2.1 SKIN

2.1.1 ANATOMY OF SKIN

The skin is the largest organ in the human body. It constitutes an effective barrier between the organism and the environment, preventing invasion of pathogens and chemicals. Its composition also protects internal organs by decreasing mechanical and physical stress as well as prevents diffusion of ions and nutrients from the body. Another important property of skin is as a barrier against water loss. This is essential for most terrestrial organisms which would otherwise become desiccated and die. The skin can be usually divided into three layers of tissue (1) (figure 2.1): epidermis, dermis and hypodermis.

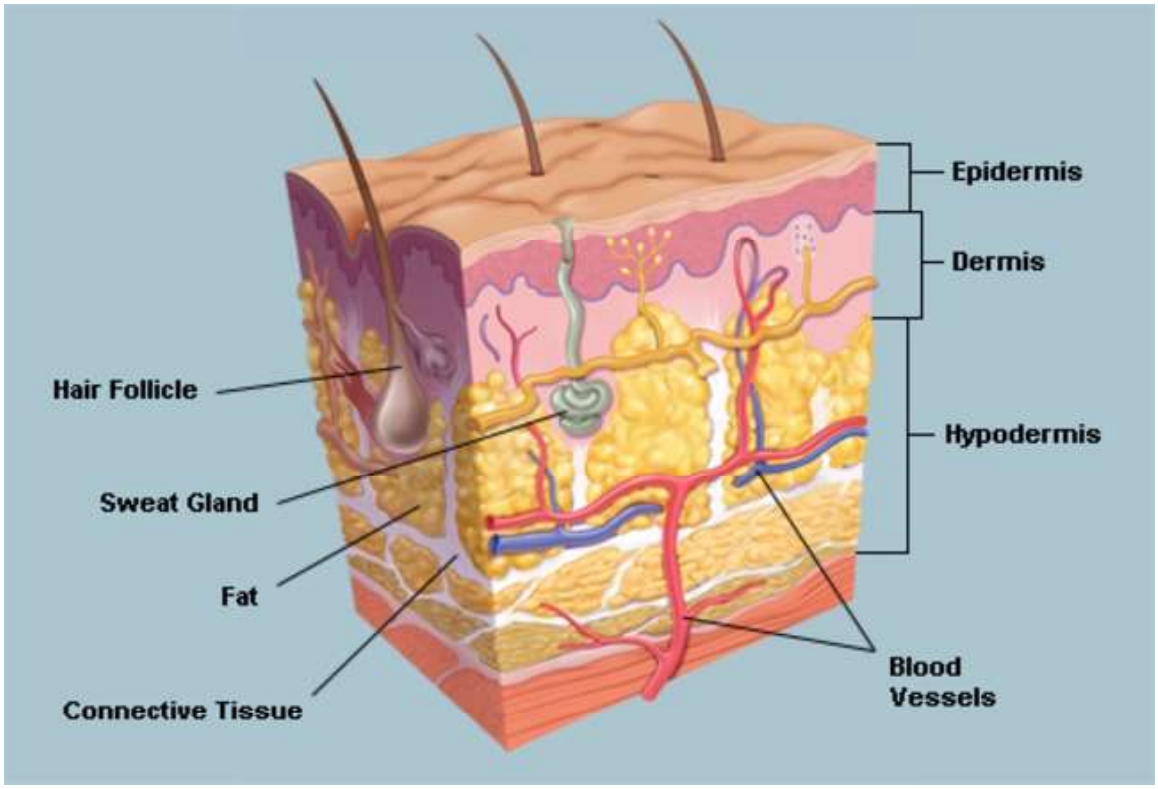

FIGURE 2.1: Anatomy of skin

2.1.1.1 **Epidermis:** The outermost layer is constituted by the epidermis. In the lower region of epidermis, the stratum germinativum, rapid cell division (mitosis) pushes older

cells towards the surface of the skin (1). Cell migration builds up the outermost layer of the epidermis, the stratum corneum (SC), during keratin formation. The SC is composed of dead cells which are continuously peeled off due to abrasion of the skin. The high proportion of keratin combined with lamellae of lipid makes the epidermis an effective barrier against water loss.

2.1.1.2 **Dermis:** The second layer is the dermis which is a fibrous layer composed mainly of collagen (1). The fiber composition enables restoration of the skin after being stretched and keeps the skin hydrated due to its ability to hold large amounts of water. Sweat glands and hair follicles are supported structurally as well as nutrition wise by the surrounding dermis. The dermis also plays a supportive and nutritive role for the epidermis which has no blood vessels of its own. This is provided through blood vessels of the dermis extending in small projections into the lower margin of the dermis.

2.1.1.3 **Subcutaneous layer:** Beneath the dermis a subcutaneous layer is situated. It is basically constituted of loose connective tissue and fat cells and serves as a nutrient supplier to the layers above (1). It also provides the body with insulation.

2.1.2 WOUND

Wound is a disruption of the cellular and anatomic continuity of a tissue and it is produced due to any accident or cut with sharp edged things; physical, chemical, thermal, microbial or immunological exploitation to the tissue as a result of the presence of an underlying medical or physiological condition (2). Wound can be described as separation of tissues of the body, an injury due to external violence, or an imperfection. Wound can be without tissue loss (e.g. in surgery) or with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments e.g. venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and dermal abrasions (3).

- Generally, wounds can be classified into acute or chronic wound based on the nature of the repair process (4).

i. Acute wounds: They are usually tissue injuries which heal completely with minimal scarring within 8 – 12 weeks (5, 6). The causes of acute wounds are mainly mechanical injuries for e.g. abrasions and tears by frictional contact between the skin and hard surface. There are different types of acute wounds such as abrasion, puncture, incision, avulsion, laceration, burns and chemical injuries. Graphical illustration of different types of wounds is shown in figure 2.2

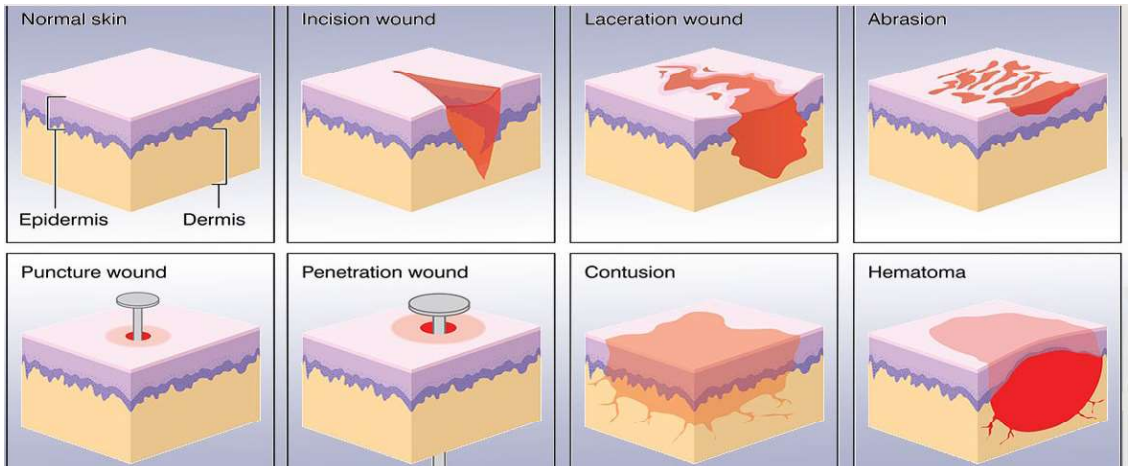

FIGURE 2.2: Illustration of different types of wound

ii. Chronic wounds: Chronic wounds arise from tissue injuries which heal slowly (more than 12 weeks) and often reoccur due to repeated tissue damage or underlying physiological condition such as diabetes, persistent infection, malignancies, poor treatment that disturbs normal sequence of events during the wound healing. Diabetic, venous stasis, pressure ulcer, decubitis ulcers and leg ulcers are some examples of chronic wounds.

➢ Wounds are also classified by the number of skin layers involved, as mentioned below (7).

i. Superficial wounds involve only the epidermis,

ii. Partial thickness wounds involve epidermis and dermis, while

iii. Full thickness wounds also involve the subcutaneous fat or deeper tissue.

A wound can have a significant impact on a person's life. Wounds can lead to prolonged periods of disability in addition to suffering, pain and discomfort, and may

even prevent a person from performing everyday activities such as walking and bathing. This inactivity may in itself lead to further health problems. Some wounds are associated with odor and excessive drainage and require frequent attention as they may impede social interactions. A non-healing wound may prevent a return to work which can have psychological as well as economic ramifications.

2.1.3 WOUND HEALING

Wound healing is a natural physiological body response after injury. It is a complex and dynamic process of repairing devitalized and missing cellular structures of tissue and involves a complex events between different cells, cytokines, vascular system and mediators (8). 2200 BC's medical manuscripts, a "CLAY TABLET" describes wound healing as "THREE HEALING GESTURES" which are: wash the wound, making plasters, and bandaging the wound(9). There is a significant advancement in wound management but the basic theme seems to be similar. Wounding destroys various layers of skin and the underlying soft tissues. Following injury, wound healing follows a series of tightly regulated, sequential events. The wound healing process can be divided into four interconnected phases which are homeostasis, inflammation, proliferation or granulation and remodeling or maturation shown in figure 2.3 (10). For complete wound healing, these phases need to be in proper sequence with specific time duration and intensity.

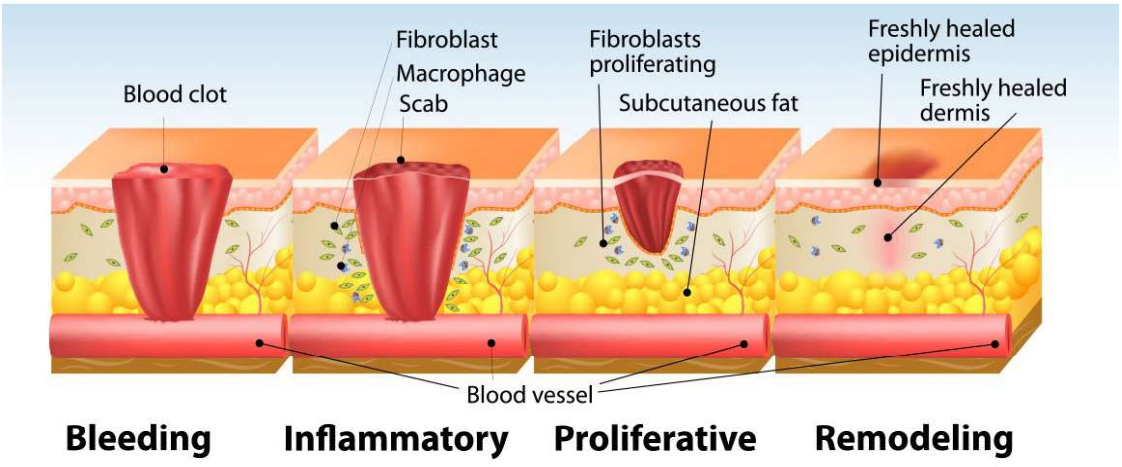

FIGURE 2.3: Phases of wound healing process

2.1.3.1 Haemostasis phase:

After tissue injury, wound healing process immediately starts and the mechanisms are triggered to control bleeding. This initial process of bleeding control is haemostasis phase which consists of two major events: (i) fibrin clot formation and (ii) coagulation. Haemostasis occurs within minutes of the initial injury unless there are clotting disorders. In wound healing, the *platelet* is the cell which acts for sealing off the damaged blood vessels. The blood vessels, themselves constrict in response to injury but this spasm ultimately relaxes. The platelets secrete vasoconstrictive substances to aid in this process but their prime role is to form a stable clot sealing the damaged vessel. Under the influence of ADP (adenosine diphosphate) leaking from damaged tissues, the platelets aggregate and adhere to the exposed collagen. They also secrete factors which interact with and stimulate the intrinsic clotting cascade through the production of *thrombin*, which in turn initiates the formation of fibrin from fibrinogen. The fibrin mesh strengthens the platelet aggregate into a stable haemostatic plug. Finally, platelets also secrete cytokines such as platelet-derived growth factor (PDGF), which is recognized as one of the first factors secreted in initiating subsequent steps (11).

2.1.3.2 Inflammation Phase:

Inflammation presents as erythema, swelling and warmth often associated with pain. This stage usually lasts up to 4 days post injury. The inflammatory response causes the blood vessels to become leaky releasing plasma and Poly Morpho Nucleocytes into the surrounding tissue. The neutrophils phagocytize debris and microorganisms and provide the first line of defense against infection. They are aided by local *mast cells*. As fibrin is broken down as parts of this clean-up, the degradation products attract the next cell involved. Macrophages plays major role in this phase. They phagocytize bacteria and provide a second line of defense. They also secrete a variety of chemotactic and growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor beta (TGF and interleukin-1 (IL-1) which appears to direct the next stage (11).

2.1.3.3 Proliferative Phase (Proliferation, Granulation and Contraction):

The granulation stage starts approximately four days after wounding and usually lasts until day 21 in acute wounds depending on the size of the wound. Inflammatory responses to injury provide the necessary framework to the production of a new functional barrier. It is characterized clinically by the presence of pebbled red tissue in the wound base and involves replacement of dermal tissues and sometimes subdermal tissues in deeper wounds as well as contraction of the wound. Fibroblasts secrete the collagen framework on which further dermal regeneration occurs. Specialized fibroblasts are responsible for wound contraction. Pericytes regenerate the outer layers of capillaries and the endothelial cells which produce the lining. This process is called as angiogenesis. The keratinocytes are responsible for epithelialization. In the final stage of epithelializtion, contracture occurs as the keratinocytes differentiate to form the protective outer layer or stratum corneum (11). This complex event incorporates many processes such as angiogenesis, formation of granulation tissue, collagen deposition, epithelialization and wound contraction which occur simultaneously.

2.1.3.4 Remodeling or Maturation Phase:

Wound repair or the healing process involves remodeling the dermal tissues to produce greater tensile strength. The principle cell involved in this process is the fibroblast. Remodeling can take up to 2 years after wounding. In healthy individuals with no underlying factors, an acute wound should heal within three weeks with remodeling occurring over the next year or so. If a wound does not follow the normal trajectory, it may get stuck in one of the stages and the wound becomes chronic. Chronic wounds are defined as wounds, which have “failed to proceed through an orderly and timely process to produce anatomic and functional integrity, or proceeded through the repair process without establishing a sustained anatomic and functional result” (11).

Kane’s analogy connects the wound healing process with repair of a damaged house shown in table 2.1 which wonderfully explains the basic physiology of wound repair (12).

