DETERMINATION OF PHYTOCHEMICAL IN EXTRACTED PUNICA GRANATUM PEELS AND ITS EFFICACY OF HEALING BURN INJURIES

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I declare that this thesis entitled “Determination of Phytochemical in Extracted Punica Granatum Peels and Its Efficacy of Healing Burn Injuries” is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Natural plants have been used as a traditional medicine all over the world. One of these natural plants is pomegranate (punica granatum). This study aimed to determine the phytochemicals from Pomegranate peels that can heal burn wounds by extracting the peels. Additionally, this study is investigating the efficiency of Pomegranate (Punica granatum) in curing the burn wounds compare to the commercial ointment. Extracting the pomegranate start with drying the peels under room temperature, followed by drying in the oven for 48 hours with the temperature of 50^{o}C, then grinding them. However, after the preparation of the ointment from the peels fine powder, four different samples of rats were burnt and treated. The result of the extract and the analysis showed that pomegranate is reach by an active constituent such as Saponin, Glycosides, Steroids and Terpenoid which play the main role in healing the burn wounds. This claimed is supported by the result of the treatment observations of the rats' samples which proof that the efficiency of using pomegranate ointment in curing the wounds is higher and faster than the commercial ointment. As conclusion the objective of this study has been achieved, and positive result has been obtained.

ABSTRAK

Tumbuhan semula jadi telah digunakan sebagai ubat tradisional di seluruh dunia. Salah satu daripada tumbuh-tumbuhan semula jadi adalah delima (Punica granatum). Kajian ini bertujuan untuk menentukan fitokimia dari kulit delima yang boleh menyembuhkan luka terbakar dengan mengekstrak kulitnya. Tambahan lagi, kajian ini menyiasat kecekapan Delima (Punica granatum) dalam menyembuhkan luka terbakar berbanding dengan ubat komersial. Prosedur untuk mengekstrak delima bermula dengan pengeringan kulit di bawah suhu bilik, diikuti dengan pengeringan dalam ketuhar selama 48 jam dengan suhu 50^{o}C, selepas itu dikisarkan. Walau bagaimanapun, selepas penyediaan ubat dari serbuk halus delima, empat sampel tikus yang berbeza telah dibakar dan dirawat. Hasil ekstrak dan analisis menunjukkan bahawa delima mengandungi konstituen aktif seperti Saponin, glikosida, Steroid dan terpenoid yang memainkan peranan utama dalam penyembuhan luka terbakar. Dakwaan ini disokong oleh hasil pemerhatian rawatan sampel tikus yang terbukti bahawa potensi ubat hasil dari ekstrak delima dalam menyembuhkan luka adalah lebih tinggi dan lebih cepat daripada ubat komersial. Sebagai kesimpulan, objektif kajian ini telah dicapai, dan keputusan yang positif telah diperolehi.

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CHAPTER I

INTRODUCTION

1.1 Background of Study

The life is filled by the different types of diseases. However, human has been searching for the solutions for those diseases till today. Natural plants have been used as a traditional medicine all over the world. The uses of the natural plants were investigated in term of its ability to cure a specific type of diseases (Nizamuddin et al., 1982; Kapoor, 1990; Barthakur and Arnold, 199, Fengshu et al., 1992, El-Mekkawyet al., 1995). Till today, the researches on the application of natural plants as medicine and their aptitude to cure the diseases are developing. Nature has been a source of medicinal agents for thousands of years and last recent years, plenty inventions and researches has been done to analyzed the hidden value inside these nature herbs. These plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The idea of developing medicines from plants used in aboriginal medical system is much older, while in some cases direct links between a local and biomedical use exists, in other cases the relationship is much more complex (Heinrich and Gibbons 2001). Wounds and particularly chronic wounds are major concerns for the patient and clinician as well; chronic wounds affect a large number of patients and seriously reduce their quality of life. Balick and Cox (1996) reported that only 1–3% of medicines listed in Western pharmacopoeia are intended for use in the skin and for wounds, by comparison, at least one third of herbal remedies are for such use.

1.2 Research Background

Pomegranate (PG) or Punica granatum, is normally drink as juice in some countries. Pomegranate is one of the antique fruit that is widely consumed as fresh fruit and juice. The use of pomegranate fruit dates back to Biblical times and hearsays of its therapeutic qualities have echoed throughout the millennia (Longtin, 2003). Pomegranate, also easily acquired from traditional medicine markets, was usually used as an astringent agent, for eliminating parasites and as an antipyretic. The pharmacological functions of pomegranate include antioxidants, anti-tumors, anti-hepatotoxicity, anti-lip peroxidation and anti-bacteria properties (Alper & Acar, 2004), (Mudzhiri, 1954; Raj, 1975), (Lansky & Newman, 2007), (Khan, Afaq, Kweon, Kim, & Mukhtar, 2007; Lansky & Newman, 2007), (Kaur, Jabbar, Athar, & Alam, 2006) , (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007) , (Menezes, Cordeiro, & Viana, 2006).

Punica granatum is widely used as medicine. Tzulker et al. (2007) reported that the homogenates prepared from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the aril or seed sacs (fleshy or brightly colored cover of seed) juice. Wound healing involves a chain of well-orchestrated, biochemical and cellular events, leading to the growth and regeneration of wounded tissue. In coetaneous wound healing, the inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulations and improvement in the components of the collagen fibers that increases the tensile strength. Although the rate of collagen synthesis slow

down after about three weeks, collagen cross-linking and reorganization occur for months after injury in the remodeling phase of repair (Beanes et al. 2003).

Simple extraction method of pomegranate peels has been reviewed to make sure the objective targeted is achievable. The extracts of the Pomegranate differ in composition according to the starting material and extraction method selected. These were proved with several studies done before with different by different types of method of extraction and resulted of different types of constituents.

1.3 Objectives of The Study

1- To extract and obtain the phytochemicals from Pomegranate peels that can heal burn wounds by carry out Pomegranate ointment application.

2- To investigate the efficiency of Pomegranate (Punica granatum) in curing of burn wounds compare to the commercial ointment.

1.4 Scope of Study.

This study was focusing in the pomegranate efficiency in healing the wounds through carrying the extraction and application. In addition, the application was carried out in two different phases, one is the soap and the second phase is ointment. The efficiency of pomegranate was investigated by applying the ointment on the sample rats.

CHAPTER II

LITERATURE REVIEW

A review of previous researchers and studies in the Medical Plants field will be explained in this chapter, which including Medical Plants, introduction and history of medical plants, wounds treatment procedure and types, and Pomegranate as medical Plant.

2.1 Medicinal Plants

2.1.1 Introduction

Herbal Medicine occasionally referred to as Herbalism or Botanical Medicine, is the use of herbs for their healing or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Kaur et. al, 2006). Herbalists use the leaves, flowers, stems, berries, and roots of plants to inhibit, relieve, and treat illness. From a "scientific" viewpoint, various herbal treatments are measured experimental. The actuality is, however, that herbal medicine has a long and respected history. Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations. For example, vincristine

(an antitumor drug), digitalis (a heart regulator), and ephedrine (a bronchodilator used to decrease respiratory congestion) were all originally discovered through research on plants.

Figure 2.1 Some Types of Medical Plants.

2.1.2 History of Medical Plants

In the last few years, people start to look for the last resort of healing diseases which is, nature herbs. This nature herbs or known as medicinal plants among the scientist are the main ingredients to heal up variety of sickness for those who lives at rural area. In 1993, the World Health Organization reported that 80% of the world's population rely primarily on traditional medicine and a major part of the traditional therapies involve the use of their active constituents (Thomas, 1999).

