganisms like bacteria, one of the most important function of the skin is the barrier function, which protects us against microbial invasion. When the skin is wounded this protective function is more or less disturbed and the susceptibility for infections increases. Therefore wounds, either acute or chronic, are always associated with an elevated risk for infections. These infections can lead to severe consequences like amputations, sepsis, toxic shock syndrome and even to death. So, one of the main goal of wound treatment is to avoid wound infections and therefore the above-mentioned consequences. Infection is also associated with a delay in wound healing. All chronic wounds are colonized with more or less bacteria. A distinction is drawn between contamination, colonization, local infection and systemic infection. While within contaminated wounds bacteria do not replicate, the bacteria within colonized wounds are able to replicate. Compared to infections colonization is not associated with tissue damage. It is important to distinguish these terms, because contamination and colonization do not delay the wound healing process [16]. 1978 Lookingbill showed that the higher the bacterial count in a wound, the higher the risk that it develops into a chronic non-healing wound [17]. The severity of an infection can not be classified by the bacterial count alone. It also depends on the type of bacteria. Infections with multi-resistant bacteria like **methicillin-resistant *Staphylococcus aureus* (MRSA)** are for example very dangerous, because there is a lack of treatment options. Therefore, new treatment options are desperately required to solve this problem.

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INFLAMMATION

Inflammation is the second phase of the wound healing process and is therefore a normal physiological and also inevitable process. Gallucci *et al.* showed the importance of inflammation on wound healing by comparing the healing time of IL-6-deficient transgenic mice with wild type control animals. It can be shown that the healing time of full-thickness punch biopsies of IL-6-deficient transgenic mice was up to three times higher than the healing time of wild type control animals [18]. Hartmann *et al.* investigated the effect of the wound dressing Acticoat® with the aid of acute full-thickness excision wounds in porcine models. They came to the unexpected conclusion that Acticoat® stimulates inflammation but has even though a beneficial effect on wounds, because it increases the rate of epithelium and blood vessels compared to control group [19]. Although inflammation is essential, resolution of inflammation is absolutely necessary for complete wound healing. Different factors can prolongate the inflammation phase, which is associated with delayed wound healing and chronic non-healing wounds. Therefore, it is absolutely necessary to disrupt the pro-inflammatory cycle to protect tissue from chronic inflammation and prevent the development of acute wounds into chronic non-healing wounds, which are associated with decreased quality of life for the patient on the one hand and with extremely high costs for the health care system on the other hand.

ROLE OF ESSENTIAL OILS IN WOUND TREATMENT

Essential oils are powerful herbal substances, which have numerous, well studied medicinal properties and are therefore commonly used to treat various diseases. The above mentioned medicinal properties include some properties which can affect the wound healing process in a positive manner. There are some essential oils which have a potent antimicrobial activity - especially the antibacterial activity is remarkable. Bacterial infections represent major problems during the wound healing process. Because there is a lack of treatment options for infections with multi-resistant bacterial strains, bacteria are favored targets to discover new wound treatments. Essential oils may have the potential to fill this gap. Furthermore, some essential oils have a potent anti-inflammatory activity which is a quite desirable activity for wound treatments. Although inflammation belongs to the natural wound healing process, severe problems can occur, when this phase is prolonged. Both, the antimicrobial as well as the anti-inflammatory activity of essential oils can reduce the risk for wound associated complications. Although this two activities may be the main activities which are responsible for the positive effect of essential oils on the wound healing process, there are other effects which are supposed to act synergistically. One of these activities is the anti-oxidative activity of several essential oils. Although there are some studies which confirm that anti-oxidant agents can promote the wound healing process *in vivo* [20, 21], the role of the anti-oxidant activity of essential oils on the wound healing process is poorly studied. In conclusion, essential oils possess numerous activities, including some activities which are desirable for wound healing agents. Especially the antimicrobial and the anti-inflammatory activities are remarkable and well studied. Nevertheless, there have to be made more studies, especially *in vivo*, to confirm the beneficial effect of essential oils on the wound healing process.

BLUMEA BALSAMIFERA ESSENTIAL OIL

Blumea balsamifera oil is obtained by steam distillation of the leaves of *B. balsamifera* L., which belongs to the *Asteraceae* family and is commonly used in Traditional Chinese Medicine (TCM). Gas chromatography-mass spectrometry (GC-MS) showed that the main constituent of the essential oil is L-borneol (43.5%), followed by D-camphor (9.5%) [22]. Agar microdilution method and disc diffusion assay exhibited that the essential oil obtained by *B. balsamifera* has a higher potency against pathogenic bacteria and pathogenic fungi compared to different *B. balsamifera* extracts. The minimum inhibitory concentration (MIC) of the essential oil against the two human pathogens *Candida albicans* and *Staphylococcus aureus* is 1.2 mg/mL, respectively [23]. Due to its excellent antibacterial and anti-fungal activities *in vitro*, *B. balsamifera* essential oil might affect wounds in a positive manner. Furthermore, the analgesic and anti-inflammatory activities of the main constituent L-borneol, which are explored *in vivo* [24], may also play an important role on the wound healing process. In addition to several *in vitro* studies which suggest beneficial effects of *B. balsamifera* on wounds, there exist some *in vivo* studies which reassure this property. In one of them 98 mice were anesthetized, wounded and randomly divided into seven equal groups. One group received no treatment and the other groups were treated with pure olive oil, basic fibroblast growth factor (bFGF), cream of Jing Wang Hong or with *B. balsamifera* oil in different concentrations (10%, 20% and undiluted). Within 10 days several parameters were evaluated. The two diluted essential oils increased blood circulation, capillary regeneration, collagen deposition, granular tissue formation, epithelial deposition, wound closure, wound contraction and wound healing rate. Furthermore, the expression of collagen, hydroxyprolin and neuropeptide substance P was elevated and the number of inflammatory cells was significantly reduced. All

these effects positively influence the wound healing process. However, undiluted *B. balsamifera* essential oil was shown to have contrary effects on the wound healing process. Because it leads to reduction of collagen syntheses, skin hardening and skin thickening it rather delays the healing of wounds [22]. Another *in vivo* study investigated the effect of *B. balsamifera* essential oil on the wound healing process of burn wounds. Therefore, deep second degree burn injuries were created in 144 rats which were randomly divided into six equal groups. Group one received no further treatment and served as control group. The other five groups were treated with the vehicle (80% ethanol), with standard burn ointment or *B. balsamifera* essential oil in three different concentrations (100%, 50% and 10%) once daily for 21 consecutive days. *B. balsamifera* essential oil increased the expression of growth factors like bFGF, TGF- β and VEGF. Furthermore, it reduces scabs dropped time, time of re-epithelialization, water content of burn tissue and plasma levels of inflammatory factors like IL-1 and tumor necrosis factor- α (TNF- α). So, it can be concluded that *B. balsamifera* oil might be an effective new burn-treating drug [25]. Unfortunately, there is relatively less known about potential toxic side effects of *B. balsamifera* essential oil. Pang *et al.* examined hepatotoxic effects caused by transdermal absorption of three different concentrations (100%, 50% and 20%) of *B. balsamifera* essential oil on normal rat skin and wounded rat skin. The undiluted essential oil leads to an increase in liver toxicity markers like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin level. Because the diluted essential oils did not show such effects and there were no significant differences in liver histopathology, it is assumed that short term usage of *B. balsamifera* essential oil on normal as well as on wounded skin is safe for the liver [22].

