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Antibacterial activity of plants used in Samoan traditional medicine for wound healing

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Abstract

In developing countries, the chronic wounds affect 1-2 % of population, whereas conditions such as skin ulcers are typical for Pacific region. Despite the rich tradition of the medicinal plant use for wound healing in Samoa, their antibacterial effect has poorly been studied. In frame of this thesis, the antimicrobial activity of extracts from medicinal plants used in folk medicine for healing of wounds was tested using broth microdilution against microorganisms related to infectious skin conditions such as *Bacteroides fragilis*, *Clostridium difficile, Clostridium perfringens, Escherichia coli, Finegoldia magna, Propionibacterium acnes, Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Omalanthus nutans* was the most effective with minimum inhibitory concentration (MIC) of 128 – 256 µg/ml against standard strains of *S. aureus*, 256 µg/ml against *P. acnes* and 4 µg/ml against *P. aeruginosa.* Other plant extracts in order of antimicrobial activity are as follows – *Psychotria insularum*, *Cerbera manghas, Mikania micrantha* and *Schizostachum glaucifolium* (MIC ≥ 4 µg/ml). On the other hand, the least effective extracts were obtained from *Premna serratifolia, Plectranthus scutellarioides, Barringtonia asiatica* and *Inocarpus fagifer.* The supposed results of the thesis should be used by pharmaceutical and cosmetic industries for development of new antimicrobial preparations for wound and burn healing.

Key words: Wound healing, Samoa, Samoan medicinal plants, Antimicrobial activity

Abstrakt

V rozvojových zemích je chronickým poraněním postiženo 1-2 % populace, pro které jsou typické zejména kožní rány. Navzdory dlouholeté tradici samojské bylinné medicíny, antimikrobiální účinky tamních rostlin jsou velmi málo prozkoumány. V rámci této diplomové práce byla studována antimikrobiální aktivita extraktů z rostlin používaných v tradiční medicíně pro hojení ran pomocí bujónové mikrodiluční metody. Testovány byly bakterie způsobující infekce kožních ran a popálenin, jako jsou např. *Bacteroides fragilis, Clostridium difficle, Clostridium perfringens, Escherichia coli, Finegoldia magna, Propionibacterium acnes, Pseudomonas aeruginosa* a *Staphylococcus aureus*. Nejvíce aktivní byl extrakt z rostliny *Omalanthus nutans* s minimální inhibiční koncentrací (MIK) 128–256 µg/ml proti standardním kmenům *S. aureus,* 256 µg/ml proti *P. acnes* a 4 µg/ml proti *P. aeruginosa.* Za ním následovaly *Psychotria insularum, Cerbera manghas, Mikania micrantha* a *Schizostachum glaucifolium* (MIK ≥ 4 µg/ml). Naproti tomu, nejméně aktivními extrakty byly z rostlin *Premna serratifolia, Plectranthus scutellarioides, Barringtonia asiatica* a *Inocarpus fagifer.* Předpokládané výsledky této práce by mohly být využity buď v kosmetickém nebo farmaceutickém průmyslu, pro vývoj nových antimikrobiálních přípravků na hojení ran či popáleniny.

Klíčová slova: Hojení ran, Samoa, Samojské léčivé rostliny, Antimikrobiální aktivita

Certification

I, Martin Pazdera, declare that this thesis, submitted in partial fulfilment of the requirements for the degree of MSc, in the Faculty of Tropical Agrisciences on the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

In Prague 15. 4. 2019

Martin Pazdera

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Contents

1. Wound healing

Skin, as the largest organ founded on of human body creates the protective barrier against the outer environment and saves us against harmful conditions. It protects us from harmful effects, such as chemicals, ultraviolet radiation and pathogenic organisms (Sood *et al*., 2014). Wound healing is a biological process that dynamically leads to skin reparation of epithelium layer after injury. This process involves multiple cell types executed by several growth factors and cytokines (Barrientos *et al*., 2014). There are four main precise phases, through which wound healing is achieved. Such phases are haemostasis, inflammation, proliferation and remodelling. To have a properly healed wound, all four processes must occur in right sequence and on right time. Factors having bad influence on wound healing process are oxygenation, infection, age and sex hormones, stress factors, diabetes diseases, obesity, medications, alcohol abuse, smoking, and nutrition (Guo & DiPietro, 2010). Acute or chronic wounds, both have been examined with the purpose of finding a new method improving cutaneous wound care. Treatment of acute wounds of small scale is effective, however chronic wounds suffer from high protease activity, infection, inflammation and hypoxia and remain a clinical problem. Although the wound care is improving, the large number of wound repairs are still poorly understood and some of them have need for higher financial support (Das & Baker, 2016). Difficulties may occur in case of presence of microorganisms that can lead to infection which is prolonging recovery time and make treatment more expensive. The abundance and diversity of microorganisms depends on wound type, depth, location and quality, the level of tissue perfusion, and the antimicrobial efficacy of the host immune response (Bowler *et al*., 2001).

1.1. Definition, characterization and classification of wounds

Definition of wound can be described as a disruption of the skin entirety. There are two types of wound, such as acute and chronic ones. External factors are always present in case of an acute wound. These factors are responsible for damaging skin and soft tissues that are underneath. By the aetiology, there are distinguished four types of acute wounds: mechanic, chemical, radiation and the thermic ones. In contrary, the chronic wounds are described as the secondary healing wound that does not, despite suitable treatment, seems to heal in 6-9 weeks. Primary suture is used as a main agent in acute wound treatment. In case when is no possibility to join the edges of the wound together, then skin grafting is used, which is also used in treating chronic wounds. In need of skin grafting, there could be expected disruption of reparation process in wound healing. Widespread chronic wounds are leg ulcers coming from venous aetiology, decubitus, skin ulcers in lymphedema, arterial and neuropathic skin ulcers (Stryja, 2008). Every wound is an acute one at first, however can potentially skip to chronicity firstly thanks to affiliated infections and secondly by microtraumatization of the skin surface which is predisposed to be healed with difficulties (Sood *et al*., 2014). The third possibility is depression of the skin necrotization based on disease such as obliterans atherosclerosis of the lower limb. Once the wound lasts open too long, it is a sign of possible presence of external microorganisms in a body and that can be leading to spread other infectious complications, for e.g. abscesses, lymphadenopathy and systemic infections. Large number of the skin ulcers cause a lot of pain and due to secretion of fluids lead to lose of proteins and other substances. Through the duration of healing process, a presence of local infection or bacterial colonization is there. Soft tissue ulcers are slowly filled with granulation tissue and are epithelized from rims. This process takes longer period and demands enough nutrients and is ended with creation of perceptible scar. Character of the wound bed is key parameter for classifying chronic wounds. Classification of the chronic wounds is listed below (Stryja, 2008).

- phase I: flesh-wound (epidermis, dermis)
- phase II: deep wound (interfering to subcutis)
- phase III: damage of fascia
- phase IV: damage of musculature
- phase V: damage of tendons, ligaments and bones
- phase VI: damage of large cavities

Demidova-Rice *et al*. (2012) divided the chronic wounds by to its origin. There are vascular (e.g. venous and arterial ulcers), diabetic, and pressure ulcers. Prolonged or excessive inflammatory phase, persistent infections, formation of drug-resistant microbial biofilms, and the inability of dermal and/or epidermal cells to respond to reparative stimuli are common features of these types (Demidova-Rice *et al*., 2012). When infection is the main problem of slowing wound healing, then the wound requires a paraclinical examination and choice of a proper wound cover. Critical colonization of wound serves as a precursor of local infection in chronic wounds. Self-defence abilities of patient collapses and healing process is slowed down (Stryja, 2008).

1.2. The physiology of wound healing process

The optimal wound healing should consist of these processes such as rapid homeostasis, appropriate inflammation, mesenchymal cell differentiation, migration to the wound site proliferation, suitable angiogenesis, prompt re-epithelialization (re-growth of epithelial tissue over the wound surface), cross-linking, proper synthesis and alignment of collagen providing strength to the healing tissue. Eventual prolongation or interruption during wound healing can lead to delayed wound healing or a non-healing chronic wound (Guo & DiPietro, 2010).

1.2.1. Haemostasis phase

The beginning of wound healing starts right after the wound´s platelets meet exposed collagen. During the aggregation of process, the organism releases clotting factors making a fibrin clot at the site of injury. The fibrin clot serves as a matrix and sets surroundings for the next stages of healing, providing a scaffold for the migration of different cell players (Landén *et al*., 2016). However, platelets are much more important. They provide a sequence of chemical signals, also known as cytokines or growth factors that initiate healing response. The most important ones are so called platelet-derived growth factor (PDGF) which is released from degranulating platelets upon injury and is present in wound fluid and transforming growth factor-beta (TGF-ß). Chemotaxis of neutrophils, macrophages, smooth muscle cells and fibroblasts are results of the PDGF. Also, the mitogenesis of the fibroblasts and smooth muscle cells are stimulated by PDGF (Barrientos et al., 2014, Diegelmann *et al*., 2004).

Signals of TGF-β attract the macrophages and make them to secrete another cytokine with fibroblast growth factor (FGF), PDGF, tumour necrosis alpha (TNFα) and interleukin-1 (IL-1). FGF, including FGF-2, FGF-7 and FGF-10 have been shown to be integral in cutaneous wound healing and is produces by keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells. This whole process initiates the wound healing (Barrientos *et al*., 2014). TGF-β makes fibroblast and smooth muscle cells stronger and modulates collagen and collagenase expression. Rapid deposition of new connective tissue at the injury site is a result haemostasis phase, which is followed by inflammatory phase (Diegelmann *et al*., 2004).

1.2.2. Inflammatory phase

This phase is responsible for activating immune system and for rapid movement of neutrophils and monocytes into injured skin. It rivals with haemostasis and is the early stage of wound healing (Landén et al., 2016). Within 24 hours, neutrophils occur in the injury site. They are responsible for removing foreign material, bacteria and non-functional host cells and also damaged matrix components that might be present in the wound. Neutrophils are then ingested phagocytosis process by the bacteria. A tripeptid called f-Met-Leu-Phe (formed by methionine, leucine and phenylalanine), provokes activation and chemotactic migration of neutrophils. However, it shows no reaction with lymphocytes and it is released as a waste product during the bacterial protein synthesis which attracts inflammatory cells. Neutrophils will absorb themselves until they are filled with bacteria and create a formation called laudable pus (Dixon *et al*., 1995).

The mast cells are another marker cells in wound healing which releases granules filled with enzymes, histamine and other active amines that are responsible for the characteristic signs of inflammation around the wound site. They make surrounding vessels leaky and allow speed transfer of the mononuclear cells into the injury area and fluid is accumulated at the wound site. The signs of inflammation are rubor (redness), calor (heat), tumor (swelling) and dolor (pain).

Monocytes tissue is fixed by 48 hours and is activated to become wound macrophages. These macrophages are the most important cells in the normal healing process. Inhibition of macrophage function will postpone the healing response. Nowadays, macrophages also release PDGF and TGF-β that can bring fibroblasts and smooth muscle cells to the wound site. They are also responsible for removing non-functional host cells, bacteria-filled neutrophils, damaged matrix, foreign debris and any other remaining bacteria from the injury area. Once the macrophages are present in the wound, it is the sign that the inflammatory phase is near the end and proliferative phase is beginning. At the later stage, also lymphocytes appear but their precise role in the wound healing remains unclear. It usually takes 2-5 days of wound healing in inflammatory phase. The immune system plays the important role the whole wounds healing process (Diegelmann *et al*., 2004).

1.2.3. Proliferative phase

Inflammatory phase is followed by Proliferative phase which is focused on overring the wound surface and restoring the vascular network and forming granulation tissue (Landén *et al*., 2016). The TGF-β, macrophages and T-lypmhocytes becomes an important signal during this phase where TGF-β is considered as a the most important signal for the regulation of fibroblast function. This signal has a three-pronged effect on extracellular matrix deposition. At first, transcription of the genes for collagen is increased while proteoglycans and fibronectin thus increasing the overall production of matrix proteins. During this whole process, the secretion of proteases which is responsible for the breakdown of the matrix is decreased by TGF-β and it also stimulates the protease inhibitor, tissue inhibitor of metallo-protease (TIMP). Interleukins, fibroblast growth factors and tumour necrosis factor-alpha are the other cytokines considered to be important ones (Diegelmann *et al*., 2004).