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledgebase. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.

Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine. Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from periwinkle. The opium poppy yields morphine, codeine, and paregoric, a treatment for diarrhea Laudanum, a tincture of the opium poppy, was the favored tranquilizer in Victorian times. Till today, morphine-the most important alkaloid of the opium poppy-remains the standard against which the new synthetic pain relievers is measured.

Regarding the randomly use of antimicrobial drugs the microorganisms have developed resistance to many antibiotics that will lead to massive clinical problem in the treatment of infectious diseases (Davis, 1994). Meanwhile, Idose et al. in their study added to this problem, antibiotics are sometimes related with adverse effects on host which include hypersensitivity, exhaustion of beneficial gut and mucosal microorganisms and immunosuppression. Consequences from that, the need to develop alternative antimicrobial drugs for the treatment of infectious diseases becomes a priority.

Figure 2.2 Egyptian Herbal Treatment.

The use of plants as medicine is older than recorded history. As mute witness to this fact marshmallow root, hyacinth, and yarrow have been found carefully tucked around the bones of a Stone Age man in Iraq. These three medicinal herbs continue to be used today. Marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membranes, such as a sore

throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. Yarrow is a time-honored cold and fever remedy that may once have been used much as aspirin is today.

In 2735 B.C., the Chinese emperor Shen Nong wrote an authoritative treatise on herbs that is still in use today. Shen Nong recommended the use of Ma Huang (known as ephedra in the Western world), for example, against respiratory distress. Ephedrine, extracted from ephedra, is widely used as a decongestant. You'll find it in its synthetic form, pseudoephedrine, in many allergy, sinus, and cold-relief medications produced by large pharmaceutical companies.

Figure 2.3 Chinese Herbal Treatment

The records of King Hammurabi of Babylon (c. 1800 B.C.) include instructions for using medicinal plants. Hammurabi prescribed the use of mint for digestive disorders. Modern research has confirmed that peppermint does indeed relieve nausea and vomiting by mildly anesthetizing the lining of the stomach.

The entire Middle East has a rich history of herbal healing. There are texts surviving from the ancient cultures of Mesopotamia, Egypt, and India that describe and illustrate the use of many medicinal plant products, including castor oil, linseed oil, and white poppies. In the scriptural book of Ezekiel, which dates from the sixth century B.C., we find this admonition regarding plant life: "and the fruit thereof shall be for meat, and leaf thereof for medicine." Egyptian hieroglyphs show physicians of the first and second centuries A.D. treating constipation with senna pods, and using caraway and peppermint to relieve digestive upsets.

Throughout the middle Ages, home-grown botanicals were the only medicines readily available, and for centuries, no self-respecting household would be without a carefully tended and extensively used herb garden. For the most part, herbal healing lore was passed from generation to generation by word of mouth. Mother taught daughter; the village herbalist taught a promising apprentice. By the seventeenth century, the knowledge of herbal medicine was widely disseminated throughout Europe. In 1649, Nicholas Culpeper wrote A Physical Directory, and a few years later produced The English Physician.

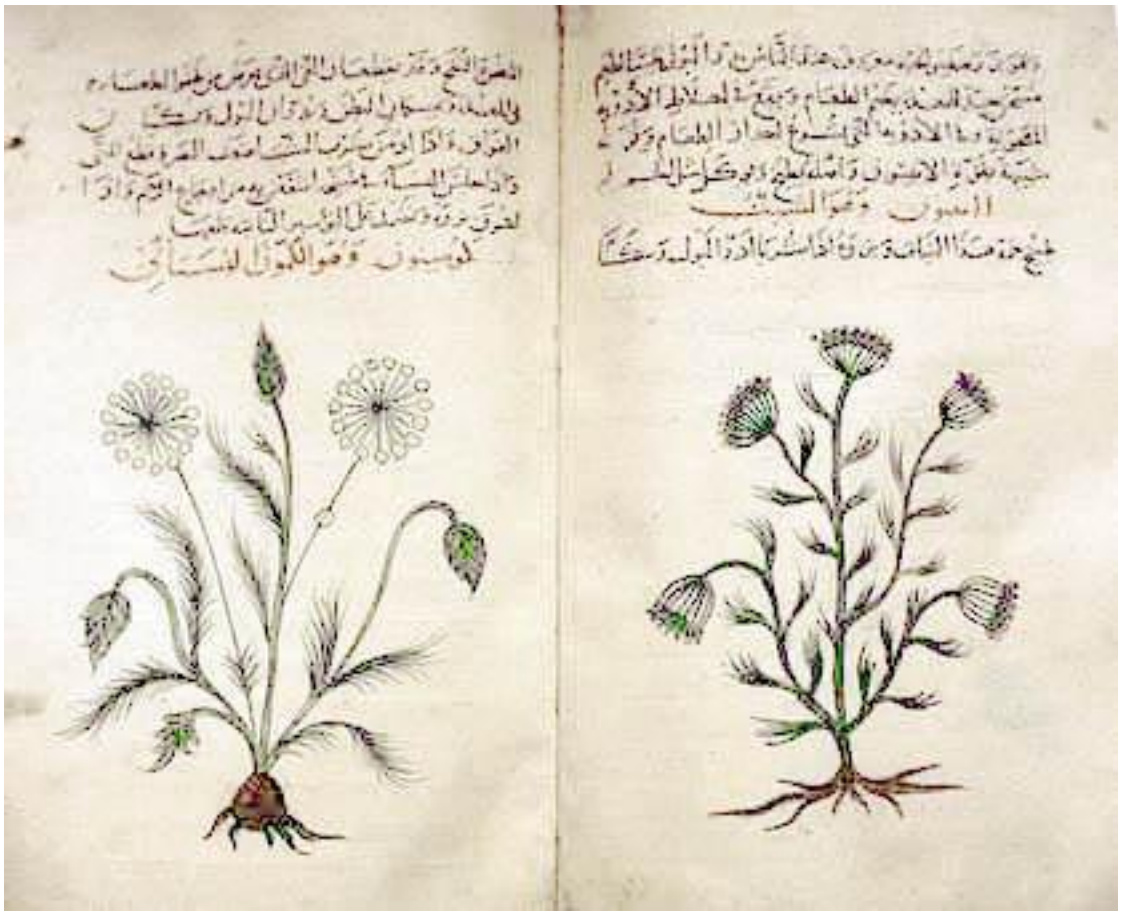


Figure 2.4 Traditional Arabic Medical Book

This respected herbal pharmacopeia was one of the first manuals that the layperson could use for health care, and it is still widely referred to and quoted today. Culpeper had studied at Cambridge University and was meant to become a great doctor, in the academic sense of the word. Instead, he chose to apprentice to an apothecary and eventually set up his own shop. He served the poor people of London and became known as their neighborhood doctor. The herbal he created was meant for the layperson.

The first U.S. Pharmacopeia was published in 1820. This volume included an authoritative listing of herbal drugs, with descriptions of their properties, uses, dosages, and tests

of purity. It was periodically revised and became the legal standard for medical compounds in 1906. But as Western medicine evolved from an art to a science in the nineteenth century, information that had at one time been widely available became the domain of comparatively few. Once scientific methods were developed to extract and synthesize the active ingredients in plants, pharmaceutical laboratories took over from providers of medicinal herbs as the producers of drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific, or at least unconventional, and to fall into relative obscurity.

Table 2.1 Important potential interactions between herbal preparations and conventional drugs.

