The Healing Effect of *Fagonia indica* and *Coriandrum sativum* on Second Degree Burn Wounds in Rat Models

By

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NATIONAL UNIVERSITY OF SCIENCES AND TECHNOLOGY
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This thesis is submitted to the Atta-Ur-Rahman School of Applied Biosciences in partial fulfillment of the requirements for the degree of
Bachelor of Applied Biosciences

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National University of Sciences and Technology (NUST)

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We would like to dedicate this thesis to our beloved parents and our loving teachers

DECLARATION

We Ayesha Saad, Iqra Naz, Maryam Hamid and Mirza Zafar Nausherwaan Ahmad declare that the work presented in this thesis titled "The Healing Effect of *Fagonia indica* and *Coriandrum sativum* on Second Degree Burn Wounds in Rat Models" is our own and has been generated as a result of our research. Where information has been derived from other sources, we confirm that this has been indicated in this thesis.

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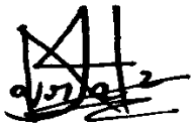

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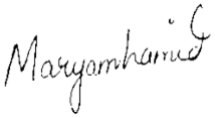

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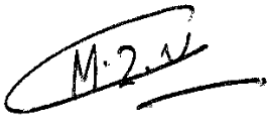

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LIST OF ABBREVIATIONS

AgNP	Silver nanoparticles
ARDS	Acute Respiratory distress syndrome
ASAB	Atta-Ur-Rahman School of Applied Biosciences
BMP	Bone Morphogenetic Protein
Cu	Elemental symbol of Copper
EGF	Endothelial Growth Factor
FDA	Food and Drug Authority
FGF	Fibroblast Growth Factor
GPx	Glutathione peroxidase enzyme
H2O	Water molecule
H2O2	Hydrogen peroxide molecule
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon gamma
IL-10	Interleukin 10
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
iNOS	Inducible isoform nitric oxide synthase
IRB	Institutional Review Board
IV	Intravenous
LB media	Lysogeny Broth media
LPS	Lipopolysaccharide

MRSA	Methicillin Resistant Staphylococcus aureus
NO	Nitric oxide molecule
O2	Atmospheric oxygen molecule
OH	Hydroxide molecule
PDGF	Platelet Derived Growth Factor
SOD	Superoxide dismutase enzyme
TNF-α	Transforming Growth Factor alpha
TGF-β	Transforming Growth Factor beta
VRE	Vancomycin Resistant Enterococcus
WHO	World Health Organization
Zn	Symbol of element zinc

ABSTRACT

Burn wounds are among one of the most common and disturbing infirmities of the body, which in many cases cause permanent disabilities. Treating these injuries so that they heal faster and preventing them from infection remains a challenging area of medical research. Current available synthetic burn treatments produce adverse effects such as erythema and pruritis which pose a hindrance in burn treatment. For this purpose, *Coriandrum sativum* and *Fagonia indica* extracts were proposed to formulate a gel that could be used as an organic alternative. Extracts from plants were obtained using Soxhlet apparatus and maceration. The plant extracts were lyophilized and used to formulate gels; 5% coriander gel, 5% fagonia gel, 5% corainder and fagonia gel and 5% coriander and fagonia gel in a known antimicrobial base. The gels were then used to treat second degree burns induced on rats via dry heat. The antimicrobial activity, skin irritant ability and burn wound healing competencies of gels were determined. Wound healing capacity was calculated by comparing the decrease in wound size on alternate days. *In silico* analysis was carried out to further corroborate the results. It was concluded that the 5% coriander gel was the most efficacious in healing of burn wounds due to its superior antioxidant concentration and its activity was comparable to the standard clinical treatment (Silver sulfathiazole).

Keywords: Burn wounds, Natural therapy, *Coriandrum sativum*, *Fagonia indica*, Wound contraction

CHAPTER 01

INTRODUCTION

Burns are a common problem all over the world. Occurrence of severe burn injuries is high as compared to the collective incidence of HIV infections and tuberculosis (Aliasl & Khoshzaban, 2013). Skin burns can have devastating consequences, the destructive outcomes of which limit not only to physical disabilities but also to the emotional and mental disturbances. An estimated 180,000 causalities occur due to burn injuries annually. Burn injuries are a major cause of death in South Asia. About one half of the total number of deaths due to burn injuries occur in South Asia. In the South Asian region, the mortality rate is 5.6 per 100,000 cases in one year (Othman & Kendrick, 2010).

Fires, flames, touching with hot liquids, electricity, acids, steam, heat, and chemicals are the agents that cause burns. Various degrees of burns include first degree burns that are minor and heal fast. Second degree burns are either superficial or deep partial thickness burns, and they often require surgical debridement and antimicrobial agents. Third degree burns are the deepest and involve full thickness of skin and require skin grafting. Fourth degree burns are extremely detrimental and often result in disability. They are difficult to heal and may require amputation (Li et al., 2011).

Management and treatment of burns is an important responsibility and its ultimate purpose is faster healing of wound and epithelialization. It is necessary to prevent the wound from infection and to avoid aesthetic complications. Wound healing is a complex process and involves various stages: inflammation followed by proliferation and remodeling of epithelium (Haghdoost et al., 2016).

During the inflammation process, to avoid infection since integrity of external barrier has been compromised bioactive molecules such as reactive oxygen species (ROS) are released to prevent infection and act as secondary messengers to non-lymphoid cells and immunocytes (Roy et al., 2006). These free radicals when present in excess can interact with lipids, DNA and proteins under conditions of high oxidative stress and damage them. Scavenging of excess ROS is achieved by non-enzymatic and enzymatic antioxidants. Non-enzymatic antioxidants include Vitamin C, Vitamin E, glutathione and carotenoids. Enzymatic oxidants responsible for scavenging ROS are catalase

(CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) which convert ROS into less harmful compounds within the cell to prevent damage (Kurahashi & Fujii, 2015).

First aid and early treatment is critical for burn wound healing. Prevention from infection is essential in order to heal the wound faster. If microbes invade the wound, they can slow the process of epithelialization and also weaken the immune function (Dai et al., 2010). For this purpose, various antimicrobial and antifungal creams are used as topical agents. Proper nutrition is a key tool in aiding the recovery from burn wounds as poor diet may result in anemia or malnutrition which prevents healing.

Several treatments to prevent infections are present. Despite the advancement in health science, frequency of burn injuries is still high globally. Most of these synthetic drugs present problems like skin rashes, allergies, resistance against drugs. However, herbal medicines have known to play essential role in wound healing from ancient times. They help in preventing infection, debridement and offer a moist area for normal healing process (Aliasl & Khoshzaban, 2013).

For centuries the practice of combating diseases and pathological conditions with plants has been very common across the world. Herbal medicinal plants could be highly preferable because of their accessibility. Researchers have also retrieved vast experiential data from the traditional medicine which supports the therapeutic effects of herbal compounds.

Coriandrum sativum generally known as coriander is a herb that belongs to the family Apiaceae. It grows in all over the world and has two main species *Coriandrum sativum L* and *Coriandrum tordylium Bornm.* It has excellent antifungal, antibacterial and antioxidant properties. It contains terpenoids, alkaloids, steroids, glycosides, tannins, flavonoids, essential fatty acids, vitamins and oils. Secondary metabolites play role in digestion, health and healing of wounds.

Figure 1.1: Coriandrum sativum (Önder, 2018)

Fagonia indica commonly known as suchi booti and dhamasa. It belongs to the family Zygophyllaceae and grows in arid regions of the world. Leaves of *Fagonia indica* are used for the treatment of several skin diseases such as acne, pimples and scabies. Its extract is used on open wounds and burn affected skin areas for its antimicrobial and antiviral properties. This plant has high nutritional value and the presence of antioxidants, minerals, and vitamins makes it pharmacologically important. It has Vitamin C and zinc present in it. It is rich in tannins and saponins. Flavonoids and alkaloids are also present but in lower quantity.

Figure 1.2: Fagonia indica flower (Azam et al., 2018)

1.1. Rationale

Burns present a serious problem for public health globally affecting the patient's physical, physiological and mental health. Over 180,000 deaths per annum are caused by burn injuries and non-fatal burns are the leading cause of morbidity including prolonged hospitalization and disability (Othman & Kendrick, 2010). Burn injuries require critical care and appropriate treatment. The pharmaceutical ointments currently used have been reported to be poor in treating bacterial infections post burn injuries since they cause allergy and skin irritation in patients. Therefore, there is a requirement for substitute methodologies to avoid and combat infections and to treat the burn injuries. The research is focused on developing an ointment from natural sources which include active ingredients.

1.2. Problem statement

1. Fire, flame, electricity, heat, hot liquids are common causes of burn injuries and infection in these wounds slows down the healing process and weakens the immune system.
2. Burn treatments available are synthetic and have side effects, which can cause irritation and allergies.
3. There is a need for an effective and appropriate ointment from natural sources in order to prevent and combat the infectious microbes and to avoid the side effects of chemical treatments.

1.3. Impact

1. Micro-organisms flourish quickly in burn wounds, primarily in large and severe wounds and cause infections and damage to the immune function.
2. Burn injuries result in disabilities and death.

1.4. Objectives

Objectives of research are:

1. To analyze the antimicrobial and healing competencies of *Coriandrum sativum* and *Fagonia indica* and formulate an effective organic gel for burn wound treatment.

2. To validate the results of wet lab using *in silico* analysis.

CHAPTER 02

LITERATURE REVIEW

2.1. Epidemiology of burn injuries

Burn injuries fall among the most devastating of all injuries and are a major public health crisis. Every year an estimated 180,000 casualties occur globally due to burn injuries. Although accurate statistical data of Pakistan is unclear, according to WHO 17% of the children face temporary disability and 18% of the children have permanent disability owing to burn wounds. Burn injuries are a major cause of death in South Asia accounting for roughly half of the deaths occurring worldwide. In the South Asian region, the mortality rate is 5.6 per 100,000 cases in one year. Studies that have been published by South Asia report more cases of injuries in females than in men (Othman & Kendrick, 2010).

In Pakistan, the reasons for such discrepancies include the traditional role of females in our society as well as the poor safety measures, poor economics and unsafe kitchen environment which places females at a higher risk. The danger posed by squatter settlements cannot be underestimated. But poor infrastructure is not the only reason. The practice of wearing loose clothing often plays a major role in burn injuries. This also has deep ties with the patriarchal mindset of the society. Females often sustain homicidal and suicidal injuries on suspicion of infidelity and are often killed to save the honor of the family (Khaliq et al., 2013).

2.2. Mechanisms of injury

2.2.1. Thermal injuries

Thermal injuries are injuries that occur due to contact with objects that are excessively hot.

- **Scalds**

  In children 70% of the burns are caused by scalds. Sometimes they also occur in the elderly. The common mechanisms include spilling of hot liquids or exposure to extremely hot water. Superficial dermal burns are caused by the scalds.

- **Flames**

  50% of the adult burn injuries are flame burns. They are often associated with inhalation injury and other associated traumas. Flame burns are usually deep dermal or full thickness burns.

- **Contact**

  These burns occur when the object is either too hot or the contact is too long. People with epilepsy or drug abusers usually get these types of burns. They have also been seen in elderly people after becoming unconscious. Contact burns are deep dermal or full thickness (Benson et al., 2006).

2.2.2 Electrical injuries

Electric current travels through the human body creating entry and exit points, damaging the tissue between these points. Voltage determines the degree of tissue damage. Electrocution injuries can be divided into the following.

- **Domestic electricity**

  It is a low voltage current and tends to cause small, deep contact burns at the exit and the entry sites of the current. Arrhythmias can occur due to the interference of the current with the cardiac cycle of the individual.

- **True High Tension**

  These injuries are caused by the high voltage current passing through the body i.e. 1000 v or greater. The tissue damage is extensive and limb loss can also occur. There is also bony tissue necrosis.

- **Flash injuries**

  In this type of injury, no current actually flows through the body and it is a tangential exposure to high voltage current arc. Heat from the arc causes the superficial burns. Face and hands of the individuals are mostly affected (Hettiaratchy & Dziewulski, 2004).

2.2.3. Chemical burns

These types of burns usually occur due to industrial accidents but may occur with household products as well. They are caused by acids and bases. In case of bases, liquefactive necrosis can occur due to skin coming in contact with the bleaches. They have the ability to penetrate into the tissues causing protein and

collagen denaturation. Acid burns cause coagulative necrosis and are very painful. E.g. the hydrofluoric acid penetrates deeply into the tissues and causes fatal systemic toxicity even in small burns (Benson et al., 2006).

The initial management of all chemical burns is the same. The contaminated clothing is to be removed and the affected area is thoroughly washed with water. This limits the burn depth. In case of any eye injury, referral to an ophthalmologist is mandatory.

2.4. Classification of burns

Burns are classified on the basis of their severity and depth into four major types:

1. First degree

The affected area is red, dry and painful. These areas of skin also tend to blanch, turning white as the blood flow is restricted with compression. The healing time is usually 3 to 7 days.

2. Second degree

Second degree burns are divided into two categories as follows:

A. Superficial partial thickness burns

They extend into the superficial papillary dermis. The affected area becomes red with blisters, the wound is wet or weeping and the skin still blanches. They are more painful than the first-degree burns. It usually heals within 3 weeks with minimal scarring.

B. Deep partial thickness burns

They extend deep into the reticular dermis. They may vary in color from yellow, white and red. They have blisters; which can be dry or wet. Since there is damage to the nerve endings, burns of this degree may not blanch. The pain is minimal due to decreased sensation. It usually heals within 3 to 8 weeks with scars present on the affected area of skin (Schaefer & Nunez Lopez, 2020).

3. Third degree

They involve the full thickness of skin and the subcutaneous structures. They appear waxy white to leathery grey or black and dry. There is no blanching and the pain feels like a deep pressure. It is relatively painless due to a decrease in sensation. Burns of this type require skin grafting and heal by contractures. The

subject takes more than 8 weeks to recover completely (Schaefer & Nunez Lopez, 2020).

4. Fourth degree

They appear charred black, skeletonized and dry. The pain only from deep pressure but can be painless from the complete destruction of the nerve endings. Fat, muscle and bones are affected. They may require amputation as they have no healing capacity (Rachel Warby & Christopher V. Maani., 2019).