TABLE 2.1: The phase of wound healing (Kane's Analogy of wound healing)

Phase of healing	Days post injury	Cell involved	Function or activity	Analogy to house repair
Haemostasis	Immediate	Platelets	Clotting	Utility workers cap-off broken utilities
Inflammation	Day 1 – 4	Neutrophils	Phagocytosis	Unskilled laborers clean up the site contractors direct activity
Proliferation (Granulation and contraction)	Days 4 -21	Macrophages Lymhocytes Angiocytes Neutrocytes Fibrinocytes Keratinocytes	Fill defect Re-establish skin function closure	Subcontractors start work: Framers, plumbers, electrician, roofers and sliders
Remodeling (Maturation)	Days 21- 2 year	Fibroblasts	Develop tensile strength	Interior finishing

2.1.4 WOUND MANAGEMENT

Wound management is careful and accurate assessment of the wound with the use of proper wound care products. Over the years, the market has moved from traditional (gauze based) products to advanced (moist wound healing) products to actives (antimicrobials, mechanical devices). Until 1960, advances in the design and efficacy of wound management products have been spasmodic and limited to the adaptation of available materials that were being used for other purposes. The products were primarily of the plug and conceal variety, and could be considered passive products that took no part in the healing process. Very little attention was paid to the functional performance of a product and minimal consideration was given to the healing environments required for

different wound types (13, 14). A new generation of products was potentiated by the advances in knowledge. The humoral and cellular factors associated with the healing process and the realization that a controlled microenvironment was needed if wound healing was to progress at the optimum level (13).

A systemic approach for assessing and managing chronic wounds, referred to as wound bed preparation has been developed. This approach comprises four individual steps described with the acronym "TIME": tissue assessment and management of tissue deficits, inflammation and infection control, moisture balance and enhancing epithelial advancement of wound edges (15).

Factors to be considered while optimizing wound management

- **Decreased dehydration and cell death:** As described earlier, the task of wound repair requires the activity of a host of cells from neutrophils and macrophages to fibroblasts and pericytes. These cells cannot function in a dry environment (16).
- **Increased angiogenesis**: Not only do the cells required for angiogenesis need a moist environment but also angiogenesis occurs towards regions of low oxygen tension such that occlusive dressings may act as a stimulus in the process (16).
- **Enhanced autolytic debridement:** By maintaining a moist environment, neutrophil cell life is enhanced and proteolytic enzymes are carried to the wound bed allowing for painless debridement (17). Further, these fibrin degradation products also stimulate macrophages to release growth factors into the wound bed.
- **Increased re-epithelialization** (18)
- **Bacterial barrier and decreased infection rate: D**ressings with good edge seals can provide a barrier to migration of microorganisms into the wound (19).
- Decreased pain
- Decreased costs

2.1.5 ADVANCED WOUND MANAGEMENT

Tremendous strides have been made in wound management since the mid-1980s when transparent films and hydrocolloids began to replace traditional gauze pads and non-adherent dressings as primary coverings for acute and chronic wounds. Today, Advanced Wound Management dressings including hydrocolloids, alginates, gels and foams allow healthcare professionals to manage moisture at the wound surface and reduce the frequency of dressing changes from several times a day to several times a week (20, 21). New wound care technologies are being developed at an increasingly rapid pace in recent years. These innovations could significantly reduce overall costs for treating complex and chronic wounds, while offering greater savings in preventing wounds and their recurrence. Advanced wound management includes products for managing difficult-to-heal wounds. These include chronic wounds such as pressure and diabetic foot ulcers as well as burns and post operative wounds. The management of chronic wounds is a challenging task for the healthcare system. Aggressive management of the underlying conditions causing chronic wounds as well as patient individualized goal setting is central to cost effective wound care. Once these objectives are achieved, topical management of the wound with appropriate dressing to support healing and avoidance of infection are vital adjutants (22).

Dressings such as hydrogels, hydrocolloids and films may efficiently maintain moisture or manage an excess amount of moisture due to exudates in a more resource effective manner than moist gauze dressings. These products are designed to help speeding up the healing process and improving patient's quality of life. Advanced polymeric gels, films and foams that create a moist environment, encourage healing, and antimicrobial dressings and ointments can be used for treating and preventing wound infection (23). Potential benefits of advanced wound management products to patients include fewer reapplications of dressings, less discomfort and pain, faster healing and reduced risk of complications such as infection and amputation. Treatment with such products would be clinically effective and less time-consuming and therefore more cost effective.

2.1.6 WOUND DRESSINGS

Wound dressings have developed over the years from the crude applications of plant herbs, animal fat and honey to tissue engineered scaffolds. Wound dressings are an essential component of every wound care treatment plan (23).

It is widely accepted that a warm, moist wound environment encourages healing and prevents tissue dehydration and cell death. These conditions also allow the interaction of the cells and growth factors involved in the in healing process. Therefore, ideal wound dressing should (24, 25):

- Maintain a moist environment at the wound surface
- Provide debridement action
- Improves epidermal migration
- Provide thermal insulation.
- Provide mechanical protection and protect against secondary infection
- Be non-adherent and easily removed without trauma
- Leave no foreign particles in the wound
- Prevent infection
- Allow gaseous exchange (water vapor and air)
- Absorb and remove excess exudates, blood
- Be cost effective and offer effective pain relief
- Improves angiogenesis and connective tissue formation
- Must be safe and non-allergic

Over the past few years, an ever-expanding list of dressing products has come onto the market in an attempt to meet these conditions. Among them are the transparent film dressings, hydrogels, hydrophilic foams, alginates, hydrocolloids and the newer antibacterial and biological dressings or devices (24).

2.1.5.1 Classification Of Dressings

Wound dressings can be classified in a number of ways depending on their function, type of material, physical form and nature of action. It is also classified as traditional dressings (cotton pads, bandages and gauze), modern dressings (hydrocolloid, hydrogels,

foams, films and alginates), advanced dressings, skin replacement products and wound healing devices (25).

❖ **Based on its nature of action**

i. Passive dressings: Passive dressings simply provide cover, while active dressings serve to actively change the e0nvironment of a wound. Traditional dressings like gauze and tulle dressings that account for the largest market segment are passive products.

ii. Bioactive dressings: Biologic dressings add materials which may be depleted. Biologic dressings are constructed from materials having endogenous activity. These materials include proteoglycans, collagen, non-collagenous proteins, alginates or chitosan (24).

iii. Active dressings / Medicated dressings: Active dressings comprise of synthetic polymeric films and forms, which are mostly transparent, permeable to water vapor and oxygen but impermeable to bacteria. These films are recommended for low exuding wounds. These types of dressings have drugs which plays an important role in the healing process (24).

❖ **Based on type of materials used**

i. **Synthetic Dressings** Synthetic polymers derived from petroleum products can be easily manufactured using conventional technology into films, fibers, sheets, and sponges. For this reason these materials have received attention as potential wound dressings for deep wounds. Synthetic polymers have several advantages such as ability to adhere to the wound edges, ability to drape to the wound contour and ease of use (24). The major disadvantage is the lack of biological properties such as enhancing wound healing via attraction of cells involved in healing process. These dressings are used as coverings for deep (full-thickness) burns and skin ulcers. In these applications, synthetic polymeric dressings create an inert environment that controls water and heat passage from the wound while preventing bacterial infiltration (26). Some examples paraffin gauze dressings (open mesh nylon fabric,

Nonwoven pad coated with aluminum) Polyurethane foam layers, polyacrylamide and polyurethane films ,silicone rubber membrane and knitted nylon fabric

ii. **Biological Dressings:** Biological dressings are derived from natural tissues usually consisting of various formulations and combinations of collagen, elastin and lipids. Examples of biodegradable dressings are collagen dressings and alginate dressings. They are far superior to synthetic dressings because they (24):

- ✓ Restore water vapor barrier and prevent dehydration of the wound;
- ✓ Decrease evaporation heat loss;
- ✓ Decrease protein and electrolyte losses in wound exudates;
- ✓ Prevent bacterial contamination of the wound and hence protect the wound and patient from sepsis;
- ✓ Permit less painful dressing changes;
- ✓ Permit painless movement over joints;
- ✓ Facilitate debridement of wounds;
- ✓ Create good granulation tissue bed for auto grafting of deep wounds;
- ✓ Can be used to test for successful subsequent auto graft;
- ✓ Decrease healing time of partial thickness burns and donor sites and
- ✓ Improve quality of healing, inhibit excessive fibroblasts and decrease contraction

2.1.5.2 Different types of wound care products

Following are the different types of wound care products which are available in market.

1. **Hydrocolloid dressing:** These consist of a combination of gel forming agent, elastomer and adhesives. They generally occur as thin films or sheet or as combination dressings. The hydrocolloids are impermeable to water in their general state, but when they absorb the exudates it result in the formation of a gel sheet. In the gel form, they rapidly become permeable to air and water. They do not cause pain on removal, maintain moist environment, adhere to dry as well as moist sites and can be used in the

management of both acute as well as chronic wounds (24). E.g. Granuflex™ by Convatec, UK; Tegasorb™ by 3M

2. **Alginate dressing:** It is either in the form of flexible fibers or foam. They have high absorption capacity due to strong hydrophilic gel formation. They limit wound secretion and also minimize bacterial infection. The ions present in the alginate fiber are exchanged with those present in the exudates to form a protective layer. This layer helps in the maintenance of an optimum environment required by the wound. The calcium ions present in the alginate provide the gelling property and also help in the production of a slow deagrading polymeric gel (26). According to a study, the calcium ions present in the alginates increase the proliferation of fibroblast (27). E.g. Sorbsan™ by Pharma-Plast ltd, Kaltostat™ by Convatec, Tegagen™ by 3M

3. **Hydrogel Dressing:** Hydrogel dressings are made from polymers such as poly(methacrylate), polyvinyl alcohol, polyethylene oxide, chitosan, and polyvinylpyrrolidine (26). Hydrogel dressings contain significantly large amounts of water and as a result they do not cause excessive absorption of the exudates. Hydrogels have low mechanical strength and hence can lead to accumulation of fluid and destruction of healthy tissues (24). Hydrogels are suitable for cleansing of dry, sloughy or necrotic wounds, are nonreactive with biological tissue, permeable to metabolites, are nonirritant, leave no residue, are malleable and improve re-epithelization of wounds (28). E.g. Nu-gel™ by GD Medical , Purilon™ by Coloplast

4. **Semi-permeable adhesive film dressing**: The traditional film dressings were originally created from nylon derivatives supported in an adhesive polyethylene frame which provided them with an occlusion property. The disadvantages were that they had limited ability to absorb sufficient quantities of wound exudates, which led to edema caused due to the presence of excessive fluids. These resulted in the destruction of skin cells and proliferation of bacterial cells and increase in the risk of infection and therefore required regular changing of the dressing (29). It is more porous and permeable to water

vapor and gases, but not permeable to the liquid from exudates. E.g. Cutifilm™ by Smith & Nephew, Tegaderm™ by 3M, Opsite™ by Smith & Nephew

5. **Foam dressing:** These dressings are made up of polyurethane and have adhesive borders. Foam dressings maintain an optimum moist environment with thermal insulation, good absorbent properties and are convenient to wear, making them patient compliant (24). They can be used for either partially thick or fully thick wounds (26). They can also be used for minimal, highly exuding wounds because of high absorbency. Foam dressings are also used in the treatment of granulating wound. E.g. Lyofoam™ by Molnlycke, Allevyn™ by Smith & Nephew

6. **Biological dressing (Tissue engineered products):** These are also called as bioactive dressings. They are made from biological materials that play an active part in the wound healing process. These technologies usually combine polymers such as collagen, Hyaluronic acid, chitosan, alginates and elastin. They are generally incorporated with antimicrobials and growth factors for better therapeutic activity. Hyaluronic acid-modified liposomes as bioadhesive carriers for delivering growth factors to wound sites have been studied(30). Chitosan leads to the acceleration of granulation during the proliferative stage of wound healing (31). Collagen is the major structural protein of an organ. It helps in the migration of endothelial cells, activation of clotting factors, formation of fibroblats and also plays a vital role in the appearance of the final scar (32). Collagen also plays a major role in wound healing (32). Main advantages of biodegradable dressings are that they play an active part in wound healing are biocompatible, non-toxic, result in formation of new tissues and are more superior to conventional and synthetic dressings.

7. **Tissue-engineered skin substitutes:** Both traditional and modern dressings cannot regenerate or replace the damaged and lost tissue. In advanced application, ‘smart’ polymers have been developed. Advancements in the fabrication of biomaterials and the culturing of skin cells have led to the development of a new generation of engineered skin substitutes. Tissue engineered skin substitutes can be categorized as acellular and

cell-containing matrices (33). Acellular matrices are produced either form synthetic collagen or in combination with hyaluronic acid. Collagen and glycosaminoglycans act as scaffolds onto which skin cells can be seeded for the growth of new tissues (33). They provide anatomic characteristics similar to that of the tissue they replace. When introduced into the body, they start degrading and leave behind a matrix of connective tissues having similar properties as that of the dermis. The disadvantages include high costs of production, ethical issues regarding stem cultures, risk of infection and antigenicity (34). Some examples of marketed products shown in table 2.2

TABLE 2.2: Marketed products used as skin substitutes

Sr. no.	Name	Components	Company
1	Biobrane	Accelular dressing, collagen bound to nylon fabric	Mylan Bertek pharmaceutical, USA
2	Epicel	Autologous epidermal graft	Sanofi Genzyme, USA
3	Alloderm	Accellular, allogeneic dermal graft	Allergan, USA
4	Integra artificial skin	Bovine collagen and chondroitin-6-sulfate	Integra lifesciences, India
5	Oasis	Pig intestinal mucosa	Cook biotech, USA
6	Trancyte	Devitalized fibroblast on nylon mesh	Advanced tissue sciences, USA
7	Dermagraft	Human fibroblasts in an absorbable matrix	Smith & Nephew, UK
8	Apigraf	Human fibroblasts and kerationcytes in bovine collagen matrix	Novartis, Switzerland

8. **Negative pressure wound devices:** Negative-pressure wound devices (NPWDs) are now being used to treat acute wounds. Topical negative pressure therapy help in wound close by applying localized negative pressure on a wound to promote wound contraction, removal of excess fluid and angiogenesis. According to the system, negative pressure of between 80 mmHg and 150 mmHg is required (35).