CALAMUS ESSENTIAL OIL

Calamus essential oil is obtained by *Acorus calamus* L., which belongs to the family of *Araceae*. Whereas the essential oil of the leaves is mainly composed of β -asarone and linalool, the main constituents of the essential oil of the rhizome are β -asarone and α -asarone [26]. Gas chromatography (GC) and GC-MS showed that the essential oil obtained by the roots differs significantly from the essential oil obtained by the leaves or rhizome. The principal components of the root oil were β -gurjunene which is also known as calarene, (*Z*)-asarone, aristolene and (*E*)-asarone [27]. *A. calamus* is known for several medicinal properties, e.g. antimicrobial [28, 29], hypolipidemic [30], radioprotecting [31] and spasmolytic [32] activities. Although *A. calamus* is commonly used as wound healing agent in folk medicine, there are only few studies which confirm the scientific evidence of this claim. In an *in vivo* study, full thickness excision wounds (2cm²) were created on the back of 48 male Wistar rats, which were divided into a treated group and a control group. While the treated group received topical ethanolic extract of the rhizomes from *A. calamus*, the control group was treated with phosphate buffered saline. Then granulation tissue was removed and several parameters, which are related to wound healing, were biochemically analyzed, e.g. by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Compared with phosphate buffered saline, the ethanolic extract of the rhizomes from *A. calamus* significantly increased collagen (especially type III collagen), hexosamine and uronic acid. *A. calamus* also increased tensile strength and reduced lipid peroxide levels, which leads to the conclusion that *A. calamus* possesses anti-oxidant activities. Complete healing was achieved after 14 days in the treated group versus 21 days in the control group. Because ethanolic extract of *A.*

calamus showed shorter period of epithelialization and higher rate of wound contraction, Ponrasu *et al.* came to the conclusion that it might be a potent dermal wound healing agent [33]. Another *in vivo* study investigated the wound healing effect of aqueous extract obtained by the roots and rhizome of *A. calamus* on excisional wounds 0.8cm in diameter in rats. Histological analysis of the granulation tissue showed less inflammatory cells and more blood vessels, collagen, hair follicles and fibroblasts in the treated group. So, the aqueous extract of *A. calamus* increased wound healing rate compared to the vehicle group *in vivo*. Because the extract reduced the expression of TNF- α , IL-1 β , IL-6 and inducible nitric oxide synthase (iNOS) *in vitro*, there is also an evidence for an anti-inflammatory activity of *A. calamus* [34]. Jain *et al.* also studied the wound healing property of *A. calamus*, not of the rhizome, but of the leaves. Therefore, excision wounds (500mm²) and incision wounds (6cm) were created in rats. The wounds were daily treated with simple ointment base, povidone-iodine ointment or with an ointment which contains different concentrations (20% or 40%) of ethanolic extract of the dried sword-shaped leaves of *A. calamus*. The extracts lead to a reduction of epithelialization time and to an increased content of hydroxyproline. Furthermore, wound contraction and wound closure was enhanced. Therefore, it has to be supposed that *A. calamus* is a potent agent, which can positively affect the wound healing process [35]. Nevertheless, more studies are required to confirm the results and to investigate the main principle which leads to the above-mentioned effects. Because this main principle is unknown by now and the components of the essential oil differs from the components of the extracts, there is currently no evidence for wound healing properties of the essential oil obtained by *A. calamus*. Additionally it remains unclear if the topical application of calamus essential oil can lead to toxicological side effects due to the main constituent β -asarone.

CHAMOMILE ESSENTIAL OIL

Chamomile essential oil is obtained by steam distillation of the dried flower heads of the plant *Chamomilla recutita* L., also known as *Matricaria recutita* L., which belongs to the *Asteraceae* family and is one of the most commonly used medicinal plants. The main constituents of chamomile essential oil are α -bisabolol, bisabolol oxides, spiroethers and chamazulene which is responsible for the characteristic blue color of the essential oil. Even though chamomile is an extensively used home remedy, there are comparatively less studies which analyzed the relevancy of this famous plant in wound treatment.

Especially the antiphlogistic properties of chamomile essential oil might be beneficial on the wound healing process. Unfortunately, there is only less information about the exact mechanism of action of chamomile essential oil. In contrast to the essential oil the mechanism of action of different chamomile extracts are well studied. Oliveira *et al.* investigated the effect of chamomile extract on oral ulcers in diabetic rats and postulate that chamomile extract inhibits the expression of TNF- α and is able to reduce apoptosis in the epithelium [36]. While an aqueous chamomile extract does not affect the activity or expression of cyclooxygenase-1 (COX-1), it suppresses the expression of COX-2 gene and inhibits the activity of COX-2 enzyme. This leads to a decreased release of pro-inflammatory prostaglandin (PG) E₂. The effects of the aqueous chamomile extract might be due to the main component apigenin and its glucoside [37]. α -Bisabolol, bisabolol oxide A and guaiazulene which are the principal substances of ethanolic chamomile extracts are also strong inhibitors of COX-2 enzyme [38]. Because the above-mentioned components of ethanolic chamomile extracts are also

main constituents of chamomile essential oil, it can be supposed that the essential oil of chamomile may act in a similar way. Nevertheless, there has to be made more studies which confirm this assumption and compare the antiphlogistic property of chamomile essential oil with the antiphlogistic property of different chamomile extract types. The majority of the available studies, which investigated the effect of different preparations of *C. recutita* on the wound healing process, primarily refer to extracts and not to the essential oil.

A short randomized controlled study investigated the effect of *M. chamomilla* extract on 3cm incisional cutaneous wounds in 30 male Wistar rats. The rats were divided into three groups: group one received no treatment, group two was treated with olive oil twice daily and group three was treated with *M. chamomilla* extract dissolved in olive oil twice daily. There was no difference in percentage of wound healing between group one and two. In group three there had been shown a significant faster rate of healing. So Jarrahi *et al.* came to the conclusion that *M. chamomilla* extract possesses a certain wound healing potential and may be beneficial in treating surgical cutaneous wounds [39]. Jarrahi *et al.* also investigated the effect of *M. chamomilla* extract on burn wounds. Therefore, 30 male Wistar rats with second-degree burns, involving twenty percent of the total body surface, were divided into three groups. The control group received no treatment, the vehicle group received topical treatments with olive oil and the treatment group received topical treatments with chamomile extract dissolved in olive oil. Because the results of the wound healing percentage measurements of chamomile oil were superior to olive oil, the study came to the conclusion that chamomile exerts a positive effect on the complex process of burn wound healing [40]. Another study on experimental cutaneous burn injury compared the effect of *Rosmarinus officinalis* extract, *M. recutita* extract and a combination of both. Each extract was topically applied

three times daily in form of a cream base. The rosemary group (48.2%) as well as the chamomile group (56.5%) achieved much better results concerning percentage of wound healing than the control group (9.8%). However, the combination of both - *R. officinalis* extract and *M. recutita* extract (88.5%) - showed the highest efficacy [41].