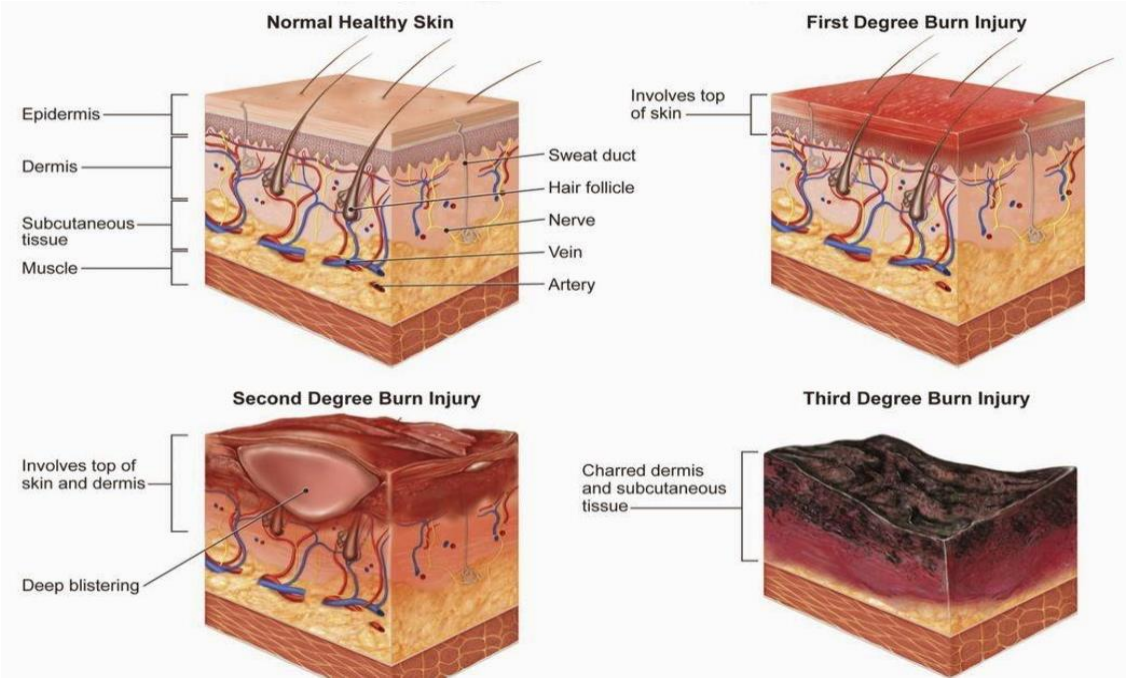

Figure 2:Varying degrees of burn (Rachel Warby & Christopher V. Maani., 2019)

2.5. Burns Pathophysiology

2.5.1. Local response of burn

1. **Zone of coagulation**

   This is the point where maximum damage has occurred due to the exposure of skin to the heat or chemical. Due to the coagulation of the constituent proteins there is irreversible tissue loss.

2. **Zone of stasis**

   There is decreased tissue perfusion. The main aim of the burn resuscitation here should be to increase the tissue perfusion and prevent any damage from

becoming irreversible. If prolonged hypotension or edema occurs in this region it can also become irreversibly damaged.

3. **Zone of hyperemia**

   This is the outermost zone in which there is increased tissue perfusion. The tissue recovers after variable time unless there is prolonged hypoperfusion or severe sepsis (Hettiaratchy & Dziewulski, 2004).

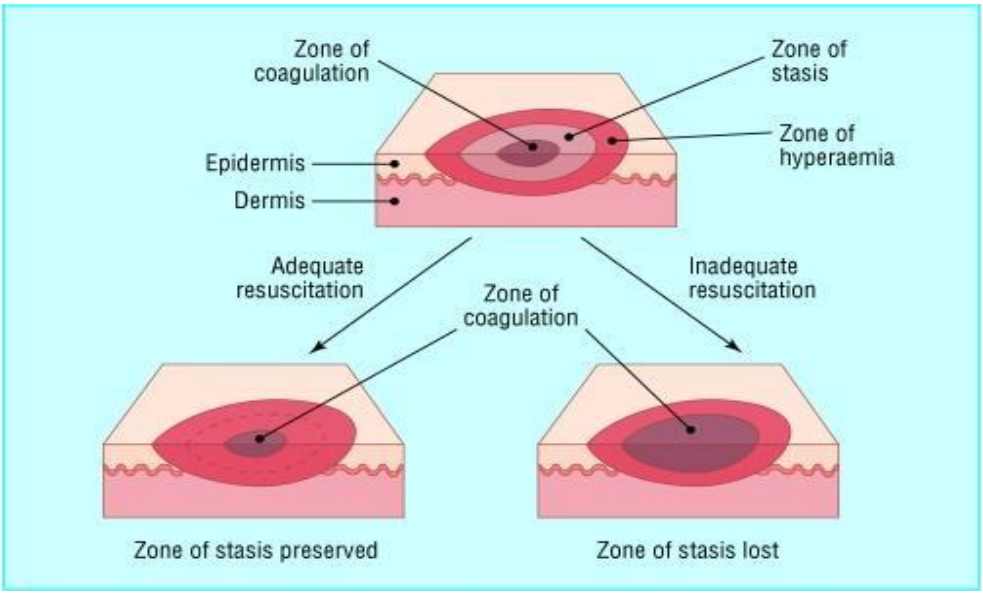

Figure 3:Jackson's burn zones (Hettiaratchy & Dziewulski, 2004)

2.5.2. Systemic Effects of Burns

Cytokines and other inflammatory inhibitors that are released at the site of a burn injury have a systemic effect. This happens when the burn reaches 30% of the total body surface.

Heart

Following the burn injury there is a depression in the cardiac output. Even when the plasma volume, arterial pressure and the cardiac output gets back to normal, a prolonged reduction in the cardiac output remains. During burn traumas the cardiomyocytes secrete TNF-α and IL-6. These cytokines tend to increase the cardiac dysfunction. The flux of Calcium ions between the cytoplasm and the sarcoplasmic reticulum is altered during major burn injuries. LPS induces cardiac apoptosis (Nielson et al., 2017).

Liver

It provides the metabolic substrates to the organisms. The inflammatory response is mounted by the liver. Inflammatory cytokines activate the specific transcription of the genes present in the liver. This includes the complement, kinin, clotting factors and fibrinolytic protein systems. In severe cases of burns hepatomegaly is commonly found. Cholestasis and the presence of large fat droplets within the hepatocytes cause hepatomegaly (Nielson et al., 2017).

Lungs

They present susceptibility to edema formation. In severe cases of burn injuries, all subjects develop generalized edema which is related to the burn size and composition. After an individual gets large burns on his body, his pulmonary wedge pressure increases that derives from a general vasoconstriction of microcirculation. Pulmonary edema also develops due to hypoproteinemia. Due to vigorous fluid resuscitation, ARDS which is known as the acute respiratory distress syndrome can occur. There are certain physiological consequences of such injuries which include reduction in airway compliance and impaired gas exchange. Morbidity in severe burn injuries increases when it is accompanied by the inhalation of smoke as it can cause ARDS (Nielson et al., 2017).

Renal

Reduced blood flow and cardiac output leads to decreased renal blood flow and glomerular filtration rate. Acute tubular necrosis can occur due to the toxins that are released from the wound. In case of electrical injury myoglobin that gets released from the muscles is very damaging to the kidneys causing renal impairment. Hypovolemic shock state, sepsis and rhabdomyolysis can occur. Massive hemolysis may result in hemoglobinuria (Nielson et al., 2017).

Metabolic rate

The basal metabolic rate increases thrice its original rate. There is an increased use of glucose and an increase in CO2 production. Glycogenolysis, lipolysis and proteolysis occur. There is futile substrate cycling. The metabolic changes include a deficiency in the essential vitamins and electrolyte imbalance. Metabolic acidosis due to hypoxia and lactic acid can also occur (Hettiaratchy & Dziewulski, 2004).

2.6. Current interventions for burn injuries

Burn wounds are among one of the most distressing injuries. They have a great influence on the patient's physical, physiological and psychological health. They remain a serious problem and require critical care and proper treatment. These injuries are intricate and present many problems that necessitate life-long rehabilitation. Currently various therapies and treatments are available. However, burn injuries are still one of the topmost reasons of death and disability globally. After stabilization and before the reconstruction of burn wounds, these therapies are aimed at treating the complications of burns, promoting healing and prevention of further complications. Specified facilities concentrate on preventing infection and improving functional recovery of wounds. Patients with severe and larger burns can have a lengthier period of hypermetabolism, chronic inflammation, weight loss and slow wound healing. Also enhanced susceptibility to infection can lead to sepsis and systemic inflammation.

2.7. Management of burn wound:

Management and treatment of burns is an important responsibility that includes many constituents from the initial first aid, evaluation of the burn depth and size, fluid restoration, wound excision, grafting and coverage, infection control to nourishing support. Management of wounds is as important as treatments. If the wound is not managed properly, the burned surface area and risk of infection can increase. Morbidity and mortality rates increase if burned surface area is large particularly in old people, for whom even small burns can be lethal. Essential wound management measures include:

1. **Rule of 9s**

   Rule of 9s is used for the assessment of the burned surface area. Body parts are distributed into anatomical sections that represent 9% (or multiples of 9) of the entire body surface. While outstretched palms and fingers represents about 1% of the body surface.

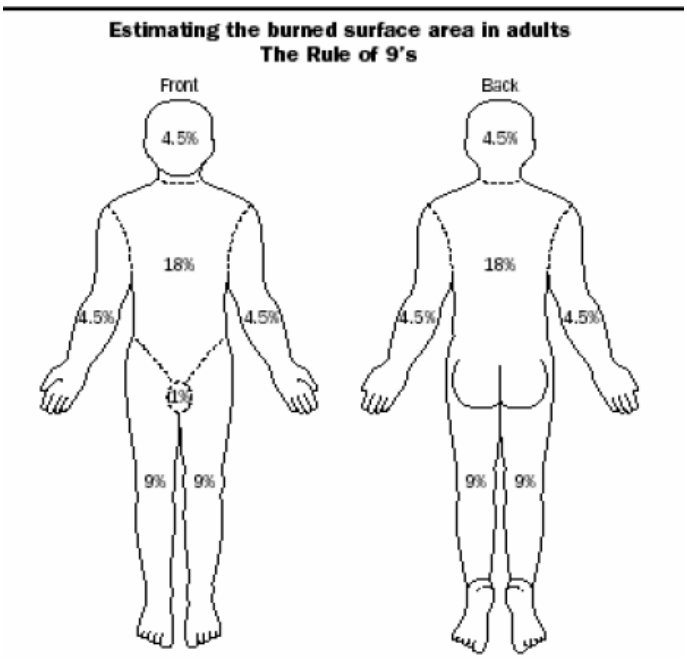

Figure 4:Estimation of burned surface area in adults (Kumar, 2007)

Rule of 9s is not accurate to assess the burned surface area of skin in children since a child's head and lower extremes represents the different magnitudes of the surface area than in an adult.

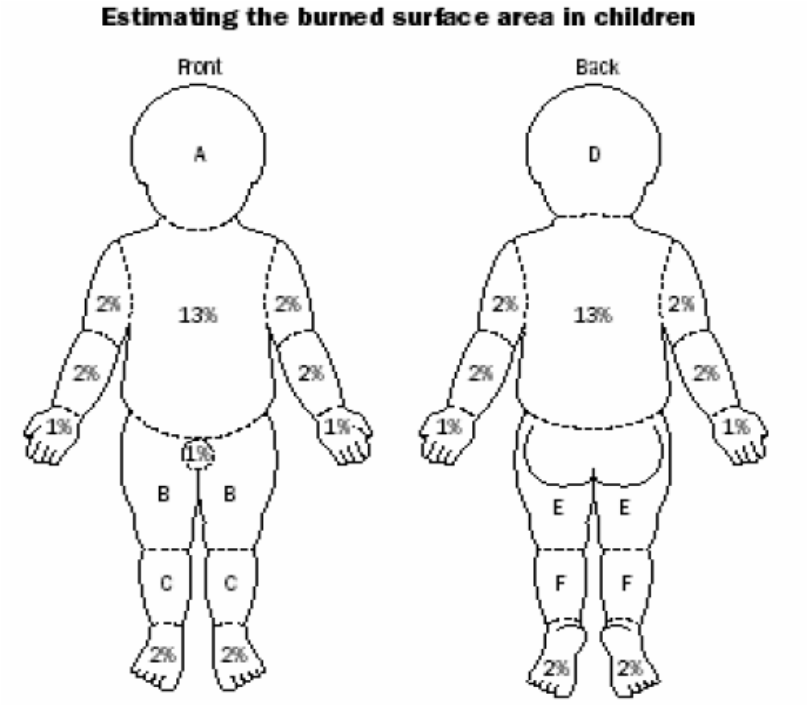

Figure 5:Estimation of burned surface area in children (Kumar, 2007)

2. **First aid**

   To assess the severity of wound it is vital to evaluate the depth of burn to make strategy for future wound care. Patients with burn are provided with first aid. Burn is drenched with cool water and burned clothing is removed to prevent further damage. First six hours are very critical for patients with severe burns.

3. **Initial care & treatment**

   Initial and daily care and treatment is important. Burns are initially sterile, and treatments aims to enhance healing and to prevent infection. Change of dressing daily is necessary to prevent seepage through the dressing.

4. **Care during healing phase**

   Depth of wound and surface involved influence the duration of healing. Superficial burns heal rapidly. These are cured with regular dressing and care of local wound up until epithelialization takes place. Full efficient recovery is the rule. To prevent infection antibiotics and topical treatments are used. Specialized care is required for severe burn injuries.

5. **Nutrition**

   Proper nutrition is necessary for healing. Patients need high energy and proteins for the catabolism of trauma, loss of heat, infection and regeneration of tissue. Anemia and malnutrition avert healing so balanced diet is essential. Importance of various nutritional components must be considered during burn injuries, as intake of excess carbohydrates may cause hyperglycemia that can aggravate inflammation and muscle deterioration. While excess fat intake may exacerbate immunosuppressed state that increases the risk of infection and sepsis (Rowan et al., 2015). Therefore, food consumption must be closely monitored in burn patients.

Treatments for 1st degree burn

These burns are minor and they do not need surgical involvement. They are usually cured with topical moisturizers, skin care products like Aloe Vera cream, antibiotics, pain medication, and avoidance of recurrent injury. The healing time is usually 3 to 7 days.

Treatments for 2nd degree burn

Treatments for second degree burn aims at debridement of blisters that are intact and are at the risk of rupture to get rid of the fluid. Covering of wound with topical antimicrobial mediators or synthetic dressings on wounds.

1. **Surgical debridement (excision and grafting)**

Full-thickness burns that cover about 25% of the body surface area are mostly treated with surgery (Dogra, 2004). For this adequate autologous skin grafts are required to close the wounds. Early surgical debridement is used to remove non-viable tissue so that wound bed becomes relatively infection free. In addition, removal of tissues that are dead has great potential to decrease the chemical mediator's production as theses mediators ((EGF, FGF, PDGF, TGF-β), cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α, IFN-γ), and chemokines can stimulate inflammatory cascade that may lead to the failure of organ (Pereira Beserra et al., 2019). So thorough debridement should advance timely even if donor sites are not sufficient to provide covering of total wound then biological dressings (allograft) should be used (Arthur P Sanford, David N Herndon, 2011).