As the healing process carry on, much more important responses are being activated such as epithelization which is stimulated by epidermal growth factor (EGF) and transforming growth factor alpha (TGFα). Once the epithelial bridge is complete, the scab is removed by enzymes. Due to high metabolic activity at the wound site, there are a higher demand for sufficient amount of oxygen and nutrients. Low pH, reduced oxygen tension and increased lactate are typical signs of local environment and initiate the release of factors needed to bring in a new blood supply. It is called angiogenesis or neovascularization and is stimulated by vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (bFGF) and TGF-ß and are produced by epidermal cells, fibroblasts, macrophages and vascular endothelial cells (Diegelmann et al., 2004, Landén et al., 2016). Similarly, to FGF, VEGF also includes other members, such as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E where VEFG-A promotes the early events in angiogenesis, particularly endothelial cell migration and proliferation (Barrientos et al., 2014). One signalling pathway including the role of low oxygen tension that in turn stimulates the expression of a nuclear transcription factor termed "hypoxia-inducible factor" (HIF) by vascular endothelial cells. Specific sequences of DNA which regulate the expression of VEGF thus stimulating angiogenesis is fringed by the HIF. Oxygen blocks the HIF activity when new blood vessels enter the wound repair area and the oxygen tension is back at a normal level. This leads to a decreased synthesis of VEGF. During the proliferative phase the prevailing cell in the injury area is fibroblast. This whole cell produces the new matrix which is necessary for restoring and structure and function of the injured tissue.

Fibroblasts are attached to the cables of the provisional fibrin matrix and begin to produce collagen. There have been identified at least 23 types of collagen but only type I is predominant for cicatrisation. After transcription and processing of the collagen messenger ribonucleic acid, it is attached to polyribosomes on the endoplasmic reticulum where are produced new chains of collagen. In this process is important part involving hydroxylation of proline and lysine residues. During the process of glycosylation, the collagen molecule begins to form its typical triple helical structure. After that, the procollagen molecule is secreted into the extracellular spaces to the further processing. Hydro proline, which can be found in collagen, gives the molecule its stable conformation. When hydroxyproline is absent, the collagen has an altered structure and can undergo denaturation much more quickly and at a lower temperature. Collagen which is released into the extracellular space undergoes further processing by cleavage of the procollagen N and C-terminal peptide. In the extracellular spaces, there is an important enzyme called lysyl oxidase which acts on the collagen creating stable cross-links. As the collagen matures and becomes older, more of these intramolecular and intermolecular cross-links are placed in the molecules. This important cross-linking step gives collagen its strength and stability later (Diegelmann *et al*., 2004, Landén *et al*., 2016).

1.2.4. Remodelling phase

Once the granulation tissue development is finished, the remodelling phase comes into effect. However, collagen is firm and organized cell, collagen fibres in scar tissue are smaller, have a random appearance and tends to break apart faster than the surrounding skin. Maximum tensile strength is of 20 % lower than of normal skin. Also, collagen degradation occurs during the process of collagen remodelling. Specific collagenase enzymes are responsible for degradation and breakdown to collagen fragments that undergo further denaturation. Normal healing cascade begins with hemostasis and fibrin deposition leading to an inflammatory cell cascade, characterized by neutrophils, macrophages and lymphocytes within the tissue. Attraction and proliferation of fibroblasts and collagen deposition follow this process and after that follows final remodelling of collagen forming a scar tissue. From time to time, some pathologic responses such as chronic ulcers or ones that lead to fibrosis may occur. This happens particularly after altercation of some part in healing process (Diegelmann *et al*., 2004, Landén *et al*., 2016). In the wound-healing process, several different cell types are involved. The cellular activities of any cell type may vary during different stages of repair. The complexity and coordination of the healing process are major struggles to therapeutic approaches, since any therapeutic must effectively be sequenced to the appropriate stage (Guo & DiPietro, 2010). Debridement should be done in the wound site before any exogenous growth factor is applied onto the wound because the cells from the non-healing wound edge have reduced responding capacity to wound healing stimuli (Barrientos *et al.*, 2014).

1.3. Chronic wound and burns care

Understanding of the molecular and cellular components present within each wound bed is crucial for successful treatment. TIME is multistep acronym used in nowadays medicine. At first, the nonviable tissue (T) is formed in and around the wound site and needs to be is removed by debridement or debriding agents, such as bacterial collagenase. Minimalization of infection and inflammation (I) with antibiotics and anti-inflammatory properties is a next step. Moisture (M) within the wound is drained with appropriately chosen dressing and for the last, epithelization (E) and granulation tissue formation are supported by particular agent like growth factors (Demidova-Rice *et al.*, 2012). However, complete examination of patient is required to detect eventual hematologic conditions (e.g. anaemia), cardiopulmonary problems (e.g. congestive heart failure), endocrine diseases (e.g. diabetes), gastrointestinal problems that cause malnutrition and vitamin deficiencies, obesity, and peripheral vascular pathology (e.g. lymphedema, atherosclerotic disease) (Daley *et al.*, 2016). Next important step is to assess the wound. Factors for examination are following: size, edge and site of the wound, depth, wound bed, necrotic tissue, slough and eschar, surrounding skin, infection and pain. There is used a wide scale of possible treatment methods according to features of the wound. In case of infected wound, need of debridement and appropriate systemic antibiotic treatment. Due to interference with wound healing, topical antiseptics are avoided because of its toxicity to healing cells. Another vital thing is to remove all foreign objects and material within the wound. Those foreign materials include road debris and retained fragments of dressing materials or suture material (Grey *et al.*, 2006).

1.3.1. Debridement

Debridement is the longest known method how to remove necrotic tissue however many patients are worried about it. Debridement is defined as a removal of foreign material or necrotic tissue from the wound and its purpose is to detect the healthy tissue on the wound bed and make it healthy again (Stryja, 2008).

The most important act of eventual appearance of necrosis is aerial humidity. In the dry conditions, it is of dry, black and solid appearance. Especially patients with ischemic leg diseases or diabetics with neurochemical ulcer are threatened by this type of gangrene. On the other hand, in wet conditions the softening of eschar happens and gets brighter colour – from brown and yellow to grey. With the ongoing wet environment, necrosis splits and create a form called slough which is a muddy necrotic tissue of grey yellow colour adherent to wound bed and often stinks. This whole process of conversion from dry to wet necrosis is known as autolysis (Stryja, 2008).

Debridement process could be divided into two phases. In the first one, there is need of removing the necrotic tissue. Largely used is surgical debridement. Other possibilities are hydro surgery or larvae therapy. The second stage can be considered as an effort to keep the wound clean and without necrosis. Range depends on width of the wound. Removal of bacterial burden, removal of necrotic tissue (the source of toxins), reducing of inflammatory reaction of the surrounding, wound secretion reduce, odour reduce, and availability of growth factors are positives features of debridement. Nowadays, large number of debridement methods are available, such as mechanical, autolytic, chemical and enzyme debridement (Stryja, 2008).

1.3.2. Burns

A burn is a type of injury damaging the skin which is the barrier from outer environment. Damaged skin is a possible and suitable area for growth of bacteria and possible infection. Burns are one the most devastating injuries having, effect primarily physical, physiological and psychological in patients (Kwang *et al*., 2014). There are some aspects that need to be considered to classify the seriousness of burn injury. The deepest spot of the burn usually appears in the middle of the damaged area. The skin has three layers. The deeper the burn gets, the greater number of layers that are affected. Current classification of burns is derived according to this knowledge. In current medicine there are used four degrees of burns according to depth of the injury. These four degrees are as follows - superficial (first degree) burn, which is mostly by sunburn, partial thickness (second degree) burn and usually leaves scars, full thickness (third degree) burn destroys all three skin layers and fourth degree burns extended through the skin into underlying tissues, such as muscles. Penicillin based antibiotics is the first treating therapy and is used in less serious cases (Anonymous, 2016 a) however, the most common problem in burns are staphylococcal and streptococcal infections (Kwang et al., 2014). The purpose of the antibiotics use is to prevent from the enter of infection and reduce the size of scar. Bandages and topical therapies are another treatment of burned areas. Compound of the bandages are silver compounds (silver sulfadiazine) or salts. Their task is to defend tissue against infection, reduce heat and loss of water vapor from burned skin, protect the burned skin due to its sensitivity, maintain the burned parts (fingers, limbs, etc) in a proper position and to collect the drainage from the wound site. In harder cases, next treating method can be raising the limbs due to its swelling and reduce the blood flow. Surgical cuts can be used to relieve the pressure from swollen limbs. To ensure the scar will not be thin and will not limit movement, it is crucial to exercise the wound site. Pressure garments also help to treat hypertrophic scarring. These scars form themselves beyond or above the injured area, although this occur is not fully understood. The pressure helps the scar to form properly and choke off the amount of hypertrophic scarring. Xu *et al.* (2019) tested Alginate-Chitosan hydrogel that promotes forming wound surface by self-crosslinking without adding any chemical crosslinking component. Skin grafting is commonly used technique and is also used in the harder cases (partial and full thickness burns) and is often applied after the debridement. It also reduces the number of days in the hospital. Best donors for skin graft are patients with healthy and unburned skin. Skin often comes from less visible parts like buttocks or upper thighs because donor sites will be affected and will not look normal once it is healed, but it depends on the size and burn location where the grafts will be taken from (Anonymous, 2016 b).

1.3.3. Recovery

This paragraph includes both, recovery of wounds and burns. For proper healing, there is need for keeping the environment moist, but it is undesirable to moist the wound bed. The moist saline dressing should be applied once debridement is over. Optimal wound coverage needs wet-to-damp dressings, which support autolytic debridement, absorb exudates and protects neighbouring skin. Polyvinyl film dressing is suitable for wounds, which are not dry neither highly exudative. Hydrocolloid dressings is good choice for covering dry wounds. For exudative wounds are appropriate absorptive and hydrofiber dressings and impregnated gauze dressings are perfect for highly exudative injuries. Sometimes, in cases of very high exudation, twice-daily dressing changes may be required. Hydrogel sheets and nonadhesive forms are suitable for securing a wound dressing when the surrounding skin is fragile (Daley *et al*., 2016). Nowadays nanofibers are widely used in the medical industry, mainly in tissue reparation, as scaffolds in wound healing and wound dressing. Chitosan/polyethylene oxide nanofibers can be a suitable replacement for common wound coverages (Rahimi *et al.*, 2019). Another crucial element in wound healing is proper diet and sufficient amount of energy supply. In patient with chronic wounds, nutrition disorders are quite common. Can be result of another disease of states such as malnutrition. Symptoms of malnutrition take shape in reducing of body weight, fat reserves, decreasing of serious proteins (albumin, prealbumin, transferin etc.). Vitamins take an essential part in lot of processes in organism. Hypovitaminoses C is the most common disorder. This disorder causes defective collagen, increased fragility of capillaries and is important for creating strong ligament, also is anti-infectious and vitamin C increases biological availability of iron. Not enough supply of vitamin B causes disruption of energy metabolism of cells, microcyte anaemia and amiss crosslinked of collagen. Hypovitaminosis A causes insufficient creation of proteoglykan, mucopolysaccharides, collagen, and disrupted epithelization. As a dietary supplement, vitamin A can antagonize negative influence of steroids on healing. Homeostasis is disrupted by lack of vitamin K. Next problem is tissue oxidation. Oxygen, which is important for tissue healing, is transported by blood-vessels. Insufficient amount of oxygen leads to distortion of healing process because of violation in collagen synthesis. Another consequence is a predisposition of bacterial infection, caused by violation of bactericidal reaction of macrophages and granulocytes (Stryja, 2008).

1.4. Wound microbiology

Healthy intact skin controls population of microorganisms living on its surface and prevent them from getting into underlying tissue and cause eventual infection. Distortion of skin entirety and exposing tissue below create a suitable environment for microbial reproduction. The diversion and abundance of the microbial organisms depends on the wound type, depth, location. Despite sterile surgical intervention, every single wound is a possible place for microbial colonization. Since 1960s, the dressings like polyurethane foams, polyurethane films, and hydrocolloids were invented as proper treatment and to prevent bacteria from multiplication and as an agent that keep the optimal wound environment. These types of dressings are more effective than the dry ones. In case of acute wounds, it is expected quick natural healing without any special surgical interventions. However, more serious injuries such as burns with the presence of nonviable tissue and foreign material are expected to have microbial colonization and need debridement and antimicrobial therapy to catalyse the healing process. Chronic wounds are usually caused by endogenous mechanisms connected with predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue. There are three main sources of wound contaminants. The first source is the environment (microorganisms in the air and those introduced by traumatic injury). The second origin is from the surrounding skin, involving members of the normal skin microflora such as *Staphylococcus epidermidis*, micrococci, skin diphtheroids, and propionibacteria. he third type is endogenous sources involving mucous membranes (primarily the oropharyngeal, gastrointestinal, and genitourinary mucosae). Current statements claim that aerobic or facultative pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and beta-hemolytic streptococci are the primary causes of prolonged healing and infection in acute and chronic wounds (Bowler *et al*., 2001). A large number of bacteria including *Staphylococcus*, *Streptococcus* and *Pseudomonas* with its exotoxins are responsible for destroying the hosts cells and disrupting normal cellular metabolism (Rhoads *et al*., 2012) However, anaerobes are aim of scientists interests due to the fact, that anaerobic bacteria were probably the cause of postoperative complications. The problem in recognizing the prevail of anaerobic bacteria in wounds may have main three reasons. First, anaerobes are not considered as being harmful to classic wound healing. Secondly, comparison with aerobic and facultative microorganisms, the culture, isolation, and identification of anaerobic bacteria is more time demanding, labour-intensive, and expensive and is often regarded to be very demanding for many diagnostic microbiology laboratories. The third reason is that anaerobes are supposed to die quickly in aerobic conditions which makes collecting and transporting them to the laboratory is difficult to keep the collected specimen viable (Bowler *et al*.,2001). Microbial biofilm represents another problem, due to its presentation of natural environment for bacteria and affects every organ system. Biofilms tends to be more resistant to antimicrobial agents and resistance to host immunity defences is also there. Multi-species biofilms are commonly used in chronic wounds thanks to the moist adherent environment in which bacteria are inserted in a self-secreted exopolysaccharide matrix. The occurrence of such biofilm is a result of antibiotic treatment that was inefficient (Han *et al*., 2011, Rhoads *et al*., 2012).