Herb	Conventional drug	Potential problem
Echinacea used longer than 8 wks	Anabolic steroids, methotrexate, amiodarone, ketoconazole	Hepatotoxicity
Feverfew	Nonsteroidal anti-inflammatory drugs	Inhibition of herbal effect
Feverfew, garlic, ginseng, ginko, ginger	Warfarin sodium	Altered bleeding time
Ginseng	Phenelzine sulfate	Headache, tremulousness, manic episodes
Ginseng	Estrogens, corticosteroids	Additive effects
St John's wort	Monoamine oxidase inhibitor and serotonin reuptake inhibitor antidepressants	Mechanism of herbal effect uncertain. Insufficient evidence of safety with concomitant use—therefore not advised
St John's wort	Antiretrovirals, digoxin, theophylline, cyclosporin, oral contraceptives	Decreased clinical effect
Valerian	Barbiturates	Additive effects, excessive sedation

Table 2.1 Continued

Kyushin, licorice, plantain, uzara root, hawthorn, ginseng	Digoxin	Interference with pharmacodynamics and drug level monitoring
Evening primrose oil, borage	Anticonvulsants	Lowered seizure threshold
Shankapulshpi (Ayurvedic preparation)	Phenytoin	Reduced drug levels, inhibition of drug effect
Kava kava	Benzodiazepines	Additive sedative effects, coma
Echinacea, zinc (immunostimulants)	Immunosuppressants (such as corticosteroids, cyclosporin)	Antagonistic effects
Kelp	Thyroxine	Iodine content of herb may interfere with thyroid replacement
Licorice	Spironolactone	Antagonism of diuretic effect
Karela, ginseng	Insulin, sulfonylureas, biguanides	Altered glucose concentrations. These herbs should not be prescribed in diabetic patients

Data from Miller LG. Herbal medicinal: selected clinical considerations focusing on known or potential drug-herb interactions. *Arch Intern Med* 1998; 158:2200-2211.

The presence and concentration of a biologically active component was followed to decide the genuineness of the drug or formulation. The presence of any other component apart from the biomarkers indicates adulteration. Knowledge of these compounds and their specific analytical methods will facilitate the herbal industry in checking adulteration and thus raise its standards (Khan et. al, 2007). Punica granatum is a deciduous tree belonging to family Punicaceae. It is known for its edible fruit. During the last two decades, the drug has been subjected to extensive phytochemical, pharmacological and clinical investigations, with many interesting findings reported in various fields. Punica granatum is found to contain hydrolysable tannins as major active chemical constituents namely punicalagin, punicalin, gallic acid, ellagic

acid and ellagic acid derivative such as ellagic acid, 3, 3'-di-O-methyl, ellagic acid, 3, 3', 4'-tri-O-methyl, ellagic acid, 3'-O-methyl-3, 4-methylene; phenolic compounds such as pedunculagin, punicacortein A–D, granatin A and B, punicafolin, punigluconin, corilagin. The phytochemical constituents namely corilagin, ellagic acid, kaempferol, luteolin, myricetin, quercetin, quercimetrine, quercetin-3-O-rutinoside were previously isolated from the fruits of Punica granatum.

2.2 Wound

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue (Bennet, 1988). Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The human body is truly remarkable. Wound healing is an ability possessed by the body to repair damaged parts – this process is sometimes visible (a cut improves) or microscopic (damaged cells are replaced) but both occur on a daily basis, and are given the term: regeneration. In the animal kingdom – a starfish can rebuild a new tentacle that is cut off, an earthworm can replace much of its body that is lost, and crabs can rebuild a new claw when one is lost. There are three phases of wound healing: the inflammatory, fibroblastic, and maturation stages.

Table 2.2 Characteristics and Uses of Wound-Dressing Materials

Category	**Examples**	**Description**	**Applications**
Alginate	AlgiSite, Comfeel,	Alginate dressings are made of seaweed extract contains guluronic and	These dressings are highly absorbent and

	Curasorb, Kaltogel, Kaltostat, Sorbsan, Tegagel	mannuronic acids that provide tensile strength and calcium and sodium alginates, which confer an absorptive capacity. Some can leave fibers in the wound if they are not thoroughly irrigated. These dressings are secured with secondary coverage.	useful for wounds have copious exudate. Alginate rope is particularly useful to pack exudative wound cavities or sinus tracts.
Hydrofiber	Aquacel, Aquacel-Ag, Versiva	An absorptive textile fiber pad, hydrofiber is also available as a ribbon for packing of deep wounds. This material is covered with a secondary dressing. The hydrofiber combines with wound exudate to produce a hydrophilic gel. Aquacel-Ag contains 1.2% ionic silver that has strong antimicrobial properties against many organisms, including methicillin-resistant*Staphylococcus aureus* and vancomycin-resistant enterococci.	Hydrofiber absorbent dressings used for exudative wounds.
Debriding agents	Hypergel (hypertonic saline gel), Santyl (collagenase), Accuzyme (papain urea)	Various products provide some chemical or enzymatic debridement.	Debriding agents are useful for necrotic wounds as an adjunct to surgical debridement.
Foam	LYOfoam, Spyrosorb, Allevyn	Polyurethane foam has absorptive capacity.	These dressings are useful for cleaning granulating wounds with minimal exudate.

Table 2.2 Continued.

Hydrocolloid	CombiDERM, Comfeel, DuoDerm CGF Extra Thin, Granuflex, Tegasorb	Hydrocolloid dressings are made of microgranular suspension of natural or synthetic polymers, such as gelatin or pectin, in an adhesive matrix. The granules change from a semihydrated	Hydrocolloid dressings are useful for dry necrotic wounds, wounds with minimal exudate and for clean granulating wounds.

		state to a gel as the wound exudate is absorbed.	
Hydrogel	Aquasorb, DuoDerm, Intrasite Gel, Granugel, Normlgel, Nu-Gel, Purilon Gel, KY Jelly	Hydrogel dressings are water-based or glycerin-based semipermeable hydrophilic polymers; cooling properties may decrease wound pain. These gels can lose or absorb water depending upon the state of hydration of the wound. They are secured with secondary covering.	These dressings are useful for dry, sloughy, necrotic wounds (eschar).
Low-adherence dressing	Mepore, Skintact, Release	Low-adherence dressings are made of various materials designed to remove easily without damaging underlying skin.	These dressings are useful for acute minor wounds, such as skin tears, or as a final dressing for chronic wounds that have nearly healed.
Transparent film	OpSite, Skintact, Release, Tegaderm, Bioclusive	Transparent films are highly conformable acrylic adhesive films with no absorptive capacity and little hydrating ability. They may be vapor permeable or perforated.	These dressings are useful for clean, dry wounds with minimal exudate. They also are used to secure an underlying absorptive material, to protect high-friction areas and areas that are difficult to bandage (eg, heels) and to secure intravenous catheters.

If we take a simple laceration or cut inflammation begins after injury and the wound site swells as the biochemical ingredients needed for wound healing gather: leukocytes and monocytes fibrinogen, histamine, prostaglandins, and vasoactive substances. A great deal happens during this stage – it must occur to prepare the wound for the succeeding phases of wound healing. In fact, conventional drugs that limit inflammation such as non-steroidal anti-inflammatories (NSAID's) will slow the healing of a wound! Next, fibroblasts begin to proliferate and position themselves for collagen synthesis. As collagen content increases, the

wound site strengthens. The third and final stage of wound healing lasts the longest. This maturation, or remodeling phase, may continue for weeks or several years (depending on the severity of the injury), with gradual improvements in wound appearance. After operations or surgical procedures, the body may take time to adjust and achieve the harmonious balance it had before and gradual wound healing should be supported. Take your time to recover and follow your chosen medical professional's advice.