2. **Topical antimicrobial agents:**

Micro-organisms thrive speedily in burn wounds, mainly in those wounds that are severe and damage the immune function. Topical antimicrobial mediators play a significant role. Infections are considered a major problem up until the integrity of the skin, lungs and gut can be reestablished and post burn immunosuppression takes place. In topical antimicrobial therapy, mediators are used to control localized infection at the site of injury, but this will not prove to be useful if substantial amounts of devitalized tissue remain.

Gentamicin nitrofurazone, sulfate, povidone-iodine, mafenide acetate, silver sulfadiazine and silver nitrate are agents that can infiltrate into the burn infected tissue in order to grasp the microbial cells that gets invaded in the wound.

For many years' silver sulfadiazine (SSD) has been used as a regular topical antimicrobial for wounds caused by burn. Nano-crystalline silver (AgNP) hydrogel is also used for managing burn wounds (Cartotto, 2017). Metallic silver has a distinctive place as a powerful antimicrobial agent against which no resistance has been found yet.

Mafenide acetate 11.2% ointment (e.g., sulfamylon) is among one of the most active topical antimicrobial agents; it has a broad spectrum for antimicrobial activity, along

with some antifungal properties. Aloe Vera gels are also used as topical antimicrobials for burn wounds. Quench (active component: SSD) is a common and standard cream used as topical treatment for burn injuries.

Treatments for 3rd degree burn

They need surgical therapy and grafting of the skin if the burned surface area is larger than 2cm. Severe burns injuries that cover large parts of the body may require more intensive treatments such as antibiotics through intravenous (IV) route or IV fluids to manage fluid deficiency.

Treatments for 4th degree burn

Precise treatment of fourth degree burn depends upon the extent of tissue damage. It affects the bones, muscle and nerves. These burns are treated with pain medications and ointments for infection prevention. Cosmetic reconstruction, such as skin grafting may be required as these burns have no healing capacity. Sometimes these burns may result in disability (Kristeen, 2019).

2.8. Drawbacks of current treatments

Various treatments and antimicrobial agents are available for burn injuries but still 265,000 deaths occur every year due to various types of burns (Othman & Kendrick, 2010). There are some drawbacks of these treatments. Skin grafting and surgery is a painful process and incurs high cost. Auto-graft for skin is effective but if it is not available allograft is used which can result in graft rejection. One of the common agents causing bacterial infections and death during burn injuries are Gram-negative and Gram-positive bacteria.

Table 1 Gram negative and Gram positive bacterial agents responsible for burn wound infection (Norbury et al., 2016)

Gram negative bacteria responsible for burn wound infection	Gram positive bacteria responsible for burn wound infection
Pseudomonas P. aeruginosa	Staphylococcus aureus (*Methicillin-resistant* S. aureus (MRSA))
Acinetobacter	Streptococcus (*S. pyogenes*) and (*S. agalactiae*)
Enterobacteriaceae (Escherichia coli, Klebsiella, Enterobacter, Proteus)	Enterococcus (vancomycin-resistant *Enterococcus* (VRE))

For aggressive fungal infections (by Candida albicans, Aspergillus, Fusarium, and zygomycetes) effective topical treatments do not exist, and infections in wound due to fungus are linked with high death rates especially in case of larger burns (Adhya et al., 2014). Patients often get infections that are resistant to drugs. Reasons behind this include: longer stay in hospital, hindrance in healing of wound, greater costs, and higher death rates.

Some of the adverse effects of Silver sulfadiazine (SSD) include leucopenia, argyria, renal toxicity and hepatic illnesses. Ointments cause toxicity to body cells like fibroblasts and epithelial cells, it also causes skin rashes and skin allergy. Mafenide is the inhibitor of enzyme carbonic anhydrase and can cause metabolic acidosis, therefore it is not recommended as a first-line antimicrobial ointment for burn injuries. The adhesive nature of these topical preparations can make the process of dressing change painful. Sometimes topical antimicrobial creams cause a local rash, skin allergies and irritation (Cartotto, 2017).

2.9. *Coriandrum sativum*

Coriandrum sativum (coriander) is a member of family Apiaceae and is known as cilantro, Arab parsley or Chinese parsley. It is an annual herb used in Indian, Middle

Eastern and Mediterranean cuisine. The plant grows all over the world but it is cultivated mainly in the tropical areas such as Russia, India, Ukraine, Morocco and Mexico. *Coriandrum sativum* has long history of being used as a culinary herb. Sanskrit literature before 5000BS and Greek Ebers Papyrus earlier 1550 BC have mentioned Coriander. Coriander contains terpenoids, steroids, cardiac glycosides, tannin, flavonoids, saponin and alkaloids. The fatty acids present in coriander are petroselinic acid, linoleic acid, oleic acid and palmitic acid. It is also reported that it is a rich source of vitamins, minerals and iron along with thiamine and zinc. Coriander extracts are considered to be antibacterial, antifungal, antioxidant, cardio protective, and exhibit anti-aging properties (Önder, 2018).

2.9.1. Botanical characterization of the plant

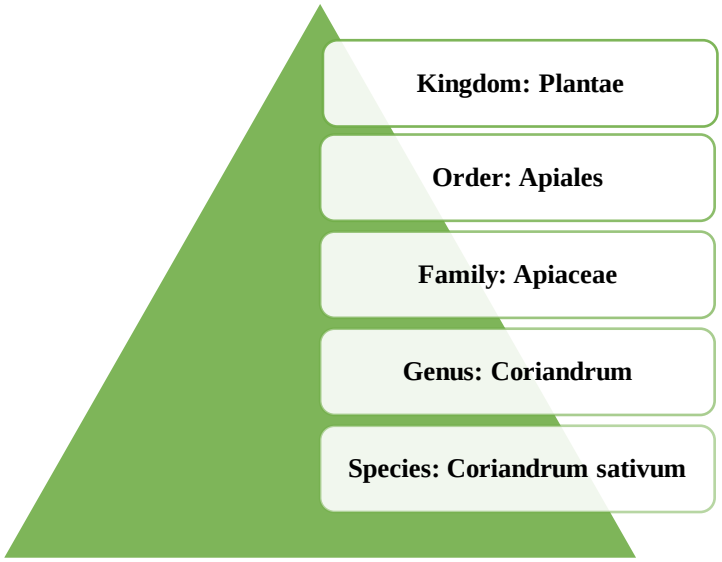

Figure 6:Botanical classification of Coriandrum sativum (Önder, 2018)

2.9.2. Morphology of *Coriandrum sativum*

Two distinct morphological types are found: One erect and tall with strong shoot and short branches. The other is bushy with weak shoot and long spreading branches. It is a plant with erect stems about 1 to 3 feet high, slender and branched. The segments of the uppermost leaves are linear and more divided. The flowers are in short-stalked umbels, five to ten rays, pale, almost white, the seed clusters are very symmetrical, and the seeds fall as soon as ripe. The lowest leaves are stalked and pinnate, the leaflets roundish or oval, slightly lobed.

2.9.3. Geographical Distribution

Coriandrum sativum is believed to be originated around the Mediterranean. Two species are found however *Coriandrum sativum L* is the most cultivated specie. Major countries where Coriandrum sativum L is cultivated are India, Turkey, Spain, Italy, Morocco, Mexico and Argentina (Bhuiyan et al., 2009).

2.9.4. Nutritional Value

The nutritional importance of coriander is because of its green leaves. Like all other green vegetables coriander's leaves are also rich in vitamins, minerals and iron. Leaves of coriander contain high amounts of Vitamin A (Carotene Beta) and Vitamin C. The green herbs contain Vitamin A and Vitamin C in quantity of 12mg/100 g and 160mg/100 g respectively. Coriander is very low in saturated fats and cholesterol and a very good source of dietary fiber, thiamine and zinc. Green coriander contains approximately 84% water (Bhuiyan et al., 2009).

Vitamins and Antioxidants

Green coriander leaves and stem are rich in vitamins and other antioxidants. Vitamin that is found most abundantly in coriander is vitamin C. Approximately a 100g portion of coriander contains about 27mg of vitamin c. Vitamin A and Vitamin E are also found in significant quantities. Other phytochemicals which are important for dietary needs and normal function of body such as carotenes, zeaxanthin, choline and pantothenic acid are also found. Most of these phytochemicals other than playing their role specifically in wound healing signaling pathway are also antioxidants and reduce the number of oxidative radicals in the tissue.

Table 2 Bioactive compounds present in *Coriandrum sativum*. Vitamins and phytochemicals present per 100 grams in coriander ("USDA Food Composition Database")

Vitamins and Phytochemicals	Per 100 g portion size
Vitamin C	**27 mg**
Niacin	**1.1 mg**
Choline	**12.8 mg**
Vitamin A (IU)	**6748 IU**
Vitamin E	**2.5 mg**
Carotene beta	**3930 ug**
Carotene Alpha	**36 ug**
Lutein + Zeaxanthin	**865 ug**
Pentothenic Acid	**0.57 mg**

Mineral Composition

Composition of minerals in the leaves of coriander is discussed here.

Table 3 Minerals found in the raw Coriander. ("USDA Food Composition Database", 2019)

Minerals	Per 100 g portion
Calcium	**67 mg**
Iron	**1.7 mg**
Magnesium	**26 mg**
Phosphorus	**48 mg**
Zinc	**0.5 mg**
Copper	**0.22 mg**
Manganese	**0.42 mg**

2.10. *Fagonia indica*

Fagonia indica is a plant belonging to family Zygophyllaceae. It is mainly found in arid and semi-arid regions of the world. It is a desert plant. In different regions of Pakistan, India and Afghanistan it is known as Suchi booti and Dhamasa booti. *Fagonia indica* is a small shrub with stiff branches and striate, glandular and slender twigs.

This plant is mainly found in dry regions of Asia such as Pakistan, India and Afghanistan, some parts of Arabia and Africa which includes Saudi Arabia and Egypt respectively. The leaves of *Fagonia indica* are boiled and the water is applied and consumed for the treatment of multiple skin diseases such as acne, pimples and scabies. The extract is also used on open wounds for its antimicrobial and antiviral properties. Recent studies have shown the effectiveness of plant against several tumors (Anil et al., 2012; Soomro & Jafarey, 2003). (floraof pakistan)

It is rich in tannins, saponins and cardiac glycosides. Anthraquinones, flavonoids, coumarins, alkaloids and triterpenoids are also present in relatively low quantities (Soomro & Jafarey, 2003).

2.10.1. Botanical characterization of the plant

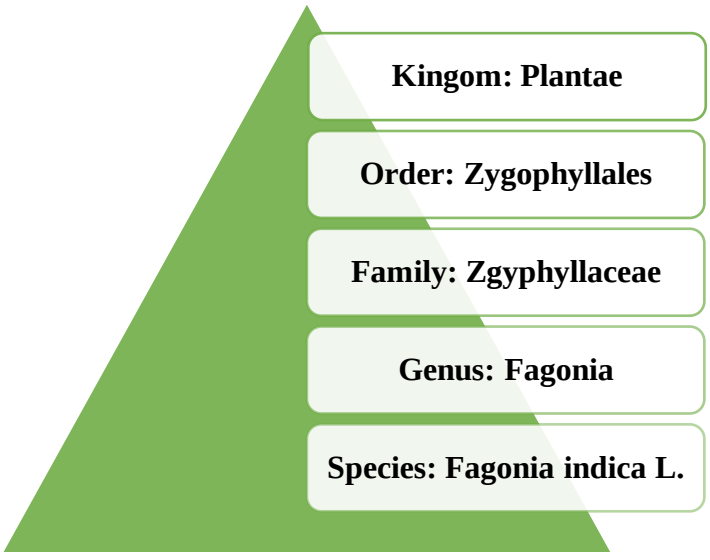

Figure 7:Botanical classification of Fagonia indica (Soomro & Jafarey, 2003)

2.10.2. Morphology of *Fagonia indica*

It is a small, erect and spiny shrub with glabrous, terete and slender branches. Flowers of the herb are purple and rose colored. Each flower has 5 petals which are approximately 6 mm in length. Leaves of the plants are opposite with 1-3 foliates. Length of the petioles vary from 3-30 mm. Stipule has 2 pair of sharp thorns exceeding

12 mm in length. It is a herb that branches as it moves away from its roots. It has woody structure at the base and has branches at the top (Anil et al., 2012).

Figure 8:Morphology of Fagonia indica plant (Anil et al., 2012)

2.10.3. Geographical distribution

Fagonia is mainly found in arid and desert areas throughout the globe. In Asia *Fagonia indica* is found in dry areas of Pakistan (Sindh, Baluchistan and southern Punjab), India (Rajasthan, Gujrat and south western Punjab) and Afghanistan. Other than Asia it is also found in parts of Africa such as Egypt and in Americas as different species of same genus.

2.10.4. Nutritional value

Suchi booti is a plant with high nutritional value. It is a plant with especially high pharmacological importance because of presence of antioxidants, vitamins and minerals. It is a source of vitamin C (ascorbic acid) and a rich source of Zinc which also acts as an antioxidant in animals (Anwar Ali Shad, Hamidullah Shah F.K. Khattak Nabeela G. Dar and Jehan Bakht, 2002).

Table 4 Constituents of nutritional value present in different parts of the plant Fagonia indica (Ghulam et al., 2014)

Plant Parts	Constituents						
	Moisture Content (%)	**Ash Content (%)**	**Protein Content (%)**	**Fat Content (%)**	**Fiber Content (%)**	**Carbohydrate (%)**	**Gross Energy (Kcal/g)**
Roots	7.4	6.7	9.0	3.7	62.3	10.9	428.9
Stem	9.1	11.9	8.6	4.9	54.3	11.2	400.8
Leaves	8.4	16.5	10.0	8.9	41.3	14.9	399.5
Fruits	7.4	10.7	10.2	6.2	55.2	10.3	423.1
Mean	8.0	11.45	9.45	5.9	53.27	11.8	413.0

Chemical composition

Table 5 Mineral composition present present in Fagonia arabica a closely related plant to Fagonia indica from Fagonia species (Anwar Ali Shad, Hamidullah Shah F.K. Khattak Nabeela G. Dar and Jehan Bakht, 2002)

Minerals	
Nutrients	**Quantity ug/g**
Zinc	28.4
Manganese	35.4
Iron	644.3
Copper	39.2
Phosphorus	766
Potassium	55097
Sodium	730
Calcium	6190

Vitamins and Antioxidants

In suchi booti significant number of phytochemicals with antioxidant ability. These include sapogenins, saponins and flavonoids. The antioxidants described here have O2, OH and NO scavenging capability However only one vitamin is found in significant quantity and that is Vitamin C. Vitamin C is found to be in 8.13 mg per 100 grams of plant (Anil et al., 2012).