2.2 BURN

A burn is tissue damage caused by heat, steam, fires, gases, chemicals, electricity, sunlight, radiation. Burns are defined by how large an area they cover. Burns can cause swelling, blistering, scarring and in serious cases shock and even death. They also can lead to infections because they damage skin's protective barrier. Types of burns include (36):

i. First-degree burns (superficial burns) damage the outer layer (epidermis) of the skin. These burns usually heal within a week. A common example is sunburn.

ii. Second-degree burns (superficial partial thickness burns) damage epidermis and dermis. These burns might need a skin draft, natural or artificial skin to cover and protect the body while it heals and they may leave a scar.

iii. Third-degree burns damage (deep partial thickness burns) or completely destroy both layers of skin including hair follicles and sweat glands and damage underlying tissues. These burns always require skin graft.

iv. Fourth degree burns (full-thickness burns) extend into fat, fifth degree burns into muscle, and sixth degree burns to bone.

2.2.1 Management of Burns: Burn treatment varies depending on the cause and severity. Before dressing selection for burn treatment, the moistness, depth, area and size of the burns needs to be taken into consideration. Different types of dressings used in burns injury are mentioned in table 2.3. The main goal of burn treatment includes (37):

- Prevention of infection
- Promoting reepitheliazation

- Avoiding moisture and heat loss
- Decreasing pain
- Allowing for movement and function

Ideal dressing for burn injury should have following characteristicces:

- Prevent wound from drying and maintain moist environment
- Easy to apply without pain
- Easy to remove without pain
- Non adherent to wound
- Absorb wound exudates
- Biocompatible
- Non-toxic

TABLE 2.3: Commonly used wound dressings for burns treatment

Sr. No.	Active agent	Formulation Details	Advantages	Disadvantages
1	Silver sulfadiazine	Salt of silver sulfadiazine in water based cream	Painless use, Long shelf life, Wide-spectrum antimicrobial action against gram -ve and gram +ve microbes, Delyas eschar separation to a lesser degree, Used for deep partial and full-thickness wounds	Stains tissue, Delays healing, Contraindication in sulfa allergy, pregnant women, nursing mothers and newborns
2	Bacitracin	Topical cream	Painless, Can be used on face	May cause urticaria, burning;

			or near mucous membranes, Inexpensive	Requires frequent dressing changes, Does not penetrate eschar
3	Mafenide acetate	Soft white, non staining cream	Penetrates thick eschar, Used for deep burns and exposed cartilage, Effective against pseudomonas	May delay healing or cause metabolic acidosis, Can be painful on application
4	Mupirocin	Cream	Painless Good activity against gram +ve bacterial infection, Can be used on face, Active against most strains of methicinlin-resistant *S. aureus*	Requires frequent dressing changes, Expensive
5	Impregnated nonadherent gauze	Semi-occlusive Nonabsoptive dressing	Used for partial thickness burns, Provides a nonadherent barrier over the burn, Maintains a moist environment deodorizing agent,	No antimicrobial activity

			Clings and conforms to all body contours	
6	Hydrocolloids	Hydrophilic absorptive dressing	Shorter time to wound closure, Less pain, Decrease dressing changes, Inexpensive, Keep underlying tissue moist	Can't be used with large exuding wounds
7	Silicone dressing	Nonabsorptive dressing	Painless, Decrease dressing changes Highly transparent, Protect skin from additional trauma, May be left in place for 14 days	Expensive No antimicrobial activity
8	Silver impregnated dressing	Silver-impregnated absorbent or nonabsorbent dressing	Broad-spectrum antimicrobial dressing, Reduce pain, Decrease use of pain medications, Faster wound closure than with standard therapies Decrease total cost	May dry out and adhere to wound Don not use with oil-based products Do not used during MRI

9	Collagenase	Enzymatic debriding ointment	Remove nonliving tissue without harming granulation tissue	No antimicrobial activity, Cannot use dressings containing silver or iodine
10	Hydrogels	Gel, Sheet, Impregnated dressing	Maintains wound humidity, Facilitates autolytic debridement, Absorbs excess exudates, Allows evaporation without compromising humidity, Maintatins warmth, Decreases pain Remove toxic components	Permeable to bacteria, Dehydrates easily, No adhesive

2.3 CHALLENGES

Although there is technological advancement in wound care field but still wound management is a clinical challenge. The significance of wound management can be described by following facts:

- Thick exudates from wound worsen the condition of healing where major novel dressing don't have the capabilities to manage exudates (38).
- Removal and control of scar formed after wound healing is a challenges and emerging area of research. The prolonged inflammatory phase leads excessive scar formation (39).
- All wound are not same in term of healing rate, problems, and healing challenges.
- As per report published by Moulik et al., in 2013, 20% of diabetic patient with foot wounds faces a major lower limb amputation even with advanced therapies (40).
- The old age people mostly suffered from chronic wounds. In USA, 3% of the population (>65 year age) have open wound. The US government estimates that 55 million elderly population will be there by 2020 and 2% of total population to be affected by chronic wounds (41).
- In India, a recent study estimated a prevalence rate of chronic wounds at 4.5 per 1000 population. The incidence of acute wounds was at 10.5 per 1000 population which is more than double form chronic wound.
- Globally, it is projected that annual cost for wound care products will be $ 3.5 billion in 2021 compare to $ 2.8 billion in 2014 (42).
- As per 2018 market research report, the global wound closure products market will more than $ 15 billion by 2024 (42).
- Diabetic foot ulcers are the greatest clinical problem which leads to amputations and infections of these ulcers can spread to the bones of the food which has the greatest clinical impact associated with diabetes (43).
- Comorbidities such as obesity, diabetes, autoimmune diseases, malnutrition, en-stage renal disease, cancer and cardiovascular disease induce adverse effects on wound healing process. There will be about 82 million people (>65 year -old) in

developing countries and 48 million people (>65 year -old) in developed countries suffered from diabetics by 2030 which indicates a critical need for advance wound management to better control the future global burden of chronic wounds (44).

- Globally, burns injuries are the 4th most common type of trauma.
- The risk of burns tends to increase with lower socioecomic status. About 90% burns cases found in low- or middle income countries (45, 46).
- In 2016, about 4,86,000 people sought care for burns injury in USA as per the Burn Incidence Fact Sheet of the American Burn Association (47).
- About 1, 80,000 deaths per year due to burn injury were estimated in low-and middle-income countries (45, 48, 49).
- Burns is a potential public health problem in the world. As per the Global burden of disease study (2013), there were 33.5 million thermal burn injuries reported I all over the world, resulting 237500 deaths. 90% burn deaths occur in low/middle-income countries (50, 51). According to National program for prevention of Burns injuries, approximately 7 million people sustain burn injuries in each year in India, out of which 0.7 million need hospitalization, 0.25 million get crippled, and 0.14 million succumb (52). According to global Health data (2017), over 61,000 deaths in 2015 due to burns in India.

2.4 HYDROGELS FOR WOUND HEALING

Hydrogels are three-dimensional hydrophilic polymeric networks which are capable of holding large quantity of water. They are composed of water insoluble, cross-linked polymers with a high affinity of water. The water absorption capacity of hydrogel depends on the cross-linking density of polar functional group such as amino, hydroxyl, amide and carboxyl in the polymer structure. Hydrogels have unique properties such as high water content, non-adhesive nature, swelling - deswelling reversibly in aqueous solution, malleability and a resemblance to living tissues in terms of biocompatibility (53).

Winter GD. explained important role of moist wound environment for faster and better wound healing in 1962 (54). From then, hydrogel, hydrocolloids and gel have been used in wound management to provide a moist environment and in debridement (53). Hydrogels promote wound healing (55) by their moisture exchanging activity that develops a balanced microclimate between the dressing and wound bed (56). Hydrogel dressing provide cooling, soothing effect; reduce pain associated with dressing changes due to its high water content (57). It can be easily removed from the site of application without causing further pain to the healing tissue (58). Hydrogels have good stability and mechanical strength due to the cross-linked polymeric network. Some transparent hydrogels also allow clinical assessment of healing process. Hydrogels can control diffusion and release of loaded active moiety which make them truly interactive dressing. Hydrogel dressings are used for different types of wounds such as dry wounds with necrotic tissue, burn wounds, diabetic foot ulcers, pressure ulcers, chronic leg ulcers and low to moderately exudating wounds (59, 60).

Hydrogels offer great advantages such as the incorporation of bioactive agents and/or cells due to mild processing conditions which can be delivered in a more prolonged mode, which represents a great advantage in relation to their topical administration. Depending on use, Hydrogel properties (composition, sensitivity to wound stimuli, etc.) can be tailored to deliver specific mediators depending on final used. Hydrogels can be used to deliver bioactive molecules known to accelerate healing, or to support and maximize the therapeutic potential of skin or stem cells promoting an increase in vessel

density and re-epithelialization as well as new ECM production and maturation, ultimately aiming to achieve full skin regeneration

2.4.1 DIFFERENT TYPES OF HYDROGELS: The hydrogel products can be classified in many ways as shown in table 2.4.

TABLE 2.4: Classification of different types of hydrogels

Hydrogel classification	Types of hydrogel
Based on source	Natural hydrogel
	Synthetic hydrogel
Based on polymeric composition	Homopolymeric hydrogel
	Copolymeric hydrogel
	Multipolymer interpenetration polymeric hydrogel
Based on biodegradability	Biodegradable hydrogel
	Non-biodegradable hydrogel
Based on type of cross-linking	Chemically cross-linked hydrogel
	Physical gel
Based on network electrical charge	Nonionic hydrogel
	Ionic hydrogel
	Amphoteric hydrogel
	Zwitter ionic hydrogel

2.4.2 METHODS FOR PREPARATION OF HYDROGELS: Various synthetic and natural polymers are used for preparation of hydrogels.

1) **Physically cross-linked hydrogels:** Hydrogels which are crosslinked by physical method are known as physical gels or physically cross-linked hydrogels. This is normally achieved via utilizing physical processes such as ion-polymer complexation, hydrophobic association, chain aggregation, crystallization, polymer-polymer complexation, and hydrogel bonding which are mentioned in table 2.5. Physical hydrogels are reversible due to the conformational changes. This method of preparation requires relatively mild conditions and simple purification procedures.

TABLE 2.5: Different physical methods of crosslinking for the preparation of hydrogel

Physical method for cross linking	Examples	Ref
Hydrophobic association	• Isopropyl groups in poly(n-isopropyl acrylamide), • Methyl groups in methyl cellulose, • Propylene oxide blocks in (ethylene oxide)-(propylene oxide)-(Ethylene oxide) terpolymers	(61)
Ion-polymer complexation	• Acrylic-based hydrogels treated with calcium, aluminum, iron, • Sodium alginate treated with calcium and aluminum • Poly (vinyl alcohol) treated with borax	(62-64)
Polymer – polymer complexation	• Alginate and chitosan • Gum Arabic and gelatin	(65)
Chain aggregation	Heat treatment of hydrocolloidals in water	
Hydrogen bonding	• PVA/PVA chains; • Poly(acrylic acid)/polyacrylamide chains	(66, 67)
Thermodynamic changes	heating or cooling of polymer solution, lowering pH	(68-70)
Crystallization	Freeze-thawing	(71)
Protein interaction	Collagen-mimetic protein with PEG	(72)

2) **Chemically cross-linked hydrogels:** Hydrogels which are prepared by chemical cross-linking method are known as a chemical hydrogels or chemically cross-linked hydrogels**.** A chemical process i.e. chemical covalent crosslinking is utilized to prepare a chemical hydrogel (Table 2.6). Chemical hydrogels are permanent and irreversible as a result of configurationally changes.

TABLE 2.6: Different chemical crosslinking methods for the preparation of hydrogel sheet

Chemical crosslinking method	**Example**	**Ref.**
Covalent bonding crosslinking	Covalent crosslinking using olefinic crosslinkers containing unsaturated bonds or reactive functional groups	(73)
Simultaneous polymerization and crosslinking	Acrylic acid or acrylamide, crosslinked with methylene bisacrylamide, ethylene glycol diacrylate, ethylene glycol dimethyacrylate, poly (ethylene glycol dimethacrylate)	(74, 75)
Post polymerization chemical crosslink	Alginate chitosan hydrogel Chitosan hyaluronic acid hydrogel	(76, 77)

3) **Hydrogel prepared by high energy radiations:** In this technique, high energy radiations are used to polymerize unsaturated compound for hydrogel synthesis. It is free from use of toxic chemical as crosslinking agents. It is a cost effective technique as separate sterilization of hydrogel not required. On exposure to high energy radiation, radicals are formed on polymer chains in an aqueous solution which initiates free radical polymerization. Some examples of hydrogel prepared by gamma irradiation technique mentioned in table 2.7.

TABLE 2.7: Example of hydrogels prepared by γ-rays irradiation techniques

Preparation method	Example	Rag
Gamma irradiation	Silver nanoparticles loaded AMPS-Na hydrogel	(78)
	PVA/Gum acacia	(79)
	Nano silver/gelatin/carboxymethyl chitosan hydrogels	(80)

2.4.3 CHARACTERIZATION OF HYDROGELS: Hydrogels are characterized for following parameters

- Morphology,
- Chemical structure
- Swelling property
- pH
- Mechanical properties
- Rheology properties
- Spreadability
- Preservative efficacy test or sterility test
- Microbial load

2.4.4 Types of hydrogel dressings available in market for wound healing

a) **Amorphous hydrogel:** Amorphous hydrogels are made up of polymers, water and other ingredients. It has no shape and specific design. It is generally in viscous form which flows freely. These hydrogels can be evenly applied on wound using an applicator. These are mostly indicated for uneven or cavity wound. These types of dressing need to be covered with a secondary dressing. Amorphous hydrogels packed into the tubes, foil packets and spray bottles. E.g. Meghaheal by Aristo pharma, Nemiheal by Mil laboratories pvt ltd., Aquasite® by Derma Sciences Inc., Intrasite ® gel by Smith & Nephew

b) **Impregnated hydrogel gauze dressing:** Impregnated hydrogels are saturated and/ or permeated with an amorphous hydrogel onto gauze pads, nonwoven sponge and gauze strips. These dressings can be applied into a deep or uneven wound or laid over the wound. These types of dressing also need to be covered with a secondary dressing. These types of dressings are available in various sizes and are used for necrotic, sloughy and granulating full thickness wounds. E.g. Aquastie ® impregnated gauze dressing by Derma Science Inc., Intrasite® conformable hydrogel gauze dressing by Smith & Nephew.

c) **Hydrogel sheet dressing:** These act as advanced wound dressing by keeping the wound bed moist thus offering a soothing effect and facilitate wound healing as well as providing a physical barrier between the wound and the external environment. These are transparent dressings so that allows easy monitoring of the healing process**.** The sheet can be cut into different sizes according to the wound. Hydrogel sheets come with or without adhesive border. They are non-adhesive against the wound for easy removal. These dressings are used for the treatment of deep cavity and partial thickness wounds such as ulcers, pressure sores, skin donor site and 1st & 2nd degree burns. E.g. Aquasite® Hydrogel sheet dressing by Derma science Inc., Flexigel® sheet, by Smith & Nephew

2.5 COLLAGEN DRESSINGS FOR WOUND HEALING

Collagen is a biodegradable and biocompatible protein mostly found in connective tissue. One key component of chronic wounds is an elevated level of matrix metalloproteinase (MMPs). At elevated levels, MMPs not only degrade nonviable collagen but also viable collagen. In addition, fibroblasts in a chronic wound may not secrete tissue inhibitors of MMPs (TIMPs) at an adequate level to control the activity of MMPs. These events prevent the formation of the scaffold needed for cell migration and ultimately prevent the formation of the extracellular matrix (ECM) and granulation tissue. Collagen based wound dressings are uniquely suited to address the issue of elevated levels of MMPs by acting as a 'sacrificial substrate' in the wound. In addition, collagen based dressings have the ability to absorb wound exudates and maintain a moist wound environment (32).