Perfusion of blood is an important agent providing minimal opportunity for microorganisms for colonization and proliferation because they deliver oxygen, nutrients and cells of immune system to the site of the wound. There is a simple equation, that with decreasing amount of oxygen within the tissue, the higher chance of infection appearance is there. Due to this, chronic wounds are characteristic with poor blood perfusion (Bowler *et al*., 2001).

1.4.1. Wound infection

Infection appears once virulence factors of extraneous microorganisms start an immune response of host organism and contribute factor in chronic wound development and maintenance. Every single wound is colonized to some level and main role of the inflammatory phase of wound healing process is to bring microbes down to steady-state levels that can be tolerated and cleared by healthy tissues (Bowler *et al*., 2001, Eming *et al*., 2014). Both, acute and chronic wounds are combined with aerobic and anaerobic origin. In the first case, there is a surgical infection where the possibility of eventual infection depends on the sensitivity of the surgical wound contamination of microorganisms. Proper surgical intervention represents just from 1 to 5 % of postoperative wound infection. On the other hand, in dirty and non-sterile procedures, there is at least 27% chance of microbial contamination and infection. Surgical wound infections have polymicrobial aetiology, in both, aerobic and anaerobic microorganisms however, this not include clean surgery interventions. The most common microbial organisms in type of such wound infection are *S. aureus* coagulase-negative *staphylococci*, *Enterococcus* spp*.*, *Escherichia coli*, *P. aeruginosa*, and *Enterobacter* spp. It requires proper asepsis and antisepsis mediums to minimize the possibility of the postoperative infection. Acute soft tissue infection is the second type in which traumatic wounds, cutaneous abscesses, and necrotizing infection are present as well. *S. aureus* is responsible about for 30 % of cutaneous abscesses. However, there are some studies claiming that cutaneous abscess or traumatic wounds are to 50 % and necrotizing soft tissue infections are about 47 % combined with mixed types of aerobic and anaerobic microflora. Other pathogens are *Bacteroides fragilis*, *Streptococcus pyogenes*, *Peptostreptococcus* spp., *Clostridium perfringens*, and *Prevotella* spp. Necrotising soft tissue has five points of classification. These are the supposed causative microorganism(s), the type and level of tissue involved, the initial clinical findings, the rate of progression, and the type of therapy required. Bite wound infection is another infection type. There is from 10 to 50 % chance to get infection from bites, depending on the severity and location of the bite wound. From dog bite, it is up to 20 % and from cats from 30 to 50 %. In 74 % are represented by both, aerobic and anaerobic microorganisms such as *S. aureus, Peptostreptococcus* spp. and *Bacteroides* spp. However, most of the pathogens are of anaerobic origin, because of oral microflora. For example, these could be *Bacteroides, Prevotella, Porphyromonas,* and *Peptostreptococcus* spp., uncommon potential pathogens such as *Bartonella henselae, Capnocytophaga canimorsus Pasteurella multocida,* and *Eikenella corrodens* may be involved as well. In burns, most deaths (about 75 %) are caused primary by infections. There is a prevalence of aerobic pathogens such as *E. coli, P. aeruginosa, S. aureus, Klebsiella* spp., *Enterococcus* spp., and *Candida* spp. In anaerobic microflora there are microbial organisms such as *Peptostreptococcus* spp., *Bacteroides* spp., *and Propionibacterium acnes*. (Bowler *et al*., 2001).

Here is list of some microorganisms which appear in both, acute and chronic wounds. These are *S. aureus, Streptococcus* spp., *Corynebacterium* spp., *E. coli, Enterobacter cloacae, Proteus mirabilis, Peptostreptococcus micros, P. anaerobius, P. magnus C. perfringens, C. panaveris Bacteroides fragilis, B. cadaveris, B. uniformis, Prevotella oralis, P. oris* etc. Acute wound microorganisms are following *Bacillus* spp., *Escherichia hermanii, Clostridium septicum, C. limosum C. tertium, Bacteroides ovatus, Prevotella* spp., *Veillonella* spp. etc. Microorganisms in chronic wound are for example *Micrococcus* spp., *Corynebacterium xerosis, Citrobacter freundii, Clostridium ramosum, Enterobacter aerogenes, Peptostreptococcus indolicus, Streptococcus intermedius, Eubacterium limosum, Bacteroides stecoris, B. capillosus, B. caccae. Fusobacterium necrophorum* etc. (Bowler *et al*., 2001).

There are more types of wound infections like diabetic foot ulcer infections and leg and decubitus (pressure) ulcer infections however they do not belong to the scope of this work.

2. Plant-derived natural products

It has been known for a long time that medical plants are valuable source of therapeutic agents and many nowadays medicine systems use their plant-derived products. However, during last few years, the pharmaceutic industry is focused mostly on synthetic compounds. They are easier to produce and resupply but on contrary, the results did not meet with the excepted results. That is why natural plant products are new scientific aim of research nowadays, despite its high complexity, which in turn necessitates broad interdisciplinary research approaches (Atanasov *et al*., 2015).

Medicaments based plant products are still essential in health care. For example, capsaicin from *Capsicum* spp. is used as a topical analgesic. As local anaesthetics and analgesics are used cocaine from *Erythroxylum coca*, codeine and morphine from *Papaver somniferum*. Nicotine is extracted from *Nicotiana* spp. has its use in smoking cessation therapy. Verapamil based on papaverine also extracted from *Papaver somniferum* found its usage in treating hypertension. The latter plant is better known as being the source of painkillers such as morphine and codeine however possibly best-known example of ethnomedicine are antimalarial drugs, like quninine and artemisine (Cragg & Newman, 2013, Raskin *et al.,* 2002). Large number of plant species produces bioactive compounds with different chemical structure. Just few plant species, about 6 %, have been systematically studied for its pharmacological features and just around 15 % phytochemically. Unfortunately, ongoing climate changes and anthropogenic factors contributes decreasing of the vegetative species thus destroying new possible sources of plant-derived natural products (Atanasov *et al*., 2015).

Besides conventional antibiotic preparations, over-the-counter pharmaceuticals and cosmetics in the forms of creams, deodorants, gels, lotions, ointments, soaps and shampoos recommended against skin infections such as acne, dandruff, dermatophytoses, and seborrheic dermatitis containing antimicrobially active plant extracts, essential oils, compounds and their derivatives are currently available on the international market. For instance, Whitfield's ointment, composed commonly of 3% salicylic acid, 6% benzoic acid and a suitable base (e.g. lanolin or vaseline), is available as an over-the-counter preparation traditionally used to cure athlete's foot (tinea pedis) and other fungal infections of the skin. *Myroxylon balsamum, Styrax benzoin, Styrax paralleoneurus*, and *Styrax tonkinensis* are trees producing resins and oleoresins containing benzoic acid (Kokoška *et al*., 2019). Noless, various plant secondary metabolites are important agents in wound healing. Since this is a focus of my thesis, further, I will describe some more specific plant-derived therapies and substances used in wound healing. They include therapeutic wound covers, flavonoids, enzymatic therapy and curative cosmetics.

2.1. Alginates

Alginates are derived from brown seaweeds such as *Ascophyllum nodosum* (Fucaceae) and *Macrocystis pyrifera* (Laminariaceae). *A. nodosum* forms single bladders centrally in long, strap-like fronds. The fronds hang downwards, gently draping sheltered intertidal rocks. The species has two-meter-long fronds with large yellow egg-shaped air bladders at regular intervals A number of fronds grow from each basal holdfast, and the plant generally regenerates new fronds from the base when one of the larger fronds are damaged, so that the stands you see on the shore may be very persistent indeed. In rocky shores of northern Europe and Canada, this is very common seaweed (Guiry, 2017). *Macrocystis pyrifera*, which is also known as the giant kelp, may exceed 30 metres in length, characterize benthic habitats on many temperate reefs. Blades absorb nutrients from seawater to support rapid growth primarily from their basal (frond producing) and apical (basal producing) meristems. Large number of algae growing on the rocky reefs are harvested for food, fertilizer, and pharmaceuticals (Kingsford, 2016, Manley, 1981).

Alginates are being used for therapeutic wound covers including impregnated gauze covers and alginates themselves consist of compression fabric woven from cotton fibres containing other active substances. There are three types of these covers. The first one is hypertonic content of NaCl gauze, also known as Mesalt, which is applied on exuding wounds with the need of debridement. Iodine impregnated gauze, or hyiodine and iodine-povidone is the second type. This gauze is used on the infected skin ulcers. The third and the last type is vaseline impregnated gauze, which performs wound and its surrounding protection. It also supports hydration of the wound bed. Cover forms could be either sterile or non-sterile of variation of size with nonocclusive, adhesive and absorption features. There are some undesirable effects like wound edges maceration, adhesion and healthy granulation tissue drying. Sometimes, during the wound healing process, the wound needs to be rebandaged, so the extra cover is required. As advantages could be considered cheapness and is suitability to those types of patients who requires frequent rebandages due to an early infection.

Alginates are sterile, soft and not woven wound cover. It is also nonocclusive, nonadhesive, humidity keeping absorption cover consisting of highly absorption alginate fibres from brown seaweed. Sodium and calcium salts of alginic acid are in a various ratio. Alginate covers are applied on the surface ulcers and have bacteriostatic and haemostatic effect. Thanks to releasing Ca^{2+}, the local haemostasis is supported and are suitable for the after-debridement bleeding wounds. Ca2+ or K+ ions replace Na+ gradually in the exudate. By this process, the alginate fibres fall apart and convert themselves to a hydrophilic viscous gel covering the affected site and creating suitable humid environment. These are primary covers for flesh and deep wounds including infected ones. However, alginate covers are not recommended for dry and dry wounds covered with necrosis, neither for patients with insufficient secretion and to the alginate hypersensitivity. There could occur side effect such as allergy, however the huge advantage is large absorption of exudate. The proper use is to apply them only on the wound bed and cannot overlap to the edges and during rebandages, it is important to remove residues. Rebandages are usually done every 2 or 3 days, depending on the secretion. These wounds Requires secondary cover (gauze or film cover in the dry wounds). Specific types of alginates are alginates with additives which provide sterile and nonwoven wound cover with a silver and alginate addition. They differ according to specific product and can be classified as follows:

- SilverCel – alginate fibres, carboxymethylcellulose (CMC) and X-STATIC fibres covered with Ag^0
- Acticoat absorbent – Ca-alginate, nanocrystalline Ag^0 SILCRYST
- Askina Calgitrol Ag – Ca-alginate, Ag^+ ionts and polyurethane foam

For widespread ulcers, the alginate covers have bacteriostatic, bactericidal and hemostatic effect, and form suitable humidity on the wound bed. Another important thing is that they are applied onto flesh, deep infected wounds with medium to heavy secretion (except of Askina Calgitrol Ag) and are not suitable for dry wounds. Side effects appeared such as adhesion of cover on the wound bed or wound bed pigmentation with Ag^+ ions. As an advantage could be considered antibacterial effect with protection against the exudates. Applied only on the wound bed without edges overlap and requires secondary cover (Stryja, 2008). In medicine, alginates are used as a Ca-alginate covers on post-operative scars, burns, bedsores and chronic ulcers. In food industry can be used as a thickener, stabilizer or as a gellant (E404) (Adams *et al*., 2012).

2.2. *Aloe vera* gel

A. vera is medicinal cactus-like plant belonging to family Xanthorrhoeaceae. It is a succulent herb, reaching from 50-80 cm height. *Aloe* grows in hot dry climates (Ernst & Vogler, 1999), has yellow flowers and the leaves are from pale to grey-green arranged in a rosette configuration, covered with white spots up to 50 cm long with branched floral stem (Hoskovec, 2007, Shelton, 1991). There are other varieties such as *Aloe ferox* which is a perennial succulent plant that can be 5 m high. On the top of the straight woody 30 cm thick stem grows lanceolate leaves up to 90 cm long. The leaves are dull green and from time to time with bluish or reddish tinge. The fruits are capsules up to 3 cm long (Svobodová, 2012). Medicinal properties of this plant have been known for a long time. Through the years it attracted of many attentions of medical scholars. Each part of the plant has its special application (Molazem *et al*., 2015). The *A. vera* gel, chemically mannose-6-phosphate, is consists of amino acids, lipids, sterols and polysaccharides, such as pectin and hemicellulose and is stored inside the leaves and is used for healing of different types of wounds. This substance is derived from the leaf pulp of the plant has become a big industry worldwide due to its application in the food industry and on the surface of the leaves is a yellowish bitter sap that has laxative properties and contains anthraquinones compounds (Ahlawat & Khatkar, 2011).