Many herbal and homeopathic remedies have been formulated with specific ingredients to promote wound healing and support the body's ability to resist infection. Herbs such as Agrimonia eupatoria (a well-known astringent which helps to tighten and constrict tissues) and Achillea millefolium (named after Achilles, the Greek mythical figure, who used it to stop the bleeding wounds of his soldiers) can help to heal wounds naturally. Calendula officianalis is an effective first-aid solution for all minor burns and scalds as well as any skin abrasion or cuts Calendula will also benefit the wound healing process from the inside out by stimulating the natural process of healthy cells and tissue regeneration. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing. Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences (Myers et al, 1980). Attention should be directed towards discovering an agent, which will accelerate wound healing either when it is progressing normally (Mather et al, 1989), or when it is suppressed by various agents like corticosteroids (Ehrlich & Hunt, 1968), anti-neoplastics (Raju & Kulkarni 1986), or non-steroidal anti-inflammatory agents. Medical treatment of wound includes administration of drugs

either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair (Savanth & Shah, 1998). The topical agents used include antibiotics and antiseptics (Chulani, 1996), desloughing agents (chemical debridement, e.g. hydrogen peroxide, eusol and collagenase ointment) (Savanth & Mehta, 1996), wound healing promoters (e.g. Tretinoin, aloe vera extract, honey, comfrey, benzoyl peroxide, chamomilia extract, dexpanthenol, tetrachlordecaxide solution, clostebol acetate and the experimental cytokines. Various growth factors like platelet derived growth factor, macrophage derived growth factor, monocyte derived growth factor (Mather et al, 1989) etc. are necessary for the initiation and promotion of wound healing. Many substances like tissue extracts (Udupa et al, 1991), vitamins & minerals and a number of plant products (Dahanukar et al, 2000) have been reported by various workers, to possess pro-healing effects. Wound healing herbals encourage blood clotting, fight infection and accelerate the healing of wounds. Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds. In this direction a number of herbal products are being investigated at present. Various herbal products have been used in management and treatment of wounds over the years.

2.3 Pomegranate (Punica Granutm)

2.3.1 Pomegranate Plant History

According to legend, pomegranates grew in the Garden of Eden, and the fruit has been used as a folk medicine for thousands of years. More recently, it has been promoted as a "superfood" that can relieve symptoms of many diseases. In laboratory tests, pomegranate shows

antiviral, antibacterial, and antioxidant properties. But there is not yet strong evidence that it works in humans to treat or prevent any condition. In addition, there is some concern that pomegranate juice might interact with medications -- much like grapefruit juice does -- making some less effective.

Pomegranate fruit extract is a rich source of polyphenols, chemicals in plants that provide their flavor and color. Polyphenols are also antioxidants, meaning they help protect cells from damage and may lower inflammation in the body. Pomegranate fruit is also high in vitamin C. One pomegranate provides about 40% of the daily requirement of this vitamin. The bark, fruit, root, and rind of the pomegranate tree are used as medicine in Asia and the Middle East, but in the West the fruit and its juice are usually the parts being studied. The juice and rind have antioxidant properties, while the juice, rind, and oil from seeds contain isoflavones similar to the ones in soy.

Due to the lack of side effects compared to synthetic drugs, approximately 60% of the world's population relies almost entirely on plants for medication, and natural products have long been recognized as an important source of therapeutically effective medicines. Indeed, many plants have been shown to possess therapeutic potential as promoters of wound healing for example, Jatropha curcas, Aloe barbadensis, Centella asiatica (Villegas et al. 1997; Shukla et al. 1999). These plants exhibited often antifungal, antimicrobial, antioxidant, anti-inflammatory activities (Turkoglu et al. 2007). North African folklore and tribal medicines employ a number of plants and animal products for treatment of cuts, wounds and burns (Ahmed et al. 1995). Some of these plants have been screened scientifically for the evaluation of their wound healing

activity in different pharmacological models and human subjects, but the potential of most of the plants remain unexplored. Punica granatum L. (Punicaceae), commonly called pomegranate, is a large deciduous shrub or small tree used medicinally in Europe, Indo-China, the Philippine Islands, North Africa, and South Africa. The plant is used in folklore medicine for the treatment of various diseases, such as ulcer, hepatic damage, snakebite, etc. The rind of the fruit is antihelminthic, useful in dysentery and ulcer (Lansky and Newman 2007). The plant also shows high antioxidant and antiartherogenic activity (Aviram et al. 2000). Modern uses of pomegranate derived products now include treatment of acquired immune deficiency syndrome (AIDS) in addition to use for cosmetic beautification and enhancement hormone replacement therapy, resolution of allergic symptoms, cardiovascular protection, oral hygiene, ophthalmic ointment weight loss soap and as an adjunct therapy to increase bioavailability of radioactive dyes during diagnostic imaging (Aviram et al. 2000; Lansky and Newman 2007). There are only few prospective randomized controlled trials that have proved the clinical efficacy of the traditional wound healing agents. Accordingly, based on its ethnopharmacological profile and reputed medicinal use in traditional practice, the present study was undertaken to (i) evaluate, the in vitro, antioxidant and antimicrobial activities (against various wound pathogens) of the methanolic extract obtained from the peels of P. granatum fruits (PgME) (ii) to evaluate systematically the possible in vivo wound healing potential of a 5% ointment formulated using this extract.

2.3.2 Medicinal Uses and Indications

1. Cancer

Because it is high in antioxidants and other nutrients, some people think that drinking pomegranate juice regularly may help prevent cancer. There is no specific evidence of that, however. In test tubes, pomegranate extracts made from juice, rind, and oil slow down the reproduction of cancer cells and may hasten their death. Some extracts also help reduce blood supply to tumors, starving them and making them smaller. Most studies have focused on breast and prostate cancer cells. In one other study, pomegranate juice extract given to mice slowed down the growth of lung tumors. However, most of these studies have been in test tubes or in animals, not humans. In one human study, men who had surgery or radiation for prostate cancer lengthened the amount of time it took for their PSA levels to double by drinking 8 oz. of pomegranate juice each day. Men whose PSA levels double in a short period of time are more at risk for death from prostate cancer. Those who drank pomegranate juice increased the time it took for their PSA levels to double from about 15 months to 54 months, a significant increase. If you are being treated for any cancer, be sure to ask your oncologist before you take pomegranate or any herb or supplement. Some may interact with cancer medications, making the medications less effective.

2. Heart disease

Pomegranate's high antioxidant content has also made researchers wonder if it could treat heart disease. So far, the scientific studies have been small and mostly done either in test tubes or animals. Pomegranate juice seems to protect LDL cholesterol from damage. Some scientists think that damage to LDL cholesterol causes plaque to build up in arteries, so stopping the damage might help keep arteries clear. One study of mice with atherosclerosis found that pomegranate juice slowed the growth of plaque formation. And a few small studies in people found that pomegranate juice improved blood flow and kept arteries from becoming thick and stiff. However, more and better studies are needed to see exactly what benefit pomegranate juice might offer. There is some preliminary evidence that drinking pomegranate juice every day may help lower systolic blood pressure (the top number in a blood pressure reading) but not diastolic blood pressure (the bottom number).