The first medical usage of collagen in humans was reported by Knapp et al. (1977) and was used to provide co-reaction of contour deformities (81). Bovine collagen was used as suture and haemostatic agents after years. In 1980, Zyderm 1 was released, a suspension form containing sterilized fibrillar bovine collagen that was used for injecting under the dermis in wounds (82). Today, collagen is used in numerous biomedical applications. These include collagen suspensions for dermal injection, topical haemostatic agents, wound dressing materials, collagen suture and catguts, collagen gels for periodontal reconstruction, collagen sponges for the homeostasis and coating of joint, and collagen rich pig skin wound dressing materials.

2.5.1 FORMULATIONS AVAILABLE IN MARKET: Various types of collagen formulations are available such as freeze-dried sheet, pads, pastes, powder, film and gels (table 2.8).

TABLE 2.8: Marketed products of collagen dressings for wound

Sr. No.	Brand name	Physical form	Main Component	Manufacture
1	BIOSTEP collagen matrix	Sponge like sheet	Collagen, sodium alginate, CMC and EDTA	Smith & Nephew, USA
2	Promogran Prisma™ Matrix	Sponge like sheet	Collagen, oxidized regenerated cellulose, Silver	3M
3	Puracol® Plus	Sponge like sheet	Type I bovine, native Collagen	Medline, USA
5	Nemigen (Dry collagen sheet)	Sponge like sheet	Type I bovine, native Collagen	Mil Laboratories Pvt. Ltd., India
7	Collograft (Dry collagen sheet)	Sheet	Bovine collagen	Cologenesis Healthcare Pvt. Ltd., India
8	Colldrex-D (sterile collagen sheet dressing)	Transparent film	Bovine collagen	Synerheal Pharmaceutical , India
9	Diacoll-S (Collagen dry sheet with gentamicin)	Transparent film	Bovine collagen, Gentamicin	Cologenesis Healthcare Pvt. Ltd., India
10	Stimulen™ collagen Lotion sachet	Lotion	Bovine Collagen	Southwest Technologies, Inc., USA
11	GenColl (Collagen in gel form)	Gel	Bovine collagen	Cologenesis Healthcare Pvt. Ltd., India
12	Stimulen™ collagen gel	Gel	Bovine Collagen	Southwest Technologies, Inc., USA
13	CellerateRX® Gel	Gel	Hydrolyzed Collagen	Sanara MedTech, USA
14	CellerateRX® powder	Powder	Hydrolyzed Collagen	Sanara MedTech, USA
15	Stimulen™ collagen powder	Powder	Bovine Collagen	Southwest Technologies, Inc., USA
16	Collofibre (Collagen sterile powder)	Powder	Bovine collagen	Cologenesis Healthcare Pvt. Ltd., India

2.5.2 INDICATIONS: Collagen dressings are indicated for following wound conditions;

- Partial- and full-thickness wounds
- Wound with minimal to heavy exudates
- Skin graft and skin donation site
- Second-degree burns
- Chronic no healing wounds

2.5.3 CONTRAINDICATIONS: Collagen dressings are not advisable in following wounds

- Third-degree wounds
- Patient having allergy to bovine, porcine, or avian product
- Wounds covered in dry scar

2.6 POT MARIGOLD (*Calendula officinalis L.)*

Calendula officinalis L. is commonly known as marigold. It is also known as "poet's marigold" as many poems have been written about it and "pot marigold" referes to its ease of cultivation in pots. The name 'CALENDULA' comes from the Latin word "*KALENDAE"* (Middle English "CALENDS") which means the first day of each month, when the flowers bloom. Since 12th century, calendula has been used medicinally in Europe and the Mediterranean. It is also known as "HERB OF THE SUN" because calendula flowers bloom in the morning and close in the evening. The "Mary's Gold" name comes from its resemblance to the rays of light that radiate like the Virgin Mother's head (83). Marigolds were first discovered by the Portuguese in Central America in the 16th century. They introduced these flowers to Europe and India. Marigolds are now widely cultivated in the sub-continent. The marigold variety that delivers health benefits is calendula.

About 20 species come under the genus *Calendula* from which only *Calendula officinalis* is used for its medicinal or culinary benefits (84). The dried, whole or cut petals of calendula flower are used medicinally (85, 86). Calendula was used in food such as butter, cookies, soups, custard, bread and rice dishes for color and flavor so that it was also known as "poor man's saffron" (84, 87). In 1550 – 1660, two British herbalists John Gerard and Nichloas Culpepper mentioned, the topical use of petals as a local remedy for insect stings (88). In 19th century, the Ecletic physicians of America used calendula internally to treat stomach ulcers, conjunctivitis, liver problems, and externally for bruises, wound and superficial burns (89).

The topical and internal use of calendula flower to treat inflammation of the mucous membrane of the mouth and throat and topical use for the treatment of poorly healing wound, food ulcers was approved by the German Commission E monograph 2000 (89). It is also approved in United Kingdom for external use only as herbal medicine in the general sale list (85). Calendula is considered as an active ingredient in Natural Health product (NHP) and requires premarketing authorization and product licensing in Canada. In America, it is used in dietary supplements, cosmetics, homeopathic remedies and also

recognized as safe for food use (GRAS 182.10) at 11 to 44 ppm (84, 85). Calendula was chosen as the international herb of the year for 2008 by the International Herb Association.

2.6.1 DESCRIPTION

Calendula officinalis L. has orange to yellow flowers with female ray florets and hermaphrodite, tridentate, tubular disc florets; sickle-shaped, curved and ringed achenes shown in figure 2.4 (b). The plant grows between 30-80 cm in height with elegant yet tough and gently green stems. The leaves are alternately arranged, get progressively smaller as they progress up the stem and range from 3-15cm in length shown in figure 2.4 (a)

(a) Plant **(b) Flower**

FIGURE 2.4: *Calendula officinalis L.* (Pot marigold) – (a) plant and (b) flower

- **Botanical name:** *Calendula officinalis L*
- **Classification**
  - Kingdom: Plantnae
  - Subkingdom: Tracheobionta
  - Division: Magnoliophyta
  - Class: Magnoliopsida
  - Subclass: Asteridae
  - Family: Asteraceae.
  - Tribe: Calenduleae
  - Genus: Calendula
  - Species: *C. officinalis*
- **Synonyms:** *Calendula aurantiaca* Kotschy ex Boiss., *Calendual eriocarpa* DC., *Calendula hydruntina* (Fiori) Lanza, *Calendula officinalis* var. *prolifera* Hort., *Calendula prolifera* Hort. Ex Steud., *Calendula santamariae* Font Quer, *Calendula sinuate var. aurantiaca* (Klotzsch ex Boiss.) Boiss., *Caltha officinalis* (L.) Moench (nom. Illeg.)
- **Common Names:** Marigold, Bull's Eye, Calendula, Herb of the Sun, Holligold, poet's Marigold, Poor Man's Saffron, Pot marigold, Water dragon, Fresh marigold, African marigold (English), Sthulapushpa, sandu, ganduga (sanskrit), Genda, gultera (Hindi), genda (Bengal), Guljharo, makhanala (Gujarat), Tangla, mentok, genda (Punjab), Gul-E-Ashrafi (Urdu), Sendigai Poo (Tamil),
- **Origin:** A native of the Mediterranean area
- **Agroecology:** It grows well in full sun and tolerates most soils such as acidic, loamy, sandy clay soilds with pH 4.5-8.3. It grows well in well-drained moisty, loamy soil.
- **Edible plant parts:** Flowers and leaves are edible.

❖ **Botany (table 2.9):**

TABLE 2.9: Botany of *Calendula officinalis* L.

Height	20–50 cm (8–20 in.). Stem ascending–erect, hairy all over, also glandular hairy. Repulsive smell.
Flower	Flowers form 4–7 cm (1.6–3 in.) wide, single flowerlike capitula surrounded by involucral bracts. Capitulum's yellow–orange–reddish yellow ray florets tongue like, 3-toothed at tip; Disk florets yellow–brownish, tubular, small (sometimes all flowers tongue like. Stamens 5. Gynoecium composed of 2 fused carpels. Involucral bracts 2 rows, virtually linear. Capitula solitary, terminating the stem.
Leaves	Alternate, stalked–stalkless, lower stalks winged, upper leaves amplexicaul. Blade Narrowly obovate–narrowly elliptic, roundish–gently tapering tip, with entire margins–sparsely toothed, hairy.
Fruits	Longbeaked or curved, spinebacked achene

❖ **Phytochemicals:** It contains various phyto-chemicals such as carbohydrates, amino acids, lipids, fatty acids, carotenoids, terpenoids, flavonoids, quinines and coumarins.

- Terpenoids : Sitosterolds, stigmasterols, diesters of diols, 3-monoesters of taraxasterol, lupeol, ertyrodiol, brein, ursadiol, faradiol-3O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate, arnidiol-3-O-palmitate, arnidiol-3-O-larurate, arnidiol-3-O-laurate, calenduladiol-3-O-palmitate, calenduladiol-3-Omyristate, oleanolic acid saponins: calenduloside A-H. Olenane triterpene glycoside: Calendulaglycoside A, calendulaglycoside A 6'-*O*-methyl ester, calendulaglycoside A 6'-*O*-n-butyl ester, calendulaglycoside B, calendulaglycoside B 6'-*O*-n-butyl ester, calendulaglycoside C, calendula glycoside C 6'-*O*-methyl ester,

calendulaglycoside C 6'-*O*-n-butyl ester, calendulaglycoside D, calendulaglycoside D2, calendulaglycoside F, calendulaglycoside F 6'-*O*-butyl ester, calendulaglycoside G 6'-*O*-methyl ester, calendasaponins A-D; glucosides of oleanolic acid (mainly found in roots of grown and senescing plants) I, II, III, VI, VII , and glucuronides (mainly found in flowers and green parts) F, D, D2, C, B and A (90, 91). One new triterpenic ester of olanane series has been isolated from flowers was cornulacic acid acetate from flowers.Triterpenoids present in Calendula are believed to be responsible for the anti-inflammatory effects of Calendula extracts, particularly faradiol monoester

- Triterpene alcohols: Free and esterified (with fatty acids) monols, diols and triols of ψ-taraxastane-type including ψ-taraxasterol, faradiol, heliantriol B0, heliantriol C, taraxastane-type including taraxasterol, arnidiol, heliantriol B1, lupine-type including lupeol, calenduladiol, heliantriol B2, ursane-type including α-amyrin, brein, ursadiol, ursatriol, oleanane-type including β-amyrin, maniladiol, erythrodiol, longispinogenin, heliantriol A1 (92, 93).
- Flavonoids: Quercetin, isorhamnetin, kaempferol, rutin, hyperoside, isoquercitrin, astragalin, quercetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-neohesperidose, quercetin 3-*O*-2G-rhamno-sylrutinoside, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside, isorhamnetin 3-*O*-neohesperidoside, iso-rhamnetin 3-*O*-2G-rhamnosylrutinoside (91).
- Ionone glucosides: Officinosides A and B; sesquiterpene glycosides: Officinosides C and D; carotenoids: Lutein, zeaxanthine, flavoxanthin, auroxanthin, β-carotene, luteoxanthin, violaxanthin, β-cryptoxanthin, mutaxanthin (94).
- hydroxycoumarins: Scopoletin, umbelliferone, esculetin; phenolic acids: Chlorogenic acid, caffeic acid, coumaric acid, vanillic acid;(Cetkovic et al., 2003) volatile oils: α-cadinol, T-cadinol (95); α-cadinene, limonene, 1,8-cineol(96).

- quinones: α-tocopherol, phylloquinone(97); fatty acids: Calendic acid, dimorphecolic acid (98).
- Carotenoids: There have been nineteen carotenoids identified in Calendula and these are considered to have antioxidant activity. These are lutein and zeaxanthin, often paired together, and lycopene & betacarotene. Lycopene is reported to reduce the risk of prostate cancer and heart disease. High iodine, carotene and manganese, which promote skin cell regeneration, have also been found in the plant.
- Others: Sterols, mucilage, carbohydrates, resin, tannins, amino acids, bitter principle calendin (99).