Table 6 This table represents the presence of different phytochemicals that have antioxidative effect (Anil et al., 2012)

Antioxidants	
Sapogenins	Nahagenin
	Hedragenim
	Ursolic acid
Saponins	23,28-di-*O*-β-D-glucopyranosyltaraxer-20-en-28-oic acid
	3β,28-di-*O*-β-D-glucopyr acid
	21,22α-epoxy-23-*O*-β-D- glucopyranosyl-nahagenin
	3-*O*-{[β-d-glucopyranosyl- (1→2)]-[α-l-arabinopyranosyl-(1→3)]-α-l- arabinopyranosyl}-ursolic acid-28-*O*-[β-d-glucopyranosyl] ester (indicasaponin A)
	3-*O*-{[β-d-glucopyranosyl- (1→2)]-[α-l-arabinopyranosyl-(1→3)]-α-l- arabinopyranosyl}-oleanolic acid-28-*O*-[β-d-glucopyranosyl] ester (indicasaponin B)
Flavonoids	Quercetin
	Isorhamnetin-α-3-O rhamnoside
	Quercetin 3-O-β-D- glucopyranosyl -(1''-6''')-β-D-glucopyranoside
	quercetin 3-O-β-D-galactopyranosyl -(6''-1''')- α-L-2''' acetyl rhamnose- (3'''-1'''') β-D-glucopyranoside
Vitamin C	Ascorbic Acid (8.13 mg/100g)

2.10.5. Antioxidant activity

Extract of *Fagonia indica* has shown to be involved in decreased levels of free radicals because of increased Cu-Zn SOD expression. Expression of SOD causes the activation of GPx enzymes and causes the conversion of H2O2 produced by SOD from superoxide (Anil et al., 2012; Nimse & Pal, 2015). GPx then converts H2O2 to H2O. Zinc is found in the suchi booti which is required for the activation of SOD. Extract of suchi booti is involved in decreased expression of iNOS.

CHAPTER 03

MATERIALS AND METHODS

3.1. Preparation of plant extracts

Extraction of plant materials from *Fagonia indica* was conducted using Soxhlet method. 25g of powdered *Fagonia indica* was added to a porous cellulose thimble. Isopropyl alcohol, methanol and n-hexane were used as solvents. 250 ml of each solvent was used against 25g of plant material for extraction. The Soxhlet apparatus was run for 16 hours to maximize extraction.

Plants contain a multitude of compounds with varying functional groups. This results in differences in their polarity which in turn effects their solubility. The general rule of thumb for solubility is “like dissolves like”, meaning polar solutes will dissolve in polar solvents and non-polar solutes will dissolve in non-polar solvents. Methanol and isopropyl alcohol are polar in nature while n-hexane is non-polar. The purpose of using these three different solvents is to extract all active compounds from plants, so the composition of extract is as close to the total chemical composition of plant (Andri Cahyo Kumoro et al., 2008).

A vacuum rotary evaporator was used to concentrate the crude extract solution at temperature of 70 °C. Removal of solvent was conducted at this temperature to avoid degradation of components due to high temperature. The extract was stored overnight in -80°C and frozen extract was then lyophilized to achieve a fine powder.

Maceration was carried out to obtain extract solution from fresh plant material of *Coriandrum sativum*. The fresh C. *sativum* was washed to remove dirt and other materials on the surface of the plant. The leaves were picked from the stems and 25g of leaves were blended with isopropyl alcohol, methanol and n-hexane respectively. The plant material was then placed into a stoppered flask along with the solvent and left to seep for 24 hours. The flask was frequently agitated until most of the plant material had dissolved. Finally, the mixture was strained to remove any insoluble plant material and was concentrated using a rotary evaporator (Shedoeva et al., 2019).

3.2. Formulation of gel

The three extract powders (from methanol, isopropyl alcohol, and n-hexane) of *Fagonia indica* were combined to form one homogenous powder mixture. The extract powders of *Coriandrum sativum* were also combined to form one homogenous powder mixture. These extracts were labelled as F3 and C3 respectively and closely resemble whole chemical composition of respective plants.

The topical gel was formulated by using 5% of powdered extracts in white petroleum jelly. Petroleum jelly was melted gently over a hot plate and powdered extracts was added slowly and mixed thoroughly to achieve a homogenous gel. The gel was stored in an airtight container at 4°C. The following four gels were formulated using the powder extract.

a) ***Fagonia indica gel*** *5%* (namely gel A)
b) ***Coriander sativum gel*** *5%* (gel B)
c) ***F. indica and C. sativum gel****:* constitutes 2.5% *F. indica* and 2.5% *C. sativum* in 95% petroleum jelly. Both plant extracts were used in this gel to determine whether they would have a synergistic effect when combined. (gel C)
d) ***F. indica and C. sativum gel****:* constitutes 2.5% *F. indica* and 2.5% *C. sativum* in 95% polyfax (active constituents polymyxin B sulphate and bacitracin zinc). The purpose of using polyfax as a base in one of the formulations was to elucidate whether a base of known antibiotic properties will improve the efficacy of gel. (gel D)

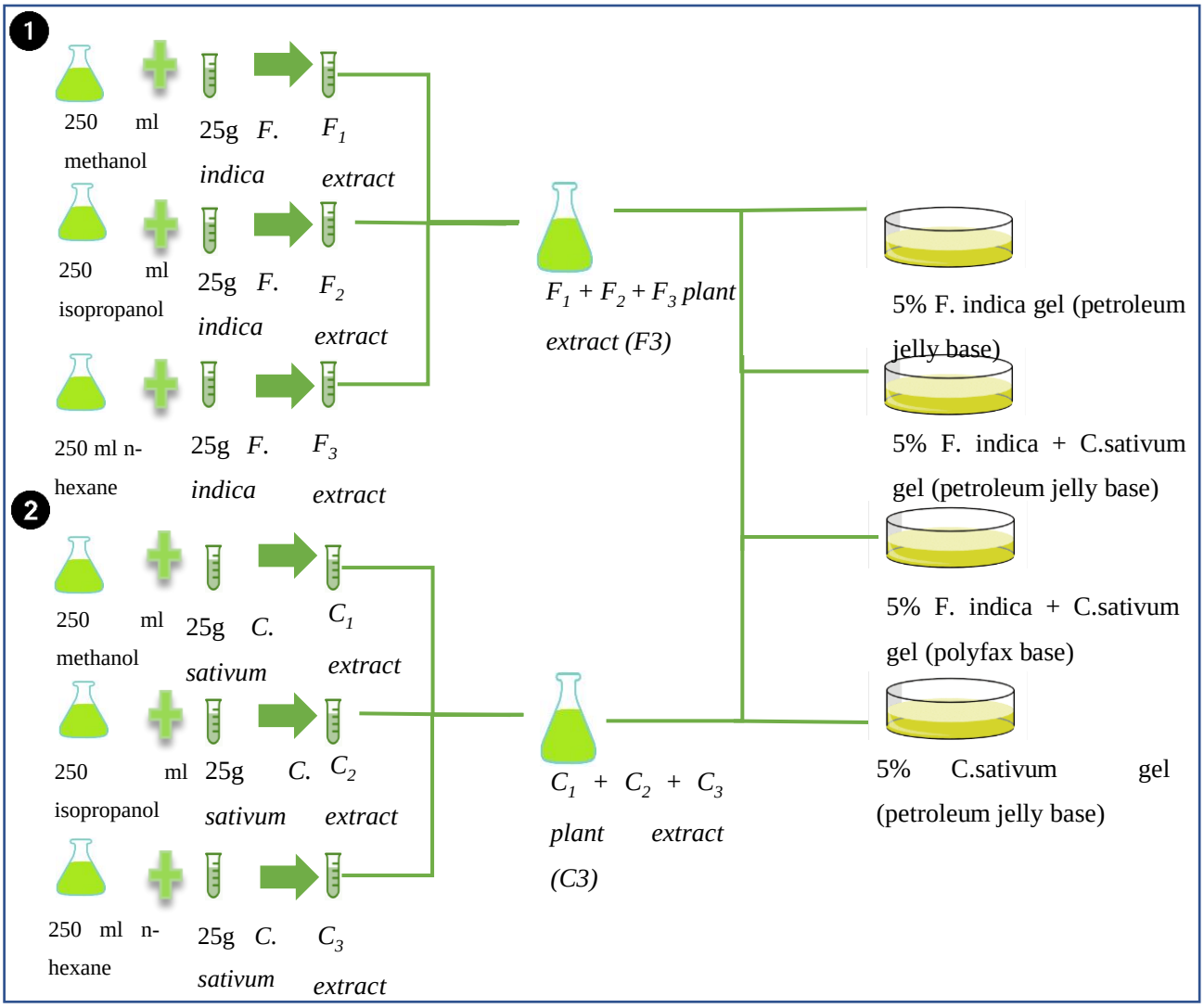

Figure 9:Formulation of gels. Step 1 indicates the usage of three different solvents for extraction of plan material from F. indica which are then combined to form a single mix of extracts called F3. Step 2 shows the usage of three different solvents for extract from C. sativum using ,maceration method and combining them together to make C3 which is then used to formulate gels.

3.3. Experimental protocol

The experiment was conducted on 18 male Wistar rats aged 10 weeks and weighing approximately 250-300g. The rats were caged in controlled conditions with food and water ad libitum and 12 hours light-dark cycle.

The rats were randomly assigned to either one of the four experimental or to the two control groups. Each group constituted of 3 rats. The rats were marked in the six groups as follows:

- Group 1: The rats were treated with *F. indica* extract.
- Group 2: The rats were treated with *C. sativum* extract.
- Group 3: The rats were treated with *F. indica* and *C. sativum* extract.
- Group 4: The rats were treated with *F. indica* and *C. sativum* extract in a polyfax base.
- Group 5: The rats were treated with silver sulfathiazole and served as a positive control.
- Group 6: The rats were left untreated and served as a negative control.

This was a seven-day experimental trial.

3.4. Ethical approval

Prior to conducting the experiment ethical approval was sought from Institutional Review Board, Atta-Ur-Rahman School of Applied Biosciences (IRB, ASAB). The rats were housed, fed and given anesthesia and analgesics according to internationally determined standards.

3.5. Burn protocol

Rats were anesthetized using chloroform as general anesthesia followed by subcutaneous injection of 7mg/kg lignocaine for local pain relief. After anesthesia dorsal trichotomy of area measuring approximately 3 cm^2 was performed using a razor and antisepsis with 1% polyvinylpyrrolidone-iodine was carried out (Cai et al., 2014).

Burns were inflicted on depilated areas through contact of skin with an aluminum bar of area 2.5cm^2 preheated to a 100°C and kept in contact with the rat's skin for 30 seconds

to induce a symmetrical full thickness second-degree burn (Yuniarti & Lukiswanto, 2017).

Analgesics were orally administered for pain management. Ibuprofen 15mg/kg was administered through water of rats. The dose was modified slightly as per weight of each rat. Water was strictly monitored to determine if sufficient amount is being consumed (Hayes et al., 2000).

3.6. Antibiotic disk diffusion test

The antimicrobial activity of *F. indica* and *C. sativum* extracts was determined against *Methicillin Resistant-Staphylococcus aureus* (MRSA) and *Escherichia coli* which are gram-positive and gram-negative bacteria respectively. Metronidazole- a broad spectrum antibiotic, was used as a positive control in the experiment.

3.6.1 Principle

The antibiotic disk diffusion test or Kirby-Bauer test is used to determine the sensitivity of a pathogenic aerobic or non-aerobic bacterium to an antimicrobial compound. Agar is impregnated with pathogenic bacteria to be tested; filter disks saturated with antimicrobial compound are then placed on agar plate (Bauer et al., 1966).

Antimicrobial compound diffuses into the agar gel due to interaction of filter disks with water within the gel. The rate of diffusion of individual compounds depends on the molecular weight and the solubility properties of antimicrobial compounds (Jorgensen & Ferraro, 2009). The concentration of antimicrobial compound is highest near the disk and reduces as the distance from disk increases.

After the gel has been inoculated with bacterium and disks have been placed over the gel, petri dish is incubated for 18-24 hours at 36±2°C after which zone of inhibition is observed. The zone is measured using a Vernier caliper and the sensitivity of bacteria to antimicrobial compound can be determined by comparing it with a standard zone (Jan, 2008).

3.6.2. Protocol

MRSA and *E. coli* bacterial stock of concentration 10^{-1}, 10^{-2} and 10^{-3} was spread on LB agar (also called lysogeny or Luria broth), in petri dishes. Then filter disks were dipped in *F. indica* and *C. sativum* extracts and placed onto the petri dish. Metronidazole was

used as positive control in this experiment. After incubation for 18 hours at 37°C the zones of inhibition were measured using a Vernier caliper.

3.7. Skin sensitivity test

All formulations of gel i.e. Gel A, B, C and D underwent testing for their irritant ability. A 10-day trial was conducted to determine whether gel would cause edema or erythema in subject after topical application. This test was carried out to ensure safety of treatment.

3.7.1. Principle

The skin irritation test is used to determine the safety of a topical treatment. The treatment is applied to the skin of a subject and observed over marked intervals of time to subjectively assess whether subject shows signs of erythema, edema or any other uncommon symptoms of allergies (Aiyalu et al., 2016).

3.7.2. Protocol

Four male rats were housed in separate cages in controlled conditions with food and water ad libitum and 12 hours light-dark cycle. 24 hours prior to skin sensitivity test the hair on the backs of rats were shaved off clearing a patch of around $5cm^2$. A thin film of gel was applied to each rat and subjectively assessed and scored after marked intervals of 1 hour, 24 hours, 72 hours, 7 and 10 days for signs of erythema or edema (Aiyalu et al., 2016). The score was given based on a scoring chart prescribed in "Assessing the Irritation and Sensitization Potential of Transdermal and Topical Delivery Systems for ANDAs Guidance for Industry" which is a draft guidance given by FDA.

Table 7 Skin response score

Skin appearance	Scores
No evidence of irritation	0
Minimal erythema that is barely perceptible	1
Definite erythema that is readily visible and minimal edema or minimal papular response	2
Erythema and papules	3
Definite edema	4
Erythema, edema, and papules	5
Vesicular eruption	6
Strong reaction spreading beyond the application site	7

3.8. Wound contraction

Wound contraction is a measure of wound healing capacity. It measures the percentage reduction in the wound area (Fahimi et al., 2015).

3.8.1. Principle

Wound contraction occurs as a result of movement of fibroblasts into the wound and consequent production of collagen, which causes the open wound to close. The rate of wound contraction indicates how fast the wound is healing (Swaim et al., 2001).