Many studies have been done on the wound healing effect. Scientists found out that it reduces wound and inflammation of the mucous membrane of the mouth and *A. vera* mouthwash was effective in healing of the wound and reducing the inflammation. Very effective is also on the first- and second-degree burning wounds without any side effects and accelerates the dermic injuries such as wounds, frostbites, inflammations, and cutaneous infections. Furthermore, supports forming of collagen and epithelial cells and reduces inflammation and desiccation. As a conclusion, it can be said that *A. vera* gel is appropriate and economic dressing for a lot of wounds and can be also used in suntan screens, shaving foams, lipbalms and facial masks (Sharma *et al*., 2013, Molazem *et al*., 2015). Another benefit is in the healing of haemorrhoidectomy. In people with chronic wounds, one trial found no statistically significant difference in pressure ulcer healing with *A. vera* and in a trial of surgical wounds healing by secondary intention *A. vera* significantly delayed healing. The low quality of the included trials shows that the trial results must be viewed with extreme caution as they have a high risk of bias (Dat *et al*., 2012). *A. vera* gel is a beneficial treatment and is very effective for patients with chronic ulcers. The use of *A. vera* gel in chronic ulcer is recommended in developing countries due to bad financial situation (Avijgan *et al*., 2016). In contrast, Schmidt & Greenspoon (1991) in one study reported delay in wound healing after the application of *A. vera*.

2.3. Flavonoids

Flavonoids belong to large group of polyphenolic compounds having a benzo-γ-pyrone structure, are ubiquitously present in plants, and commercially extracted from citruses belonging to family Rutaceae, such as *Citrus limon* and *Citrus sinensis*. *C. limon* is a small, evergreen tree grows to 3-6 m high with green branches, oblong leaves and flowers leaves intensely fragrant. The fruit is a berry sizes up to 14 x 8 cm and are harvested 3 times a year. *C. sinensis* is evergreen tree with its height from 8-15 m. Flowers are intensely fragrant as well as *C. limon*. The whole tree is very similar to *C. limon* (Rak, 2009). Studies that have been made so far show that secondary metabolites of phenolic compounds including flavonoids are responsible for the variety of pharmacological activities. Synthetization of flavonoids is mediated by plants as a response to microbial infection. Their main task is to catch free radicals by chelating metal ions preventing the generation of radicals that are damaging biomolecules. According to these facts, they are important anti-oxidants and induce human protective enzyme system. Many reports say that flavonoids are protecting organisms against degenerative diseases like cardiovascular diseases, cancers and other age-related problems (Kumar & Pandey, 2013). Many exotic plant species are rich on anti-oxidants such as flavonoids however they are underutilized or unknown. Some of these plants and fruits of course, are consume safe and some of them are developed as medicines. The wild species of fruits are full of flavonoids and anthocyanins. Luckily, in last few years there is an increasing attention wild fruit study. The investigations showed its bioactive impact, such as antioxidant, antimicrobial anti-inflammatory, and anticancer effects and the wild fruit wild fruits could have the potential to prevent and treat some chronic disease (Li *et al*., 2016).

For this thesis, the most important feature of flavonoids is the antimicrobial activity. Several flavonoids including apigenin, chalcones, galangin, flavone and flavonol glycosides, flavanones and isoflavones showed to possess potent antibacterial activity and can have multiple cellular targets, but their antimicrobial activity can be related to their ability in inactivation of microbial adhesins, enzymes, cell envelope transport proteins, and so on. Some flavonoids such as catechins, neringerin and sophoraflavanone G acts against *Streptococci* and *Staphylococci*. Different study shows inhibitory activity of quercetin, apigenin, and 3, 6, 7, 3′,4′-pentahydroxyflavone against *Escherichia coli* DNA gyrase (Kumar & Pandey, 2013). Some flavonoids like diosmin synthetized from hesperidine can be found in citrus pericarp and have positive effect on microcirculation and lymphatic drainage, fragility, permeability or venous tonus and inflammatory process inhibition. Another way of usage of flavonoids is for instance for reducing vascular resistance as well as for treating haemorrhoids, varicose ulcers and for wounds or ulcers oedema are used in tableted or gel cures. In general, flavonoids can be accepted in dietary sources, such as parsley, blueberries, citruses, cocoa etc. Another source are gels, pills and creams (Tong et al., 2013).

2.4. Enzymes

In wound healing, there are two important enzymes such as bromelain and papain. Bromelain comes from *Ananas comosus*, a tropical herbaceous perennial plant up to 150 cm tall with an edible fruit belonging to family Bromeliaceae. Stem grows from the centre of the rosette and can reach to 30 m in lenght. Flowers are blue and later purple. It is cultivated in several varieties. Its origin is probably in the South America and still grows in this area (Kovář, 2007). Papain of *Carica papaya* (Caricaceae) is extracted from latex. *C. papaya* is large, tree-like perennial herb, up to 10 m tall and up to 75 cm long leaves with straight and unbranched stem with greyish cinnamon colour. Its surface is covered with visible scars from old leaves petioles. The fruit is a pear-shaped berry weighing 2-10 kg. Originally grew in the Central America, however, currently is spread in tropics and subtropics (Kovář, 2008). Bromelain is from group of protein digesting enzymes obtained from pineapple stem or fruit and is considerably absorbable in the body keeping its proteolytic activity and without producing any major side effects. Bromelain is present in lot of therapies treatments such as angina pectoris, bronchitis, sinusitis, surgical trauma, and thrombophlebitis, debridement of wounds, enhanced absorption of drugs and particularly antibiotics. Bromelain also relieves osteoarthritis, diarrhoea, and various cardiovascular disorders and has anticancerous effect. Another feature regarding wound healing is reversible inhibition of platelet aggregation. Clinical studies have shown that bromelain may help in the treatment of several disorders. Bromelain has positive effect on cardiovascular and circulation, on immunogenicity, blood coagulation and fibrinolysis, diarrhoea, cancer cells, relieves osteoarthritis and has role in surgery and in burn debridement. Another important feature is influence on blood coagulation by increasing the serum fibrinolytic ability and by inhibiting the synthesis of fibrin, a blood clotting involved protein. Several studies have been made in rats where the reduction of serum fibrinogen level by bromelain is dose dependent. This substance also reduces the average number of days for disappearance of pain and post-surgery inflammation. Nowadays, use of bromelain is extended to treat sports injuries by reduce swellings and bruising (Pavan *et al*., 2012). In injuries, soft tissue disruption or inflammation can be healed and protects muscle against ischemic injuries (Rathnavelu *et al*., 2016). Bromelain is very effective agents in burns as well, applied as a cream and is suitable for debridement of necrotic tissue and acceleration of healing containing escharase, which is responsible for this effect. Enzymatic debridement using bromelain is better than surgical debridement. It is mainly because surgical incision is painful, nonselective and exposes the patients to the risk of repeated anaesthesia and significant bleeding. There is a potential of use for oncology patients in the future, from the development of oral enzyme therapies. Very good therapy is debriding gel dressing, which is a bromelain-based enzymatic medical grade agent derived from the stems of pineapples that results in rapid and selective debridement of the necrotic eschar. This method represents new alternative way of surgical and non-surgical eschar removal strategies which is minimally invasive to organism. This gel is sold under commercial name Nexobrid® (Pavan et al., 2012, Rosenberg et al., 2015).

Papain is a phytotherapeutic agent extracted from unripe fruit of *Carica papaya*. This substance is being used in treating eschars and as a debriding chemical agent to remove or necrotic tissue of pressure ulcers and gangrene. Leaves of *C. papaya* are used to treat infected wounds and burns, especially the green papaya is used for dressing the ulcers and is recommended to other chronic skin ulcers treatment. On the other hand, in spite of its extensive use, the following disadvantages were described, as problems concerning the availability of green papaya and difficulties in preparing and storing papaya. Papain is equivalent to human enzyme pepsin. The literature states that papain interacts with partially degraded collagen in the necrotic tissue of carious lesions, causing additional softening of this tissue, especially in the necrotic one (Júnior *et al*., 2015, da Silva *et al*., 2010). Papain ointment is used for enzymatic debridement and together with bromelain, are used in treatment of arthritis and arthrosis and there are ongoing studies on topical applications on burns, irritations, and wounds. It has also been used for ulcers and bedsores. Traditional cultures in Hawaii and Tahiti made poultices out of the skins of papaya, as in this part of fruit is very high concentration of papain. The inhabitants applied it to the skin to heal wounds, burns, rashes and bug stings and it also serves as a powerful antioxidant. There are a lot of products on the market, that are sources of papain, such as dietary supplements, creams and other drugs (Edward, 2011, Sharma et al., 2013).

2.5. Benzoic acid

This chemical substance can be extracted from tree called *Styrax benzoin* (Styracaceae), also known as Gum benjamin tree, Kemenyan, Onycha or from a tree called *Myroxylon balsamun* (Fabaceae). *Styrax benzoin* is an evergreen tree that can grow up to 10 meters tall. The bark is grey or brown and the leaves are pointed, oval, around 14 cm long and 5 cm wide with fine greyish hairs on the lower surface and flowers form small clusters and are white, bell-shaped and fragrant (Storluson, 2017). *Myroxylon balsamum* is a large and beautiful tree with a valuable wood like mahogany, and a straight smooth trunk; the last is coarse grey, compact, heavy granulated and a pale straw colour, containing a resin which changes from citron to dark brown; smell and taste balsamic and aromatic. Leaves alternately, abruptly pinnate, leaflets two pairs mostly opposite, ovate, lanceolate with the end blunt emarginate; every part of the tree including the leaves abounds in a resinous juice. The mesocarp of the fruit is fibrous, and the balsamic juice, which is abundant, is present in two distinct receptacles, one on each side. The flowers of *M. balsamum* have very intensive fragrance (Grieve, 2017). Pure benzoic acid is colourles or needles of leaflets. This compound is one of the oldest chemical preservative used in the cosmetic, drug and food industries. In nature can be found in several foods and commodities like fruit, spices, milk and fermented products, such as beer, black teas and wines. Benzoic acid is responsible for antimicrobial activity as the undissociated molecule and as a result, it is reported that antimicrobial effect is efficient in a strongly acidic solution than in the neutral medium. It inhibits microorganisms such as *B. cereus*, *E. coli*, *S. aureus*, *Pseudomonas* spp., *Aspergillus* spp., and *Penicillium* spp. Together with sorbic acid and with physical environment changes like temperature and pH, the benzoic acid can get even better antimicrobial results (Davidson *et al*., 2005). Medicinal usage of B. acid is present in chemical debridement as a cream and solutions in combined with other compounds such as malic, salicylic acid and propylene glycol through chemical degradation of necrotic tissue and wound disinfection. Another reason of using is found food industry as a preservative (E210). As this agent is added to drinks, fruit products, mustard, chemically yeast dough and spices with pH lower than 4, 5 (Rosen *et al*., 2015).

2.6. Extract of *Centella asiatica*

This species is known as Asiatic pennywort or Indian pennywort. It is a small, herbaceous, frost-tender perennial plant belonging to Apiaceae family growing in tropical swampy areas, for instance in Sri Lanka, Madagascar and India. It is a thin perennial with long internode, simple and heart-shaped leaves at the base with long petioles. Flowers are brown and small with fruit containing two seeds. As a rich source of natural bioactive substances, triterpenoid saponins, flavonoids, phenolic acids, triterpenic steroids, amino acids and sugars, extracts from this plant are widely used. *Centella*´s free radicals show anti-inflammatory activity and affect *Stratum corneum*, which is the outermost layer of the epidermis, consisting of dead cells, by hydration and epidermal barrier function. Important isolated constituents are terpenoids such as asiaticoside, centelloside, madecassoside, moside, thankuniside, sceffoleoside, centellose, as well as triterpenic acids such as asiatic, centellic, madecassic and terminolic acids. From this list of chemical constituents, asiaticoside, madecasosside, asiatic acid and madecassic acid are very important due to their dermatological and pharmacological activity. Other compounds, such as flavonoids, phenolic acids, amino acids, sugars, vitamins and essential oils and other volatile constituents in about 0.1% concentration are present in *C. asiatica*. The most important part of therapeutic activities of this plant are antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic effects and in dermatology is used for treating small wounds, hypertrophic wounds as well as burns, psoriasis and scleroderma. The constituents of *C. asiatica* can increase the blood microcirculation in the skin and prevent excessive accumulation of fat in cells (Ratz-Lyko *et al*., 2016). No less important feature is supporting the formation of collagen and decreasing formation of stretch marks and inflammatory reactions in curative medicine by ointments. Notable activity of 1% *C. asiatica* extract cream has been reported because of improving wound healing of chronic ulcer in width, length and depth after 7, 14 and 21 days of use of the product. In oral application as a capsule, it has been proved to be effective in promotion of wound healing and suppressing scars in patients with diabetes-related wounds, with no serious side effects (Somboonwong et al., 2012). Because of antioxidant functions, *C. asiatica* affects the human nervous system as it reduces oxidative *in vivo* and *in vitro* stress. *In vitro* promotes dendrite arborisation and elongation and protects the neurons from apoptosis. *In vivo* studies have shown that the whole extract and individual compounds of *C. asiatica* have a protective effect against variety of neurological diseases. Alzheimer's disease, Parkinson's disease, learning and memory enhancement, neurotoxicity and other mental illnesses such as depression and anxiety, and epilepsy were the aims of most *in vivo* studies. There are a lot of products on the market, that are sources of *C. asiatica* extract such as creams, oils and other drugs e.g. Cicapair cream (Lokanathan *et al*., 2016).