❖ **Pharmacological properties:** Various pharmacological properties have been attributed in preclinical research to various constituents, including anti-inflammatory, immunostimulating, antibacterial, antiviral, antiprotozoal, and antineoplastic properties (100).

a) **Antibacterial effects**: Hydroacetonic extract from fresh plants inhibited the growth of *Staphylococcus aureus* at a concentration of 1mg/mL *in vitro (101) (102).*

b) **Anti-inflammatory effects**: The active components of calendula's anti-inflammatory activity are thought to be the triterpenoids, particularly faradiol monoester. Free ester faradiol is the most active and exhibits the same effects as an equimolar dose of indomethacin (103-106). Calendula's glycosides have also inhibited lipooxygenase activity *in vitro (107).*

c) **Antioxidant effects**: Extracts of *Calendula officinalis* have anti-oxidant activity and demonstrate strong abilities to scavenge reactive oxygen species (108). Plants of the genus *Calendula* are natural sources of betacarotene (109), which may contribute to potential antioxidant effects. Nineteen carotenoids have been identified in extracts of *Calendula officinalis* petals, including flavoxanthin and isomers of lycopene, carotene, and rubixanthin (110).

d) **Antiprotozoal effects**: Oxygenated terpene alcohols and terpene lactones from calendula have been observed to possess trichomonacidal activity (111).

e) **Antiproliferative effects**: In a mouse model, dietary lutein derived from calendula extract has been found to suppress mammary tumor growth, increase tumor latency, and enhance lymphocyte proliferation (112). Saponins isolated from calendula express *in vitro* antimutagenic and tumor cell cytotoxic activity (113, 114). *C. officinalis* does not exert a direct mitogenic effect on human lymphocytes *in vitro* and exhibits inhibitory effects on lymphocyte. Proliferation.

f) **Antispasmodic and spasmogenic effects**: Crude extracts of *Calendula officinalis* flowers have been shown to contain both spasmolytic and spasmogenic constituents in rabbit jejunum(115).

g) **Antiviral effects**: Anti-HIV activity of calendula has been demonstrated *in vitro* specifically involving the inhibition of HIV-1 (IIIB) induced cytopathogenicity in CD4+ lymphocytic Molt-4 clone 8 cells. Triterpenoid saponins from *Calendula arvensis* have inhibited multiplication of vesicular stomatitis virus and rhinovirus *in vitro (116).* Triterpene and flavonol glycosides isolated from calendula have also demonstrated inhibitory activity against Epstein-Barr virus activation (91).

h) **Cytotoxic effects**: A novel laser-activated calendula extract is reported to activate lymphocytes and cytotoxicity *in vitro*, and may have important anti-tumor effects *in vivo (117).*

i) **Deodorant effects**: The components of marigold and other medicinal plants may act as oral deodorants by inhibiting salivary protein putrification (118).

j) **Hepatic effects**: *Calendula officinalis* extracts have been shown to have both protective and cytotoxic effects in rat hepatocyte cultures (119). Calendula extracts have also been shown to reduce hepatocytolysis and steatosis in CCl_4-intoxicated liver in Wistar rats (120).

k) **Hypoglycemic effects**: Hypoglycemic, gastric emptying inhibitory, and gastroprotective properties have been attributed to calendasaponins A, B, C, and D, two additional ionone glycosides (officinosides A and B), and two sesquiterpene oligoglycosides (officinosides C and D) (100).

l) **Wound-healing effects**: It has been proposed that *Calendula officinalis* extract may aid in wound healing by promoting epithelial growth and by enhancing

immune responses (121). Rao et al. observed a reduction of epithelialization time, an increase in wound strength, and improvement of wound contraction in rats with experimental incision wounds that were topically treated with calendula (122). The effects may also be mediated by the stimulation of phagocytosis, by increased granulation, and via effects on metabolism of glycoproteins, nucleoproteins, and collagen proteins in tissue regeneration (123).

m) **Other effects**: *In vitro*, calendula exhibited moderate "uterotonic" effects in isolated rabbit and guinea pig uterine tissues (124). In early (1964) animal studies, high doses of calendula preparations were reported to act as sedatives and hypotensive agents (125).

TABLE 2.10: Summary of pharmacological action of *Calendula officinalis* L.

Sr. No.	Pharmacological action	References
1	Wound healing activity	(123, 126-131)
2	Cell growth stimulating activity	(132)
3	Exfoliative Cheilitis Therapeutic activity	(133)
4	Anti-inflammatory activity	(104, 134-140) (91, 106, 141, 142)
5	Anti-oedematous activity	(105)
6	Antioxidant activity	(143-148)
7	Antidermatitic/Radio protective/Skin therapeutic activities	(149-157) (158-161)
8	Antimicrobial activity	(101, 102, 162-169)

❖ **Examples of combination marketed products:** About 200 cosmetic formulations are available which contain *Calendula officinalis* extract

1. Estromineral® Gel (isoflavones, *Lactobacillus sporogenes*, *Calendula officinalis* extract, and lactic acid);
2. Herbadermal® (calendula, garlic, and St. John's wort);

3. NHED® solution (*Calendula flores*, garlic (*Allium sativum*), *Verbascum thapsus*, St. John's wort *Hypericum perfoliatum*), lavender (*Lavandula angustifolia*), and vitamin E);
4. Otikon Otic® solution (herbal extract of calendula (*Calendula flores*), garlic (*Allium sativum*), mullein (*Verbascum thapsus*), and St. John's wort (*Hypericum perforatum*) in olive oil);
5. Traumeel®; and IND 61,164 mouthwash

- **Traditional medicinal uses:** It is used medicinally in Europe, China and India among several other places in the world. Calendula officinalis has been widely used as an anti-inflammatory agent to treat minor skin wounds, infections, burns, bee stings, bites, sunburns, sprains, sore eyes and ulcers
- **Other uses:** It can be grown as an intercrop or used as soil amendments to deter insect and soil pests. Jankowska et. al., found that intercropping with pot Marigold afforded the most effective pest control on white cabbage (170). Studies found that when used as a soil mulch (compost), the plant can significantly reduce root-knot nematode, *Meloidogyne incognita* , population in quito orange (*Solanum quitoens* e) plantings(171) Pérez et al. reported that amending soil with *C. officinalis* flowers significantly reduce reproduction rate of *Meloidogyne artiellia* on chickpea compared to the non-amended treatment(172). In eastern countries, Marigold flowers are used in garlands for social and religious purposes. The flowers also provide a yellow dye used for fabrics, cosmetics and food. Potted Marigold plant extracts are widely used in cosmetics presumably due to presence of compounds such as saponins, flavonoids, resins and essential oils. Potted Marigold is also an important dietary and medicinal source of carotenoids such as lutein, lutein esters, zeaxanthin, auroxanthin and fl avoxanthin.

2.6.2 RESEARCH WORK ON *CALENDULA*

1. Justyna et. al, (2020) prepared hydrogel delivery system containing *Calendula flos* lyophilized extract with chitosan for wound healing application. They confirmed synergism action of the active compounds present in the Calendula flowers and chitosan against hyaluronidase inhibition and antimicrobial properties (173).
2. Zahra pedram Rad et. al., (2019) prepared *Calendula officinalis* extract/PCL/Zein/gum Arabic nanofibrous bio-composite scaffolds using electrospining technique for skin tissue engineering(174). They prepared multilayer scaffold which showed more tensile strength than other *C. officinalis*-loaded PCL/Zein/GA scaffolds. Their results confirmed that PCL/Zein/GA/*C.officinalis* nanocomposite scaffold had favorable proliferation and adhesion against fibroblast cell as compared to PCL/Zein/GA scaffold for regenerating skin. They concluded that *C. officinalis*-loaded PCL/Zein/GA scaffold indicated better antibacterial properties and biocompatibility than PCL/Zein/GA scaffold.
3. Paula et. al., (2020) reported bacterial cellulose hydrogels containing calendula officinalis extract as dressing for wound healing(175). According to their *in-vivo* study results, bacterial cellulose/Calendula officinalis hydrogel promoted a better wound healing and statistically significant difference in the tissue repair. They also verified that a statistically significant difference between the treatments in the evaluation of the quality, quantity and orientation of the collagen fibers. The results of the histological evaluation demonstrated a statistically significant difference for tissue inflammatory reaction between the treatments in the 3-day period.
4. Christoph et. al., (2017) analyzed the molecular mechanism of the wound healing effect of calendula flower extract in human immortalized keratinocytes and human dermal fibroblasts(176). Their results showed that calendula flower influenced the inflammatory phase by activating the transcription factor NF-kB and by increasing the amount of the chemokine IL-8, both at the transcriptional and protein level in keratinocytes.

5. Buzzi M. et. al., (2016) carried out small clinical trial to evaluate the therapeutic benefits of the Phenusdermax® which contains calendula officinalis flower extract (4%) for pressure ulcer healing. They noted that the proportions of patients who were completely healed after 15 and 30 weeks of treatment were 63% and 88%, respectively, and the mean healing time was 12.5±7.8 weeks. No adverse events were observed during treatment (177).
6. Millan et. al., (2016) preclinical evaluated collagen type I scaffolds including gelatin-collagen micro particles and loaded with a hydroglycolic Calendula officinalis extract in a lagomorphs model of full-thickness skin wound. Histological and histomorphometric results indicated that grafting of SGC alone favored wound healing and showed a better clinical outcome than grafting SGC-E. In vitro collagenase digestion data suggested that the association of the C. officinalis extract to SGC increased the SGC-E cross-linking, making it difficult to degrade and affecting its biocompatibility (178).
7. Arana et. al., (2015) prepared *Calendula officinalis* extract loaded solid lipid nanoparticles for ophthalmic uses. In-vitro cell cytotoxicity and wound healing activity of *Calendula* loaded solid lipid nanoparticles were evaluated using a conjuctival epithelium cell line WKD. They determined effect of free *Calendula* officinalis extract on wound reepithelialization in the conjuctival epithelium using WKD cells and report that the 5 ppm of free *Calendula* extract was able to promote 20% are healing after 45 h incubation (179).
8. Okuma et. al., (2015) developed lamellar gel phase emulsion containing marigold oil as a potential modern wound dressing and evaluated their stability and activity on experimental wound healing in rats. In addition, in vitro and in vivo studies were carried out on wound healing rats as a model. They reported that LGP emulsion promoted better quality wound healing in a rat skin wound model than the other groups (180).
9. Different concentration (5%, 7.5% & 10%) of *Calendula officinalis* topical gel for cutaneous wound treatment were prepared and evaluated by Shafeie et. al., (2015). In this investigation, the effects of different concentrations of Calendula officinalis gel on histological and biomechanical changes of skin were studied. They reported that

animals treated with the 7% gel resulted in a better tissue alignment, collagen fibris differentiation and maturation compared to other concentration (181)

10. Dinda et. al., (2015) investigated the role of *Calendula officinalis* tincture (CDOT) on cell viability and wound closure. They reported C. officinalis tincture stimulated both proliferation and migration of fibroblasts in a statistically significant manner in a PI3K-dependent pathway. The increase in phosphorylation of FAK(Tyr 397) and Akt (Ser 473) was detected after treatment of CDOT. Inhibition of the PI3K pathway by wortmannin and LY294002 decreased both cell proliferation and cell migration. HPLC-ESIMS revealed the presence of flavonol glycosides as the major compounds of CDOT. Altogether, their results showed that CDOT potentiated wound healing by stimulating proliferation and migration of fibroblast in a PI3K-dependent pathway, and the identified compounds are likely to be responsible for wound healing activity (182).
11. Neda Babaee et. al., (2013) carried out randomized controlled clinical study to check the effect of *Calendula officinalis* flower extract mouthwash gel as oral gel on radiation induced oropharyngeal mucositis in patient with head-and-neck cancer. Calendula mouthwash significantly decreased the intensity of OM compared to placebo at week 2 (score: 5.5 vs. 6.8, $p = 0.019$), week 3 (score: 8.25 vs. 10.95, $p < 0.0001$) and week 6 (score: 11.4 vs. 13.35, $p = 0.031$). Total antioxidant, polyphenol and flavonoid contents and quercetin concentration of the 2% extract were 2353.4 ± 56.5 µM, 313.40 ± 6.52 mg/g, 76.66 ± 23.24 mg/g, and 19.41 ± 4.34 mg/l, respectively (183).
12. Mayur et. al., (2013) carried out clinical trial on 240 patients within the age group of 20 -40 years to evaluate the efficacy of *C. officinalis* in reducing dental plaque and ginigival inflammation(184). They found that test group showed a statistically significant reduction in the scores of plaque index, gingival index, sulcus bleeding index and oral hygiene index-simplified ($p < 0.05$). They concluded that calendula mouthwash is effective in reducing dental plaque and gingivitis adjunctive to scaling.

13. Pommier et al., (2014) performed phase III randomized trial of petroleum jelly of calendula extracts compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. Their result showed the occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% *v* 63%; *P* _ .001) with the use of calendula than with trolamine. Moreover, patients receiving calendula had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain (185).
14. Leelapompisid et. al., (2014) prepared cream containing nanostructured lipid carriers loaded marigold (*Tagetes erecta* linn) flowers ethyl acetate extract for anti-wrinkles application. All of ME-NLCs containing creams exhibited no skin irritation in healthy volunteers. They concluded that the developed nano-cosmeceutical creams of Marigold flower extract could be regarded as the effective anti-wrinkles formulation (186).
15. Lam et. al., (2014) prepared calendula officinalis oil and extract loaded gelatin/collagen reservoir and matrix nanoparticles respectively for treatments of cancer using D-glucose as a modifying agent. They reported that the C. officinalis powder loaded nanoparticles significantly strengthened the anti-cancer effect towards human breast adenocarcinoma MCF7 cells and human hepatoma SKHep1 cells when compared with the free powder (187).
16. Demir et. al., (2014) prepared nanoemulsion enriched with gold nanoparticles and marigold extract loaded phytosome. The AuNP–phytosomes exhibited significant antioxidant and wound healing activity (188).
17. A quasi-experimental, prospective, longitudinal study was conducted at the National Hospital of Nebaj, Guatemala, to determine the effectiveness of calendula tincture to 20% in the treatment of furunculosis and contact dermatitis and atopic (189). Their results showed that the 88.1% of patients in the study group had successful outcomes dermal lesions compared with 71.4% in the control group. They reported that the calendula tincture was effective in the treatment of furunculosis, contact and atopic dermatitis.
18. Aruna et al., (2012) studied the effects of calendula essential oil-based cream on biochemical parameters of skin of albino rats against ultraviolet B radiation (190).

They found that the treatment with creams containing 4% and 5% of calendula essential oil caused a significant decrease in the malonyldialdehyde level, whereas the levels of catalase, glutathione, superoxide dismutase, ascorbic acid, and the total protein level were significantly increased after 1 month of daily irradiation and treatment when compared to untreated control groups. Their results suggest that the cutaneous application of the essential oil of Calendula prevents UV-B-induced alterations in the level of antioxidants in skin tissue.

19. Mishra et. al., (2012) evaluated effect of 4-5% calendula oil based cream on biochemical parameters of skin albino rats against ultraviolet B radiation. Their results indicated that the treatment with creams containing 4% and 5% of Calendula essential oil caused a significant decrease in the malonyldialdehyde level, whereas the levels of catalase, glutathione, superoxide dismutase, ascorbic acid, and the total protein level were significantly increased after 1 month of daily irradiation and treatment when compared to untreated control groups. The results suggest that the cutaneous application of the essential oil of Calendula prevents UV-B-induced alterations in the level of antioxidants in skin tissue.(190)
20. Bernatoniene et. al., (2011) prepared hydrophilic cream containing *Calendula officinalis* L. extract and checked antioxidant activity (148). They determined concentration of extract which provided significant antioxidant effect ($p < 0.05$). They mentioned that cream with the best properties (0.9% of Calendula extract) contained 0.73 ± 0.04 mg/100 g of total carotenoids expressed as carotene (148).
21. Parente et. al., (2011) studied angiogenic activity of calendula officinalis flowers ethanolic, hexanic and dichloromethane extract in 90 embryonated eggs and 30 rats (130). The angiogenic activity of the extract and the fractions was evidenced in both experimental models.
22. Patrick *et al.* (1996) carried out a study with European *C officinalis* flowers by the use of the chorioallantoic membrane (CAM) of chicken fertilized egg. They demonstrated the inductive role of the plant on the microvascularization(191).
23. M. Fronza et. al, (2009) determined the wound healing effect of calendula extracts (ethanolic and hexane extract) using the scratch assay with Swiss 3T3 mouse

fibroblasts. They concluded that both extracts of Calendula officinalis stimulated proliferation and migration of fibroblasts (129).