3. Osteoarthritis

Flavonols (a kind of antioxidant) similar to the ones found in pomegranate fruit have been suggested as treatments for osteoarthritis. Osteoarthritis happens when the cartilage in joints wears down and causes pain and stiffness. Researchers believe flavonols can help block inflammation that contributes to the destruction of cartilage. In test tubes, pomegranate extract blocked the production of an enzyme that destroys cartilage in the body. The results were promising; however, more studies and studies that look at the effects in humans are needed

CHAPTER III

METHODOLOGY

3.1 Overview

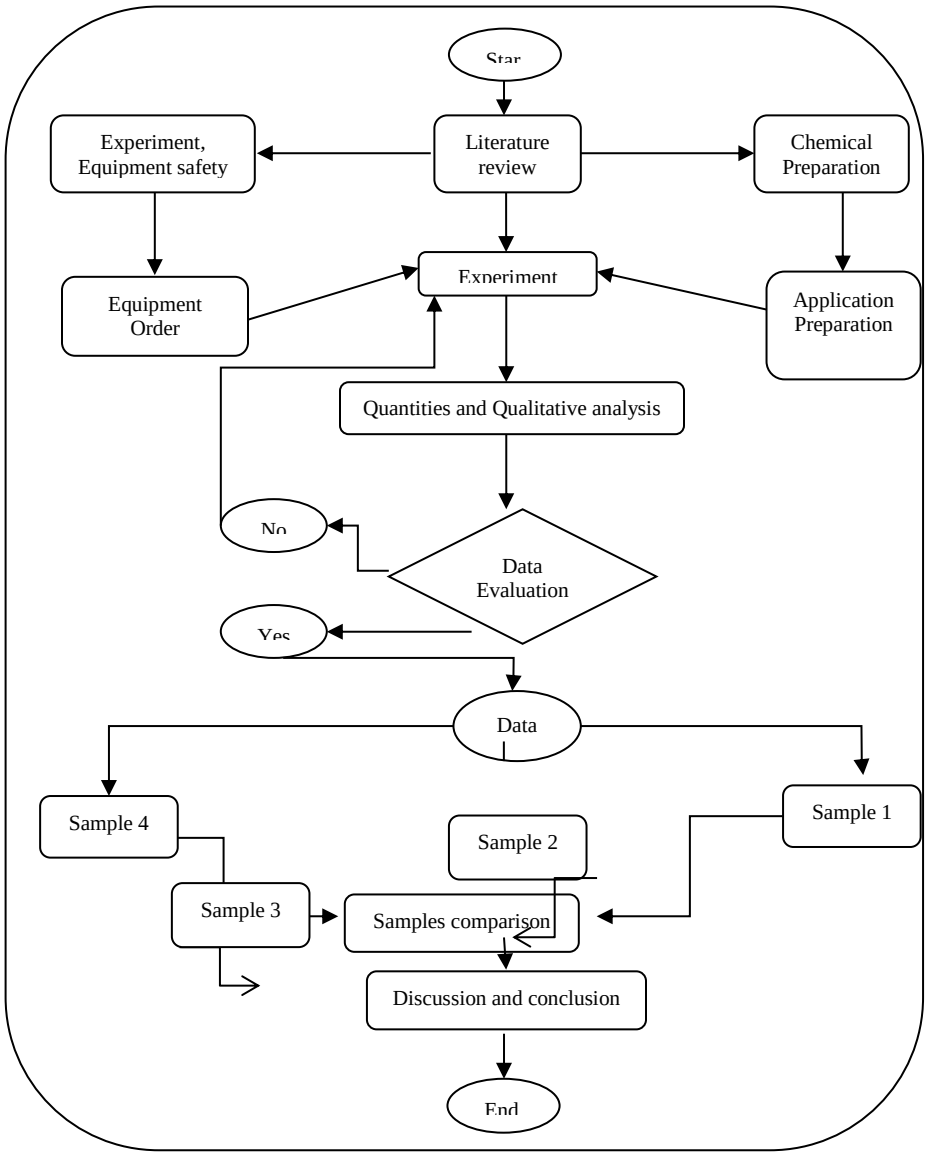


Figure 3.1 Workflow diagram

Figure 3.1 above shows the chronology which followed in order to achieve the objective in this study. The method applied is dividing into four significant parts, Part 1 through 4 which differ in term of type of treatment of the sample. Sample 1 was applied by ointments extraction from Pomegranate while Sample 2 by commercial skin cream. For Sample 3, the treatment was using Vaseline and Sample 4 without applying anything (self-recovery system). In order to measure the efficiency between two models, the method applied for burn wounds and observations of the improvement was recorded. However the details of the procedure or the strategy are going to be described in the next subtopics.

3.2 Materials and Equipment Including the Procedure

This section is describing the materials and equipment that used in order to achieve the desired result.

3.2.1 Materials

3.2.1.1 Plant Material

The plant was used in this study is Pomegranate (Punica grantum). In this study, Pomegranate plays the main role to achieve the objectives and in order to carry out the applications of ointment from this plant. However, only one part from this plant was considered for the application which is the peels. Firstly, the pomegranate was collected from the market in Gambang Pahang, and then was separated into peels and fruit. All the collected peels of

pomegranate was dry at room temperature for 1 day and within this day; the peels was kept at room temperature whole the day without exposing to the sunlight then dried in an oven at 60 ˚C for 48 hours. Dried peels then was grinding to powder using grinder (Hayouni et al, 2011).

3.2.1.2 Analysis of the Continuance of Pomegranate for healing wounds

This section extraction analysis was conducted using aqueous methanol and water as the solvent. There are four main phytochemical tests were conducted 'for the purpose of analyzing' as following:

3.2.1.2.1 Saponin Test with Fresh Sample

This test was conducted through a sequence steps. A 10 gram of fresh sample (Blend powder of pomegranate), was prepared and then dissolved in 100ml distilled water (1:10). After that the sample was filtered and purred into a test tube. The filtrate in the test tube was warmed in water bath. The stable persistent froth then, was mixed with three drops of olive oil and shaken vigorously using test tube shaker, than observed for the formation of emulsion, indicate the presence of saponins (Edeoga et al, 2005), with some modification.

3.2.1.2.2 Glycosides Test with Fresh Sample

The Glycosides test was done in order to determine the presence of Glycosides. In this test, 0.5 grams of crude powder was dissolved in 5 ml of methanol followed by the addition of 10ml of 50% HCl to 2 ml of methanolic extract in test tube. The mixture then was heated in a boiling water bath for 30 minute. After that, 5 ml of Fehling's solution was added and the mixture was boiled for 5 minute, to observe a brick red precipitate as an indication for the presence of glycosides (Harborne, 1973).

3.2.1.2.3 Terpenoid Test (Salkowski Test)

In this test, 5 ml of water extract from plant was mixed in 2 ml of chloroform, and then was concentrated with 3 ml of H_2SO_4. After that the mixture was carefully added to form a layer and then was placed on the test tube shaker for 30 minutes. A reddish brown coloration of the inter face was formed to show positive result for the presence of terpenoid (Edeoga et al, 2005).

3.2.1.2.4 Steroids Test

The Steroid test is consisted of some steps. The test was achieved by dissolving 0.5 grams of crude powder in 5 ml of methanol. One mL of the extract was then treated with 0.5 ml of acetic acid anhydride and cooled for 3 minutes in refrigerator. Accordingly the mixture then was mixed with 0.5 ml of chloroform. After that one ml of concentrated sulphuric acid was added carefully by means of a pipette. At the separations level of the two liquids a reddish brown

ring was formed, as indication of the presence of steroids (Kola wok et al, 2006) and (Majaw and Moirangthem, 2009).