2.7. Tea tree oil

Melaleuca or Ti tree oil is distilled from an evergreen tree or a tall shrub named *Melaleuca alternifolia* of family Myrtaceae. It is consisting of monoterpenes, sesquiterpenes, and their associated alcohols. Originating in Australia, this species is up to 5 meters tall with leaves 2-5 cm long and blooms with white flowers. This very durable deep-rooted tree became popular in recent decades for its antiseptic and anti-inflammatory effect. Process of gaining tea tree oil is steam distillation from the leaves and terminal branches. From the distillate, the clear to pale yellow oil is separated with typical yield of 1 to 2% of wet plant material weight. Methods such as the use of microwave technology have been considered, however has not been utilized on a commercial scale. As an antimicrobial agent, the crushed leaves were inhaled to treat coughs and cold or were sprinkled on wounds, after which a poultice was applied (Carson *et al*., 2006). Tea tree oil also has abilities to inhibit bacteria such as *S. aureus* and *E. coli*. Clinical studies with tea tree oil products have shown efficacy for a range of superficial infections including acne, cold sores, tinea, and oral candidiasis plus *M. alternifolia* has antiviral and antifungal activity too (Hammer *et al*., 2012). In curative cosmetics, tea tree oil is used as creams, gels, body milks and shampoos for treating small wounds, burns, acne, insect bites and as a disinfection (Thomas *et al*., 2016).

3. Samoan traditional medicine

Samoa (officially Independent State of Samoa) is country in the central South Pacific Ocean, among the westernmost of the island countries of Polynesia and according to legend, Samoa is often called the ´´Cradle of Polynesia´´. Its culture is the main source the whole Polynesian culture and the dance, music, and visual art is remarkable throughout Southeast Asia. The country's international image is that of a tropical paradise inhabited by tourist-friendly flower-wreathed peoples. In 1962 gained Samoa independence on from New Zealand however remains under the government of Commonwealth. The country was known as Western Samoa until 1997. Its capital and main commercial centre is Apia, on the island of Upolu (Briney, 2018, Foster, 2018). The largest island of Samoan archipelago is Savai´i covering area of 1,707 km^2 and reaching maximum elevation of 1, 858 m at Mount Silisili, a volcano located in the middle of the island. Upolu, the other large island, lies about 16 km to the east across the Apolima Strait. Upolu is more elongated and uneven in shape than Savai‘i and has lower average elevations. Samoan islands are rocky and are formed by volcanic activity. Coastline is lined by coral reefs and lagoons. Samoa's volcanic soils support lush vegetation but are easily eroded by runoff. Large number of local plants, animals, winds etc. have their own local names. Samoans traditionally had a pantheistic religion, where family elders performed most rituals; they appear not to have had a dominant priestly class. However, they adopted Christian teaching thanks to contact with Europeans and even the more remote villages-built churches, often of grand proportions. Since the region was first settled, the most of inhabitants still live on coastal villages. This means that only 20 % of all people here lives in urban area. (CIA, 2018, Foster, 2018).

Traditional medicine is described as a group of health care practices and products with a long history of use referring to medical knowledge developed by indigenous cultures, incorporating plant, animal and mineral-based medicines, spiritual therapies and manual techniques designed to treat illness or maintain wellbeing. The major herbal medicines are used as ointments and dressings and are applied onto wounds and to treat skin problems. The World Health Organization (WHO) defines traditional medicine as the summary of the knowledge, skills and practices based on the theories, beliefs and experiences from indigenous to different cultures. Whether explicable or not, they are used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. Traditional medicine is practiced sometimes outside of allopathic medicine, which is known as biomedicine or Western medicine that is mainly used in developed world. Many cultures use it as a comprehensive system of health care refined over hundreds or thousands of years. Commonly known systems of traditional medicine are Indian (Ayurveda) medicine, traditional Chinese medicine, and traditional Arabic (Unani) medicine. In the Pacific regions such as, Melanesia, Micronesia and Polynesia, traditional medicine is primarily used for wound healing and treating ghost sicknesses. Nevertheless, folk remedies are also used for treating classic ailments, such as diarrhoea, cough, fever etc. (Abbott, 2014, WHO, 1998).

3.1. History of Samoan traditional medicine

There is a very little information about the early practices of Samoan medicine. The first significant encounters were missionaries; however, their reports were very brief. Before arrival of Europeans, health of ancient Samoans was generally very good. This was thanks to long isolation from the rest of the world so infectious diseases like measles, mumps, and whopping cough were completely absent. Noticeable diseases that were not present are for instance cholera, smallpox and venereal diseases. Despite the Europeans got quite used to them, for Samoans were much deadlier. Before accepting Christianity, Samoans believed in many gods. Every village had its own god but, similar were ´´ghosts´´. It appears that in pre-European era, most of the healing was by messages, incantations and consultation with the gods believed to be responsible for the illness. Prior to 1830, the healing process was in the hands of a priestly class called *taulaitu* (´´anchor of the gods´´), because they were believed to have connection with the gods. Once a person was afflicted with an illness of unknown origin, the patient was taken to the priest-healers. If this was impossible, the *taulaitu*s were supposed to come to the patient´s home. Payment was presented by giving a gift to a healer (Whistler, 1996). Turner (1861) described a healing process of priestess of Alaiva.

Her usual mode of acting the doctor was, first of all, to order down all the cocoa-nut leaf window-blinds of one end of the house. She then went into the darkened place. Presently that end of the house shook as if by an earthquake, and when she came out, she declared what the disease was, and ordered corresponding treatment – the result was that some recovered, and some died.

There where many ways and many variations of treatments of every single healer. On the other hand, common way of every *taulaitu* was, that they somehow communicated with gods, or made it to look so and praised them to spare the patient. However, a little is known about ancient Samoan religion. Ailments affecting ancient Samoans can be divided into two broad categories. At first wounds, burns, rashes and all medical problems, where the cause was obvious. Secondly it was ailments occurring within the body and origins were unclear. First category could be healed by almost everyone and could be characterized as folk medicine, on the other hand, the second group was handled by specialists, who had to make diagnosis and its cause. It was very difficult process and nowadays, despite all the modern technology, the causes of some ailments remain a mystery. In history, the most serious infectious diseases were elephantiasis and its cause (filiaris) and tetanus. Diseases such as hypertension, diabetes and cancer were also present (Whistler, 1996, 2000). Herbal medicine itself possess lack of information on what ancient Samoans used medicinal plants. Something different is painted on a picture by Heath (1840), where plants were used primarily on external ailments and just a little on the internal ones. It looks like, that prior to European presence in Samoa, most of the healing was by massage, incantations, and consultations with the gods, who were believed to be responsible for the illnesses. Medicine taken internally were primarily purgatives or to repel so called *aitu* – those that were believed to cause sickness or impede recovery. Very wide spread plant used in traditional medicine was *Bambusa vulgaris* or *Phyllostachys edulis*. Its bamboo shoots were used for treating of burns and rashes. Sea water presented another powerful agent. It was used for healing of cuts by washing them and after that, they were bounded with leaves, most likely leaves of medicinal plants (Whistler, 1996).

3.2. Traditional medicinal practices

Very traditional and old practice are massages. They are applied mostly on sprains, fractures, strains and body pains such as headache and backache. The general term for massage is *fofo* as well as for the people practising it. However, for everyday pains, the Samoans are taught the basic of treatments. Oils are usually involved in massages for reducing friction. Specialist called *fogau* are healers who treats fractures. These healers are mostly men, in contrast of the herbal practitioners who are females. The reason is because manipulation with broken limbs requires more strength. They set the limbs by manipulating with them to proper position and angle. Sometimes they are immobilized by wrapping it with stiff rods split from coconut frond stalk. The injured area is then massaged and covered with wet compresses to reduce swellings. Before the massage itself begins, *fofo*s feel the injured or painful area with fingertips. There are three or four types of massages, depending of the diagnosis. The most physical is called *tu´itu´i,* including vigorous hitting of the affected area with the fist, the palms or the sides of the open hand. *Lomilomi* is less severe and is characterized by application of pressure or kneading with the knuckles of the fists. Knuckles are moved between the muscles in circles. The gentlest kind is *milimili.* This technique involves pressure with the fingertips or palms, with Samoan oils to reduce the friction. It is usually applied to boils which cause them to burst and in cases, where more vigorous massage would increase the pain. The fourth type is called *eneene* or *ene*, described as a ´´light touching, almost pinching of the skin with the fingertips´´. Incantation is another typical practice in healing, practised by the priest-healers. It was used at least for ailments, such as *mo´omo´o*, which a headache located on the side the head. Although this practice is over 140 years old, it is still used today with, according to Moyle (1974), spear, staff of pair of knives. Moyle also stated, that usage of incantations in treating hiccups (*to´omaunu*), a headache called *fe´e*, a fishbone stuck in throat (*lava*), a swelling in the groin (*puga*), etc. Nevertheless, incantations are still used in present days. (Whistler, 1996, 2000). Treatment of Possession (*Ma´i Aitu*) is one of the most studied type of Samoan medicinal care. There are used mostly non-medicinal treatments but the religious ones. At first, the healer must find whether the origin of possession is genuine or the patient fakes it. Once the healer finds it real, he/she will try to find who is the ´´ghost´´ (*aitu*). The healing process is dangerous to the healer as well as he/she is supposed to apply on skin a plant infusion as a repellent agent. *Aitu*s are believed to residue in four places in the body – ears, armpits, back of the knees, top of the toes. Medicinal plants are firstly applied to ears, believing to move the ghost to the armpits, then to the knees and so on. When a proper prayer is said, then the healer tries to talk with the ghost trying to identify it and learn the reason of possession. When there is no response, the medicinal plants are dripped into eyes, ears, mouth and nose and/or the infusion is given as a sponge bath. Sometimes, the patient is wrapped into old clothes in believes that the ghost will be ashamed of talking (Whistler 1992, 1996). Samoa has two different systems of medical care – Samoan and the Western one. However, nowadays the Western systems is officially sanctioned and in the recent years, there has been an effort for combining these two medical cares to improve the health practices. Samoan medicine itself can be divided into two categories – in the first one, the practice is done by local healers. The second group consists of untrained individuals and is known as a folk medicine. Folk medicine is based upon common body knowledge, such as first aid, massages and properties of medicinal plants, that is shared in community. However, when the ailment is more serious or of chronic aetiology, than the visiting expert is on place (Whistler, 1996). Regardless of recent improvements, major part of the medical care is still handled by local healers, because most Samoans still do not believe that many indigenous diseases can be successfully treated with Western medicine. Samoan ailments are divided into three classes called *mumu, ma´i* or *ila*. The general word for sickness is *ma´i* and *ila* includes number of unrelated childhood ailments however having no precise medical definition. It is difficult to categorize all ailments in Samoa, or even those treated by means of traditional medicine, but it is required to what is being treated with herbal medicine. First category can be named as boils. Thanks to warm and humid Samoan climate, the incidence of boils and carbuncles (a deep-seated boil usually with more than one head) is high. Another factor is diet poor in vitamins. Though it has similar origin like bacterial infection on the skin, the Samoans give it a different name. On the upper part of the body it is called *sila´ilagi* and on the lower parts, such as buttocks, anus and genitals it is called *sila´ilalo*. Boil in the armpit is called *alo´omatua*, which means ´´old woman´´ (Dittmar, 1991, Whistler, 1996). *´Oloa* is a type of wound or boil which is thought to be of supernatural origin. Nevertheless, there are even these types of natural origin like *´oloa sami*, which are believed to be infected puncture wound from sea urchin spine but could occur on land as well. *Faoaileta* (general term for blood poisoning) is often on the soles of the feet. Another ailment probably fits the best as tetanus, called *´ona*. It is a toxic bacterial infection of muscles that results from a wound from sharp objects which was in contact with the bacteria (Whistler, 1996). Similar to boils are skin sores and rasher but they tend to be on the surface, not rooted deeply. Many of them are recognized by healers and do not have name in Western medicine. They are very common in hot and humid climate of Samoa and mainly results from cuts and mosquito bites (Whistler, 1992).