3.8.2. Protocol

The mean length and breadth of the wound was measured in centimeters using a standardized scale. The product of both means was multiplied to attain the mean area of wound. The following formula was then used to calculate the rate of wound contraction:

$$\% \text{ wound contraction} = \frac{\text{Initial wound size - specific wound size on } n^{th} \text{ day}}{\text{Initial wound size}} \times 100$$

The measurements were taken on day 1, 3, 5, and 7. The results were analyzed using one way ANOVA to determine whether the results were statistically significant or not (M. Abercrombie, M. H. Flint, D. W. James, 1954).

3.9. *In silico* analysis

Carotene α, Carotene β, vitamin C, vitamin A and lutein was docked against bone morphogenetic protein (BMP) to determine the effect that they will have during wound healing process. The protein and ligand structures were retrieved from Protein Data Bank and PubChem database, respectively. The protein and ligands were docked using patch dock and visualized in chimera. The last step was performing Plip analysis.

CHAPTER 04

RESULTS

4.1. Antimicrobial susceptibility assay

Antimicrobial activity testing of the plant extracts was performed via disk diffusion method. Zones of inhibition of the plant extracts, silver sulfadiazine and Metronidazole (positive control) were measured against *Methicillin-resistant Staphylococcus aureus (*Gram positive*) and Escherichia coli* (Gram negative) bacteria.

Figure 10: Measurement of zone of inhibition with Vernier caliper

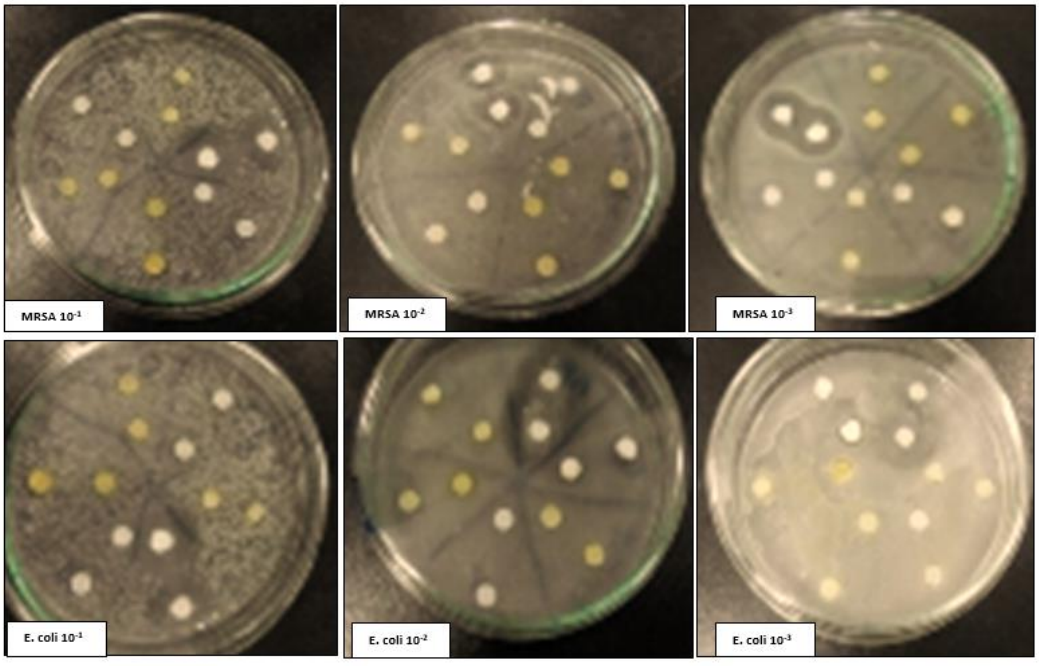

Figure 11: Figure showing the results of disk diffusion

Table 8 Antimicrobial activity of plant extracts, SSD and positive control against MRSA and *E. coli*

Micro-organisms	Conc.	Readings	Zones of inhibition (radius of zone) in mm					
			C	**F**	**C+F**	**C+F+P**	**MTZ**	**SSD**
MRSA	10^{-1}	**R_1**	2.92	4.20	3.90	5.80	4.65	8.62
		R_2	2.98	5.21	3.38	4.96	3.10	8.14
		Avg.	**2.95**	**4.70**	**3.64**	**5.38**	**3.87**	**8.38**
	10^{-2}	**R_1**	2.90	3.98	3.15	4.31	NIE	8.42
		R_2	2.80	3.41	3.55	4.22	NIE	8.54
		Avg.	**2.85**	**3.69**	**3.35**	**4.26**	**0.00**	**8.48**
	10^{-3}	**R_1**	NIE	3.90	4.27	3.33	3.10	5.87
		R_2	NIE	3.75	3.20	NIE	3.20	4.75
		Avg.	**0.00**	**3.82**	**3.73**	**1.66**	**3.15**	**5.28**
E. coli	10^{-1}	**R_1**	NIE	3.31	NIE	NIE	10.4	4.64
		R_2	NIE	3.05	NIE	NIE	11.5	3.63
		Avg.	**0.00**	**3.18**	**0.00**	**0.00**	**10.9**	**4.13**
	10^{-2}	**R_1**	NIE	2.92	3.80	3.10	2.95	7.37
		R_2	NIE	2.61	2.60	4.50	NIE	7.60
		Avg.	**0.00**	**2.76**	**3.2**	**3.8**	**1.47**	**7.48**
	10^{-3}	**R_1**	NIE	NIE	NIE	NIE	2.90	10.6
		R_2	NIE	NIE	NIE	NIE	2.7	9.10
		Avg.	**0.00**	**0.00**	**0.00**	**0.00**	**2.8**	**9.85**

Abbreviations: C. *Coriandrum sativum*, F. *Fagonia indica*, C.+F. *Coriandrum sativum*+ *Fagonia indica*, C.+F.+P. *Coriandrum sativum*+ *Fagonia indica*+ Polyfax, MTZ. Metronidazole, SSD Silver sulfadiazine, NIE no inhibitory effect, Avg. average, Conc. Concentration, MRSA *Methicillin-resistant Staphylococcus aureus* and *E. coli Escherichia coli*.

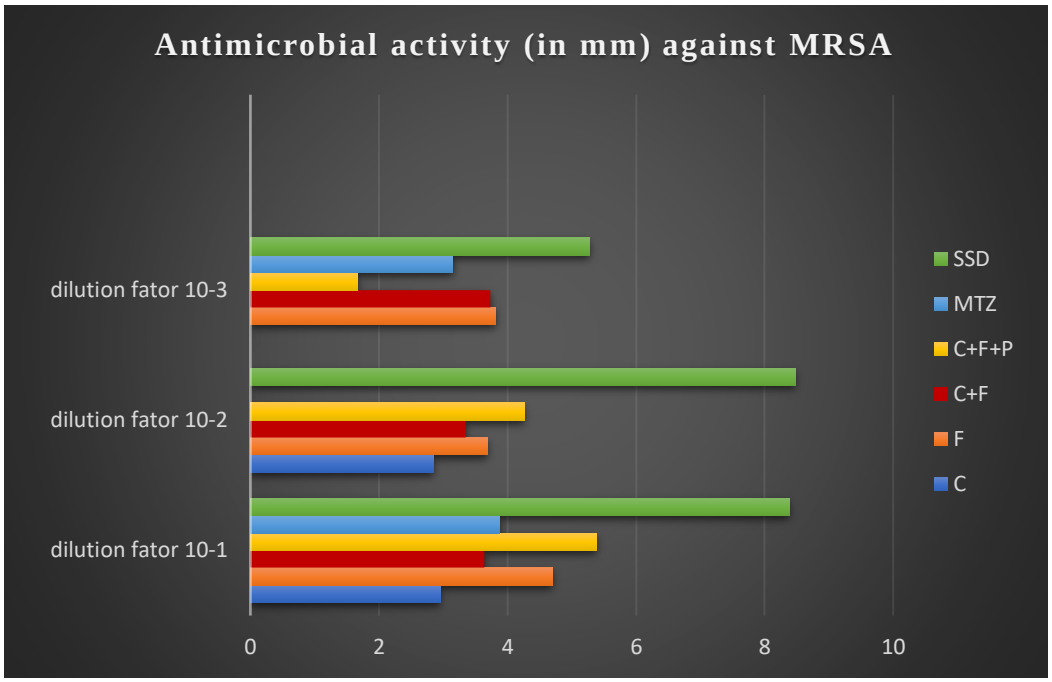

Figure 12:Graph showing the antimicrobial activity against MRSA

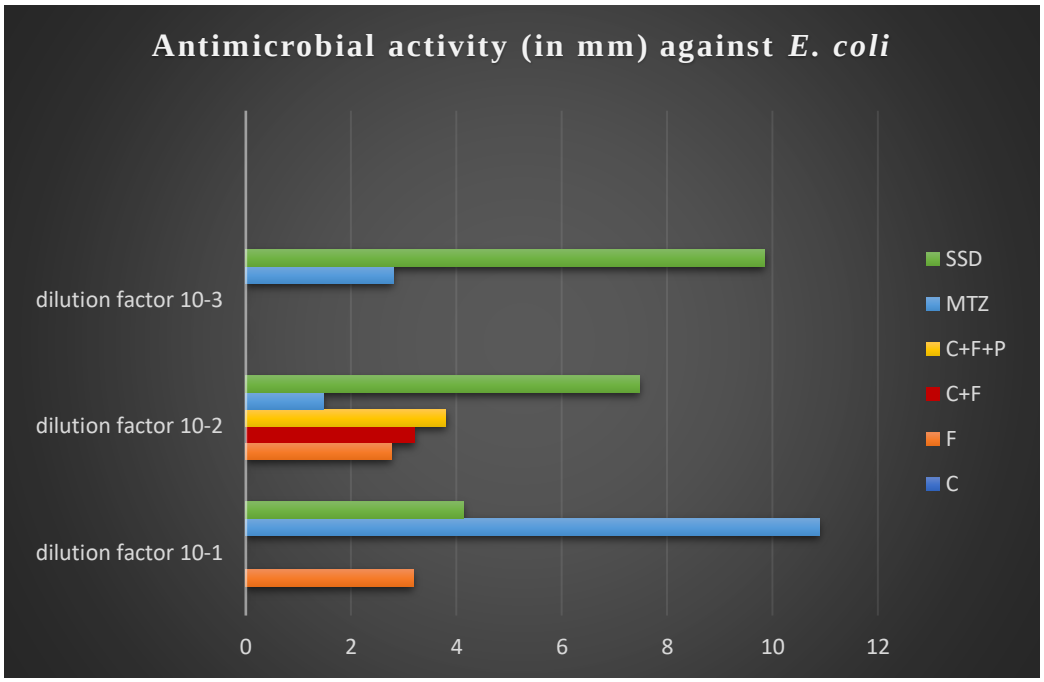

Figure 13:Graph showing the antimicrobial activity against E. coli

The gels formulated from plant extracts show adequate antimicrobial activity against *MRSA* which is the largest contributor to infections in burn wounds, 5% Fagonia gel showed the best antimicrobial activity .The gels show poor antimicrobial activity against *E. coli*, the activity is not comparable to that of commercially available Silver Sulfadiazine and Metronidazole.. This may indicate that the gels are more efficacious towards gram positive bacteria. During the course of burn healing no signs of infection such as redness, edema or pus where observed. It may be concluded that although they do not display strong antimicrobial, since no infection occurred at site of burn, they may be adequate in preventing infection.

4.2. Skin sensitivity test

Table 9 Results of skin sensitivity tests

Skin appearance	**5% *F. indica gel***	**5% C. sativum gel**	**5% *F. indica* and *C. sativum***	**5% *F. indica* and *C. sativum* in polyfax**
Observations after 1hr				
Erythema score	0.0	0.0	0.0	0.0
Edema score	0.0	0.0	0.0	0.0
Rashes score	0.0	0.0	0.0	0.0
Evidence of irritation	No evidence	No evidence	No evidence	No evidence
Observations after 24hr				
Erythema score	0.0	0.0	0.0	0.0
Edema score	0.0	0.0	0.0	0.0
Rashes score	0.0	0.0	0.0	0.0
Evidence of irritation	No evidence	No evidence	No evidence	No evidence
Observations after 48hr				
Erythema score	0.0	0.0	0.0	0.0
Edema score	0.0	0.0	0.0	0.0
Rashes score	0.0	0.0	0.0	0.0

Evidence of irritation	No evidence	No evidence	No evidence	No evidence
Observations after 72hr				
Erythema score	0.0	0.0	0.0	0.0
Edema score	0.0	0.0	0.0	0.0
Rashes score	0.0	0.0	0.0	0.0
Evidence of irritation	No evidence	No evidence	No evidence	No evidence
Observations after 7days				
Erythema score	0.0	0.0	0.0	0.0
Edema score	0.0	0.0	0.0	0.0
Rashes score	0.0	0.0	0.0	0.0
Evidence of irritation	No evidence	No evidence	No evidence	No evidence
Observations after 10 days				
Erythema score	0.0	0.0	0.0	0.0
Edema score	0.0	0.0	0.0	0.0
Rashes score	0.0	0.0	0.0	0.0
Evidence of irritation	No evidence	No evidence	No evidence	No evidence

During the 10-day trial no erythema, edema or rashes were observed, proving that no irritation was caused by these gels. We can conclude, since no signs of irritation were displayed these gels are safe to be used as a topical treatment for burn wound healing.