3.2.1.3 Preparation of Application products.

In this section, details description of the preparation of two different products from pomegranate's peels was presented. These two applications are Ointment product and Soap product. Fully description of the preparation is described in the following.

3.2.1.3.1 Ointment Preparation.

In this section the preparation of Ointment product is described. In order to prepare the ointment product, 1000 g of Vaseline and 4 drops of lavender fragrance was used. As well 125 g of fine powder of pomegranate and 30 ml of menthol was needed. The preparation was started by melting the Vaseline on a hot plate and was stirred by using magnetic stirrer after melting. During the stirring, the fine powder of pomegranate and the menthol with lavender fragrance was slowly poured into the melt stirred Vaseline. The mixture is then was mixed well. Finally the viscos mixture was then poured into an ointment cases.

3.2.1.3.2 Soap Preparation.

This section is describing the procedure for preparing the soap product. Preparation of 250 g of palm oil, 140 g of olive oil, 100g of corn oil and 30 ml of fragrance is necessary for achieving the product; meanwhile 74 g of NaOH was diluted into 210g of water. After that the diluted NaOH solution' pH was measured by using PH meter. If the pH value is not naturalized then farther dilution is needed. The prepared oils and solutions was mixed and then placed in the blender. The mixture then was blended until well mixing is achieved. The high viscose mixture was poured into a case to give the shape accordingly. The mixture was left for one day (Kazuyuki & Takuji, 1976).

3.2.2 Equipment

3.2.2.1 Test Tube Shaker

This device is designed to constantly shake the test tube. However, the operating shaking speed is enough to guarantee the well mixing of the components.

Figure 3.2 Lab Test Tube Shaker

3.2.2.2 Blender

The blending container can be made of glass, plastic, stainless steel, or porcelain, and often has graduated markings for approximate measuring purposes. In cases where the blades are removable, the container should have an O-ring or gasket between the body of the container and the base to seal the container and prevent the contents from leaking. The blending container is generally shaped in a way that encourages material to circulate through the blades, rather than simply spinning around. The container rests upon a base that contains a motor for turning the blade assembly and has controls on its surface. Most modern blenders offer a number of possible speeds. Low-powered blenders require some liquid to be added for the blender to operate correctly. This is because the lid is used to move the solids around the jar and bring it in contact with the blade as the "whirlpool" fluid movement brings items from the top to the bottom. High-powered blenders are capable of milling grains and crushing ice without such assistance.

Figure 3.3 Electrical Blender

3.2.2.3 pH Meter

The pH probe measures pH as the activity of the hydrogen cations surrounding a thin-walled glass bulb at its tip. The probe produces a small voltage (about 0.06 volt per pH unit) that is measured and displayed as pH units by the meter. For more information about pH probes, see glass electrode. This device was used in this study to measure the pH of the product application in order to guarantee the safety of the product. For very precise work the pH meter should be calibrated before each measurement. For normal use calibration should be performed at the beginning of each day. The reason for this is that the glass electrode does not give a reproducible e.m.f. over longer periods of time. Calibration should be performed with at least two standard buffer solutions that span the range of pH values to be measured. For general purposes buffers at pH 4 and pH 10 are acceptable.

Figure 3.4 pH Meter.

3.3 The Animal Samples and The Treatment Procedure.

3.3.1 The Animal Samples.

In this study four groups or models was used in order to investigate the efficacy of pomegranate prepared ointment in healing the burn wounds.

Group1: consist of four rats with the same genetic family, which was treated by the prepared ointment from pomegranate.

Group2: consist as well of four rats with the same genetic family, which was treated by the commercial ointment.

Group3: consist as well of four rats with the same genetic family, which was treated by Vaseline.

Group4: consist as well of four rats with the same genetic family, which won't be treated (Self-recovery system).

3.3.2 Treatment procedure

The samples of rats of the four groups were placed on a cage. For every sample rat, one burn wounds was inflicted by burning the rat back by hot flat steel with the area of $4mm^2$. The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. The herbal ointment prepared from pomegranate was applied to the wounds in group1 samples (enough to cover the wound) once daily for the period of 10 consecutive days. In the same time, the commercial and Vaseline was applied with same miner to group 3&4 respectively, once daily for the period of 10 consecutive days. Group4 was left without treatment for the same period of time.

3.3.3 Data Collection.

For the determination of the wound contraction, burn wounds was traced on a transparent paper having a scale, and the change in wound size at 4 day-intervals was calculated as the percentage of wound area that healed. The rate of wound contraction was expressed in terms of the percentage of wound area that had healed: % wound contraction = (healed area/total area) ×100. Additional observation was recorded daily in order to observe the improvements of the wounds recovery.

CHAPTER IV

RESULT AND DISCUSSION

4.1 Introduction

In the present chapter, the efficiency of Pomegranate Peels as burn wounds healing treatment will be introduced and discussed. In similar, the analysis of Pomegranate result will be shown and discuss too. The result of ointment application preparation will be presented and discussed as well, in this chapter. In addition, the effectiveness of using Pomegranate application ointment in healing the compare to commercial ointment observations will be presented.

4.2 Solvent Extraction Analysis of Active constituent of Pomegranate.

Solvent extraction technique is one of the most common analytical methods to determine the existence of specific constituent in materials. Solid-Liquid extraction method, the separation and isolation of desired constituent is based upon the solubility phenomena. Extracting solvent is chosen which will have highest-solubilizing power for the desired constituent. In this study, the extraction was conducted using the test tubes for different constituent.

4.2.1 Saponin Analysis

The result of the analysis is presented in the following table:

Table 4.1 Result for The Analysis of Saponin

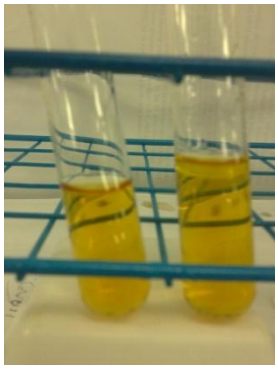
Step	Picture	Notes
Preparation of solution		2 solutions having the same amount of Pomegranate. One control solution and another one is the sample.
Mixing with extraction agent.		The drops of the extraction agent (Olive Oil) were added and shake with shaker.
Emulsion Forming		The emulsion formed indicating the present of Saponin.

Table 4.1 indicates the result obtain for the analysis of the active constituent (Saponin). Saponins are known to promote wound healing process due to their antioxidant andantimicrobial activities (Sachinet al., 2009). The presence of Saponin in pomegranate as one of its active constituents promote 56% increasein hydroxyproline, 57% increase in tensile strength, increased collagen content, and better epithelialization (MacKay and Miller, 2003).

4.2.2 Glycoside Analysis

The result of the analysis is presented in the following table:

Table 4.1 Result for The Analysis of Glycoside

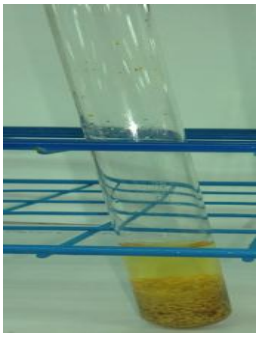
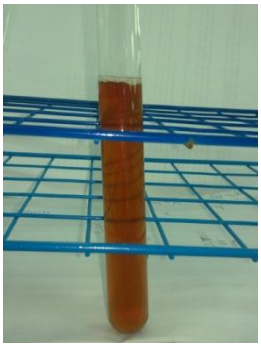
Step	**Picture**	**Notes**
Preparation of solution		2 solutions having the same amount of Pomegranate. One control solution and another one is the sample.
Mixing with extraction agent.		The drops of the extraction agent (Fehling solution) were added and shake with shaker.