3.3. Herbal medicine

There are some remedies which date to the pre-European times; some of these were widely used in Polynesia and can even be traced to Southeast Asia, including *Achyranthes aspera* (circumcision wounds), *Terminalia catappa* (stomatitis), *Sigesbeckia orientalis* (wounds), *Wollastonia biflora* (wounds), *Calophyllum inophyllum* (eye ailments), *Hibiscus rosa-sinensis* (easing childbirth), *Syzygium maluccense* (stomatitis), *Oxalis corniculata* (childhood ailments), *Morinda citrifolia* (boils and stomatitis), *Centella asiatica* (childhood ailments), *Curcuma Zonga* (sores) and *Zingiber zerumbet* (enteritis). There are other plants such as *Vigna marina* and *Euodia hortensis*, which are widely used in Polynesia for treating “ghost sicknesses" (*fakemahaki*), but evaluation of these would be difficult because of the vague and unscientific nature of the target ailments (Whistler, 1991). Minor, not life-threatening injuries, can be handled by local healers, which are commonly middle-aged or elder women. Men commonly treat injuries such as broken bones (Whistler, 1992). Basic remedies are known to nearly all Samoans however the practice of Samoan herbalism is carried on by a subspecialty of healer called *taulasea.* Samoan *taulasea* are usually women who learned their craft from their mothers or other female family relative however there are very few remaining Samoan herbalists. Still serving ones are usually very old and rarely have someone who to give their knowledge. There are significant differences between Samoan ethnomedicine and Western medicine in description of disease aetiology. As a result, many Samoan diseases are not directly translatable into Western terminology. When a healer diagnoses a disease that requires herbal treatment, he/she will immediately begin to collect the necessary plant materials, but only fresh plants are used (Cox, 1993). Onto boils, abdominal pains, to ease menstrual etc. is popular *Hernandia* spp (Dittmar, 1991, Whistler, 1996). The most common treatment is crushing leaves of some plant species and applying them onto boils, with or without the scented coconut oil called ´´Samoan oil´´. In the brackets are written traditional Samoan names. The most common used species are *Hibiscus rosa-sinensis (´aute Samoa)* or *Capsicum frutescens (polo feii).* However, *C. frutescens* is an introduced plant so it may had replaced other species. There are some other types of skin ailments like boils, such as sebaceous gland on the eyelid, which is called *matafa*. It is commonly treated with flowers and leaves of *Morinda citrifolia (nonu). Papala* is the general term for such ailments and is very similar to stomatitis or mouth sores which is called *pala* and can be treated with the bark of *mago* (*Mangifera indica*) (Whistler, 1992). General term for sores is *po´u* and variation of this is called *po´u sa* and leaves white marks on the skin. Most common plants for treatment are *Curcuma longa (ago), Cerbera manghas (leva), Coleus scutellarioides (pate)* and *Syzygium corynocarpum (seasea).* Another skin disease is yaws *(tona)* which is related to syphilis, but it is not sexually transmitted nor as serious. Treatment is made of crushed stems of *limu* (*Ramalina* sp.) which is a lichen. ´´A skin eating ailment´´ is called *manemane*. It affects skin on the palms and soles of the feet. *´Atiloto* are sores around mouth area, related to viral disease *Herpes zoster*. This can appear along a spinal cord. Large number of sores or rashes is caused by fungi. The best known is so called ringworm (*lafa*) that is treated with the leaves of plant *Senna alata (la´au fai lafa).* This remedy is effective thanks to prussic acid in the leaves. Next quite common skin disease is *Tinea versicolor(tane),* which is characterized by large and irregular blotches on the skin. Another fungal skin infection is *´utu* and is sometimes mistaken with lice, that is called the same.

The general name for rash or itchiness is *mageso*. Diaper rash of infants is called *mu* (the same word for burns). Large blister is treated with *Stephania forsteri (lau i΄atolo)*, however this plant is rare treatment is uncommon (Whistler 1992, 1996). *Mumu* is local name for swellings and inflammations but includes higher body temperature as well. Treating of inflammations is a major part of Samoan herbal medicine. Some of these ailments are not life-threatening but some are more serious and can be fatal eventually. There are *mumu* appearing only on specific part of the body, while the other types can occur everywhere. Nine types of *mumu* are currently known. Commonly used plants of treatment are *Morinda citrifolia (nonu), Psychotria insularum (matalafi), Vigna marina (fue sina), Mussaenda raiateensis (aloalo vao), Cordyline fruticose (lau ti), Hoya australis (lau mafiafia), Premna serratifolia (aloalo), Gardenia taitensis (pua Samoa), Centella asiatica (togo), Phymatosorus scolopendria (lau ΄auta)* and *Tarenna sambucina* (*ma΄anunu)*. *Nonu, matalafi* and *fue sina* are probably used mostly for inflammations though to have supernatural origin. General term for swellings is *fula* and for permanent swellings of the limbs caused by filarial worms, it is called *tupa*. Used species for treatment are *Macropiper puberulum (΄ava΄avaaitu tu), Erythrina variegata (matalafi, gatae)* and *Polyscias scutellaria (tagitagi).* As well as *nonu*, the first two species are of supernatural origin (Whistler 1992, 1996). Wounds are part of Samoan herbal culture since the ancient times. There are two local words for wounds, such as *lavea* and *manu΄a*. Two main problems of wounds are bleeding and breaching skin, which allows pathogen to enter and cause the infection. Plants applied to cuts are leaves of *Mikania micrantha (fue Saina)*, sap of *Kleinhovia hospita (fu΄afu΄a), Sida rhombifolia (mautofu)* and *Aloe vera (aloe)*. The last plant is very popular in treating wounds and burns. For circumcision wounds, plants such as *Omalanthus nutans (mamala)* and *Achyranther aspera (lau tamatama)* were applied. Now, it goes under more hygienic conditions and *lau tamatama* is quite uncommon, these usages have been largely forgotten. Burns are also very common in Samoa and it is because of cooking on open fire so it is easily accessible for little children. Very popular is mentioned *aloe* and also *Schizostachyum glaucifolium (΄ofe)*. Stings and puncture wounds are treated same as wounds and burns however there is no recognized treatment for being stung by poison fish. Eye ailments are generally called *ma΄i mata*. Most common is boil on the eyelid, which mentioned above, in the description of boils. Injury or trauma caused to the eye is named *mata pa΄ia* and is caused by foreign object in the eye, a piece of dirt or sawdust being lodged in. Torn eye *(Mata masae)* is another type of ailment and involves pain and insufficient amount of fluid. Pants used for treatment are sap from petiole of *Erythrina variegate (gatae)*, flowers and leaf stalks from *Morinda citrifolia (nonu)*, sap from the petiole of *Artocarpus altilis (΄ulu)*, whole plant of *Rorippa sarmentosa* *(a´atasi)* and *Laportea interrupta (ogoogo tea)*, leaves of *Bischofia javanica (´o´a)*, sap from the bark of *Hibiscus tiliaceus (fau)* and *Kleinhovia hospita (fu´afu´a)* (Whistler 1992, 1996). However, it is not always necessary to visit a healer in a case of commonly known issue, such as bleeding cut. Ailments such as wounds, swellings and sores were probably treated in a more scientific way (Uhe, 1974, Whistler, 1992). Likewise, in other Polynesian states, the concept of Samoan ´´ghost sickness´´ is very similar. On the other hand, Samoa appears to be weak in making diagnosis. It is usually made backwards depending on effectivity of given medicine or treatment. Sometimes the patient can be taken to the hospital until the cure is found, but it is not usual for the patient to undergo treatment at the hospital meanwhile receiving traditional medicine. Many plants that are used in Samoa are usually used in different countries like Tonga however they are used for different ailments. Inhibition of bacteria growth is not always the same as is the effect of the same plant in Tonga. Samoan and Tongan medicines are prepared similarly. Massages are practiced for strains, soreness and fracture and are important part in bone setting, as well as in the rest of Polynesia (Whistler, 1992).

4. Material and methods

4.1. Plant materials

Different parts of a total number of 15 medicinal plants were collected in various locations of the territory of Independent State of Samoa in September 2015. Prof Kokoska authenticated the species and their voucher specimens have been deposited in the herbarium of the Department of Botany and Plant Physiology of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague (CZ). The selection of plants and their collected parts was based on literature data on their uses in traditional Samoan medicine for the treatment of wounds, burns and sores (Castro and Tsuda, 2001; Sotheeswaran *et al*., 1998; Whistler, 1996, 2000). For all species assayed, the scientific names, families, local names, voucher specimen codes, collection sites GPS coordinates collected parts (plant samples) and the uses in folk medicine are given in Table 1.

4.2. Preparation of plant extracts

Although the most common form of folk Samoan treatment is crushed fresh plant material applied directly to the wound (Whistler, 1996), the ethanol has been chosen for extraction of plant samples since it is an efficient solvent for herbal drugs with well-established tradition in herbal medicine (Kelber *et al*., 2017). Each dried plant sample was finely ground into powder using Grindomix mill (Retsch, Haan, DE), of which 15 g was then extracted in 450 mL of 80% ethanol (Penta, Prague, CZ) for 24 h at room temperature using laboratory shaker (GFL, Burgwedel, DE). Extracts were subsequently filtered and concentrated using rotary evaporator (Büchi Labortechnik, Flawil, CH) *in vacuo* at 40 °C. Dried residues were finally diluted in 100% dimethylsulfoxide (DMSO) (Penta, Prague, CZ) to obtain stock solution of the final concentration 51.2 mg/mL and stored at -20 °C until their use. Dried residue yields (%) are also shown in Table 1.

4.3. Bacterial strains and media

The antibacterial activity of prepared extracts was determined against the main representatives of bacterial pathogens present in acute and chronic wounds and burns (Bowler *et al*., 2001), including 5 anaerobic and 3 aerobic bacterial species dominated by 16 strains of *S. aureus*. Standard American Type Culture Collection (ATCC) strains, namely *Bacteroides fragilis* ATCC 25285, *Escherichia coli* ATCC 25922, *Propionibacterium acnes* ATCC 11827, *Pseudomonas aureginosa* ATCC 27853, *S. aureus* ATCC (25923, 29213, 33591, 33592, 43300, and BAA 976) were obtained from Oxoid (Basingstoke, UK). German Resource Centre for Biological Material (DSMZ) (Braunschweig, DE) provided cultures of *Clostridium difficile* DSMZ 12056, *Clostridium perfringens* DSMZ 11778, and *Finegoldia magna* DSMZ 2974. Six clinical isolates (SA1-10) were provided on agar plates from the Motol University Hospital (Prague, Czech Republic). The identification of clinical isolates was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry as it is described in Rondevaldova *et al*. (2018). With exception of clostridial strains stored in Clostridial Reinforced medium (Oxoid, Basingstoke, UK) with glycerol at -80 °C, all cultures of aerobic bacteria were maintained at Mueller-Hinton agar (Oxoid, Basingstoke, UK) and stored at 4°C until use. Mueller-Hinton broth (Oxoid, Basingstoke, UK) was used as a growth medium for all tested aerobic bacteria (cation-adjusted for *S. aureus* strains), whereas anaerobes were cultured in Wilkins-Chalgren broth (Oxoid, Basingstoke, UK) enriched by 5 g/L soya peptone and 0.5 g/L cystein under anaerobic condition. The pH of media used was equilibrated to the final value of 7.6 using Trizma base (Sigma-Aldrich, Prague, Czech Republic).

4.4. Broth microdilution method

Minimum inhibitory concentrations (MICs) were determined by the standard broth microdilution method using 96-well microtiter plates according to the previously approved guidelines and recommendations for susceptibility testing of aerobic (Clinical and Laboratory Standards Institute, 2009) and anaerobic (Hecht, 1999) bacteria with slight modifications proposed by Cos *et al*. (2006) for the effective assessment of anti-infective potential of natural products. In each microplate, serial extracts dilutions were performed in appropriate growth medium (100 µl) making concentrations ranging from 4 to 512 µg/mL, by using the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, CE) and a manual multichannel pipette (Eppendorf, Wesseling-Berzdorf, DE) in case of aerobic and anaerobic bacteria, respectively. All bacterial cultures were diluted to contain 1.5×10^8 CFU/mL and the plates were inoculated with bacterial suspension using a 96-pin multi-blot replicator (National Institute of Public Health, Prague, CZ). Microplates were then incubated for 24 h at 37 °C. The plates inoculated with anaerobes were prepared and incubated in Whitley A35 Anaerobic Workstation (Don Whitley Scientific, West Yorkshire, UK). Bacterial growth was determined by the absorbance measurement in Cytation 3 Imaging Reader (BioTek, Vermont, USA) at 405 nm. The lowest extract concentration showing at least ≥80% reduction of microbial growth compared to the positive growth control was considered as MIC. The oxacillin (Sigma-Aldrich, Prague, CZ), an antibiotic commonly recommended for treatment of wounds (Bowler *et al*., 2001), was dissolved in distilled water and used as a positive control. Oxacillin was also used as marker of methicillin resistance of *S. aureus* strains. The solvents used (DMSO and ethanol) did not inhibit bacterial growth at the concentrations tested (1%). All tests were performed as three independent experiments each carried out in triplicate and the results are presented as median/modal values.