4.3. Wound contraction

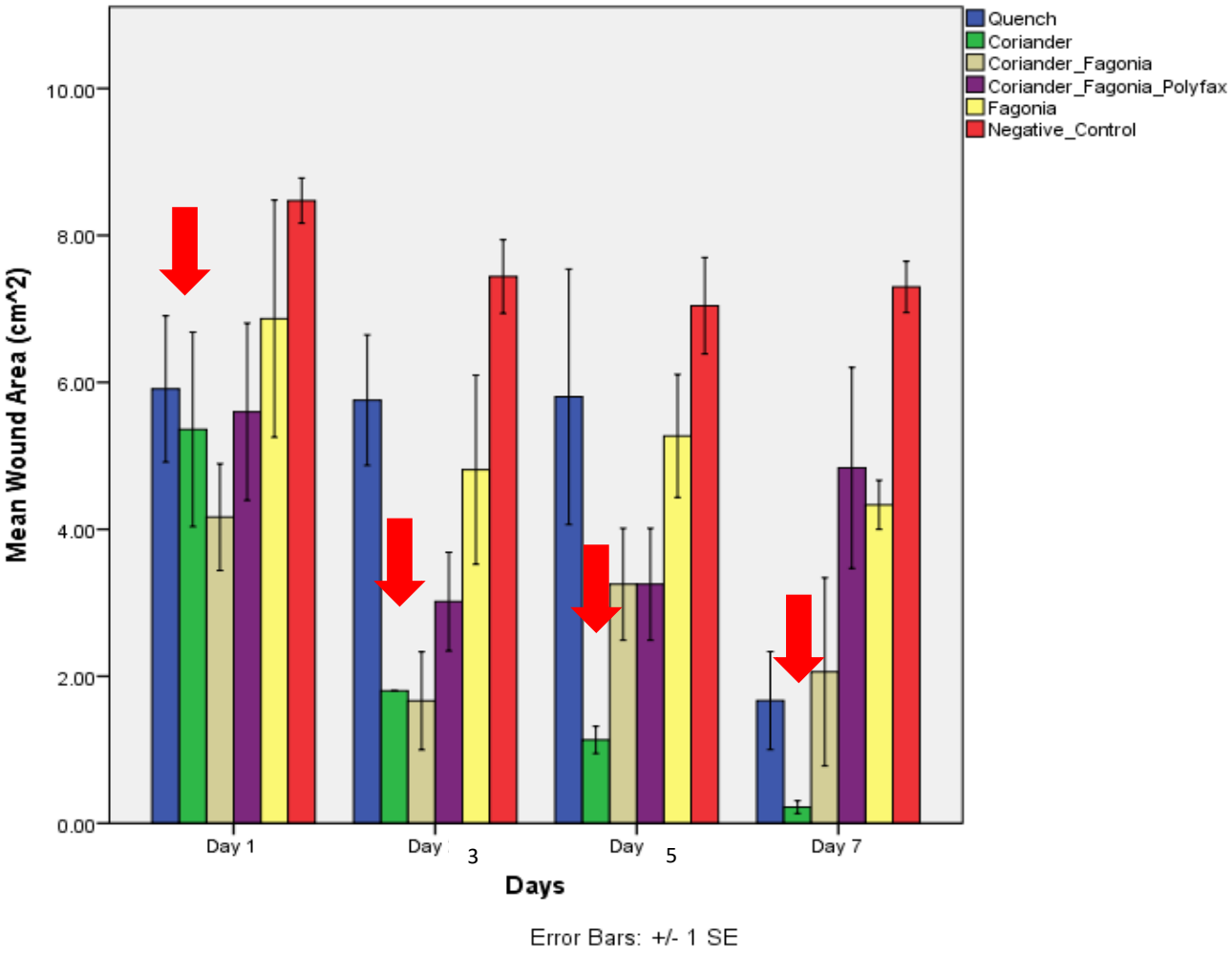

Figure 14:Wound contraction

One-way ANOVA analysis showed that 5% C. has significantly healed the wound (p=0.003). However, the recovery of wound with the treatment of 5% C. + F., 5% C.+ F.+ P. and 5% F. were not significant (p>0.05). Kruskal Willas Analysis showed that, wound treatment through 5% C. was found more significant compared to Quench(p=0.023). However, the wound treatment through 5% C. + F., 5% C.+ F.+ P. and 5% F. were found insignificant compared to Commercial Quench (p>0.05).

4.4. Molecular docking

Results of ligand-protein interaction are described here.

Compound **Carotene-Alpha** was docked with BMP 1 and the docked complex of BMP1 carotene alpha predicted had a patchdock docking score of 7048 and the area of

interaction between ligand and enzyme is 863.5 A. Desolvation energy of the interaction also called the ACE score was predicted to be -324.41. Results obtained from docking predicted that there is a strong interaction between the molecule of carotene alpha and the BMP1 protein protease domain. The results also predicted that carotene alpha interacted with the enzyme in its binding pocket as in the figures.

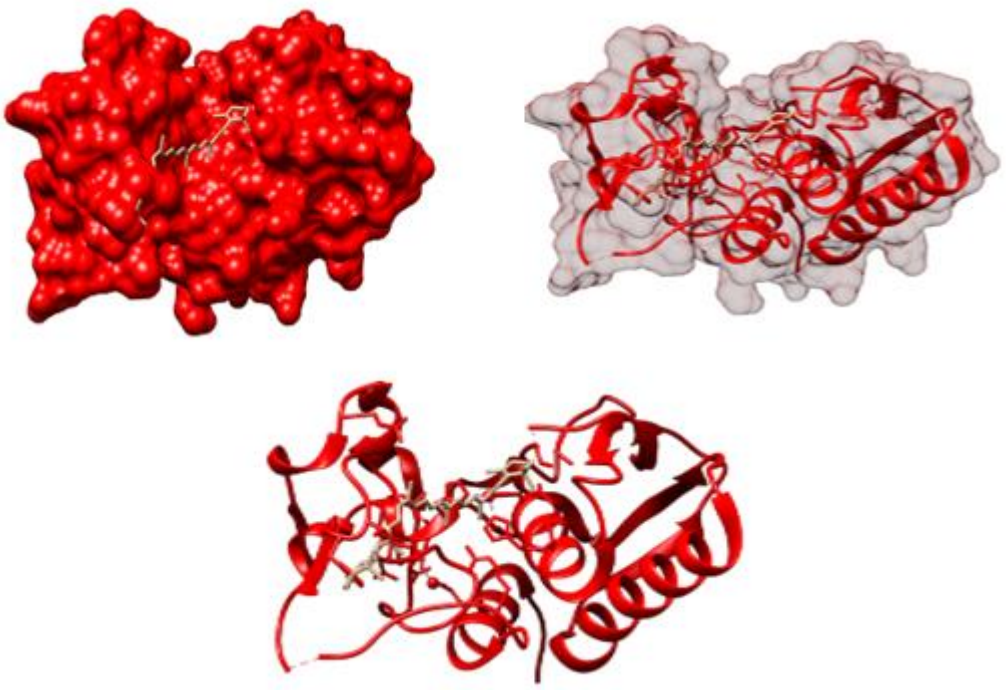

Figure 15:Interaction of Carotene-alpha molecule in the binding pocket of BMP-1 protease domain- surface model on the left- Partial transparency model on the right- Full transparent model at the bottom

PLIP analysis revealed the interaction at atomic level and revealed that there are no hydrogen interactions while there were 7 hydrophobic bonds as displayed in the image below.

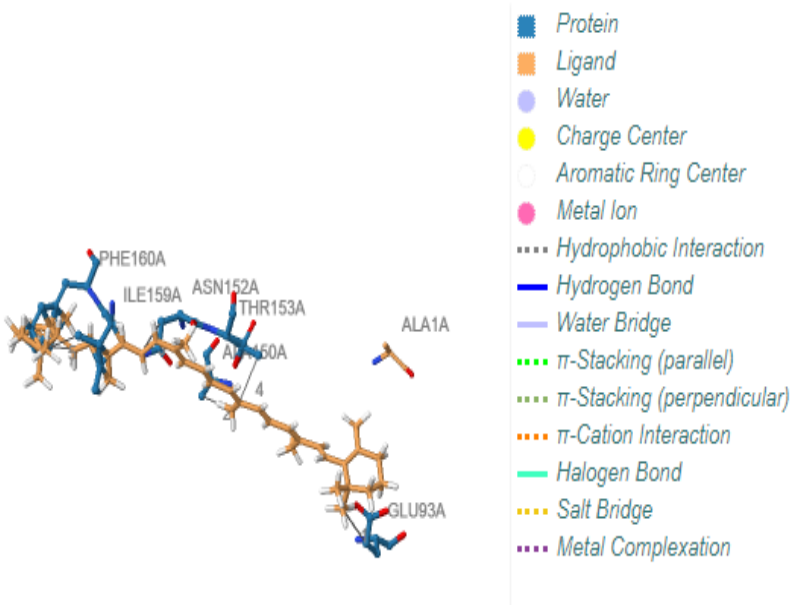

Figure 16: PLIP analysis image showing the presence of 7 hydrophobic interactions in between Carotene -alpha and BMP-1 protease domain

Second compound that was used to predict interaction with BMP1 protease domain was **Carotene-Beta**. The patchdock docking score was 6370 with area of interaction of 777.80 A. The ACE score for the interaction was predicted to be -258.71. The results obtained strongly predict that there is a strong interaction between the BMP1 protease domain and the Carotene –beta active compound as it is interacting with the binding pocket of the BMP1 enzyme.

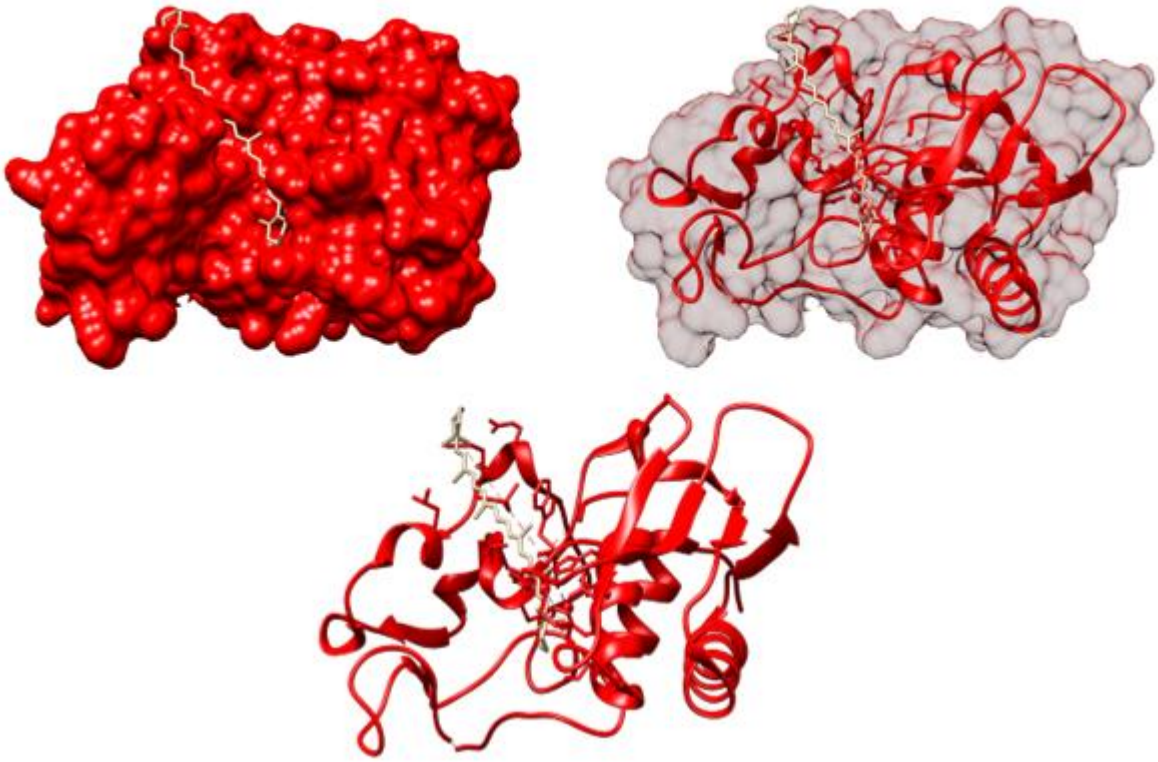

Figure 17:Interaction of Carotene-beta molecule in the binding pocket of BMP-1 protease domain- surface model on the left- Partial transparency model on the right- Full transparent model at the bottom

PLIP analysis of the interaction between Carotene-beta and BMP1 revealed that at the atomic level there are no hydrogen bonds in the interaction and the interaction between carotene beta and BMP1 is held solely on 3 hydrophobic interaction as shown in the figure below.

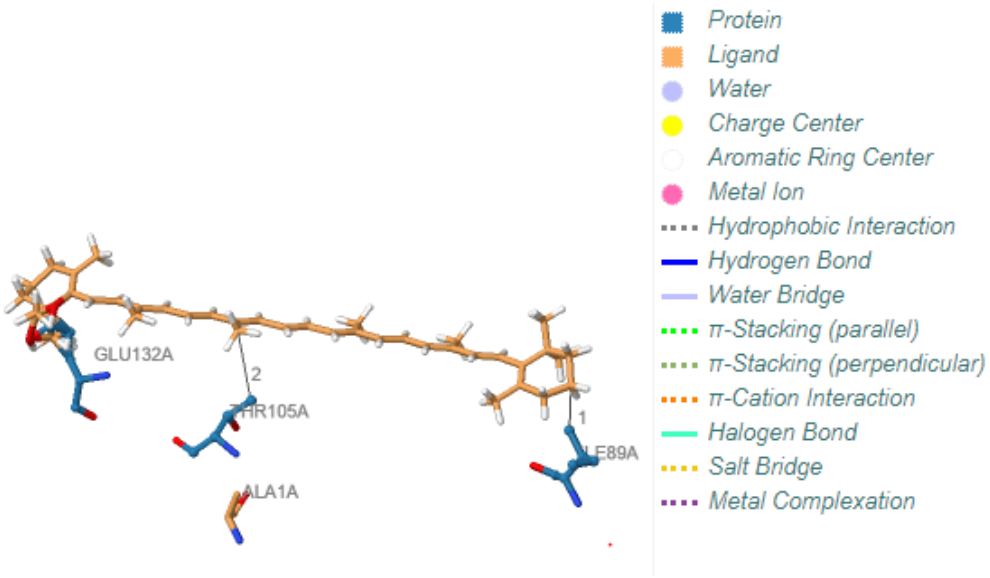

Figure 18:PLIP analysis image showing the presence of 3 hydrophobic interactions in between Carotene - beta and BMP-1 protease domain

Lutein was the third compound that was docked against BMP1 protease domain. The patchdock score for this interaction was 6278 with the area of interaction predicted to be 768.60 A. The energy of desolvation score or the ACE score for this interaction was predicted to be -244.58. The simulation predicted that lutein is interacting in the binding pocket of the enzyme.

