Medicinal plants and their products have been important resources for mankind health from long times to the present day. According to World Health organization (WHO), the most of the peoples are depends on traditional health care system for many health problems. Plants and their products are used in prevention and treatment of various chronic conditions which results in early well-being of patients. In many parts of the word, more peoples are depends on traditional medicines and long history in use of medicinal plants. Modern medicine also accepts the effectivity of traditional practice and ayurvedic preparations (Stevanovic, 2015).

With increasing interest in the field of traditional medicines, ayurvedic dosage forms and research in the field of ayurvedic formulation, it has become important to look into the area of systemic knowledge of ayurvedic drugs and use of this information in authentication, complete information and practical use of natural drugs. Modern chemical methods have led to a dramatic increase in the number of natural molecules available for pharmacological research. Most pharmacological research are mainly based on two approach, one is to demonstrate new pharmacological activities or even future clinical application from raw materials or natural substance already known and, second is the discovery of new natural substances displaying pharmacological or even new therapeutic effects (Trease and Evans, 2015). Research on natural products for wound healing is an important area in modern pharmaceutical sciences. Researcher, who are interested to develop newer formulation from natural products are looking towards the ayurveda, the Indian traditional system of medicine. Many drugs of natural origin (from plant, mineral and animal origin) as noted in the ayurveda for wound healing activity. Many of these products are obtained from plant sources.

Some of these products from plants have scientifically screened for their wound healing activity in various pharmacological models and also directly in the patient having various types of wounds including burn wounds, but the potential effects of many of these products from plant sources remain unexplored (Mukherjee, 2002).
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Number of medicinal plants have been examined for wound healing activity with the intention of screening agents with least toxicity and maximum efficacy. Many plants and plant products have shown promising results. From the available data and literature survey it was found that about one-third of all traditional medicines are used for the treatment of various types wounds or skin disorders including burns as compared modern medicines which are used up to 1-3% (Pirbalouti et al., 2010).

1. Skin

The human body has two systems which protect it from the outer environmental conditions including harmful organisms which are present the environment. The outer system prevents the entry of bacteria and microorganisms into the body. The internal immune system protects the body from microorganisms and bacteria that have already entered into the body by attacking and destroying it by various mechanisms. Skin is the biggest outer defense system. The skin completely covers the body and protects the underlying structures from injury and invasion by microbes. It contains sensory nerve endings which are responsible to enable discrimination of pain, temperature and touch. It also regulates body temperature 30 to 40°C depending on the environmental conditions (Tortora, 2011).

1.1. Structure of the skin

Skin is the largest organ in the body having surface area of about 1.5 to 2 square meter in adults. In certain areas, it contains accessory structures like glands, hairs and nails. It consists of three layers; Epidermis (outer layer which covers dermis), dermis (middle layer) and hypodermis (innermost layer) (Ross and Wilson, 2014; Raina et al., 2008).
1.1.1 Epidermis

It is the most superficial layer and is composed of **stratified keratinized squamous epithelium**. Its thickness is different at different part of the body. Blood vessels and nerve endings are absent in the epidermis, but its inner layers are embeded in interstitial fluid from the dermis, which provides oxygen and nutrients, and drains away as lymph. There are several layers (strata) of cells in the epidermis which extends from the deepest **germinative layer** to the most superficial **stratum corneum** (a thick horney layer). Epidermal cells originate in germinative layer and undergo gradual change as they progress towards the skin surface (Kanitakis, 2002).
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Types of cells that present in the epidermis are:

- **Keratinocytes**: main cell types in epidermis, covers about 95% of cells.
- **Melanocytes**: the pigment producer cells which are found in the lower layers of epidermis.
- **Langerhans cells**: important cells responsible for immunological response and also found in the mid dermis.
- **Merkel cells**: important as a one part of amine precursor and decarboxylation system these are present in the lower layer of epidermis. (Spellberg, 2000).