Table 1. *Ethnobotanical data on plant species tested*

Latin name [Family]	Voucher specimen	Collection place (GPS coordinates)	Local name	Part traditionally used and tested	Extract yield (%)	Ethnomedicinal use
Barringtonia asiatica (Lecythidaceae)	2403KBFR0	13.8657247S, 171.7597639W	Futu	Seed	13.9	Skin sores (Whistler, 2000)
Cerbera manghas (Apocynaceae)	2405KBFR0	13.8490414S, 171.7751425W	Leva	Leaf	22.2	Skin sores and infections (Whistler, 2000)
Commelina diffusa (Commelinaceae)	2406KBFR0	13.5369394S, 172.3952458W	Mauutoga	Leaf	9.7	To staunch bleeding of cuts (Whistler, 2000)
Inocarpus fagifer (Leguminosae)	2415KBFR0	13.9841172S, 171.7158883W	Ifi	Leaf	11.9	Wounds (Sotheeswaran et al., 1998)
Kleinhovia hospita (Malvaceae)	2416KBFR0	13.8968333S, 171.5730494W	Fuafua	Bark	15.2	Cuts and wounds, to staunch bleeding (Whistler, 1996, 2000)
Mikania micrantha (Compositae)	2419KBFR0	13.9954850S, 171.9223325W	Fue saina	Leaf	23.1	Wounds, to staunch bleeding and prevent infection (Whistler, 1996, 2000)
Omalanthus nutans (Euphorbiaceae)	2423KBFR0	13.9972500S, 171.7168467W	Mamala, Fanuamamala	Leaf	25.1	Circumcision wounds (Sotheeswaran et al., 1998; Whistler, 1996, 2000)
Peperomia pellucida (Piperaceae)	2424KBFR0	13.9856886S, 171.9208125W	Vao vai	Aerial part	2.8	Boils (Whistler, 2000)
Phymatosorus scolopendria (Polypodiaceae)	2425KBFR0	13.8290075S, 171.7667367W	Lau maga maga	Leaf	22.6	Hard-to-cure wounds (Sotheeswaran et al., 1998; Whistler, 2000)
Piper graeffei (Piperaceae)	2427KBFR0	13.8997278S, 172.0259856W	Fue mangoi	Leaf	16.6	Infected wounds (Whistler, 2000)
Plectranthus scutellarioides (Lamiaceae)	2436KBFR0	13.8930631S, 171.5806925W	Pate	Leaf	12.8	Skin sores (Whistler, 2000)
Premna serratifolia (Lamiaceae)	2431KBFR0	13.8296856S, 171.7547908W	Aloalo	Bark	15.5	Wounds and sores (Whistler, 2000; Castro and Tsuda, 2001)
Psychotria insularum (Rubiaceae)	2433KBFR0	13.8657247S, 171.7597639W	Matalafi	Leaf	21.9	Infected wounds (Whistler, 2000)
Schizostachyum glaucifolium (Poaceae)	2435KBFR0	13.8560900S, 171.7732883W	Ofe	Leaf	11.5	Burns (Whistler, 2000)

5. Results

5.1. Susceptibility of standard strains of *S. aureus*

Growth-inhibitory effect of extracts tested against standard strains of *S. aureus* is shown in *Table 2*. Although, the lowest value of MIC in growth-inhibitory effect against standard strains of *S. aureus* (SA 33592) was 4 µg/ml for plant extracts of *P. graeffei* and *S. glaucifolium*, the plant extracts effective against all strains tested was obtained from *P. insolarum* with MIC of 64 µg/mL against SA 25923 and SA 33592. In the other four cases, the activity was moderate with MICs ranging from 128 µg/ml to 256 µg/ml. For *P. insolarum* extract, the whole scale of active concentrations was 64 – 256 µg/ml. O. nutans was the second most effective extract. The MIC of 128 µg/ml was gained from all strains except from SA 25921 where the activity was at concentration of 256 µg/ml. The active scale in this sample was 128 – 256 µg/ml. The extract of *M. micrantha* produced similar effect as extract of O. nutans. Activity of this species is little bit lower with the MICs range of 256 – 512 µg/ml. The lower concentration activity was observed in four strains (BAA 976, SA 25923, SA 29213, SA 33592). In the other two cases, the activity was recognized at the higher concentration of 512 µg/ml. The fourth active plant extract was obtained from *K. hospita*. In this case, there is quite wide range of activity (64 – 512 µg/ml). SA 33591 was found as the most susceptible strain with MIC of 64 µg/ml, followed by SA 29213 (128 µg/ml). The rest of the strains was less sensitive, with activity of 512 µg/ml. On the other hand, the weakest activity was observed in case of extract from *P. serratifoila*, which shows activity only against SA 25923 at highest concentration tested (512 µg/ml). *P. serratifolia* was followed by *P. scutellarioides* and *I. fagifer*. The rest of the extracts show moderate activity which is shown in the table below.

Table 2. In vitro growth-inhibitory effect of ethanol extracts form Samoan medicinal plants against standard strains of Staphylococcus aureus

Strain	Plant species and antibiotics/minimum inhibitory concentrations (µg/ml)														
	BA	CD	CM	IF	KH	MM	ON	PG	PhS	PI	PP	PrS	PS	SG	OX
BAA 976	512	512	256	512	512	256	128	512	512	256	256	>512	256	512	16
25923	512	512	256	512	512	256	256	128	256	64	512	512	>512	512	0.5
29213	512	512	512	512	128	256	128	256	256	128	512	>512	>512	256	0.5
33591	256	>512	512	>512	64	512	128	>512	512	64	512	>512	>512	>512	>512
33592	512	512	512	>512	512	256	128	4	256	128	512	>512	>512	4	8
43300	512	>512	512	>512	512	512	128	>512	512	128	128	>512	>512	512	64

Abbreviations: BA – *Barringtonia asiatica,* CD – *Commelina diffusa*, CM – *Cerbera manghas*, IF, – *Inocarpus fagifer*, KH – *Kleinhovia hospita*, MM – *Mikania micrantha*, ON – *Omalanthus nutans*, OX – *Oxacillin*, PG – *Piper graeffei*, PhS - *Phymatosorus scolopendria*, PI - *Psychotria insolarum*, *PP – Peperomia pellucida*, PrS – *Premna serratifolia*, PS - *Plectranthus scutellarioides*, SA – *Staphylococcus aureus*, SG - *Schizostachyum glaucifolium*

5.2. MIC of clinical isolates of *S. aureus*

The second group of tested microorganisms was represented by 10 clinical isolates of *Staphylococcus aureus*, shown in *Table 3*. The most active plant extract was obtained from *O. nutans*, with MIC of 4 µg/ml in three isolates SA 8, SA 9 and SA 10. The whole active range of this extract was relatively wide, ranging from 4 µg/ml to 512 µg/ml (SA 3). The second most active plant extract is from *P. insularum*, with quite low MIC of 32 µg/ml in SA 3 and SA 9. SA 2, SA 8 and SA 10 were still very susceptible, with MIC of 64 µg/ml. Active scale here is narrower (32 – 512 µg/ml) than in the previous case, but it is still quite wide. SA 1 and SA 7 were the most resistant with MIC of 512 µg/ml. *P. insularum* is followed by *S. glaucifolium*, with the MIC of 4 µg/ml against three isolates (SA 8, SA 9 and SA 10). In case of SA 3, the MIC was 8 µg/ml. However, of the total activity of *S. glaucifolium* was relatively wide (≥4). Nevertheless, SA 7 was only one strain resistant at MIC 512 µg/ml, while the rest of clinical isolates have shown moderate susceptibility. The fourth most effective plant extract was *M. micrantha*, with the same scale of activity as the previous plant extract. In contrast, the weakest activity was produced by extracts form *P. serratifolia*, *S. glaucifolium* and *I. fagifer* (MIC ≥512 µg/ml). The most susceptible clinical isolates were SA 3, SA 8, SA 9 and SA 10. For e.g. in SA 9, the most sensitive isolate, the most frequent concentration was 4 µg/ml. This concentration was in the rest of the isolates as well, however, in comparison to this one, it was not so frequent.

Table 3. In vitro growth-inhibitory effect of ethanol extracts form Samoan medicinal plants against clinical isolates of Staphylococcus aureus

Strain	Plant species and antibiotics/minimum inhibitory concentrations (µg/ml)														
	BA	CD	CM	IF	KH	MM	ON	PG	PhS	PI	PP	PrS	PS	SG	OX
SA 1	512	512	512	>512	512	512	256	>512	512	512	256	>512	>512	256	32
SA 2	512	512	512	512	256	256	128	512	256	64	512	>512	>512	512	128
SA 3	512	4	4	>512	128	4	512	4	8	32	512	>512	>512	8	64
SA 4	512	256	512	512	128	256	128	512	128	128	512	512	512	256	256
SA 5	256	>512	512	512	128	512	128	512	512	256	>512	>512	512	256	4
SA 6	512	>512	512	512	>512	256	256	256	>512	256	512	>512	>512	256	128
SA 7	512	>512	512	>512	256	>512	256	>512	>512	512	512	>512	>512	>512	0.5
SA 8	512	4	4	>512	128	4	4	4	4	64	4	>512	>512	4	32
SA 9	512	4	4	>512	256	4	4	4	4	32	4	>512	>512	4	1
SA 10	512	4	4	512	256	128	4	4	4	64	4	>512	>512	4	0.25

Abbreviations: BA – *Barringtonia asiatica*, CD – *Commelina diffusa*, CM – *Cerbera manghas*, IF, – *Inocarpus fagifer*, KH – *Kleinhovia hospita*, MM – *Mikania micrantha*, ON – *Omalanthus nutans*, OX – *Oxacillin*, PG – *Piper graeffei*, PhS - *Phymatosorus scolopendria*, PI - *Psychotria insolarum*, *PP – Peperomia pellucida*, PrS – *Premna serratifolia*, PS - *Plectranthus scutellarioides*, SA – *Staphylococcus aureus*, SG - *Schizostachyum glaucifolium*

5.3. Susceptibility of other bacterial pathogens

In this case (shown in *Table 4.*), the MIC are usually not as low as in the *S. aureus* cases as well as the width of the active scale is not so wide. In anaerobic pathogens, the valuable plant extract was shown to be *C. diffusa*, with MIC of 128 µg/ml against *P. acnes.* However, in case of *B. fragilis, C. difficile* and *C. perfiringens*, the MIC was not obtained. *E. coli, F. magna* and *P. aeruginosa* have shown sensitivity on 512 µg/ml. The scale of activity was from 128 µg/ml to >512 µg/ml. *O. nutans* is the second most active plant extract with 4 µg/ml of MIC against *P. aeruginosa*. Some susceptibility has also shown *E. coli.* The highest concentration, where MIC was not detected is >512 µg/ml, detected in 4 cases, such as *B. fragilis, C. difficile, C. perfringens* and *F. magna*. The third plant extract was *I. fagifer* with the lowest active concentration of 256 µg/ml against *F. magna*. Plant extract was active also against *B. fragilis* and *C. difficile* on concentration of 512 µg/ml. Bacteria where MIC was not detected are as follows – *C. perfringens, E. coli, P. acnes, P. aeruginosa*. Total wide of active range 256 - >512 µg/ml. *C. manghas* and *P. pellucida* was very effective against *P. aeruginosa* (4 µg/ml). However, against all other bacteria, except of *F. magna* in *C.manghas* and *E. coli* in *P. pellucida* where the susceptibility was detected at 512 µg/ml, was quite ineffective and the range of activity was from 4 µg/ml to >512 µg/ml. The less effective plants extract seems to be *P. scolopendria,* that have shown zero activity against all pathogens. The most sensitive bacterium is *P. aeruginosa* which have shown susceptibility in three plant extracts, such as *C. manghas, O. nutans* and *P. pellucida.* The most resistant bacteria were *B. fragilis* and *C. perfringens.* Sensitivity was shown only on concentration of 512 µg/ml. *B. fragilis* was inhibited by *I. fagifer* and *C. perfringens* by *P. scutellarioides.*

Table 4. In vitro growth-inhibitory effect of ethanol extracts from Samoan medicinal plants of against other wound colonising bacteria

Bacterium	Plant species and antibiotics/minimum inhibitory concentrations (µg/ml)														
	BA	CD	CM	IF	KH	MM	ON	PG	PhS	PI	PP	PrS	PS	SG	OX
Bacteroides fragilis	>512	>512	>512	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	16
Clostridium perfringens	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	2
Clostridium difficile	>512	>512	>512	512	512	>512	>512	>512	>512	>512	>512	>512	512	>512	32
Escherichia coli	>512	512	>512	>512	>512	>512	512	>512	>512	>512	512	>512	>512	>512	512
Finegoldia magna	>512	512	512	256	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	8
Propionibacterium acnes	512	128	>512	>512	>512	256	256	256	>512	128	>512	512	256	256	8
Pseudomonas aeruginosa	>512	512	4	>512	>512	>512	4	>512	>512	>512	4	>512	>512	>512	>512

Abbreviations: BA – *Barringtonia asiatica*, CD – *Commelina diffusa*, CM – *Cerbera manghas*, IF, – *Inocarpus fagifer*, KH – *Kleinhovia hospita*, MM – *Mikania micrantha*, ON – *Omalanthus nutans*, OX – *Oxacillin*, PG – *Piper graeffei*, PhS - *Phymatosorus scolopendria*, PI - *Psychotria insolarum*, *PP – Peperomia pellucida*, PrS – *Premna serratifolia*, PS - *Plectranthus scutellarioides*, SA – *Staphylococcus aureus*, SG - *Schizostachyum glaucifolium*,

6. Discussion

In the frame of this thesis, the antibacterial effect of medicinal plants used in traditional Samoan medicine for treatment of wounds has been confirmed *in vitro*. The most active plant species against both standard strains and clinical isolates of *S. aureus* were *P. insularum*, *O. nutans*, *M. micrantha*, *K. hospita* and *S. glaucifolium.*

Although there are no reports on antibacterial activity of *P. insularum* leaf ethanol extract in the literature, the methanol extract of leaves of *P. microlabastra* showed a broad spectrum of antibacterial activity in previously published experiments. It has been proved that leaves contain compounds such as flavonoids, sterols, tannins, triterpenoids (Khan *et al.,* 2001). These studies performed with taxonomically related species support the findings of our study. In recent years, several classes of natural products such as alkaloids (major compounds), coumarins, flavonoids, terpenoids, tannins and cyclic peptides have been isolated from genus *Psychotria*. Among them, alkaloid quadrigemine B isolated from *P. rostrata* showed bactericidal activity against *E. coli* and *S. aureus*. From the leaves of the same species, other alkaloids like psychotrimine and psychopentamine were isolated, which showed activity against *S. aureus* (Calixto *et al.,* 2016). Based on chemotaxonomical relationship of these species, we suppose that above-mentioned compounds can be responsible for antibacterial action of *P. insularum*.