Table 4.1 Continued.

Brick red precipitate Forming		Observe of brick red precipitate formed indicating the present of Glycoside.

4.2.3 Terpenoid Analysis

The result of the analysis is presented in the following table:

Table 4.3 Result for The Analysis of Terpenoid

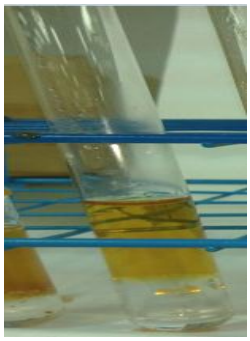
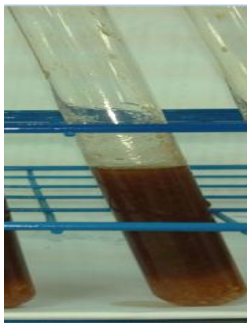
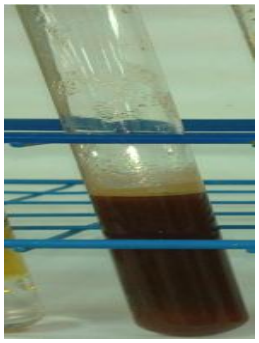
Step	**Picture**	**Notes**
Preparation of solution		Solution having some amount of Pomegranate. One control solution and another one is the sample.
Mixing with extraction agent.		The drops of the extraction agent (chloroform) were added and shake with shaker.

Table 4.3 Continued.

Reddish brown coloration Forming		A reddish brown coloration of the inter face was formed indicating the present of Terpenoid.

4.2.4 Steroid Analysis

The result of the analysis is presented in the following table:

Table 4.4 Result for The Analysis of Steroid

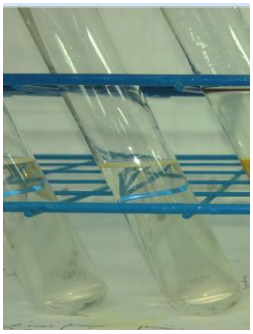
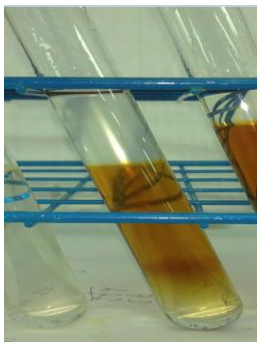
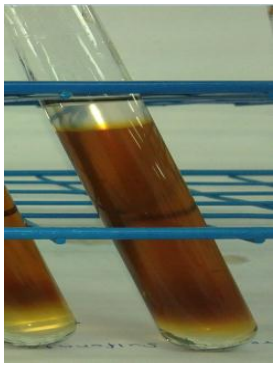
Step	**Picture**	**Notes**
Preparation of solution		Solution having some amount of Pomegranate. One control solution and another one is the sample.
Mixing with extraction agent.		The drops of the extraction agent (sulphuric acid) were added and shake with shaker.

Table 4.4 Continued.

Reddish brown ring Forming		A reddish brown ring was formed, as indication of the presence of steroids.

4.3 Living Sample (Rats) Treatment

Table 4.5 The Pomegranate Ointment treatment.

Day	Sample Progress
0	

Table 4.5 Continued.

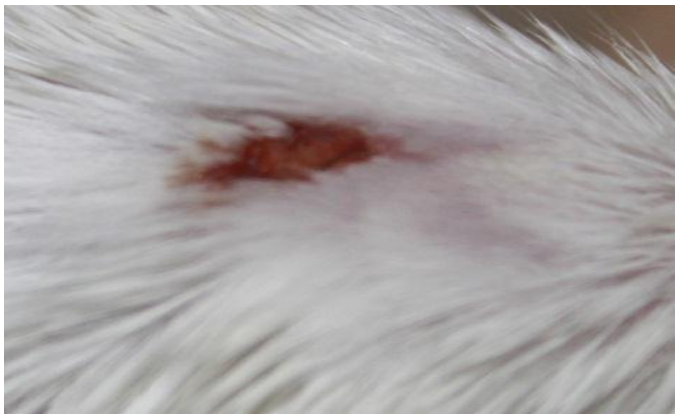
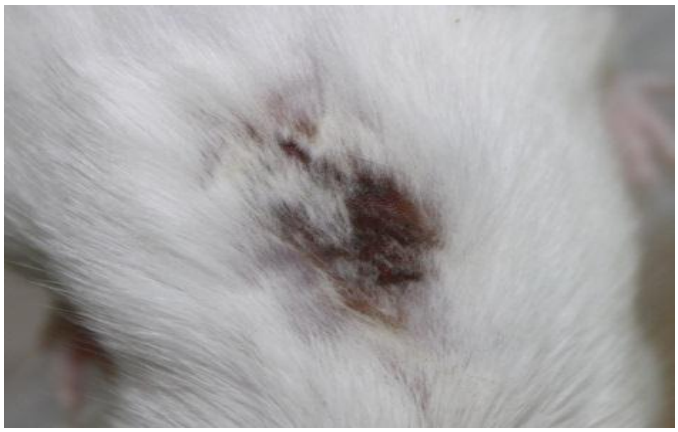
3	
6	
9	

Table 4.5 Continued.

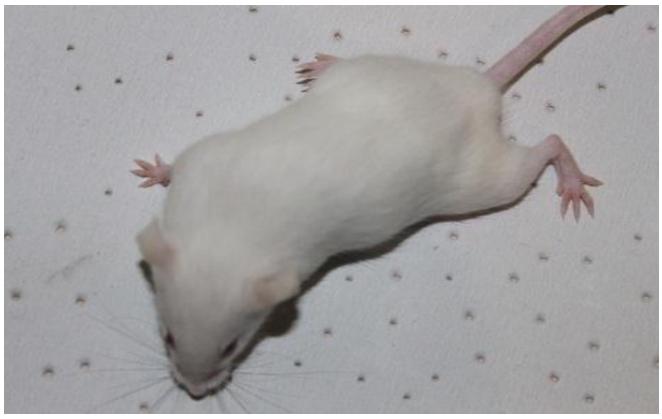
12	
15	

Table 4.6 The Commercial Ointment Treatment.

Day	Sample Progress
0	

Table 4.6 Continued.

3	
6	

9	

Table 4.6 Continued.

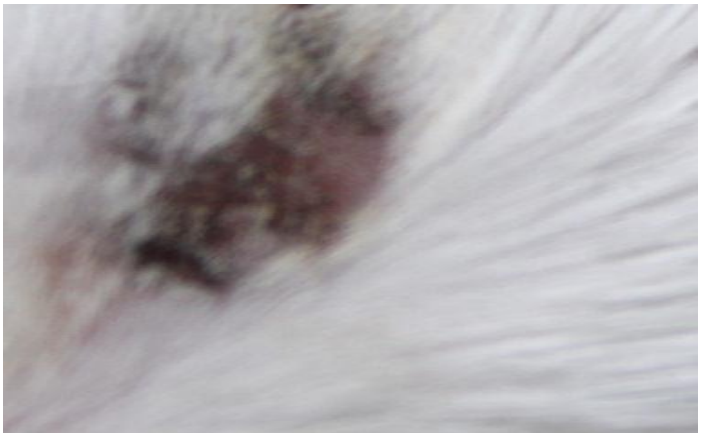
12	
15	

Table 4.7 The Vaseline Treatment.

Day	Sample Progress
0	

Table 4.7 Continued.

3	
6	

9	

Table 4.7 Continued.