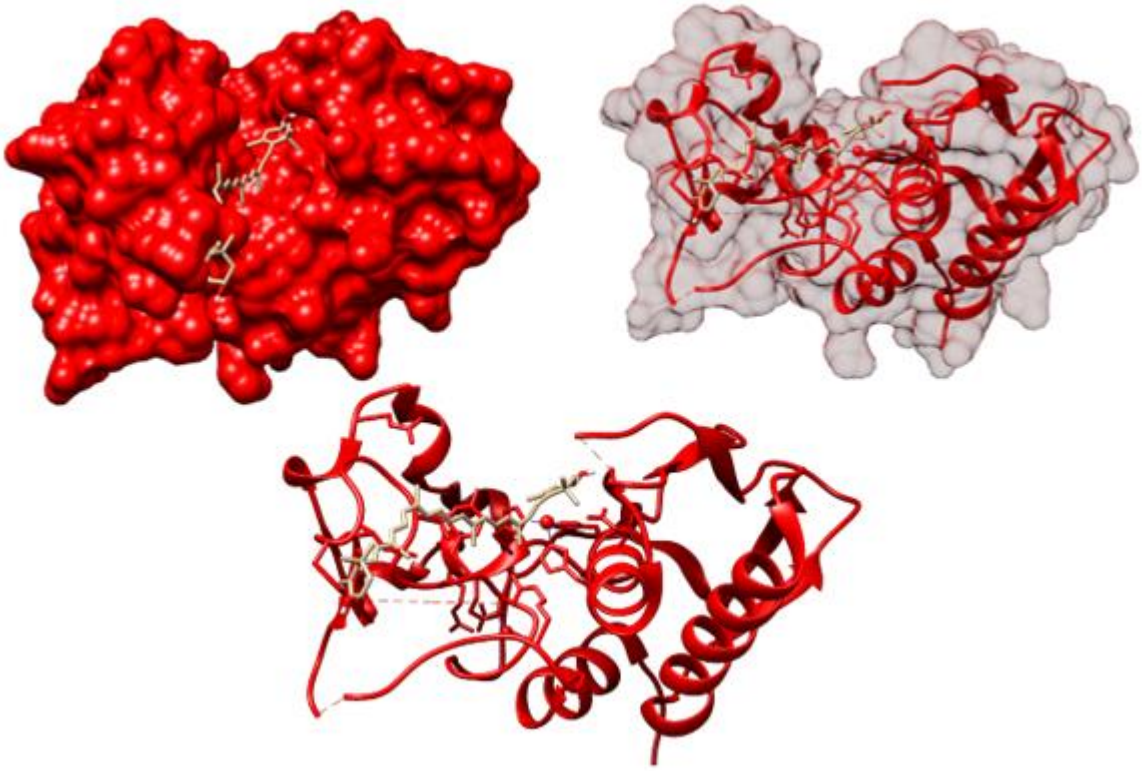

Figure 19:Interaction of Lutein molecule in the binding pocket of BMP-1 protease domain- surface model on the left- Partial transparency model on the right- Full transparent model at the bottom

After performing PLIP analysis the atomic interaction of the ligand-protein was revealed that there is one hydrogen bond and six hydrophobic interactions. These atomic interactions hold the lutein molecule in place to interact with the BMP1 protease domain as shown in the figure.

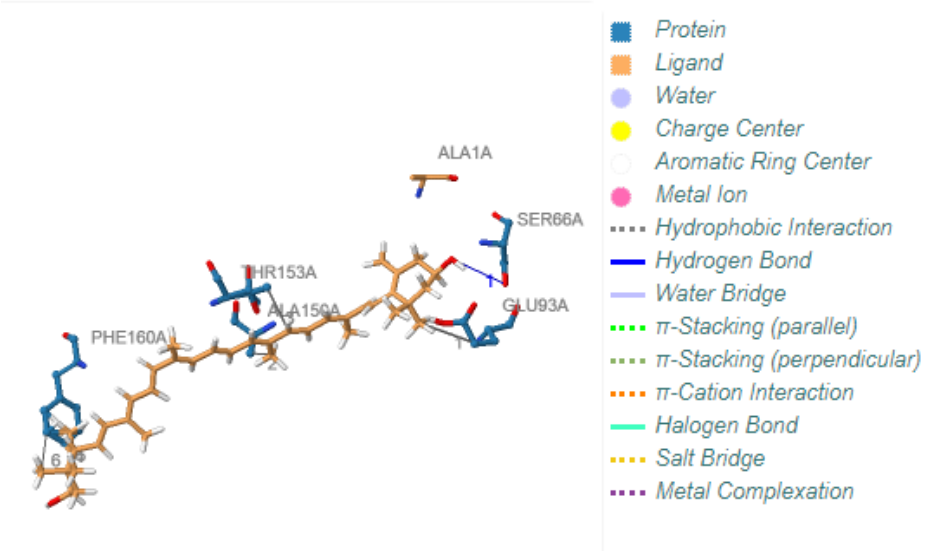

Figure 20:PLIP analysis image showing the presence of 6 hydrophobic interactions and 1 hydrogen bond in between Lutein and BMP-1 protease domain

Vitamin A is another compound that was docked against BMP1 protease domain and the patchdock score was 4356 with the predicted area of interaction being 528.20 A. The ACE score for this interaction is predicted to be -177.19. Compound of interest was predicted to be docked in the binding pocket of the enzyme.

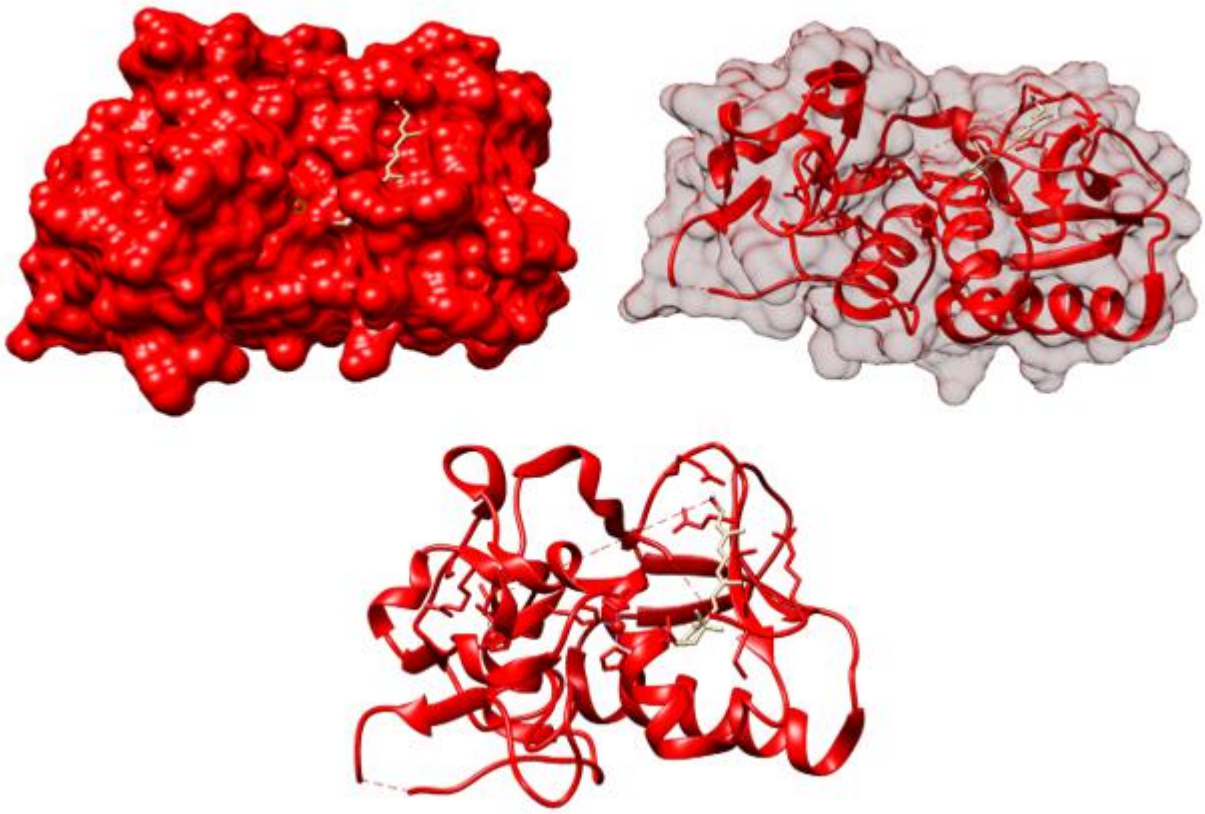

Figure 21:Interaction of vitamin A molecule in the binding pocket of BMP-1 protease domain- surface model on the left- Partial transparency model on the right- Full transparent model at the bottom

PLIP analysis of the interacting complex revealed that there two hydrogen bonds present between the interacting complex and two hydrophobic interacting bonds present. The atomic interactions are shown in the image below.

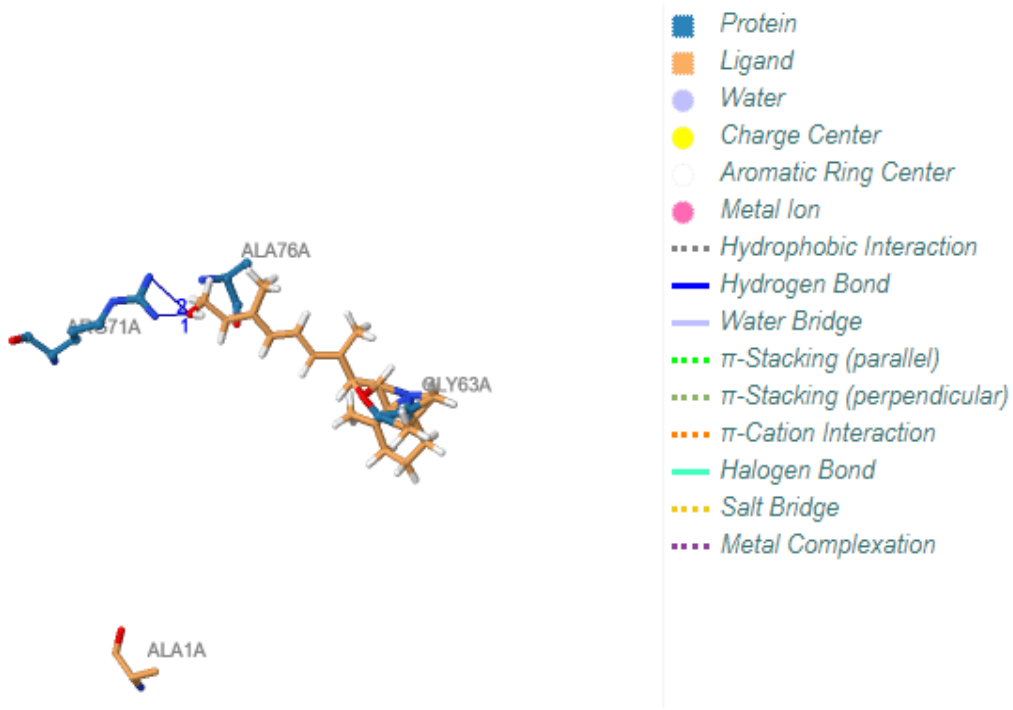

Figure 22:PLIP analysis image showing the presence of 2 hydrogen bonds in between vitamin A and BMP-1 protease domain

Vitamin C also known as ascorbic acid found in both the plants significantly. When vitamin C was run through simulations of whether it can interact with the BMP1 protease domain. The patchdock score for this simulation was 2344. The area of interaction was 264.70 A between the ligand and the protein molecule. The ACE score is predicted to be -83.80.

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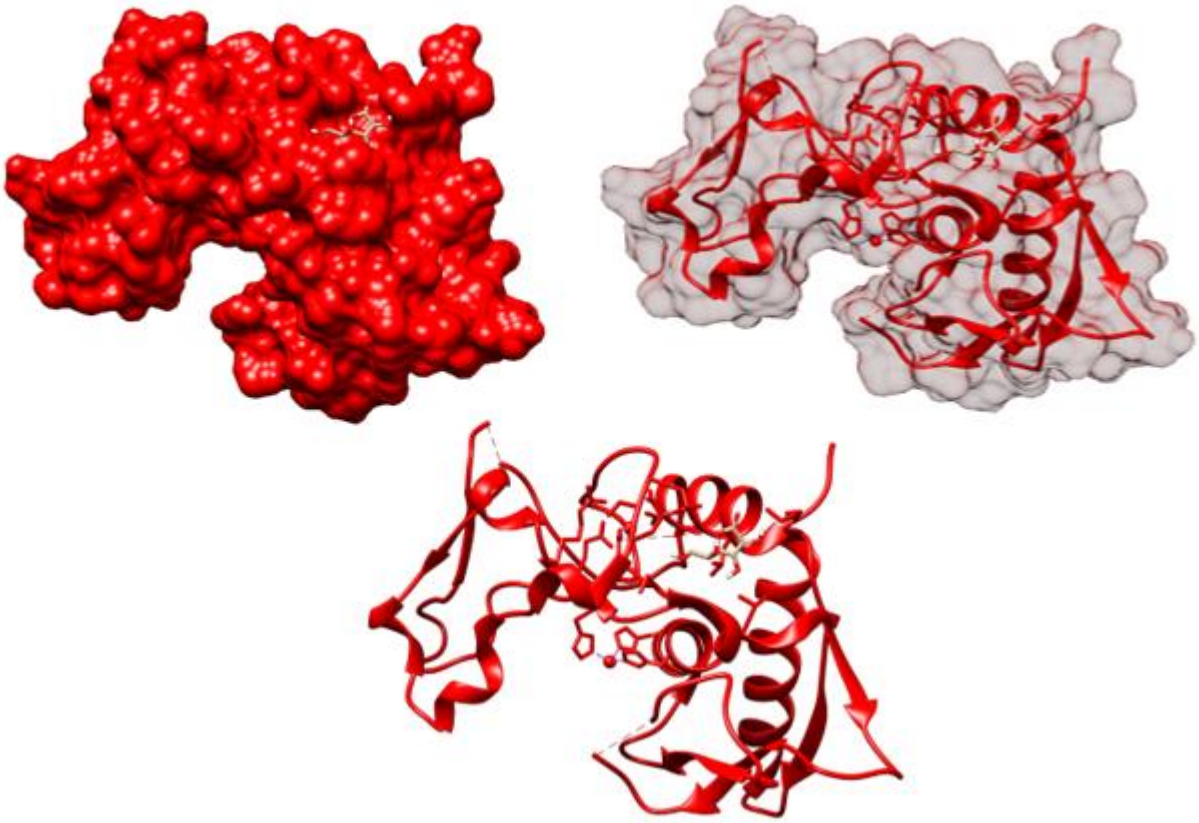

Figure 23:Interaction of vitamin C molecule in the binding pocket of BMP-1 protease domain- surface model on the left- Partial transparency model on the right- Full transparent model at the bottom

PLIP analysis revealed that vitamin C and the binding pocket of BMP1 protease domain has one hydrogen bond and one salt bridge interactions

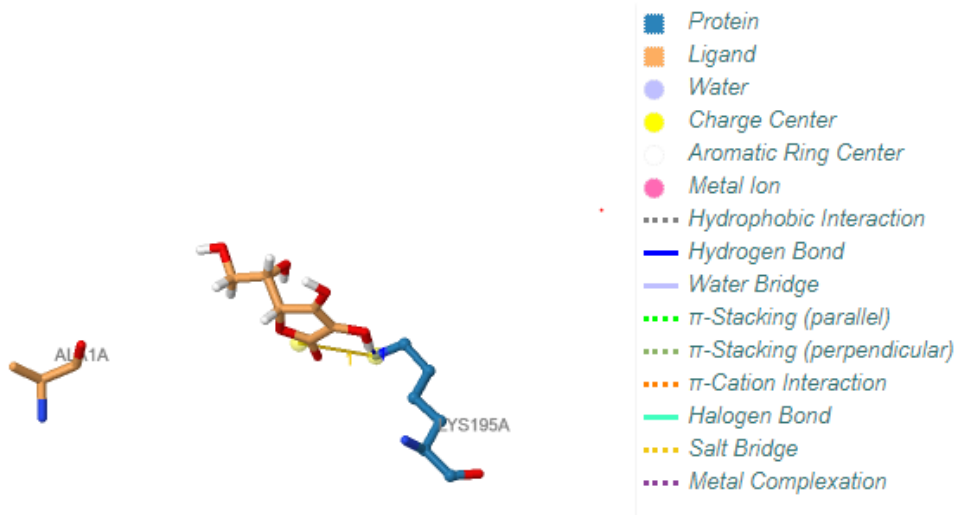

Figure 24:PLIP analysis image showing the presence of 1 hydrogen bond and salt bridge interactions in between vitamin C and BMP-1 protease domain.