According to our best knowledge, there are no studies on antimicrobial activity of *O. nutans,* which was the second most active plant against *S. aureus*. Khan *et al.,* (2001) reported antimicrobial activity of ethanol extracts of leaves, stem and root barks of *O. nervosum*. Flavonoids, triterpenoids, saponins and sterols were classes of compounds found in the sample. We suppose that these compounds can contribute to the antibacterial activity of *O. nutans* observed in our study.

Facey *et al.* (1999) published an article about plant *M. micrantha* used in Jamaican folk medicine for anti-bacterial activity. In this study, they reported extracting two sesquiterpenoids (mikanolide and dihydromikanolide) with activity against *S. aureus*. MIC of mikanolide was 45 mg/disc and of dihydromikanolide was 48 mg/disc. Although the concentrations tested in this study are very high, these two compounds may be responsible for antimicrobial activity of *M. micantha.* The same team published another work on the antibacterial activities of mikanolide and its derivatives (Facey *et al.*, 2010). Ghosh *et al.* (2008) published work on antimicrobial activity of *M. micrantha* containing compounds like alkaloids, flavonoids, essential oil, terpenoids etc.

The ethanol extract of *K. hospita* was fourth most active extract against *S. aureus*. However, there are no available records on activity against *S. aureus* of this plant. Nevertheless, Rahim *et al.* (2018) published a study in which they report isolation of novel cycloartane triterpenoid alkaloid, kleinhospitine E, six new cycloartane triterpenoids, three known cycloartane triterpenoids, and taraxerone from a methanol extract of *K. hospita*. Mo *et al.,* (2014) found two new cycloartane triterpenoids - (23R)-21,23:23,27-diepoxycycloarta-1,24-diene-3,27-dione and (3 alpha) -(alpha-L-arabinopyranosyloxy)-1 alpha-hydroxy-23-oxocycloartan-28-oic acid in this species. These alkaloids could be responsible for its antimicrobial activity. In number of studies, triterpenoids together with alkaloids have been considered as compounds responsible for antimicrobial activity (Mo *et al.,* 2014).

According to our best knowledge, there are no studies on *S. glaucifolium*. Few researches have been done on genus *Schizostachyum* where *S. brachycladum* showed contain of lignin, cellulose and carbon (Negara *et al.,* 2017). However, what causes the microbial activity is still unknown and there is need for further research on this genus to find its phytochemical composition.

P. pellucida has been studied by Sunday *et al.* (2007). His team has done research of stem and leaves essential oils. MIC as well as minimum bactericidal concentration of the essential oils was much lower (0.2 µg/ml) than in our case (aerial parts used). In stem essential oils were present monoterpenoids and sesquiterpenoids. Monoterpenoids were found also in leaves essential oils. Compounds such as terpenoids are common for medicinal plant. In leaves of *B. asiatica*, there are triterpenes - germanicol caffeoyl ester and camelliagenone (Ragasa *et al.,* 2011) and bark contins triterpene (3 beta,11 alpha)-11-hydroxyolean-12-en-3-yl palmitate (Ragasa *et al.,* 2012), that have shown antimicrobial activity against *S. aureus*.

In case of *in vitro* growth-inhibitory effect of ethanol extracts from Samoan medicinal plants of against other wound colonising bacteria the most active plant extracts were *C. diffusa, O. nutans, P. scutellarioides, I. fagifer* and *P. insularum*.

According to our best knowledge, there are no antimicrobial studies on *C. diffusa* were found. In the literature, there are only reports on *C. nudiflora* showing its antibacterial activity. Crude aqueous extract has been found to contain alkaloids, flavonoids, phenolics, saponins, steroids, tannins and carbohydrates. Regarding to its antimicrobial activity, aqueous *C. nudiflora* extract showed growth-inhibitory effect against food pathogenic bacteria with MIC 12.25 μg/ml against *P. aeruginosa* (Kuppusamy *et al.*, 2015). In the work published by Cerdeiras *et al.* (2001), the antibacterial activity of chloroform and aqueous extracts of *C. erecta* has been detected against Gram-negative and Gram-positive microorganisms. However, no particular compounds were identified. Khan *et al.* (2011) reported presence of alkaloids, anthraquinones, ketonic compounds and terpenoids in *C. diffusa*, and the activity was observed only against *S. aureus* using disc diffusion method. In my thesis, the plant extract showed activity against other pathogens as well. The differences can be caused by different methods that were used in studies.

In case of *P. scutellarioides*, no studies were found on its activity on tested pathogens; however, several studies have been performed on its compounds. Cretton *et al*. (2018) discovered six previously undescribed diterpenoids (scutellarioidone A, scutellarioidone B, scutellarioidone C, 6-acetylfredericone B, scutellarioidone D, scutellarioidolide A) with anti-inflammatory and antiproliferative effects. Kubínová *et al*. (2018) published work on new diterpenoid glucoside and flavonoids within *P. scutellarioides.* The aerial parts of the plant were collected, frozen and extracted with methanol for 24 h at room temperature. These compounds were diterpenoid (13S,15S)-6β,7α,12α-trihydroxy-13β,16-cyclo-8-abietene-11,14-dione 7-O-β-D-glucoside, flavonoid apigenin 7-O-(3″-O-acetyl)-β-D-glucuronide, flavonoid apigenin 5-O-(3″-O-acetyl)-β-D-glucuronide, caffeic acid, luteolin 5-O-β-D-glucoside, rosmarinic acid and galantamine. Wardojo *et al.* (2018) also published work on phytochemical screening and proved that *P. scutellarioides* contained flavonoid, saponin, and polyphenols. According to studies, terpenoids and flavonoids are effective against microorganisms.

According to our best knowledge, there are no reports on antimicrobial activity and chemical composition of *I. fagifer*. Studies on *O. nutans* and *P. insularum* are described above. Study on plant extract from *B. asiatica* has been reported by Ragasa *et al.* (2011, 2012), who isolated a new triterpene - (3 beta,11 alpha)-11-hydroxyolean-12-en-3-yl palmitate that showed activity against *E. coli.* The same compound, together with triterpenes germanicol caffeoyl ester and camelliagenone, produced inhibitory effect against *P. aeruginosa*. In case of my thesis, the only susceptible bacterium was *P. acnes.* Chemical analysis of leaves of *C. manghas* has been made by Zhang *et al*. (2010), who obtained compounds such as p-hydroxybenzaldehyde, benzamide, n-hexadecane acid, monoglyceride, loliolide, β-sitosterol, cerberin, neriifolin, cerleaside A and daucosterol. For example, Rageeb *et al.* (2016) published a book describing effectivity of p-hydroxybenzaldehyde, benzamide, n-hexadecane acid, cerberin, neriifolin, cerleaside A and daucosterol on skin against scabies and prurigo. It is possible that these compounds are responsible for activity of *C. manghas*. In case of *M. micrantha,* few studies have been done against *E. coli*. Perez-Amador *et al.* (2010) published phytochemical and pharmacological study on *M. micrantha* containing essential oils consisting of linalool and α-pinene. Ethyl acetate extract of aerial plant exhibited significant antibacterial and anti-inflammatory properties. According to James *et al*. (2018), the compounds such as tannins, polyphenols, alkaloids, saponins and triterpenoids may be responsible for the antimicrobial activity of the plant. The same study proved activity against *P. aeruginosa* as well. On the other hand, in my study, the sensitive pathogen was only *P. acnes.* This could be because of usage of different parts of the plant. Okoh *et al.* (2017) published article describing antibacterial properties of the leaves and stem essential oils of *P. pellucida* containing bioactive compounds such as terpenoids with antibacterial properties against *E. coli* (and *S. aureus* as well).

7. Conclusion

In the frame of this thesis, the antibacterial effect of medicinal plants used in traditional Samoan medicine for treatment of wounds has been confirmed *in vitro* against broad spectrum of wound and burn colonizing pathogenic bacteria such as *B. fragilis*, *C. difficile*, *C. perfringens*, *E. coli*, *F. magna*, *P. acnes*, *P. aeruginosa* and *S. aureus*. Considering the susceptibility of all bacteria tested, the most effective plant species were *Cerbera manghas, Mikania micrantha, Omalanthus nutans, Psychotria insularum,* and *Schizostachyum glaucifolium.* In case of clinical isolates of *S. aureus,* the most active plant extracts were from *O. nutans, P. insularum* and *S. glaucifolium*. Similarly, *P. insularum* followed by *O. nutans* and *M. micrantha* were the most effective extracts against standard strains of *S. aureus.* The supposed results of the thesis can be used by pharmaceutical and cosmetic industries for development of new antimicrobial preparations for wound and burn healing. However, detailed phytochemical and pharmacological studies are needed for identification of compounds that are responsible for antibacterial effect of the most active plant species tested in this study.

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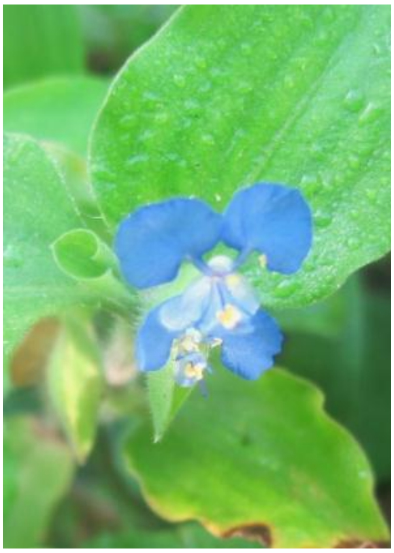
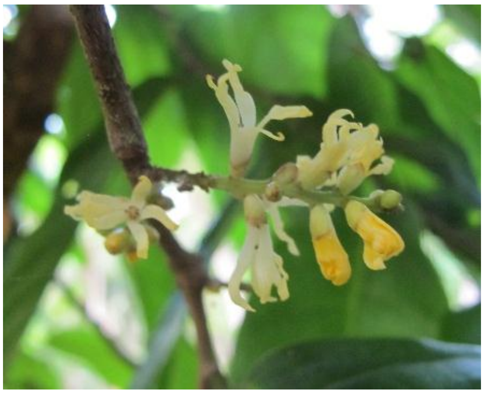
9. Appendix – photographic illustrations of tested plants

Barrintonia asiatica

Cerbera manghas



Commelina diffusa



Inocarpus fagifer

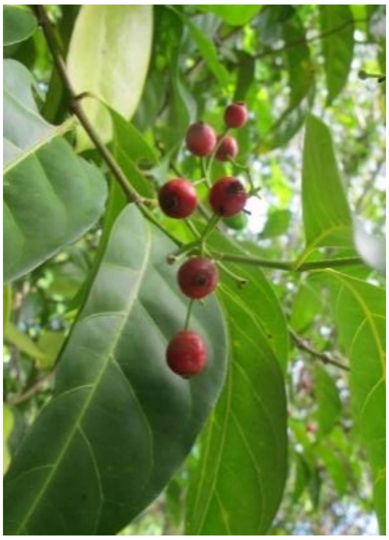
Kleinhovia hospita

Mikania micrantha

Omalanthus nutans

Piper graeffei

Phymatosorus scolopendria



Psychotria insularum

Plectranthus scutellarioides

Premna serratifolia

Schizostachum glaucifolium