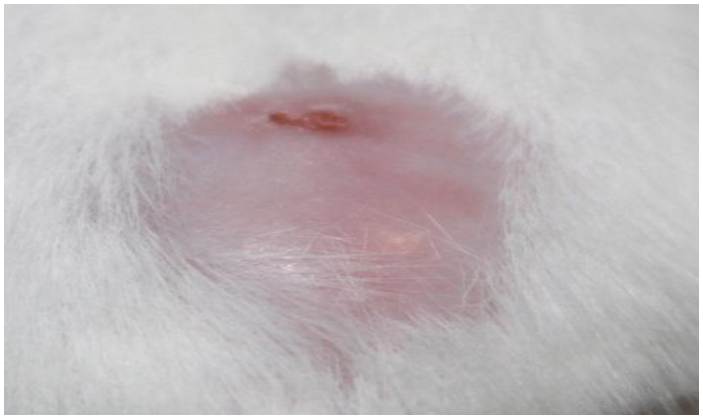
12	
15	

Table 4.8 The Control group (Without Treatment).

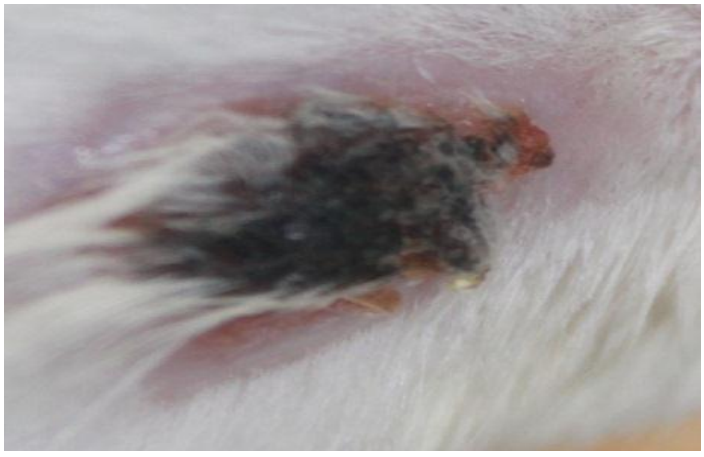
Day	Sample Progress
0	

Table 4.8 Continued.

3	
6	

9	

Table 4.8 Continued.

12	
15	

In this section, the observation of using the ointment prepared from Pomegranate is presented. The efficiency of pomegranate in healing the burn wounds was investigated by comparing four different samples.

The present investigation describes some unique features of the leaf extract from Pomegranate peels with respect to its potential wound healing capacity in infected rats. Plant products are potential wound healing agents, and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects, and effectiveness as crude preparations. Earlier it was reported that Centella asciatia and Terminalia chebula are effective in wound healing in rats. Various activities were conducted in this study to evaluate the potential of Pomegranate as a wound healing agent. One such activity is the phytochemical screening test. The phytochemical results reveal the presence of glycoside, steroids, saponins, and terpenoid in the methanolic extract. The constituents of the pomegranate, such as terpenoids and steroids, may play a major role in the wound healing process observed in this study; however, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities. This is because of the presence of terpenoid in the methanolic extract of pomegranate. Terpenoids are known to promote the wound healing process, mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and an increased rate of epithelialization. Terpenoids, or isoprenoids as they are also known, may have great antifungal or antimicrobial potential due to possible effect on the non-mevalonate pathway. This pathway is essential in fungi, protozoans, gram-negative bacteria and other micro-organisms for the synthesis of cell membrane components, prenylation proteins and as a

secondary source of carbon (Nayak et. al., 2010). Studies with other plant materials also demonstrated the presence of similar phytochemical constituents, which were responsible for promoting wound healing activity in rats (Nayak et. al. 2006). The topical application of drugs is an efficient therapy method of destroying microbial populations because the availability of the drug at the infected wound site leads to enhanced wound healing activity. The virulence capacity of microorganisms, amount of inoculums, and host immune response are important factors that can cause massive damage during infection. Normally, common wound pathogens such as S. aureus, C. albicans, and P. aeruginosa with ≥103

CFU/g tissues are classified as infections (Bergstrom et. al., 1994). From this study, it appears that pomegranate peels extract exhibits favorable antimicrobial activity against C. albicans. Fabry et al. reported that if the extracts having activities where MIC values are below 8 mg/mL, this indicates that the extract possesses some effective antimicrobial activity. In vitro anti-yeast studies and an in vivo short period of epithelialization in the treated rats provide evidence of the healing effect of pomegranate on infected wounds.

Pomegranate peel ointment not only destroys the pathogens from the wound environment; it also acts as a stimulant for wound healing because it has polyphenols and flavonoids as active constituents. After injury, revascularization of the wound bed and redevelopment of the extracellular matrix are achieved through cell proliferation and the production of granulation tissue. Wound contraction, a part of the proliferative phase of wound healing, occurs through the centripetal movement of the tissues surrounding the wound, which is mediated by myofibroblasts (Adam et. al., 1999). The increased wound contraction in the treated group may be due to the enhanced activity of fibroblasts and successful elimination of yeast

Molecules 2010, 153194 by pomegranate peels extract. The slow rate of wound closure in the control group might be attributed to the presence of microorganisms and their metabolites, which inhibit wound contraction and deteriorates the wound healing activity (Figures 2–4). A significant increase in collagen content due to enhanced migration of fibroblasts and epithelial cells to the wound site was observed during the wound healing process in the treated group. Moreover, as shown in previous studies, oil palm contains ascorbic acid, which acts as a cofactor for the synthesis of collagen as well as elastin fibers (Sauermann et. al., 2004). The decreased collagen content in the control group might be due to a prolonged inflammatory phase where the degradation of collagen will be greater than its synthesis.

A close examination of granulation tissue sections revealed that tissue regeneration was much quicker in the treated group compared to that in control wounds (Figures 5–6). The increased cellular infiltration observed from hematoxylin and eosin staining in both groups may be due to the presence of pathogens, but the antimicrobial property of pomegranate massively reduced the bacterial population, thereby indirectly reducing the inflammatory cells on the wound site. Early dermal and epidermal regeneration in the treated group confirmed that the ointment containing the pomegranate extract had a positive effect toward cellular proliferation, granulation tissue formation, and epithelialization. The well-formed collagen bundles in the treated group shown in hematoxylin and eosin staining support the efficacy of Pomegranate on fibroblast proliferation and synthesis of extracellular matrix during healing (Figure 5). Incomplete epithelialization with less extracellular matrix synthesis was observed in control rats, as shown in Figure 6. Clumps of degenerating neutrophils, necrotic changes, and the persistence of inflammatory exudates in the upper dermis with loss of epidermis were also observed up to

day 16. The treated rats showed marked epithelialization, a moderate amount of extracellular matrix synthesis, and new blood vessel formation.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The above data suggest that the application of Pomegranate ointment to an infected wound not only reduces the risk of further infection, but also improves the healing activity. The application of a methanolic extract of Pomegranate was found to improve the different phases of wound repair, including collagen synthesis and maturation, wound contraction, and epithelialization. As Pomegranate possesses an antifungal property and is traditionally used in several African countries, this study' findings may provide scientific rationale for the use of Pomegranate to promote healing of infected wounds and born injuries.

5.2 Recommendations.

Two main recommendations can be summarized base on the present study as guideline for future improvement. It is recommended to make the analysis of the phytochemicals quantitatively and qualitatively in order to obtain the optimum condition of the ointment or medicine. Additionally, for further concern of the application on human, pH test is recommended.

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