Epidermis consist of five different layers, namely from inside to outside;

- Stratum germinativum (lower layer)
- Stratum spinosum,
- Stratum granulosum
- Stratum lucidum
- Stratum corneum (outer layer) (Sherwood, 2007).

Figure 2: Epidermal and skin layer
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Stratum corneum has a structure like arrangement of bricks and mortar. Corneocytes (bricks) which are rich in keratin are settled in the matrix (mortar) which is rich in intracellular lipid. Stratum corneum arranged as a 15-20 layers which composed of 85% corneocytes and 15% intracellular lipids. It mainly contains 70% proteins, 15% lipids and 15% water (Maghraby, et al., 2008).

![Figure 3: Layers of epidermis](image)

Molecules are passes through skin by two different mechanisms. In first mechanism, which is called as trans-appendageal route, molecules permeate through sweat glands by crossing the hair follicles. But limited molecules permeate by this mechanism. In second mechanism, which is called as trans-epidermal route, molecules passed through stratum corneum which acts as a multilayered barrier. Second mechanism has two micro pathway intracellular and transcellular (Nino et al., 2010).
1.1.2 Dermis

The dermis is elastic and tough in nature. It composed of connective tissue and the matrix which contains collagen fibers interconnected with elastin fibers. Rupture of elastic fibers occurs when the skin is overstretched, resulting in permanent striae or stretch marks, that may be found in pregnancy and obesity. Collagen fibers absorb water and give proper tensile strength to the skin but its ability declines with age which results into wrinkles development.
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Dermis contains following structures:

- Blood vessels and lymph vessels
- Sensory nerve endings
- Sweat glands and its ducts
- Hairs, arrectorpili muscles and sebaceous glands.

Dermis performs an important role as connection between other skin layers. Growth of epidermis, hair follicles and skin glands are influenced by changes in metabolism in dermis.

Dermis contains following cells:

- **Fibroblast**: these are responsible for collagen production.
- **Macrophages**: these are act as a scavenger cells for capturing materials.
- **Mast cells**: these are involved in interaction with eosinophils and immunological reactions (Robson et al., 2001).

1.1.3 Hypodermis

It is the innermost layer of the skin and act as a connecting layer between skin and the underlying tissue in body such as muscles and bones (Ross and Wilson, 2014).
2. Wound

It is a break in the epithelial pattern of the skin and loss or breaking of cellular, anatomical or functional continuity of living tissue. Wounds may be arising from injury by various agents such as physical, chemical or microbial. (Raina et al., 2008). Wounds may also result from surgical incision or any type of trauma. In addition, to above conditions, skin ulcers (also known as bedsores), might also be considered as wounds. Wounds can be classified as chronic wounds and acute wounds on the basis of nature of wounds and repair mechanism.

- Acute wounds are any type of tissue injuries which heals themselves by taking its own time and systematic healing mechanism within 5 to 10 days or 30 days. The main causes of acute wounds are mechanical injuries such as friction and contact between skin and hard surfaces, chemical injuries like skin contact with hazardous and poisonous chemicals and burns which may be intentional or non-intentional (Robson et al., 2001).

- Chronic wounds are such types of wounds which fail to heal by normal healing process and therefore may be converted into the serious pathological inflammatory stage. It’s healing takes long time and also kept mark of serious scar. There may be several reasons for long healing time of chronic wound as compare to acute wound; some are patient having any type of infections, diabetic patient and incorrect starting treatment of wound (Proksch et al., 2008; Menke et al., 2007).

Types of chronic wounds
- Venous ulcers
- Arterial ulcers
- Diabetic foot ulcers
- Pressure ulcer
- Vasculitis
Acute and chronic wounds can be categorized as a complex wound if it has following characteristics:

- More loss of the integuments which consist skin, hair and associated glands.
- Tissue loss due to any type of infection.
- Tissue death due to any reason or signs of blood circulations.
- Presence of any pathological conditions (Faller, 2004).