Martins *et al.* compared the effect of an ointment which contains 10% chamomile extract with commonly used topical steroids like triamcinolone acetonide and clobetasol on experimental tongue ulcers in rats. In the chamomile group, it took 5 days until the created lesions were completely repaired in 100% of mice. Because in the other groups this result was achieved 9 days later, the study was able to show that chamomile extract can speed up the wound healing process compared to topical steroids. The fact that only in the chamomile group no abscess formation was determined, could be explained by the excellent antibacterial property of chamomile [42].

There are also *in vivo* studies in humans which confirm the predominance of chamomile extracts over topical corticoids. Charousaei *et al.* compared the effect of compresses with an aqueous chamomile extract with the effect of hydrocortisone on peristomal skin lesions in 72 colostomy patients. Group one (n = 36) was treated once daily with 1% hydrocortisone ointment and group two (n = 36) was treated twice daily with compresses soaked in aqueous chamomile extract. The lesions in the chamomile group healed more quickly than the lesions in the hydrocortisone group (8.89 days versus 14.53 days; $p = 0.001$). After 15 days, the lesions which were treated with chamomile compresses completely healed in 100% of the patients. The lesions which were treated with hydrocortisone ointment completely healed in just 76% of patients after 21 days [43]. Because topical corticoids can also cause many

non-desired side effects, including systemic adverse reactions, chamomile might be a good alternative treatment option with fewer sides effects. Nevertheless, more studies have to made to confirm this assumption. Because most of the studies, which are available by now, refer to chamomile extracts, the role of chamomile essential oil in wound treatment remains unclear. Especially studies which compare the effect of extracts and essential oils would be of general interest.

CINNAMON ESSENTIAL OIL

Due to its delicate odor and taste the dried and powdered inner bark of *Cinnamomum* is an extensively used spice in the whole world. The two most popular *Cinnamomum* species are *Cinnamomum verum* (*Cinnamomum zeylanici*), also known as Ceylon cinnamon or „true“ cinnamon and *Cinnamomum aromaticum*, also known as cassia cinnamon or Chinese cinnamon. Because cinnamon offers numerous properties, e.g. antibacterial [44, 45], chemopreventive [46], hepatoprotective [47] and tocolytic [48] properties, it has considerable medicinal potential and is commonly used in folk medicine and Ayurveda. Sun *et al.* investigated the mechanism of action of the anti-inflammatory property of cinnamon and found out that orally applied essential oil from the twigs of *C. aromaticum* inhibits carrageenan-induced production of TNF- α , IL-1 β , nitric oxide (NO) and PGE₂ in rats. Furthermore, it significantly reduces the expression of COX-2 and iNOS protein [49]. The antiphlogistic property of cinnamon is not only restricted to the essential oil. The aqueous extract of cinnamon also possesses an inhibiting effect on TNF- α . Hong *et al* found out that orally applied water extract of cinnamon significantly reduces lipopolysaccharide (LPS) induced increase of TNF- α and IL-6 serum levels [50]. There is another *in vivo* study which investigated the effect of orally applied ethanolic extract from the bark of *C. verum* on the wound healing process. Therefore, incision wounds (6cm) were created in Wistar rats which were divided into three groups. Group one received 2% tragacanth suspension and acted as control group and group two and three were treated with the ethanolic extract (250mg/kg and 500mg/kg). After the removal of the sutures on day 10 the wound breaking strength was significantly increased in the two verum groups [51].

Mice with excision wounds (500mm²) were also divided into three groups and were treated equally. It could be shown that cinnamon accelerates eschar dropping and therefore leads to an enhanced epithelialization. In addition, the rate of wound contraction was also enhanced. Furthermore, it could be shown that cinnamon increases the granulation tissue breaking strength in a dead space wound model. Therefore, Kamath *et al.* came to the conclusion that cinnamon positively affects collagenation, wound contraction and epithelialization during wound healing process [51].

In an *in vivo* study, full thickness surgical wounds were created in 24 male Wistar rats which were randomly divided into four equal groups. The first two groups were treated with an ointment containing ethanolic extracts of *C. verum* in different concentrations (1.5% and 3.0%). The third group received no treatment and acted as control group and the fourth group was treated with a placebo ointment. The results showed that ethanolic extracts obtained by *C. verum* positively affected angiogenesis and epithelialization during wound healing process. Furthermore, it achieved a significant reduction of wound area compared to control and placebo. Farahpour *et al.* estimate that the above-mentioned effects are mainly due to eugenol and cinnamaldehyde [52]. Because these two components are also part of the essential oil it has to be assumed that cinnamon essential oil may act in a similar way.

In another study excision wounds (300mm²) were created in Wistar rats. The first group received no treatment, the second group was treated with a neomycin ointment, the third group was treated with pure cinnamon essential oil and the fourth group was treated with a micro emulsion with cinnamon essential oil. Then the time until the wound completely healed was compared - in the control group it took 20 days, in group two and three it took 16 days respectively and in

the fourth group it took only 14 days. On the one hand the study showed that cinnamon essential oil is able to speed up the wound healing process and on the other hand it revealed the respectable importance of the correct formulation [53].

Unfortunately, there is a lack of studies in humans which confirm the healing potency of different cinnamon preparations. For example, there is a randomized, double-blind, placebo-controlled trial which investigated the effects of hydroalcoholic cinnamon extract on perineal pain and healing of episiotomy incision after vaginal birth. 144 postpartum women were treated either with an ointment containing 2% hydroalcoholic cinnamon extract (n = 72) or with a placebo ointment (n = 72) twice daily for ten consecutive days. The reduction of pain intensity after ten to eleven days amounts 43% in the placebo group versus 76% in the intervention group. The use of an ointment which contains 2% hydroalcoholic cinnamon extract was able to significantly lower the perineal pain intensity score and the healing score of episiotomy incision [54].

EUCALYPTUS ESSENTIAL OIL

Eucalyptus oil is obtained by steam distillation of the leaves from *Eucalyptus globulus* which belongs to the *Myrtaceae* family and is native to Australia. GC-MS showed that 1,8-cineole, also referred to as eucalyptol, is the main component of eucalyptus oil [55]. Sugumar *et al.* investigated the antibacterial and wound healing activity of eucalyptus oil nanoemulsion. *St. aureus* completely lost the viability within 15 minutes of treatment with eucalyptus nanoemulsion and showed morphological changes when observed via scanning electron microscopy. So, there is evidence for the bactericidal activity against the clinical pathogen *St. aureus*. To examine the wound healing potency excisional wounds (300mm²) were created in rats. Group number one was non-wounded, group number two was wounded and received no treatment, group number three was wounded and treated with eucalyptus nanoemulsion and group number four was wounded and treated with neomycin ointment. Within 16 days in the third group the wounds healed 100% compared to 94.2% in the control group and neomycin group, respectively. The study also includes a skin irritation test which was performed on 12 male Wistar albino rats. Group one (n = 4) received no treatment, group two (n = 4) was treated with a standard irritant (0.8% aqueous formalin solution) and group three (n = 4) was treated with the eucalyptus oil nanoemulsion. Because there were no pathological changes or skin irritations like edema and erythema noticed in the third group, it is assumed that eucalyptus oil nanoemulsion is non-irritant to the skin [56]. Although the results of the study are quite promising, a comparison with a nanoemulsion which does not contain eucalyptus oil, would be highly interesting. Another study published by Sugumar *et al.* confirmed the activity of eucalyptus oil against *St. aureus*. Therefore, chitosan was compared to chitosan

impregnated with eucalyptus oil nanoemulsion. Agar disc diffusion method and plate count assay showed effective bactericidal activity of chitosan impregnated with 5% eucalyptus oil nanoemulsion against the clinical pathogen *St. aureus*. In comparison, lower concentrations (1% and 3%) were shown to be not effective [57].