24. Preethi et. al., (2009) studied anti-inflammatory activity and possible mechanism of action of calendula flower extract against carrageenan and dextran induced acute paw edema (142). They found that the oral administration of 250 and 500 mg/kg body weight Calendula extract produced significant inhibition (50.6 and 65.9% respectively) in paw edema of animals induced by carrageenan and 41.9 and 42.4% respectively with inflammation produced by dextran. Their results showed that the potent anti inflammatory response of *Calendula* officinalis extract was mediated by the inhibition of proinflammatory cytokines and Cox-2 and subsequent prostaglandin synthesis.

25. There was a case report by Roveroni-Favaretto et. al., (2009) on treatment of exfoliate cheilitis by topical application of 10% *calendula officinalis ointment*. The results showed that *Calendula officinalis* L. is a potential therapy in cases of cheilitis exfoliative (133).

26. Guler et. al., prepared bioactive nanoemulsion enriched with gold nanoparticles, Nigella sativa oil enriched with marigold extract and lipoic acid to study their potential applications including wound healing and antioxidant activity via in vitro cell culture studies. All data confirmed that the enriched N. sativa formulations exhibited better antioxidant and wound healing activity than *N.sativa* emulsion without any enrichment (192).
27. Hadfied et. al., (2008) used marigold therapy for podiatric skin conditions. Results showed that the active group had a significant difference in appearance, pain, and size compared with the placebo group. Studies support the combined use of marigold therapy with a protective aperture pad for patients with painful medial eminence bursitis (151).
28. Manendez et al.,(2007) studied healing effect, dermal irritation effect and ophthalmic irritant effect of 1% cream of calendula officinalis on the scaring of wounds in rats and rabbits(193). In their study, the histopathology and clinical analysis showed that

the cream favors the process of the scaring in skin of rats. Their results demonstrate the possible use of the cream as phytoterapeutic agent.

29. A prospective, descriptive pilot study was conducted in a Brazilian hospital to evaluate the clinical benefits of using *Calendula officinalis* hydroglycolic extract in the treatment of DFUs. The proportions of patients who achieved complete wound closure after 11, 20, and 30 weeks of treatment was 54%, 68%, and 78%, respectively; mean healing time was 15.5} 6.7 weeks. No adverse events were observed during treatment. The study findings suggest *C. officinalis* extract is safe and has a beneficial effect on DFU healing (194).
30. Duran et. al., (2005) examined marigold ointment in the treatment of venous leg ulcers. There was a statistically significant acceleration of wound healing in the experimental group ($p < 0.05$), the suggesting positive effects of the ointment with marigold extract on venous ulcer epithelialization (121).

2.7 REFERENCES

1. Yousef H, Alhajj M, Sharma S. Anatomy, Skin (Integument), Epidermis: StatPearls Publishing, Treasure Island (FL); 2019.

2. Leaper DJ, Harding KG. Wounds: biology and management: Oxford Medical Publications; 1998.

3. Hutchinson J. The wound programme. Centre for Medical Education: Dundee. 1992.

4. Cooper P. A review of different wound types and their principles of management in Wound Healing: A systematic approach to advanced wound healing and management. Cromwell Press, UK; 2005.

5. Tallman P, Muscare E, Carson P, Eaglstein WH, Falanga V. Initial rate of healing predicts complete healing of venous ulcers. Archives of dermatology. 1997;133(10):1231-4.

6. Rijswijk V. Full-thickness leg ulcers: patient demographics and predictors of healing. Multicenter leg ulcer study group. J Fam Pract. 1993;36:625-32.

7. McGuckin M, Goldman R, Bolton L, Salcido R. The clinical relevance of microbiology in acute and chronic wounds. Advances in skin & wound care. 2003;16(1):12-23.

8. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. Skin Wound Healing: An Update on the Current Knowledge and Concepts. European Surgical Research. 2017;58(1-2):81-94.

9. George Broughton I, Janis JE, Attinger CE. A brief history of wound care. Plastic and reconstructive surgery. 2006;117(7S):6S-11S.

10. Schreml S, Szeimies R-M, Prantl L, Landthaler M, Babilas P. Wound healing in the 21st century. Journal of the American Academy of Dermatology. 2010 2010/11/01/;63(5):866-81.

11. Schultz GS, Chin GA, Moldawer L, Diegelmann RF. Principles of Wound Healing. Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists [Internet]: University of Adelaide Press; 2011.

12. Kane D. Chronic wound healing and chronic wound management. Chronic wound care: a clinical source book for healthcare professionals 4th ed Malvern, PA: HMP Communications. 2007:11-24.

13. Junker JP, Caterson E, Eriksson E. The microenvironment of wound healing. Journal of Craniofacial Surgery. 2013;24(1):12-6.

14. Kruse CR, Nuutila K, Lee CC, Kiwanuka E, Singh M, Caterson EJ, et al. The external microenvironment of healing skin wounds. Wound repair and regeneration. 2015;23(4):456-64.

15. Leaper DJ, Schultz G, Carville K, Fletcher J, Swanson T, Drake R. Extending the TIME concept: what have we learned in the past 10 years? International wound journal. 2012;9:1-19.

16. Field CK, Kerstein MD. Overview of wound healing in a moist environment. The American Journal of Surgery. 1994 1994/01/01/;167(1, Supplement):S2-S6.

17. Childs DR, Murthy AS. Overview of Wound Healing and Management. Surgical Clinics of North America. 2017 2017/02/01/;97(1):189-207.

18. Quain AM, Khardori NM. Nutrition in Wound Care Management: A Comprehensive Overview. Wounds: a compendium of clinical research and practice. 2015;27(12):327-35.

19. Percival SL, McCarty SM, Lipsky B. Biofilms and wounds: an overview of the evidence. Advances in wound care. 2015;4(7):373-81.

20. Boateng J, Catanzano O. Advanced therapeutic dressings for effective wound healing—a review. J Pharm Sci. 2015;104(11):3653-80.

21. O'Callaghan S, Galvin P, O'Mahony C, Moore Z, Derwin R. 'Smart'wound dressings for advanced wound care: a review. J Wound Care. 2020;29(7):394-406.

22. Lindholm C, Searle R. Wound management for the 21st century: combining effectiveness and efficiency. International wound journal. 2016;13:5-15.

23. Abdelrahman T, Newton H. Wound dressings: principles and practice. Surgery (Oxford). 2011 2011/10/01/;29(10):491-5.

24. Dhivya S, Padma VV, Santhini E. Wound dressings–a review. BioMedicine. 2015;5(4).

25. Shi C, Wang C, Liu H, Li Q, Li R, Zhang Y, et al. Selection of Appropriate Wound Dressing for Various Wounds. Frontiers in bioengineering and biotechnology. 2020;8:182-.

26. Mir M, Ali MN, Barakullah A, Gulzar A, Arshad M, Fatima S, et al. Synthetic polymeric biomaterials for wound healing: a review. Progress in biomaterials. 2018;7(1):1-21.

27. Loty S, Sautier JM, Loty C, Boulekbache H, Kokubo T, Forest N. Cartilage formation by fetal rat chondrocytes cultured in alginate beads: A proposed model for investigating tissue–biomaterial interactions. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and the Australian Society for Biomaterials. 1998;42(2):213-22.

28. Martin L, Wilson CG, Koosha F, Tetley L, Gray AI, Senel S, et al. The release of model macromolecules may be controlled by the hydrophobicity of palmitoyl glycol chitosan hydrogels. Journal of controlled release. 2002;80(1-3):87-100.

29. Ramos-e-Silva M, de Castro MCR. New dressings, including tissue-engineered living skin. Clinics in dermatology. 2002;20(6):715-23.

30. Yerushalmi N, Arad A, Margalit R. Molecular and cellular studies of hyaluronic acid-modified liposomes as bioadhesive carriers for topical drug delivery in wound healing. Archives of biochemistry and biophysics. 1994;313(2):267-73.

31. Patrulea V, Ostafe V, Borchard G, Jordan O. Chitosan as a starting material for wound healing applications. European Journal of Pharmaceutics and Biopharmaceutics. 2015 2015/11/01/;97:417-26.

32. Brett D. A review of collagen and collagen-based wound dressings. Wounds. 2008;20(12):347-56.

33. Bello YM, Falabella AF, Eaglstein WH. Tissue-engineered skin. American journal of clinical dermatology. 2001;2(5):305-13.

34. Vig K, Chaudhari A, Tripathi S, Dixit S, Sahu R, Pillai S, et al. Advances in skin regeneration using tissue engineering. International journal of molecular sciences. 2017;18(4):789.

35. Vowden K, Vowden P. Wound dressings: principles and practice. Surgery (Oxford). 2017 2017/09/01/;35(9):489-94.

36. Greenhalgh DG. Management of burns. New England Journal of Medicine. 2019;380(24):2349-59.

37. Liu H-F, Zhang F, Lineaweaver WC. History and advancement of burn treatments. Annals of plastic surgery. 2017;78(2):S2-S8.

38. Tickle J. Effective management of exudate with AQUACEL extra. British journal of community nursing. 2012.

39. Visscher MO, Bailey JK, Hom DB. Scar treatment variations by skin type. Facial Plastic Surgery Clinics. 2014;22(3):453-62.

40. Moulik PK, Mtonga R, Gill GV. Amputation and mortality in new-onset diabetic foot ulcers stratified by etiology. Diabetes care. 2003;26(2):491-4.

41. Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, et al. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. Systematic reviews. 2017;6(1):15.

42. Sen CK. Human wounds and its burden: an updated compendium of estimates. Mary Ann Liebert, Inc., publishers 140 Huguenot Street, 3rd Floor New …; 2019.

43. Armstrong DG, Boulton AJ, Bus SA. Diabetic foot ulcers and their recurrence. New England Journal of Medicine. 2017;376(24):2367-75.

44. Wild SH, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030: response to Rathman and Giani. Diabetes care. 2004;27(10):2569-.

45. Mortal G. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117-71.

46. Norton R, Kobusingye O. Injuries. New England Journal of Medicine. 2013;368(18):1723-30.

47. Association AB. Burn incidence fact sheet. Chicago, IL: The ABA Available online at: http://ameriburn org/who-we-are/media/burn-incidence-fact-sheet. 2016.

48. Porter C, Tompkins RG, Finnerty CC, Sidossis LS, Suman OE, Herndon DN. The metabolic stress response to burn trauma: current understanding and therapies. The Lancet. 2016;388(10052):1417-26.

49. Peck MD, Toppi JT. Epidemiology and Prevention of Burns Throughout the World. Handbook of Burns Volume 1: Springer; 2020. p. 17-57.

50. Jeschke MG, Peck MD. Burn care of the elderly. Journal of burn care & research. 2017;38(3):e625-e8.

51. Fuzaylov G, Anderson R, Knittel J, Driscoll DN. Global health: burn outreach program. Journal of burn care & research. 2015;36(2):306-9.

52. Singh AK. Burns management in India: The way ahead. Indian Journal of Burns. 2018;26(1):3.

53. Moura LI, Dias AM, Carvalho E, de Sousa HC. Recent advances on the development of wound dressings for diabetic foot ulcer treatment--a review. Acta Biomater. 2013 Jul;9(7):7093-114.

54. Winter GD. Formation of the Scab and the Rate of Epithelization of Superficial Wounds in the Skin of the Young Domestic Pig. Nature. 1962 1962/01/01;193(4812):293-4.

55. Wu D-Q, Zhu J, Han H, Zhang J-Z, Wu F-F, Qin X-H, et al. Synthesis and characterization of arginine-NIPAAm hybrid hydrogel as wound dressing: In vitro and in vivo study. Acta Biomaterialia. 2018 2018/01/01/;65:305-16.

56. Miguel SP, Ribeiro MP, Brancal H, Coutinho P, Correia IJ. Thermoresponsive chitosan-agarose hydrogel for skin regeneration. Carbohydr Polym. 2014 Oct 13;111:366-73.

57. Gonzalez JS, Ludueña LN, Ponce A, Alvarez VA. Poly(vinyl alcohol)/cellulose nanowhiskers nanocomposite hydrogels for potential wound dressings. Mater Sci Eng C Mater Biol Appl. 2014 Jan 1;34:54-61.

58. Madaghiele M, Demitri C, Sannino A, Ambrosio L. Polymeric hydrogels for burn wound care: Advanced skin wound dressings and regenerative templates. Burns Trauma. 2014;2(4):153-61.

59. Aguiar P, Amaral C, Rodrigues A, de Souza AH. A diabetic foot ulcer treated with hydrogel and hyperbaric oxygen therapy: a case study. J Wound Care. 2017 Nov 2;26(11):692-5.

60. Fleck A, Cabral PF, Vieira FF, Pinheiro DA, Pereira CR, Santos WC, et al. Punica granatum L. Hydrogel for Wound Care Treatment: From Case Study to Phytomedicine Standardization. Molecules. 2016 Aug 22;21(8).

61. Ahmed EM. Hydrogel: Preparation, characterization, and applications: A review. Journal of Advanced Research. 2015 2015/03/01/;6(2):105-21.

62. Hennink WE, van Nostrum CF. Novel crosslinking methods to design hydrogels. Advanced Drug Delivery Reviews. 2002 2002/01/17/;54(1):13-36.

63. Rezvanian M, Ahmad N, Mohd Amin MC, Ng SF. Optimization, characterization, and in vitro assessment of alginate-pectin ionic cross-linked hydrogel film for wound dressing applications. Int J Biol Macromol. 2017 Apr;97:131-40.

64. Pereira R, Carvalho A, Vaz DC, Gil MH, Mendes A, Bártolo P. Development of novel alginate based hydrogel films for wound healing applications. International Journal of Biological Macromolecules. 2013 2013/01/01/;52:221-30.

65. Kaditi E, Mountrichas G, Pispas S. Amphiphilic block copolymers by a combination of anionic polymerization and selective post-polymerization functionalization. European Polymer Journal. 2011 2011/04/01/;47(4):415-34.

66. Liu L, Gao Q, Lu X, Zhou H. In situ forming hydrogels based on chitosan for drug delivery and tissue regeneration. Asian Journal of Pharmaceutical Sciences. 2016 2016/12/01/;11(6):673-83.