**Figure 5: Difference between acute and chronic wound**
2.1. Open wound

In this type of wound, blood come outside the body and bleeding is clearly observable. It is further categorized into different types on the basis of object that created the wound.

- **Incisions or incised wounds**: It is caused due to intentional or non-intentional cut by clean, sharp-edged object such as a knife, razor or glass splinter.

- **Irregular tear-like wounds**: It is created due to some blunt trauma. It may be appear as regular incisions (linear)) or irregular incision (stellate).

- **Abrasions or superficial wounds**: in this type of wound, epidermis the uppermost layer of the skin is scraped off. Abrasions may be caused by a sliding or fall onto a rough surface by accident.

- **Avulsions**: In this type of injuries any body structure or parts forcefully detached from its normal point of insertion may be due to serious accident.

- **Puncture wounds**: this type of wounds caused due to the puncturing of skin by a pointed object such as a splinter, nail or needle.

- **Penetration wounds**: this type of wound caused by entering and coming of object from the skin such as knife.

- **Gunshot wounds**: this type of wounds caused by a bullet or similar projectile driving devices into or through the body. There may be two wounds, one at the entry site and another at the exit site. (Boateng et al., 2008).
2.2 Closed wound

In this type of wound blood partially or completely escapes the circulatory system but remains in the body. It is as dangerous as open wound and has following some categories.

- **Hematomas**: it is also called as a blood tumor which may be caused by damage to a blood vessel which results into collection of blood under the skin. On the basis of origin they are further categorized as hematomas that originate from internal blood vessel pathological changes and hematomas that originate from an external source as a trauma.

- **Crush injury**: this type of injury caused by a more or extreme amount of force applied for a long period of time (Fonder et al., 2008; Wounds, 2006).
3. Wound Healing

It is a process of complete healing of wound, generally seen after simple injury that has come back to its normal anatomical, structural and functional appearance within a given period of time. It may also show complete skin closure without fluid drainage or dressing requirements. Trauma or wound caused by any agents (mechanical, surgical, biological, or chemical) develops a wound and activates a complex mechanism of body which initiates and completes the healing process. Although normal healing process may be differ from tissue to tissue, but the overall process has a similar protective role throughout the body (Strecker, et al., 2007).

Wound healing still a challenging medical problem throughout the world to solve this problem correct and efficient wound healing is essential. Wound healing comprises of systematic approach that re-maintain the integrity of the damaged tissue. The process starts with series of programmed, separate and interdependent responses to the injury which includes inflammation (swelling of tissue), epithelization (growth of new epithelial cells), angiogenesis (regeneration of blood vessels) and the accumulation of matrix. All these steps are takes place in controlled manner which requires varies cytokines including growth factors. Many wounds are simple and heal by body itself through immune system. However some wounds are difficult to heal by body itself either because of the severity of wounds and weak health of the individual (Richardson, 2004).

3.1 Phases of Wound healing

The process of wound healing, which is initiated by tissue injury, may be comprises of four sequential phases which are time dependant.

i) Coagulation and haemostasis, which starts immediately after injury.
ii) Inflammation, which starts after coagulation and haemostasis.
iii) Proliferation, which starts after a day of the injury and involve the major healing process
iv) Wound remodeling, in this phase scar tissue formation takes place which may last for long time (a year or more than that) (Velnar et al., 2009; Attinger et al., 2006; Broughton et al. 2006).