Agar diffusion tests showed that non-diluted eucalyptus oil has moderate bactericidal activity against MRSA, vancomycin-resistant *Enterococcus sp.*, multi-resistant *Pseudomonas aeruginosa*, extended spectrum β -lactamase (ESBL) producing *Escherichia coli* and ESBL producing *Klebsiella pneumoniae*. Although eucalyptus oil showed better results than popular antiseptics, e.g. ethanol (70%) and chlorhexidine (0.1%), lemongrass oil and tea tree oil offer higher activities against the above-mentioned pathogens, respectively [58]. The assumption that eucalyptus oil is active against MRSA was reassured by disk diffusion assay and agar dilution method. The inhibition zone was 8 mm and the MIC was 85.6 $\mu\text{g/ml}$. In comparison, the essential oil of *Thymus vulgaris* showed higher activity against MRSA (12 mm inhibition zone and 18.50 $\mu\text{g/ml}$ MIC) [55]. Although the activity of eucalyptus oil against MRSA is well documented *in vitro*, there is a lack of studies which confirm this activity *in vivo*. There is just a case report of a man who suffered from chronic osteomyelitis including MRSA infection due to an open fracture of the lower tibia. After long term, intravenous antibiotic treatments with flucloxacillin and dicloxacillin was not successive, amputation was considered. As a last resort, he was treated with calcium sulphate pellets which were soaked in Polytoxinol® and directly applied into the infected bone area. Polytoxinol® is an antimicrobial preparation including several phytochemicals, e.g. clove, eucalyptus, lemongrass, melaleuca and thyme. After three months, the wound cultures were clear, the wound was completely healed and so his lower limb could be saved [59].

There is not only evidence for the antibacterial activity of eucalyptus essential oil, but also for eucalyptus extract. Al-Saimary *et al.* compared the activity of aqueous eucalyptus extract with the activity of six standard antibiotics on the Gram-negative, aerobic or facultative anaerobic bacteria *P. aeruginosa*. Therefore, growth inhibition zones were analyzed by agar diffusion method and MICs were evaluated by tube dilution method. Because *P. aeruginosa* is the predominant bacterial pathogen associated with burn infections and high doses of aqueous eucalyptus extract (1.000 mcg/ml) have anti-pseudomonal activity, eucalyptus extracts may be beneficial in treating burn wounds, especially in preventing infections of burn wounds [60]. Unfortunately, it remains unclear which components are responsible for the anti-pseudomonal activity of aqueous eucalyptus extract and if eucalyptus essential oil acts in similar way.

Because eucalyptus oil was shown to be highly active against numerous bacterial strains, it has to be assumed, that it might have a beneficial effect on the wound healing process. Especially the use in wound dressings has great potential. Although sodium alginate wound dressings possess wound healing properties, they do not exert antimicrobial properties. Therefore, antimicrobial additives are needed to boost the wound healing activity of sodium alginate wound dressings. Because essential oils might be suitable for this purpose Liakos *et al.* compared the antimicrobial activity of sodium alginate matrix with the antimicrobial activity of nine different essential oils, including eucalyptus oil, dispersed in sodium alginate matrices respectively. The antimicrobial behavior was tested against two microorganisms - *E. coli* as a model for Gram-negative bacteria on the one hand and *C. albicans* as a model for Gram-positive fungi on the other hand. Eucalyptus was not active against *E. coli*, but inhibited the growth of *C. albicans* in intermediate concentrations (50%) and high concentrations (66%). In comparison essential oils of

cinnamon, peppermint and lemongrass achieved higher inhibition zones [61].

Although the positive effect of eucalyptus oil on the wound healing process, is mainly due to its antibacterial effect, eucalyptus oil shows additional properties, e.g. analgesic and anti-inflammatory activities, which can be beneficial concerning wound healing. Acetic acid-induced writhes in mice and hot plate thermal stimulation in rats showed the peripheral and central analgesic effects of eucalyptus oil *in vivo*. Eucalyptus oil inhibits carrageenan- and dextran-induced rat paw edema and carrageenan induced migration of neutrophils into peritoneal cavities in rats. Furthermore, it reduces the cutaneous vascular permeability which was elevated by carrageenan and histamine [62]. However, further trials are warranted to assess the underlying mechanisms of action.

LAVENDER ESSENTIAL OIL

Lavender essential oil is a very popular oil obtained by steam distillation of the flowers of *Lavandula angustifolia*, which belongs to the *Lamiaceae* family. The principal components are monoterpenes - especially linalool and its corresponding ester linalyl acetate [63]. Although the effect of lavender oil on the central nervous system is well documented, there is relatively less known about the effect of lavender on the skin, especially on wounds. Mori *et al.* investigated the molecular mechanism of lavender oil. For this purpose, wounds (10mm in diameter) were created at the back of rats. After wound surgery group, one received no further treatment, group two received a topical treatment with a control solution and group three received a topical treatment with 1% lavender oil, which was dissolved in the control solution. The result of the trial is that lavender oil increases the expression of TGF- β , the proliferation of fibroblasts, the synthesis of collagen type I and collagen type III and the differentiation of fibroblasts to myofibroblasts. Compared to the control group lavender oil also progresses the wound closure. All these factors exert a positive influence on the early phase of the wound healing process [64]. There are further trials which investigated the effect of lavender oil on the expression of growth factors. In a rat model lavender oil leads to a significant increase in EGF expression, which results in a positive effect on re-epithelialization and wound closure. Because the PDGF expression is not influenced by lavender oil it has to be assumed that it is ineffective in dermis renewal [65].

Lavender oil is a popular oil in midwifery, e.g. to reduce anxiety during childbirth [66]. Dale *et al.* showed that bath additives with pure lavender oil reduced post-natal perineal discomfort in comparison with bath additives with synthetic lavender oil or with a lavender

product, which is **generally recognized as safe** (GRAS). Indeed, the results revealed no statistical significance [67]. A trial with 120 primiparous women, who underwent a spontaneous vaginal delivery including episiotomy, compared the standard treatment (povidone-iodine) with lavender oil based on a hip-bath used twice a day. Ten days postpartum the healing in episiotomy was evaluated via six different criteria, e.g. edema, pain level and redness. Concerning the number of infections and the severity of pain there was no difference between the case group and the control group. However, in the lavender oil group there was significantly less inflammation and less redness compared to the povidone-iodine group during the post-natal period [68]. One year later a similar trial with 60 primiparous women was published, which compared the standard treatment (betadine) with lavender oil, also based on a hip-bath used twice a day. Four hours and five days after episiotomy the pain intensity was significantly lower in the lavender oil group. The lower pain intensity results in a significant lower level of analgesics consumption [69]. Morris *et al.* compared the effect of different bath additives on the mood. He found out that lavender baths improve psychological well-being compared to baths with grape seed oil [70]. This may also have a positive effect on the quality of life during the post-natal period.