67. Gulrez SK, Al-Assaf S, Phillips GO. Hydrogels: methods of preparation, characterisation and applications. Progress in molecular and environmental bioengineering-from analysis and modeling to technology applications. 2011:117-50.

68. Wathoni N, Motoyama K, Higashi T, Okajima M, Kaneko T, Arima H. Physically crosslinked-sacran hydrogel films for wound dressing application. Int J Biol Macromol. 2016 Aug;89:465-70.

69. Ullah F, Othman MB, Javed F, Ahmad Z, Md Akil H. Classification, processing and application of hydrogels: A review. Mater Sci Eng C Mater Biol Appl. 2015 Dec 1;57:414-33.

70. Mukherjee D, Azamthulla M, Santhosh S, Dath G, Ghosh A, Natholia R, et al. Development and characterization of chitosan-based hydrogels as wound dressing

materials. Journal of Drug Delivery Science and Technology. 2018 2018/08/01/;46:498-510.

71. Holloway J, Lowman A, Palmese G. The role of crystallization and phase separation in the formation of physically cross-linked PVA hydrogels. Soft Matter. 2013;9:826-33.

72. Cereceres S, Touchet T, Browning MB, Smith C, Rivera J, Höök M, et al. Chronic Wound Dressings Based on Collagen-Mimetic Proteins. Advances in wound care. 2015;4(8):444-56.

73. Hua Y, Gan Y, Li P, Song L, Shi C, Bao C, et al. Moldable and Removable Wound Dressing Based on Dynamic Covalent Cross-Linking of Thiol-Aldehyde Addition. ACS Biomaterials Science & Engineering. 2019;5(8):4048-53.

74. Maitra J, Shukla V. Cross-linking in hydrogels - a review. Am J Polym Sci. 2014 01/01;4:25-31.

75. Witthayaprapakorn C. Design and Preparation of Synthetic Hydrogels Via Photopolymerisation for Biomedical Use as Wound Dressings. Procedia Engineering. 2011 12/31;8:286-91.

76. Chen H, Xing X, Tan H, Jia Y, Zhou T, Chen Y, et al. Covalently antibacterial alginate-chitosan hydrogel dressing integrated gelatin microspheres containing tetracycline hydrochloride for wound healing. Mater Sci Eng C Mater Biol Appl. 2017 Jan 1;70(Pt 1):287-95.

77. Gupta B, Tummalapalli M, Deopura BL, Alam MS. Preparation and characterization of in-situ crosslinked pectin-gelatin hydrogels. Carbohydr Polym. 2014 Jun 15;106:312-8.

78. Boonkaew B, Barber PM, Rengpipat S, Supaphol P, Kempf M, He J, et al. Development and characterization of a novel, antimicrobial, sterile hydrogel dressing for burn wounds: single-step production with gamma irradiation creates silver nanoparticles and radical polymerization. J Pharm Sci. 2014 Oct;103(10):3244-53.

79. Juby KA, Dwivedi C, Kumar M, Kota S, Misra HS, Bajaj PN. Silver nanoparticle-loaded PVA/gum acacia hydrogel: synthesis, characterization and antibacterial study. Carbohydr Polym. 2012 Jul 1;89(3):906-13.

80. Zhou Y, Zhao Y, Wang L, Xu L, Zhai M, Wei S. Radiation synthesis and characterization of nanosilver/gelatin/carboxymethyl chitosan hydrogel. Radiation Physics and Chemistry. 2012;81(5):553-60.

81. Knapp TR, Kaplan EN, Daniels JR. Injectable collagen for soft tissue augmentation. Plastic and reconstructive surgery. 1977;60(3):398-405.

82. Stegman SJ, Tromovitch TA. Implantation of collagen for depressed scars. The Journal of Dermatologic Surgery and Oncology. 1980;6(6):450-3.

83. Samaha BJM. Mary's Gold. Calendula: An Herb Society of America Guide Kirtland, OH: Herb Society of America. 2007.

84. Tucker AO, DeBaggio T. Big book of herbs: Interweave Press; 2000.

85. Wichtl M. Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis: Medpharm GmbH Scientific Publishers; 2004.

86. Monographs E. European Scientific Cooperative On Phytotherapy. United Kingdom: ESCOP. 2003.

87. Foster S, Johnson R. Publishers Desk Reference to Nature's Medicine. National Geographic Books; 2006.

88. Grieve M. Maude. A Modern Herbal. Vol. 2. 1931. Reprint. New York: Dover; 1971.

89. Blumenthal M, Goldberg A, Brinckmann J. Herbal medicine. Expanded commission E monographs: Integrative Medicine Communications; 2000.

90. Yoshikawa M, Murakami T, Kishi A, Kageura T, Matsuda H. Medicinal flowers. III. Marigold.(1): hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D, from Egyptian Calendula officinalis. Chemical and Pharmaceutical Bulletin. 2001;49(7):863-70.

91. Ukiya M, Akihisa T, Yasukawa K, Tokuda H, Suzuki T, Kimura Y. Anti-inflammatory, anti-tumor-promoting, and cytotoxic activities of constituents of marigold (Calendula officinalis) flowers. Journal of natural products. 2006;69(12):1692-6.

92. Wilkomirski B, Kasprzyk Z. Free and ester-bound triterpene alcohols and sterols in cellular subfractions of Calendula officinalis flowers. Phytochemistry. 1979;18(2):253-5.

93. Śliwowski J, Dziewanowska K, Kasprzyk Z. Ursadiol: a new triterpene diol from Calendula officinalis flowers. Phytochemistry. 1973;12(1):157-60.

94. Bakó E, Deli J, Tóth G. HPLC study on the carotenoid composition of Calendula products. Journal of biochemical and biophysical methods. 2002;53(1-3):241-50.

95. Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicine. 2nd. Montale, NJ, USA: Medical Economics Company. 2000.

96. Okoh O, Sadimenko A, Afolayan A. The effects of age on the yield and composition of the essential oils of Calendula officinalis. JApSc. 2007;7(23):3806-10.

97. Janiszowska W, Michalski W, Kasprzyk Z. Polyprenyl quinones and α-tocopherol in Calendula officinalis. Phytochemistry. 1976;15(1):125-7.

98. Ul'chenko N, Glushenkova A, Mukhamedova KS. Lipids ofCalendula officinalis. Chemistry of natural compounds. 1998;34(3):272-4.

99. Muley B, Khadabadi S, Banarase N. Phytochemical constituents and pharmacological activities of Calendula officinalis Linn (Asteraceae): a review. Tropical Journal of Pharmaceutical Research. 2009;8(5).

100. Marukami T, Kishi A, Yoshikawa M. Medicinal flowers. IV. Marigold.(2): Structures of new ionone and sesquiterpene glycosides from Egyptian Calendula officinalis. Chemical and pharmaceutical bulletin. 2001;49(8):974-8.

101. Dumenil G, Chemli R, Balansard C, Guiraud H, Lallemand M, editors. Evaluation of antibacterial properties of marigold flowers (Calendula officinalis L.) and mother homeopathic tinctures of C. officinalis L. and C. arvensis L.(author's transl). Annales pharmaceutiques francaises; 1980.

102. Modesto A, Lima K, De Uzeda M. Effects of three different infant dentifrices on biofilms and oral microorganisms. The Journal of clinical pediatric dentistry. 2000;24(3):237.

103. Della Loggia R, Becker H, Issac O, Tubaro A. Topical anti-inflammatory activity of Calendula officinalis extracts. Planta Médica. 1990;56(06):658-.

104. Della Loggia R, Tubaro A, Sosa S, Becker H, Isaac O. The role of triterpenoids in the topical anti-inflammatory activity of Calendula officinalis flowers. Planta medica. 1994;60(06):516-20.

105. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della Loggia R, Tubaro A, et al. Anti-oedematous activities of the main triterpendiol esters of marigold (Calendula officinalis L.). J Ethnopharmacol. 1997 Jul;57(2):139-44.

106. Neukirch H, D'Ambrosio M, Sosa S, Altinier G, Della Loggia R, Guerriero A. Improved Anti-Inflammatory Activity of Three New Terpenoids Derived, by Systematic Chemical Modifications, from the Abundant Triterpenes of the Flowery Plant Calendula officinalis. Chemistry & biodiversity. 2005;2(5):657-71.

107. Bezákova L, Masterová I, Paulíková I, Psenák M. Inhibitory activity of isorhamnetin glycosides from Calendula officinalis L. on the activity of lipoxygenase. Pharmazie. 1996 Feb;51(2):126-7.

108. Herold A, Cremer L, Calugăru A, Tamaş V, Ionescu F, Manea S, et al. Antioxidant properties of some hydroalcoholic plant extracts with antiinflammatory activity. Roumanian archives of microbiology and immunology. 2003;62(3-4):217-27.

109. Karadas F, Grammenidis E, Surai P, Acamovic T, Sparks N. Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. British poultry science. 2006;47(5):561-6.

110. Kishimoto S, Maoka T, Sumitomo K, Ohmiya A. Analysis of carotenoid composition in petals of calendula (Calendula officinalis L.). Bioscience, biotechnology, and biochemistry. 2005;69(11):2122-8.

111. Gracza L. Oxygen-containing terpene derivatives from Calendula officinalis. Planta medica. 1987;53(02):227-.

112. Chew BP, Wong MW, Wong TS. Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice. Anticancer Res. 1996 Nov-Dec;16(6B):3689-94.

113. Elias R, Meo MD, Vidal-Ollivier E, Laget M, Balansard G, Dumenil G. Antimutagenic activity of some saponins isolated from Calendula officinalis L., C. arvensis L. and Hedera helix L. Mutagenesis. 1990;5(4):327-32.

114. Quetin-Leclercq J, Elias R, Balansard G, Bassleer R, Angenot L. Cytotoxic activity of some triterpenoid saponins. Planta Med. 1992 Jun;58(3):279-81.

115. Bashir S, Janbaz KH, Jabeen Q, Gilani AH. Studies on spasmogenic and spasmolytic activities of Calendula officinalis flowers. Phytotherapy Research. 2006;20(10):906-10.

116. De Tommasi N, Conti C, Stein ML, Pizza C. Structure and in vitro antiviral activity of triterpenoid saponins from Calendula arvensis. Planta Med. 1991 Jun;57(3):250-3.

117. Jiménez-Medina E, Garcia-Lora A, Paco L, Algarra I, Collado A, Garrido F. A new extract of the plant calendula officinalis produces a dual in vitroeffect: Cytotoxic anti-tumor activity and lymphocyte activation. BMC câncer. 2006;6(1):119.

118. Sterer N, Rubinstein Y. Effect of various natural medicinals on salivary protein putrefaction and malodor production. Quintessence international. 2006;37(8).

119. Barajas-Farias LM, Pérez-Carreón JI, Arce-Popoca E, Fattel-Fazenda S, Alemán-Lazarini L, Hernández-García S, et al. A dual and opposite effect of Calendula officinalis flower extract: chemoprotector and promoter in a rat hepatocarcinogenesis model. Planta Med. 2006 Feb;72(3):217-21.

120. Rusu M, Tamas M, Puica C, Roman I, Sabadas M. The hepatoprotective action of ten herbal extracts in CCl4 intoxicated liver. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2005;19(9):744-9.

121. Duran V, Matic M, Jovanovc M, Mimica N, Gajinov Z, Poljacki M, et al. Results of the clinical examination of an ointment with marigold (Calendula officinalis) extract in the treatment of venous leg ulcers. International journal of tissue reactions. 2005;27(3):101-6.

122. Rao S, Udupa A, Udupa S, Rao P, Rao G, Kulkarni D. Calendula and Hypericum: two homeopathic drugs promoting wound healing in rats. Fitoterapia. 1991;62(6):508-10.

123. Klouchek-Popova E, Popov A, Pavlova N, Krŭsteva S. Influence of the physiological regeneration and epithelialization using fractions isolated from Calendula officinalis. Acta Physiol Pharmacol Bulg. 1982;8(4):63-7.

124. Shipochliev T. Uterotonic action of extracts from a group of medicinal plants. Veterinarno-meditsinski nauki. 1981;18(4):94-8.

125. Bojadjiev C. ON THE SEDATIVE AND HYPOTENSIVE EFFECT OF PREPARATIONS FROM THE PLANT CALENDULA OFFICINALIS. Nauchni Tr Vissh Med Inst Sofiia. 1964;43:15-20.

126. Patrick K, Kumar S, Edwardson P, Hutchinson J. Induction of vascularisation by an aqueous extract of the flowers of Calendula officinalis L. the European marigold. Phytomedicine. 1996;3(1):11-8.

127. Chandran PK, Kuttan R. Effect of Calendula officinalis flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. Journal of clinical biochemistry and nutrition. 2008;43(2):58-64.

128. Preethi KC, Kuttan R. Wound healing activity of flower extract of Calendula officinalis. J Basic Clin Physiol Pharmacol. 2009;20(1):73-9.

129. Fronza M, Heinzmann B, Hamburger M, Laufer S, Merfort I. Determination of the wound healing effect of Calendula extracts using the scratch assay with 3T3 fibroblasts. J Ethnopharmacol. 2009 Dec 10;126(3):463-7.

130. Parente LML, Andrade MA, Brito LAB, Moura VMBDd, Miguel MP, Lino-Júnior RdS, et al. Angiogenic activity of Calendula officinalis flowers L. in rats. Acta Cirúrgica Brasileira. 2011;26:19-24.

131. Parente LML, Lino Júnior RdS, Tresvenzol LMF, Vinaud MC, de Paula JR, Paulo NM. Wound healing and anti-inflammatory effect in animal models of Calendula officinalis L. growing in Brazil. Evidence-based complementary and alternative medicine. 2012;2012.

132. Matysik G, Wojciak-Kosior M, Paduch R. The influence of Calendulae officinalis flos extracts on cell cultures, and the chromatographic analysis of extracts. Journal of pharmaceutical and biomedical analysis. 2005;38(2):285-92.

133. Roveroni-Favaretto LHD, Lodi KB, Almeida JD. Topical Calendula officinalis L. successfully treated exfoliative cheilitis: a case report. Cases Journal. 2009;2(1):1-3.

134. Marinchev V, Bychkova L, Balvanovich N, Giraev A. Use of calendula for therapy of chronic inflammatory diseases of eyelids and conjunctiva. Oftalmologicheskii zhurnal. 1971;26(3):196-8.

135. Mozherenkov V, Shubina L. Treatment of chronic conjunctivitis with Calendula. Meditsinskaia sestra. 1976;35(4):33.

136. Bezakova L, Masterova I, Paulikova I, Psenak M. Inhibitory activity of isorhamnetin glycosides from Calendula officinalis L. on the activity of lipoxygenase. Pharmazie. 1996;51(2):126-7.