Carotene-alpha>Carotene-beta>Lutein>vitamin A>vitamin C

CHAPTER 05

DISCUSSION

The wound healing process is divided into four major phases coagulation and hemostasis, inflammation, proliferation and remodeling. The coagulation phase prevents exsanguination and protects vascular system from damage. It provides a matrix for invading calls that are required in the healing process including endothelial cells and thrombocytes. The coagulation and fibrinolysis determine the amount of fibrin and regulates homeostasis. Coagulation leads to platelet aggregation and clot formation which limits bleeding. This clot provides a matrix for cell migration during inflammatory phase. PDGF, TGF-beta and EGF that activate and attract macrophages, endothelial cells and fibroblasts are released from cytoplasm of platelets (Velnar et al., 2009).

The inflammatory phases involve activation of humoral and cellular pathways. Neutrophils infiltrate the site of wound and prevent infection by phagocytosis of bacteria and removal of damaged cells. Macrophages then migrate to the wound site, mediated by chemoattractant PDGF, TGF-beta and collagen breakdown process. Lymphocytes play a role in collagenase regulation and are required for collagen remodeling.

The proliferative phase involves tissue repair. Fibroblasts rapidly migrate and deposit new extracellular matrix which imparts integrity to the wound. Collagen is a foundation for intracellular matrix. The deposition of collagen causes wound contraction by pulling the wound edges together. Angiogenesis, adhesion and epithelization are also key phases within the proliferative phase. Remodeling phase is responsible for development of new epithelium and scar tissue formation (Adjepong et al., 2016).

Reactive Oxidative Species family includes hydrogen peroxide H2O2, peroxide ·O−2 2, superoxide anion ·O− 2, hydroxyl radicals ·OH. They are produced by oxidoreductases and in order to stabilize themselves they procure electrons from other molecules destroying their structures (Kaushal et al., 2019). ROS are produced by phagocytes as a means of pathogen destruction.

ROS are a necessary evil as they act as secondary messengers, responsible for vasodilation, vasoconstriction and protection from infection. However, ROS when they cross a certain threshold induce oxidative stress, cause degradation and modification of

extracellular matrix protein. Furthermore, ROS may induce metalloproteases causing cell death and dysfunction of keratinocytes and fibroblasts (Dunnill et al., 2017; Sharma et al., 2012).

Enzymatic and non-enzymatic antioxidants play a key role in stabilizing ROS and rendering them harmless. They can be used as a means to optimize the healing process by lowering the concentration of ROS at burn site. Enzymatic antioxidants include SOD, CAT and GPX. The non-enzymatic antioxidants include Vitamin C, vitamin E, carotenoids and lutein amongst others (Nimse & Pal, 2015).

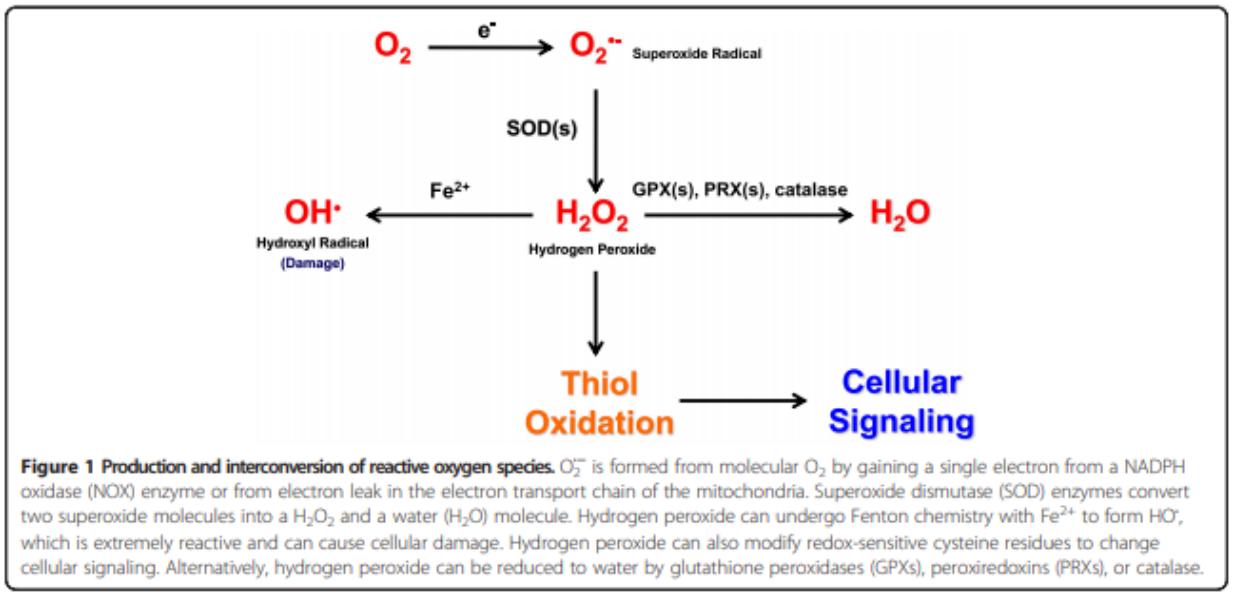

Figure 1 Production and interconversion of reactive oxygen species. $O_2^{\bullet-}$ is formed from molecular O_2 by gaining a single electron from a NADPH oxidase (NOX) enzyme or from electron leak in the electron transport chain of the mitochondria. Superoxide dismutase (SOD) enzymes convert two superoxide molecules into a H_2O_2 and a water (H_2O) molecule. Hydrogen peroxide can undergo Fenton chemistry with Fe^{2+} to form $HO^\bullet$, which is extremely reactive and can cause cellular damage. Hydrogen peroxide can also modify redox-sensitive cysteine residues to change cellular signaling. Alternatively, hydrogen peroxide can be reduced to water by glutathione peroxidases (GPXs), peroxiredoxins (PRXs), or catalase.

Figure 25:Role of antioxidants in neutralizing ROS (Ighodaro & Akinloye, 2018)

The SOD (EC 1.15.1.1) acts as a first line of defense against the ROS. It is the most powerful antioxidant enzymes present in eukaryotes. As it is a metalloprotease it needs to bind to metallic co-factors to function properly. Co-factors of SOD include Zn, Cu, Mn and Fe. SOD1 which uses Cu and Zn as co-factors is encoded by SOD1 gene on chromosome 21 and SOD2 which uses Mn as a co-factor is encoded on SOD2 gene on chromosome 6. The dismutation of superoxide anion into hydrogen peroxide and molecular oxygen is mediated by SOD (Sullivan & Chandel, 2014).

Catalase (EC 1.11.1.6) is a common antioxidant enzyme that uses Mn as a co-factor and is responsible for the catalysis of hydrogen peroxide to water. It completes the

antioxidation process initiated by SOD as hydrogen peroxide is also an extremely harmful compound (Ighodaro & Akinloye, 2018).

It can be observed in the composition of Coriander that scavenging antioxidants are in high quantity i.e. Vitamin C and Vitamin E are present in 27mg/100 kg and 6748 IU/100 kg. Carotene beta and alpha are also present in high concentration of 3930ug and 36ug respectively. They help prevent oxidative damage to the healing tissue in burn wounds.

When comparing the composition of 5% Coriander gel to 5% Fagonia gel the concentration of Zn, Cu and Mn is much higher in coriander. These metal ions act as co-factors for SOD and CAT which rapidly convert ROS into less harmful compounds. The composition of Coriander provides proof as to its efficaciousness in treating burn wounds.

Table 10 Comparison of concentration of metallic ions in Fagonia and Coriander

Concentration of Compound per 100g	***Fagonia Indica***	***Coriandrum sativum***
Cu	39.2 ug	0.22 mg
Zn	28.4 ug	1.5 mg
Mn	35.4 ug	0.42 mg

Many of the phytochemicals such as Vitamin E, Lutein, Carotene Beta and Quercetin are reactive oxygen species scavengers. These oxidant scavengers behave as recipient of reactive oxygen and prevent oxidants to damage molecules essential to maintain normal life. As they donate their electron, they become a oxidative species but are less damaging and are quickly neutralized.

Collagen content is said to be one third of the total body mass. It is found abundantly in most parts of the body which include skin, tendons, bones and the interstitial membranes. It is also the major component found in connective tissue. Collagen type I and type III are most abundantly found in skin. Up to 80% of collagen found in skin is type I and other 20% is accounted by type III. COL1A1 gene responsible for the production of procollagen type I monomer.

It is reported that the procollagen consists of Glycine-proline-X or Glycine-Hydroxyproline-Y where X and Y can be any amino acid. In collagen type I the structure

of collagen is reported to be Glycine-proline-hydroxyproline. In order to produce hydroxyproline, prolyl hydroxylase enzyme is required to convert proline to hydroxylated proline molecule, which is a major constituent of collagen type I. Vitamin C acts as a co-factor to prolyl hydroxylase increasing the efficiency of the enzyme. Coriander leaves contain significant amount of Vitamin C. It is reported that coriander leaves contain 27mg per 100 gram of vitamin C. Thus, increased and rapid wound closing, and removal of scab and scar is observed after treatment with extracts obtained from Coriander. *Fagonia indica* extracts have approximately 8.3 mg of vitamin C per 100 grams so when compared with Coriander they show slower healing properties (Prockop & Kivirikko, 1995).

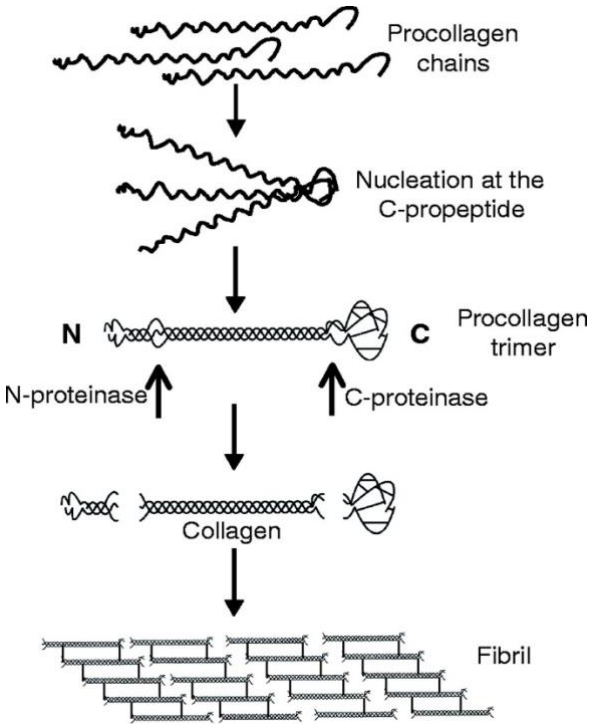

Figure 26:Cleavage of procollagen trimer to collagen trimer. Enzymes involved in cleavage are C-proteinase and N-proteinase as shown in the figure. (Canty und Kadler 2005)

Procollagen chains then form a procollagen trimer. In order to activate collagen fibril, the N-proteinase and C-proteinase enzymes cleave propeptides from procollagen trimer. Both of these enzymes belong to BMP family. They require Zinc as a cofactor for activation.

In order to verify whether there is any effect of vitamin C on BPM proteins. Molecular docking was performed using major phytochemicals such as vitamin C along with Carotene alpha and beta (Canty & Kadler, 2005).

The results proved that there is strong binding of carotene alpha and beta in the binding pocket of the BMP-1 however vitamin C had a relatively weak binding. Results obtained from molecular docking thus established that carotene alpha and carotene beta had an increased activity BMP-1. Cleavage of procollagen trimer to collagen trimer is observed to be high in rats treated with coriander extracts compared to rats treated with *F. indica* extract. Potential reasoning for this observation after the increased production of procollagen in coriander extract, the presence of Zinc which is 0.5 mg per 100g in coriander compared to 0.000285 mg per 100 g in *Fagonia indica* facilitates formation of collagen trimer. Similarly, presence of high levels of carotene alpha and carotene beta in coriander extracts 36ug and 3930ug respectively compared to *Fagonia indica* with very low levels.

Cleavage of procollagen trimer to collagen trimer is observed to be high in rats treated with coriander extracts compared to rats treated with *F. indica* extract. Potential reasoning for this observation after the increased production of procollagen in coriander extract, the presence of Zinc which is 0.5 mg per 100g in coriander compared to 0.000285 mg per 100 g in *Fagonia indica* facilitates formation of collagen trimer. Of the four gels developed only 5% *Coriandrum sativum* extract showed extra ordinary and quick formation of scab, skin regeneration and closing of the wound. The main reason as discussed above is the high ascorbic acid content and zinc content of the coriander extract.

The 5% *Fagonia indica* gel has lower efficacy as compared to that of coriander. One of the reasons is the low vitamin c and zinc metal content in the fagonia gel. The effectiveness of the gels with the mixture of both the extracts was not very satisfying. This led us to conclude that extracts obtained from *Fagonia indica* has antagonistic effect on the wound healing ability of *Coriandrum sativum.* Having a known antibiotic

component as in the case of the gel 5% coriander and fagonia in polyfax base attributed no additional advantages and the performance of this gel was not comparable to that of 5% coriander gel

CONCLUSION

Coriandrum sativum and *Fagonia indica* were used as folk medicine since few millennia. Formulation of extracts obtained from these plants into easy to use gel formulation will bring this medicine to hand of every individual. According to our research extracts obtained from *C. sativum* were found to be most effective against the treatment of burn injury as it enhances the pro-collagen formation and conversion into collagen and the extracts of *Fagonia indica* had a prominent antimicrobial activity. However, when extracts from *C. sativum* and *F. indica* were combined in a gel formulation the results were not significant and both the extracts were antagonizing each other's effect.

FUTURE PROSPECTS

1. Maximize the efficacy of antimicrobial activity of *5% C. sativum* gel against commonly found pathogens in burn wounds.

2. Further research is required to elucidate dose dependent antioxidant activity of *5% C. sativum* gel in vivo.

3. Creation of a medicated occlusive bandage impregnated with a formulation of the 5% *C. sativum* gel.

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