There is a study which showed evidence of lavender oil in treating recurrent aphthous stomatitis (RAS). In this trial, the case group (n = 58) received a topical treatment with 2% lavender oil in glycerin and the control group (n = 58) such treatment with glycerin alone. In comparison with the control group the patients of the case group showed lower pain level in the first 20 minutes after baseline, less inflammation, smaller size of the ulcer and a far shorter healing time [71].

Because most studies focus on *L. angustifolia* by now, there is less known about the effect of other lavenders e.g. *Lavandula latifolia x allardii* on the wound healing process. In an *in vivo* study with 50 rats there were no significant differences concerning wound size and wound site capillary volume between the group treated with honey and the group treated with *L. latifolia x allardii* honey. So, it can be assumed that *L. latifolia x allardii* has no beneficial effect on the wound healing process. Although further trials are required to confirm this assumption [72].

A study with 34 hemodialysis patients revealed that the topical application of lavender oil significantly reduces the pain intensity during the insertion of dialysis needles compared to placebo. So, the use of lavender oil is a simple and safe method to control pain und elevate the quality of life in hemodialysis patients especially in needle-phobic patients [73]. Especially patients who suffer from wound-associated pain could benefit from the analgesic effect of lavender oil. Nevertheless, further studies have to be made to investigate this presumption.

MYRTLE ESSENTIAL OIL

Myrtle essential oil is obtained by the evergreen, mediterranean, perennial shrub *Myrtus communis* L. which is also known as common myrtle or true myrtle and belongs to the *Myrtaceae* family. The main constituents of the essential oil are linalool, myrtenyl acetate, 1,8-cineole, α -pinene, α -terpineol and geranyl acetate. Especially the main constituents have a strong antimicrobial activity which is known as a desirable property for wound healing agents. Aleksic *et al.* investigated the effect of myrtle essential oil on wound isolates containing *Acinetobacter baumannii*. *A. baumannii* is a Gram-negative bacterium, which is a commonly and highly resistant clinical pathogen. The effect of myrtle essential oil was compared to the effect of myrtle essential oil combined with several antibiotics, e.g. ciprofloxacin and polymyxin B. Myrtle essential oil is highly active against multi drug resistant *A. baumannii in vitro* (MIC 0.25 - 4 μ l/ml). Furthermore, it increases the sensitivity of the pathogen against antibiotics. Due to the synergistic effect of myrtle essential oil to antibiotics, the minimum efficient dose and therefore the dose-dependent adverse effects, e.g. nephrotoxic and neurotoxic side effects of some antibiotics, can be reduced [74].

Al-Saimary *et al.* analyzed wound isolations from 92 burned patients. Out of the 92 burn wounds 78 were infected - this is equivalent to 84.8%. The major bacterial pathogen was found to be *P. aeruginosa*, followed by *St. aureus*. The antibacterial effect of six different standard antibiotics (cephalexin, gentamicin, kanamycin, neomycin, penicillin and tetracycline) was compared to different concentrations (50 to 1000 μ g/ml) of aqueous eucalyptus extract and different concentrations (50 to 1000 μ g/ml) of aqueous myrtle extract. Agar diffusion method showed that eucalyptus aqueous extract revealed

higher growth inhibition zones compared to myrtle aqueous extract, but minor growth inhibition zones compared to some antibiotics like gentamicin and neomycin. Whereas the two extract types inhibited bacterial growth in high concentrations of 1000 µg/ml, minor concentrations were not effective on *P. aeruginosa* and *S. aureus* [75].

In Iran myrtle essential oil is commonly used in folk medicine to treat aphthous ulcers and ulcers which are located in oral cavity. In a controlled, double-blind clinical trial 45 patients, who suffer from minor RAS, were recruited and randomly divided into two groups. The first group was treated with placebo paste and the second group was treated with oral paste containing 5% aqueous extract of *M. communis*. Each patient was told to carefully dab the paste on the oral lesions four times daily for six consecutive days. Several parameters, e.g. burning sensation, erythema level, exudation level, oral health, pain severity and size of the ulcers in millimeters were evaluated four times during the trial - before the treatment, on the second, the fourth and on the sixth day of treatment. On day zero there were no statistically significant difference between control group and treatment group concerning the above-mentioned parameters. The myrtle oral paste was able to significantly improve any evaluated parameter compared to placebo. The oral ulcers occurring in connection with RAS are associated with severe pain and therefore with difficulties concerning drinking, eating, speaking and swallowing. Because myrtle can reduce the pain severity, it has potential in improving the quality of life of patients who suffer from RAS. Furthermore, there occurred no side effects like burning sensation, hypersensitivity, or malfunction of the taste sense. So, the study showed that a paste containing 5% aqueous extract of *M. communis* is an effective as well as safe treatment option for RAS [76]. The good effectiveness of *M. communis* on RAS may be due to

the good antimicrobial activity. Fani *et al.* tested the antimicrobial activity of different concentrations of *M. communis* oil on oral pathogens including *Aggregatibacter actinomycetemcomitans*, *C. albicans*, *Porphyromonas gingivalis*, *Streptococcus mutans* and *Str. pyogenes*. Agar disk diffusion assay and broth micro dilution method showed that the essential oil obtained by *M. communis* is highly active against the above mentioned oral pathogens. Therefore, it has potential in preventing oral infections which are associated with the tested microorganisms, e.g. dental caries, periodontal diseases, pharyngitis and oral lesions [77]. Also, the ethanolic extract of *M. communis* is active against oral pathogens. Although mouth rinses with 2% myrtle extract significantly reduces the count of *Str. mutans* in saliva, it is not as effective as chlorhexidine (0.12%) [78]. The anti-fungal activity of myrtle essential oil on clinical isolates of *C. albicans* was confirmed by Mambourin *et al.* via broth micro dilution assay. Although the MIC of the essential oil obtained by *M. communis* was about six times higher compared to amphotericin B, it can be assumed that myrtle oil possesses a high activity on *C. albicans*. Furthermore, the combination of both, myrtle essential oil and amphotericin B, was evaluated by checkerboard micro titer assay. If 4µg/ml myrtle essential oil was combined with amphotericin B, the MIC of amphotericin B can be reduced from 1-2µg/ml to 0.06µg/ml. So, the study came to the conclusion that there is evidence for a synergistic interaction between myrtle essential oil and amphotericin B *in vitro* [79]. Also, the extract of *M. communis* is highly active against pathogenic fungi. Alcoholic myrtle extract showed even a lower MIC on clinical isolated and standard strains of *C. albicans* than nystatin [80].