137. Akihisa T, Yasukawa K, Oinuma H, Kasahara Y, Yamanouchi S, Takido M, et al. Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects. Phytochemistry. 1996;43(6):1255-60.

138. Sarrell EM, Mandelberg A, Cohen HA. Efficacy of naturopathic extracts in the management of ear pain associated with acute otitis media. Archives of pediatrics & adolescent medicine. 2001;155(7):796-9.

139. Sarrell EM, Cohen HA, Kahan E. Naturopathic treatment for ear pain in children. Pediatrics. 2003;111(5):e574-e9.

140. Saini P, Al-Shibani N, Sun J, Zhang W, Song F, Gregson KS, et al. Effects of Calendula officinalis on human gingival fibroblasts. Homeopathy. 2012;101(2):92-8.

141. Hamburger M, Adler S, Baumann D, Förg A, Weinreich B. Preparative purification of the major anti-inflammatory triterpenoid esters from Marigold (Calendula officinalis). Fitoterapia. 2003;74(4):328-38.

142. Preethi KC, Kuttan G, Kuttan R. Anti-inflammatory activity of flower extract of Calendula officinalis Linn. and its possible mechanism of action. Indian J Exp Biol. 2009 Feb;47(2):113-20.

143. Cordova CA, Siqueira IR, Netto CA, Yunes RA, Volpato AM, Filho VC, et al. Protective properties of butanolic extract of the Calendula officinalis L.(marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. Redox report. 2002;7(2):95-102.

144. Ćetković GS, Djilas SM, Čanadanović-Brunet JM, Tumbas VT. Antioxidant properties of marigold extracts. Food Research International. 2004;37(7):643-50.

145. Preethi K, Kuttan G, Kuttan R. Antioxidant Potential of an Extract of Calendula officinalis. Flowers in Vitro. and in Vivo. Pharmaceutical Biology. 2006;44(9):691-7.

146. Frankič T, Salobir K, Salobir J. The comparison of in vivo antigenotoxic and antioxidative capacity of two propylene glycol extracts of Calendula officinalis (marigold) and vitamin E in young growing pigs. Journal of Animal physiology and Animal nutrition. 2009;93(6):688-94.

147. Didier F, Catherine F, Odile T, Jean-Louis L. Caffeoyl derivatives: major antioxidant compounds of some wild herbs of the Asteraceae family. Food and Nutrition Sciences. 2011;2011.

148. Bernatoniene J, Masteikova R, Davalgiene J, Peciura R, Gauryliene R, Bernatoniene R, et al. Topical application of Calendula officinalis (L.): Formulation and evaluation of hydrophilic cream with antioxidant activity. Journal of Medicinal Plants Research. 2011;5(6):868-77.

149. Pommier P, Gomez F, Sunyach M, D'hombres A, Carrie C, Montbarbon X. Phase III randomized trial of Calendula officinalis compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. Journal of clinical oncology. 2004;22(8):1447-53.

150. Fuchs S, Schliemann-Willers S, Fischer T, Elsner P. Protective effects of different marigold (Calendula officinalis L.) and rosemary cream preparations against sodium-lauryl-sulfate-induced irritant contact dermatitis. Skin pharmacology and physiology. 2005;18(4):195-200.

151. Hadfield R, Vlahovic T, Khan MT. The Use of Marigold Therapy for Podiatric Skin Conditions. The Foot & Ankle Journal. 2008 07/01;1.

152. Khan T. The evidenced-based approach to treating podiatric conditions with Marigold therapy and homeopathy. European Journal of Integrative Medicine. 2008;1:21-2.

153. Re T, Mooney D, Antignac E, Dufour E, Bark I, Srinivasan V, et al. Application of the threshold of toxicological concern approach for the safety evaluation of calendula flower (Calendula officinalis) petals and extracts used in cosmetic and personal care products. Food and Chemical Toxicology. 2009;47(6):1246-54.

154. Chargari C, Fromantin I, Kirova Y. Importance of local skin treatments during radiotherapy for prevention and treatment of radio-induced epithelitis. Cancer radiotherapie: journal de la Societe francaise de radiotherapie oncologique. 2009;13(4):259.

155. Fonseca YM, Catini CD, Vicentini FT, Nomizo A, Gerlach RF, Fonseca MJV. Protective effect of Calendula officinalis extract against UVB-induced oxidative stress in

skin: evaluation of reduced glutathione levels and matrix metalloproteinase secretion. Journal of ethnopharmacology. 2010;127(3):596-601.

156. Fonseca YM, Catini CD, Vicentini FT, Cardoso JC, Junior RLCDA, Fonseca MJV. Efficacy of marigold extract-loaded formulations against UV-induced oxidative stress. Journal of pharmaceutical sciences. 2011;100(6):2182-93.

157. Akhtar N, Zaman SU, Khan BA, Amir MN, Ebrahimzadeh MA. Calendula extract: effects on mechanical parameters of human skin. Acta Pol Pharm. 2011;68(5):693-701.

158. Mishra A, Mishra A, Chattopadhyay P. Assessment of in vitro sun protection factor of Calendula officinalis L.(asteraceae) essential oil formulation. Journal of Young Pharmacists. 2012;4(1):17-21.

159. Mishra A, Mishra A, Verma A, Chattopadhyay P. Arun k. Mishra *, Amrita Mishra , Anurag Verma, Pronobesh Chattopadhyay. Effects of Calendula Essential Oil-Based Cream on Biochemical Parameters of Skin of Albino Rats against Ultraviolet B Radiation. Sci. Pharm. 2012; 80: 669–683. Scientia Pharmaceutica. 2012 09/25;80:669-83.

160. Andrade Md, Clapis MJ, Nascimento TGd, Gozzo TdO, Almeida AMd. Prevention of skin reactions due to teletherapy in women with breast cancer: a comprehensive review. Revista latino-americana de enfermagem. 2012;20(3):604-11.

161. Panahi Y, Sharif MR, Sharif A, Beiraghdar F, Zahiri Z, Amirchoopani G, et al. A randomized comparative trial on the therapeutic efficacy of topical aloe vera and Calendula officinalis on diaper dermatitis in children. The Scientific World Journal. 2012;2012.

162. Kasiram K, Sakharkar P, Patil A. Antifungal activity of Calendula officinalis. Indian Journal of Pharmaceutical Sciences. 2000;62(6):464.

163. Radioza S, Iurchak L. Antimicrobial activity of Calendula L. plants. Mikrobiolohichnyi zhurnal (Kiev, Ukraine: 1993). 2007;69(5):21-5.

164. Szakiel A, Ruszkowski D, Grudniak A, Kurek A, Wolska KI, Doligalska M, et al. Antibacterial and antiparasitic activity of oleanolic acid and its glycosides isolated from marigold (Calendula officinalis). Planta medica. 2008;74(14):1709.

165. Chakraborthy G. Antimicrobial activity of the leaf extracts of Calendula officinalis (Linn). J Herb Med Toxicol. 2008;2(2):65-6.

166. Cwikla C, Schmidt K, Matthias A, Bone K, Lehmann R, Tiralongo E. Investigations into the antibacterial activities of phytotherapeutics against Helicobacter pylori and Campylobacter jejuni. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2010;24(5):649-56.

167. Goyal M, Mathur R. Antimicrobial effects of Calendula officinalis against human pathogenic microorganisms. J Herbal Med Tox. 2011;5(1):97-101.

168. Faria RL, Cardoso LML, Akisue G, Pereira CA, Junqueira JC, Jorge AOC, et al. Antimicrobial activity of Calendula officinalis, Camellia sinensis and chlorhexidine against the adherence of microorganisms to sutures after extraction of unerupted third molars. Journal of Applied Oral Science. 2011;19(5):476-82.

169. Efstratiou E, Hussain AI, Nigam PS, Moore JE, Ayub MA, Rao JR. Antimicrobial activity of Calendula officinalis petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. Complementary Therapies in Clinical Practice. 2012;18(3):173-6.

170. Jankowska B, Poniedziałek M, Jędrszczyk E. Effect of intercropping white cabbage with French marigold (Tagetes patula nana L.) and pot marigold (Calendula officinalis L.) on the colonization of plants by pest insects. Folia Horticulturae. 2009;21(1):95-103.

171. Betancourth García C, Salazar González C, Rodriguez M. Soil coverage evaluation with calendula (Calendula officinalis L.), crotalaria (Crotalaria sp. L.) and oat (Avena sp. L.) in Meloidogyne spp. control in quito orange (Solanum quitoense Lam.). Revista De Ciencias Agrícolas. 2011;28(2):43-57.

172. Pérez M, Navas-Cortés JA, Pascual-Villalobos M, Castillo P. Nematicidal activity of essential oils and organic amendments from Asteraceae against root-knot nematodes. Plant Pathology. 2003;52(3):395-401.

173. Chanaj-Kaczmarek J, Paczkowska-Walendowska M, Osmałek T, Kaproń B, Plech T, Szymanowska-Powałowska D, et al. Hydrogel Delivery System Containing

Calendulae flos Lyophilized Extract with Chitosan as a Supporting Strategy for Wound Healing Applications. Pharmaceutics. 2020 07/07;12:634.

174. Pedram Rad Z, Mokhtari J, Abbasi M. Calendula officinalis extract/PCL/Zein/Gum arabic nanofibrous bio-composite scaffolds via suspension, two-nozzle and multilayer electrospinning for skin tissue engineering. International Journal of Biological Macromolecules. 2019 2019/08/15/;135:530-43.

175. Fontes P, Ribeiro S, Gaspar A. Bacterial cellulose/phytotherapic hydrogels as dressings for wound healing. Material Science & Engineering International Journal. 2020 01/22;3.

176. Nicolaus C, Junghanns S, Hartmann A, Murillo R, Ganzera M, Merfort I. In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts. Journal of Ethnopharmacology. 2017 2017/01/20/;196:94-103.

177. Buzzi M, Freitas Fd, Winter MdB. Pressure ulcer healing with Plenusdermax® Calendula officinalis L. extract. Revista Brasileira de Enfermagem. 2016;69(2):250-7.

178. Millan D, Jimenez RA, Nieto LE, Linero I, Laverde M, Fontanilla MR. Preclinical evaluation of collagen type I scaffolds, including gelatin-collagen microparticles and loaded with a hydroglycolic Calendula officinalis extract in a lagomorph model of full-thickness skin wound. Drug delivery and translational research. 2016 Feb;6(1):57-66.

179. Arana L, Salado C, Vega S, Aizpurua-Olaizola O, de la Arada I, Suarez T, et al. Solid lipid nanoparticles for delivery of Calendula officinalis extract. Colloids and surfaces B, Biointerfaces. 2015 Nov 1;135:18-26.

180. Okuma CH, Andrade TA, Caetano GF, Finci LI, Maciel NR, Topan JF, et al. Development of lamellar gel phase emulsion containing marigold oil (Calendula officinalis) as a potential modern wound dressing. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences. 2015 Apr 25;71:62-72.

181. Shafeie N, Naini AT, Jahromi HK. Comparison of different concentrations of Calendula officinalis gel on cutaneous wound healing. Biomedical and Pharmacology Journal. 2015;8(2):979-92.

182. Dinda M, Dasgupta U, Singh N, Bhattacharyya D, Karmakar P. PI3K-mediated proliferation of fibroblasts by Calendula officinalis tincture: implication in wound healing. Phytotherapy research : PTR. 2015 Apr;29(4):607-16.

183. Babaee N, Moslemi D, Khalilpour M, Vejdani F, Moghadamnia Y, Bijani A, et al. Antioxidant capacity of calendula officinalis flowers extract and prevention of radiation induced oropharyngeal mucositis in patients with head and neck cancers: a randomized controlled clinical study. Daru : journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2013;21(1):18.

184. Khairnar M, Pawar B, Marawar P, Mani A. Evaluation of Calendula officinalis as an anti-plaque and anti-gingivitis agent. Journal of Indian Society of Periodontology. [Original Article]. 2013 November 1, 2013;17(6):741-7.

185. Pommier P, Gomez F, Sunyach MP, D'Hombres A, Carrie C, Montbarbon X. Phase III randomized trial of Calendula officinalis compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2004 Apr 15;22(8):1447-53.

186. Leelapornpisid P, Chansakaow S, Na-Boonlong S, Jantrawut P. Development of cream containing nanostructured lipid carriers loaded marigold (Tagetes erecta Linn.) flowers extract for anti-wrinkles application. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(5):313-4.

187. Lam PL, Kok SH, Bian ZX, Lam KH, Tang JC, Lee KK, et al. D-Glucose as a modifying agent in gelatin/collagen matrix and reservoir nanoparticles for Calendula officinalis delivery. Colloids and surfaces B, Biointerfaces. 2014 May 1;117:277-83.

188. Demir B, Barlas FB, Guler E, Gumus PZ, Can M, Yavuz M, et al. Gold nanoparticle loaded phytosomal systems: synthesis, characterization and in vitro investigations. RSC Advances. [10.1039/C4RA05108D]. 2014;4(65):34687-95.

189. Pérez AH, Gutiérrez MC, de Corcho LBP. Effectiveness of calendula tincture to 20% in dermatological conditions. Acta Médica del Centro. 2014;8(3):28-36.

190. Mishra AK, Mishra A, Verma A, Chattopadhyay P. Effects of Calendula Essential Oil-Based Cream on Biochemical Parameters of Skin of Albino Rats against Ultraviolet

B Radiation. Scientia Pharmaceutica. 2012 Jul-Sep 04/1612/22/received 04/16/accepted;80(3):669-83.

191. Patrick KF, Kumar S, Edwardson PA, Hutchinson JJ. Induction of vascularisation by an aqueous extract of the flowers of Calendula officinalis L. the European marigold. Phytomedicine. 1996 May;3(1):11-8.

192. Guler E, Barlas FB, Yavuz M, Demir B, Gumus ZP, Baspinar Y, et al. Bio-active nanoemulsions enriched with gold nanoparticle, marigold extracts and lipoic acid: In vitro investigations. Colloids and surfaces B, Biointerfaces. 2014 Sep 1;121:299-306.

193. Menéndez A, Parra AL, Pavón VB, Domínguez CC, Martínez OV, Sardiñas IG, et al. Healing effects and irritant assays of a 1% Calendula officinalis cream. Latin American Journal of Pharmacy. 2007 11/01;26:811-7.

194. Buzzi M, de Freitas F, Winter M. A Prospective, Descriptive Study to Assess the Clinical Benefits of Using Calendula officinalis Hydroglycolic Extract for the Topical Treatment of Diabetic Foot Ulcers. Ostomy/wound management. 2016 Mar;62(3):8-24.