The potential benefit of *M. communis* on the wound healing process is not only limited to the antimicrobial activity. There is also evidence for a noticeable antiphlogistic property *in vivo*. After ear edema was

induced by croton oil in mice, Maxia *et al.* investigated the topical anti-inflammatory activity of myrtle essential oil. The essential oil was able to significantly decrease ear thickness, myeloperoxidase activity and migration of neutrophils into the inflamed tissue. Furthermore, the essential oil obtained by *M. communis* significantly reduced the mass of cotton pellet-induced granuloma and serum TNF- α and serum IL-6 [81]. Nassar *et al.* confirmed the anti-inflammatory activity *in vivo*. Furthermore, they found out that the antiphlogistic effects of the essential oil obtained by *M. communis* is superior to the antiphlogistic effects of different myrtle extracts [82]. Because the anti-oxidant activity of the essential oil is inferior to *M. communis* extracts [83], clinical trials which compare the effect of the essential oil with different extract types would be highly interesting. It can be concluded, that myrtle essential oil is a potent anti-inflammatory agent. Therefore, it has potential to affect the wound healing process in a positive manner. The antimicrobial activity might probably act synergistically. Nevertheless, more studies are required to confirm these assumptions.

OREGANO ESSENTIAL OIL

Oregano essential oil is obtained by *Origanum vulgare* L. which belongs to the *Lamiaceae* family and is commonly used as spice all over the world. GC-MS analysis showed that the principal constituents of oregano essential oil are oxygenated monoterpenes, e.g. carvacrol, thymol and β -fenchyl alcohol, and monoterpene hydrocarbons like γ -terpinene, α -terpinene, α -thujene. The main values which are associated with oregano are anti-oxidant and antibacterial effects. Compared to aqueous and ethanolic extracts the essential oil showed poor antioxidant activity by reducing power analysis and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging test. Nevertheless, oregano essential oil possesses higher antibacterial activity against pathogenic bacteria in comparison with different *O. vulgare* extracts [84].

There are several trials which confirm the excellent antibacterial activity of oregano essential oil *in vitro*. Özkalp *et al.* compared the MICs of oregano essential oil and ampicillin, which were obtained by broth micro-dilution technique. MIC was analyzed concerning different bacterial strains, e.g. *Bacillus cereus*, *E. coli*, *K. pneumoniae*, MRSA, *P. aeruginosa*, *Salmonella enteritidis*, *St. aureus* and *Str. pyogenes*. Oregano essential oil inhibits the growth of all tested bacteria, although the bactericidal effect on Gram-positive bacteria was superior to the effect on Gram-negative bacteria [85].

In an *in vivo* double-blind study 40 post-surgical patients were randomly divided into two groups. The surgical excisions of group one were treated with an ointment which contains 3% oregano extract and the surgical excisions of group two were treated with

petrolatum. Although petrolatum is hydrating and hypoallergenic and has therefore a positive effect on wounds, it has no antimicrobial activity. Cultures which were made on day 12 showed contamination with *St. aureus* in 41% of the petrolatum group compared to 19% of the oregano group. In group one only one patient developed cellulites versus three patients in the second group. Compared to petrolatum, the ointment which contains 3% oregano extract significantly ameliorates the scar concerning color, pigmentation and pliability [86]. Sankar *et al.* confirmed the wound healing properties of *O. vulgare* *in vivo*. After full thickness excision wounds (20mm²) were created on the back of twelve anesthetized albino rats, they were randomly divided into two groups. Group one was topically treated with *O. vulgare* aqueous leaf extract engineered titanium dioxide nanoparticles and group two was treated with physiological saline and acted as control group. After twelve days of treatment the rats were sacrificed and wound granulation tissue was analyzed by light microscope and SDS-PAGE. Whereas the rats of the control group revealed signs of inflammation, the treated rats showed no bleeding, infections, pus formation or other complications during the wound healing process. Furthermore, wound size was reduced due to faster wound closure (94% vs. 86% on the 12th day). Histopathological analysis and protein expression profiling analysis showed superior epithelialization and formation of collagen and fibroblast in the treated group. Therefore Sankar *et al.* came to the conclusion that *O. vulgare* aqueous leaf extract engineered titanium dioxide nanoparticles can positively affect the wound healing process [87]. Sankar *et al.* also investigated the antimicrobial effect of *O. vulgare* leaf extract engineered silver nanoparticles. Agar diffusion method showed that those nanoparticles possess an astonishing activity against human pathogenic bacteria. The antibacterial activity was even superior to chloramphenicol [88]. Unfortunately, it remains unclear if the promising results are mainly due to the oregano extract

or mainly due to the silver nanoparticles. Silver nanoparticles are known to have beneficial effects on the wound healing process. These effects are associated with antibacterial properties, but also with anti-inflammatory activities due to cytokine modulation [89].

To make a concluding statement, if oregano can boost the positive effect of silver nanoparticles on the wound healing process, there should be made more studies, which compare the results of silver nanoparticles and *O. vulgare* engineered nanoparticles, especially *in vivo*. Furthermore, there has to be said that even though the essential oil exerts a higher antibacterial activity compared to oregano extracts, there is no evidence for wound healing properties of oregano essential oil by now. Therefore, further studies have to be made to find out if the wound healing properties of the essential oil obtained by *O. vulgare* are superior to the wound healing properties of the aqueous extracts.

ROSEMARY ESSENTIAL OIL

Rosemary essential oil is the aromatic oil of *Rosmarinus officinalis* L., which is popularly named as rosemary and belongs to the *Lamiaceae* family. The principal components of the essential oil derived from rosemary are monoterpenes, such as 1,8-cineole, α -pinene, camphor and bornyl acetate [90]. In contrast to the essential oil the principal components of rosemary extracts are carnosic acid and its derivative carnosol. It is supposed that mainly these two phenolic diterpenes are responsible for the anti-inflammatory property of *R. officinalis*. Mengoni *et al.* investigated the underlying mechanism of action and found out that the anti-inflammatory effect is generated by the reduced expression of pro-inflammatory molecules like IL-1 β , TNF- α , nuclear factor kappa-light-chain-enhancer of activated b-cells (NF- κ B) and COX-2, but not COX-1. Furthermore, carnosic acid and also its oxidation product carnosol inhibit NO production and reduce the number of leucocytes. The anti-inflammatory effect of these two compounds is nine-fold higher than indomethacin [91]. The anti-inflammatory activity of rosemary oil was investigated using the carrageenan-induced paw edema and pleurisy test in rats. Furthermore, the antinociceptive activity was investigated by acetic acid-induced writhing test and hot plate test. All tests were performed in comparison to indomethacin. The results showed that the anti-inflammatory and antinociceptive activities of rosemary oil are similar to indomethacin. Because the antinociceptive activity against chemical stimuli was better than against thermal stimuli, it has to be assumed that the peripheral antinociceptive effect of rosemary oil is predominant versus the central peripheral antinociceptive effect [92].

Abu-Al-Basal *et al.* wanted to prove the scientific evidence for the traditional use of rosemary oil in wound treatment. For this aim 80

mice were enrolled in the study. After anesthesia and wound creation (4mm diameter) the mice were divided into four groups, each with 20 mice. Group one were non-diabetic mice which received no further treatment. Group two were diabetic mice which received intraperitoneal injections of distilled water once daily for three consecutive days. Group three were diabetic mice which received intraperitoneal injections of 10% aqueous extract instead of distilled water and group four were also diabetic mice, which received a topical application of rosemary oil twice daily, also for three consecutive days. The results showed positive effects of *R. officinalis* on angiogenesis, inflammation, re-epithelialization, regeneration of granulation tissue and wound contraction which was measured as the reduction in wound size percentagewise. Compared to the aqueous extract, the essential oil achieved better results. Also notable is the beneficial effect of the aqueous extract and the essential oil on blood glucose levels of diabetic mice. It is assumed, that this property can be explained by the anti-oxidant effect which may protect beta cells [93]. Another study with mice found out that the topical application of rosemary oil prevents flap necrosis and therefore improves the survival of skin flaps which are used in soft-tissue reconstruction. This effect might be due to increased vessel diameters and accordingly to an increased blood circulation in the flaps [94]. The flap survival can also be increased by oral application of rosemary oil [95]. By now there is no substance known which prevents flap necrosis. So, it is absolutely necessary to examine the beneficial role of rosemary oil in surgery more in depth.

SANDALWOOD OIL

Sandalwoods are slow growing, hemi-parasitic, small trees which belong to the taxonomic group *Santalaceae* and occur mainly in India, Indonesia, Australia and the Pacific Islands. Due to its high heartwood oil content (6-10% by dry weight) and its odor characteristics *Santalum album* L. is the most eligible member in the family of the *Santalaceae*. It is also referred to as Indian sandalwood or tropical sandalwood [96, 97]. Yearly about 200 tons of sandalwood oil are required [98]. Because it is extremely high in price and the chemical synthesis of the main components is uneconomically, exploitation of sandalwood trees is problematic [97]. In the list of threatened species published by the International Union for Conservation of Nature and Natural Resources (IUCN) *S. album* is classified as vulnerable. The essential oil is obtained by distillation of the fragrant heartwood [97] and is mainly comprised of sesquiterpene olefins and sesquiterpene alcohols [97]. The main components are α -santalol, β -santalol, epi- β -santalol and α -exo-bergamotol [97]. α -Santalol and β -santalol are responsible for the unique woody odor of sandalwood oil [99]. Sandalwood oil is heavily used in perfumes, cosmetics, medicine, aromatherapy and recently also in the prevention of skin cancer [100].

Sandalwood oil is active against acyclovir-resistant *Herpes simplex* virus type 1 (HSV-1). It is supposed that it inhibits the entry of HSV-1 into the host cell. The systemic application of essential oils is associated with serious problems like bioavailability and a high required dosage, which is toxic [101].

Patients with head and neck cancer frequently require a radiotherapy, which is often associated with severe side effects,

especially dermatitis. Unfortunately, there is no effective standard treatment to prevent radiation-induced dermatitis. Palatty *et al.* showed that a turmeric- and sandalwood oil-based cream is effective in preventing radiation dermatitis and reduces significantly severe dermatitis already in existence [102].

Human keratinocytes express different receptors, e.g. transient receptor potential channels, ATP receptors, endocrinology receptors and also olfactory receptors (ORs), such as OR 6M1, OR 11A1, OR 2AT4, OR 5V1 and OR 5V1. Synthetic sandalwood odorants like Brahmanol® and Sandalore® are agonists of OR 2AT4 and lead to an increase in the intracellular calcium concentration. Because this effect can be blocked by co-application of specific OR 2AT4 antagonists like oxyphenylon and phenirat, it is assumed that the effects of Brahmanol® and Sandalore® are based on the activation of OR 2AT4. Other sandalwood odorants and natural sandalwood oil do not activate this receptor and have no effect on the intracellular calcium concentration. Brahmanol® and Sandalore® activate the phosphorylation of extracellular signal-regulated kinases (ERKs) and p38 mitogen-activated protein kinases (MAPKs) and lead to a significant increased keratinocyte migration and proliferation *in vitro* [103, 104]. Because the proliferation rate is an important factor during wound healing process, especially for re-epithelialization [105], it has to be assumed, that the use of synthetic sandalwood odorants for wound treatment might be effective. However, there have to be made more studies, especially *in vivo*, to confirm the efficacy of Brahmanol® and Sandalore®.

TEA TREE OIL

Tea tree oil (TTO) is obtained by steam distillation of the leaves from *Melaleuca alternifolia*. This plant belongs to the *Myrtaceae* family and is used by Australian Aborigines for decades as traditional medicine to treat colds, coughs, sore throats, insect bites, skin infections, bruises and wounds. After studies about the antibacterial, anti-fungal, antiviral, antimycoplasmal, antiprotozoal and anti-inflammatory activities of TTO have been published, this oil has become increasingly popular in modern society [106-108]. Nowadays TTO is used to treat acne, burns, folliculitis, gingivitis, halitosis, herpes labialis, malodorous skin ulcers, oropharyngeal candidiasis, skin warts, toenail onychomycosis, vaginitis and wounds [106, 109, 110].

TTO is a clear to pale yellow oil with a distinct odor. It is obtained by steam distillation of the leaves and branches from the Australian native plant *Melaleuca alternifolia* (*Myrtaceae*). TTO consists of over one hundred components, especially monoterpenes, sesquiterpenes and their associated alcohols. The antimicrobial activity of TTO can be attributed to its main constituent terpinen-4-ol. TTO is active against a wide range of medically important microorganisms, e.g. *St. aureus* including MRSA, *St. epidermidis*, *Str. mutans*, *Str. pyogenes*, *Str. equisimilis*, *C. albicans* and *C. krusei* [106, 110].

Due to its analgesic, antimicrobial and anti-inflammatory activity TTO is supposed to have positive effects on the wound healing process and especially on infected wounds. Unfortunately, there is not too much known about the exact mechanism of action of TTO by now. The more hydrophilic compounds of TTO (terpinen-4-ol, α -terpineol and 1,8-cineole) interact with hydrophobic structures of bacteria and weaken the barrier function of bacterial cell walls [106, 107, 110,

111]. It is assumed that TTO activates white blood cells and suppresses the production of superoxide ions and inflammatory-inducing mediators, e.g. $\text{TNF}\alpha$, IL-1 β , IL-8, IL-10 and PGE_2 [106, 108, 110].

Due to its cooling effect, TTO has a positive impact on burns. Within a few minutes it reduces the skin temperature by about 2°C on burn wound sites [112]. However, the effectiveness of a TTO hydrogel dressing in cooling burn wounds is similar to the effectiveness of compresses with ordinary tap water (15°C) [113]. Another study found out that a hydrogel dressing impregnated with TTO exerts a cooling effect when applied after a burn injury, but it does not have a significant effect on re-epithelialization, histology of the scar and cosmetic appearance of the scar [114].

Sherry *et al.* investigated the effect of a water-based *Melaleuca* oil preparation on gangrenous lower limbs of patients who suffer from diabetes type 1. The results are promising, but unfortunately not significant, because the number of probands (n = 10) was far too low. Furthermore, there were no comparison to the standard treatment [115].

TTO also plays a role in oncology and modern palliative care. It is successfully used as an additive therapy to eliminate tumor-related malodor. The reduction of foul smell is associated with an increased quality of life for the patients and due to its antimicrobial activities, it may also have a beneficial effect on the wound healing process [116].

Because of the bad solubility of TTO in water it could be difficult to incorporate it in hydrophilic vehicles. The answer to this problem may be the inclusion of the essential oil in nanocapsules and

nanoemulsions. There is a study, which investigated the anti-edematogenic effect of TTO in different formulations. In this trial mice were irradiated with UVB (270-400nm) and 24 hours after irradiation the severity of the UVB-induced edema was evaluated by the increase in ear thickness. One hour before and four hours after the irradiation different hydrogels were applied to the ears of the mice. Hydrogels with TTO nanocapsules or nanoemulsions reduced the ear thickness significantly compared to the untreated group and to hydrogels without TTO or hydrogel with free oil [117]. This outcome shows the great importance of the type of formulation.

Although there are a lot of studies which confirm the activity of TTO against MRSA *in vitro*, by now there are only a few studies which confirm this activity *in vivo*. A current randomized study investigated the influence of TTO on chronic MRSA-colonized wounds, like pressure ulcers, leg ulcers and toe ulcers. While the TTO group (n = 16) received a treatment with a topical 10% TTO preparation, the control group (n = 16) received a treatment with saline gauze dressings. In the TTO group all wounds healed and MRSA was completely eradicated in 14 out of 16 patients within four weeks. In this study, no adverse or allergic reactions could be determined [118]. In another *in vivo* trial MRSA-positive wounds were treated with a wound wash solution which contains 3.3% TTO. The eradication of MRSA was not achieved in any patient, but the wound size has significantly reduced in 8 out of 11 patients after 12 weeks of treatment. Because only two participants completed the study and there were no control group, the results are not significant [119]. Maybe the results would be better if the concentration of TTO would be higher and the formulation would be supposed to be left in contact with the wound instead of washed of.

Another *in vivo* study compared the wound healing times between conventional treatment and the same treatment combined with TTO. Ten participants with cellulites and abscessed wounds infected with *St. aureus* were enrolled. The healing time could be decreased in all participants of the TTO group, except one who suffered from diabetes [120]. Although an overdose or extended exposure to TTO can lead to toxic side effects, e.g. allergic reactions and skin irritations, it is assumed that the topical use of TTO in lower concentrations is safe [121].

THYME ESSENTIAL OIL

Thyme essential oil is obtained by *Thymus vulgaris* L. or *Thymus zygis* L. (*Lamiaceae*) and has a characteristic spicy odor which is mainly due to the phenolic compounds. The main constituents of thyme oil are the two phenolic substances carvacrol and thymol. Additionally, there are other monoterpenes like p-cymene and gamma-terpene [122]. Agar diffusion tests showed that thyme essential oil is highly active against multidrug resistant bacterial strains, which are meaningful in clinical practice and often causes severe hospital acquired infections. Different bacterial strains of *Enterococcus*, *Escherichia*, *Pseudomonas* and *Staphylococcus* were kept from the hospital, from the hospital staff and from the patients, e.g. wound swabs. Among the obtained bacterial strains there were several strains which were resistant against most of the antibiotics tested by disk diffusion method. The conclusion of the study was that thyme essential oil may act as effective and safe alternative to treat bacterial infections including infections with multidrug resistant bacterial strains [123]. The bactericidal activity of thyme essential oil on *E. coli* was confirmed by Hammer *et al.* Furthermore, it could be shown that the essential oil obtained by *T. vulgaris* is highly active against *C. albicans* (MIC 0.03%) [124].

Altioek *et al.* studied the antimicrobial, anti-oxidant and physical characteristics of different concentrations (0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 1.2%) of thyme oil incorporated in chitosan films as wound dressing. The antimicrobial activity was investigated by agar diffusion method on the four pathogens *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *St. aureus*. The minimum effective concentration of thyme oil, where growth inhibition effects on the above-mentioned microorganisms can be observed, was found to be 1.2%. Because the culture supplied ideal circumstances, e.g. concerning pH or

temperature, it has to be assumed that the antimicrobial activity of thyme essential oil would be better in a natural wound surface. Compared with chitosan films without essential oils, chitosan films with 1.2% thyme oil, have an eight-time higher anti-oxidant activity. This mode of action might be due to the phenolic compounds, especially carvacrol, of thyme essential oil. The addition of the essential oil obtained by *T. vulgaris* was able to improve water vapor and oxygen transmission rates. Because these two factors are very important for wound healing materials, it can be supposed that thyme essential oil can enhance the positive effect of chitosan films on the wound healing process [125].

The antibacterial efficacy of *T. vulgaris* is well studied, but there are other properties, e.g. antiphlogistic properties, which can positively influence the wound healing process. 10 µg/ml thymol significantly inhibits the release of elastase induced by N-formyl-methionyl-leucyl-phenylalanine (fMLP). Elastase is a serine protease which acted as marker for inflammation and is released by neutrophils [126]. Yager *et al.* found out that the levels of elastase in fluids from non-healing chronic wounds are much higher than the levels of elastase in fluids from acute healing wounds. The overproduction of elastase in chronic wounds leads to damages of healthy tissue and degradation of growth factors. Therefore, elastase inhibitors are considered as potentially effective components in wound dressings for chronic stagnating wounds [127]. Nevertheless, there has to be more studies to confirm this activity of thyme essential oil *in vivo*.

Besides the antimicrobial and antiphlogistic properties of thyme, the anti-oxidant activity may also have a hand in the wound healing properties. Significant anti-oxidant activities could be shown for all of the four main compounds carvacrol, thymol, p-cymene and γ -terpinene. However, in comparison thymol possesses the highest

anti-oxidant activity in a DPPH radical scavenging test [128]. Unfortunately, the antioxidant properties of thyme are mainly studied and commonly used in food industry, but not in pharmaceutical industry. Further studies have to be made to conclude, if the antioxidant activity of thyme could be used in pharmaceutical industry, e.g. in preparations which are supposed to affect the wound healing process in a positive way.

Due to the antimicrobial, antiphlogistic and antioxidant effects thyme essential oil has great potential concerning wound treatment. Nevertheless, the amount of *in vivo* studies is surprisingly little. Dursun *et al.* investigated the effect of thyme essential oil on burn wounds. Therefore, 40 male rats were anesthetized by ketamine hydrochloride, burned and divided into five groups. Three of them received topical treatments with thyme oil diluted with olive oil, olive oil or silver din and sulfadiazine once daily for 21 consecutive days after burn injury. Two groups received no treatment and acted as control groups - one of them was burned and the other was non-burned. In comparison on the seventh day thicker dermis and thinner necrotic tissue was observed in mice which were treated with thyme oil and silver din and sulfadiazine. After 21 days thinner epidermis, minimal keratinization and higher rates of new tissue were noticed in the thyme oil group. Compared with the non-burned control group the burned rats showed a much higher NO production on days 1, 7 and 21. This overproduction could be significantly decreased by topical application of thyme oil and topical application of silverdin and sulfadiazine. So, there is evidence that thyme oil can act as protecting agent against tissue damage due to an overproduction of NO [129].

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