![Figure 6: Phases of wound healing](image)

Figure 6: Phases of wound healing
i. **Coagulation and Haemostasis:**

It starts immediately after injury to prevent extra loss of blood. It also protects the blood vessels by keeping it untouched, so that the function of the important organs remains unchanged and harmless irrespective of wound. Secondly it also provides a matrix for settling the cells that are required for further phases of healing. With haemostatic process, the coagulation phase is activated through extrinsic and intrinsic mechanism, results into platelet aggregation and clot formation to reduce blood loss (Harsh Mohan, 2015). At the site of wound, the blood corpuscles and platelets come in contact with intact collagen and other extracellular matrix which initiates the release of clotting factors from the platelets which result into blood clot formation. The cytoplasm of platelets contains α-granules which are rich in growth factors and cytokines, these molecules activate and attract neutrophils, macrophages, endothelial cells and fibroblast and act as a promoters in the wound healing. After injury to cell or cell membrane, Prostaglandins and other related products of arachidonic acid metabolism are released which have potent biological effects in immediate inflammatory phase (Toy, 2005).

ii. **Inflammatory Phase:**

It includes humoral and cellular inflammatory events which act as an immune barrier against invading microorganism. In this phase two processes occurs simultaneously firstly, blood clotting takes place due to constriction of blood vessels and aggregation of platelet and secondly, inflammation at wound site by dilation of blood vessels and phagocytosis. (Li et al., 2007). This phase further divided into two separate sub phases, namely an early inflammatory phase and a late inflammatory phase.

- **Early inflammatory phase:**

It starts immediately after coagulation; this phase prevents infection at the wound site by infiltrating neutrophils. This activity of neutrophils gradually changes during wounding days after removing contaminating bacteria. It must be eliminated from the...
wound site after completing their task. Other remaining cells and bodies are removed by macrophages by phagocytosis (Hart, 2002; Diegelmann et al., 2004).

- **Late inflammatory phase:**

  It starts 48-74 hour after making wound, phagocytosis continues as macrophages are present at wound site. These cells are basically monocytes that convert into macrophages by reaching to wound site. As compare to neutrophils macrophages have a longer lifespan which continue to work at lower pH. Lymphocytes are the last cells that enter the wound site in this phase they are attracted 72 hour after injury. (Ramasasty, 2005; Hunt et al., 2000).

iii. **Proliferative phase:**

  This phase includes the generation and repair of skeletal muscles after injury. It is starts by the 3rd day after wounding takes place and continues upto 2 weeks. In this phase migration of fibroblast takes place and simultaneously newly synthesized extracellular matrix which contain fibrin and fibronectin will gel deposited. Proliferative phase comprises of following different processes (Guo et al., 2010).

- **Fibroblast migration**

  After wounds, surrounding tissue became rich in proliferated myofibroblats and fibroblasts with in 3 days. They are attracted by various factors such as TGF-β and PDGF which are released by inflammatory cells and platelet and migrate into the wound (Goldman, 2004). At the end of this repair process many extracellular components accumulates which supports cell migration (Baum et al., 2005).
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- **Collagen synthesis**

  Collagen which is synthesized by fibroblast gives strength to all present tissue and it is essential component in proliferative as well as remodeling phase of repair. It is also essentially required for all phases of wound healing. Collagen unevenly distributed in the tissue. Type I and type II collagen present in unwounded dermis 80% and 25% respectively whereas 40 % of type III collagen is present in wound granulation tissue. (Toshikazu et al., 2010).

- **Angiogenesis and granulation tissue formation**

  Angiogenesis is a synthesis of new blood vessels which is an important and essential process in wound healing. Several factors which are essential for angiogenesis process called as angiogenic factors synthesized during first phase of wound healing that is haemostatic phase. At the same time neutrophils and macrophages are attracted toward the site of wound (Hsu et al., 2007).

  Its regulation is achieved by three mechanisms:

  a) **Chemotaxis** - ability of cells to move along a chemical gradient is called as chemotaxis. The chemical agents which are responsible for this process is called as chemotactic agents. They are responsible for cell movement by acting on cell surface receptors which is essential for angiogenesis.

  b) **Mechanotaxis** – it is the movement of cell in the influence of mechanical forces.

  c) **Haptotaxis** – it is the movement of cell under the control of immobilized ligand gradient (Li et al., 2005).
• Epithelialization

In this step movement of epithelial cells takes place which starts from the wound edges after some time of wounding. First of all a single layer of cells forms on the centre of wound and the cells moves around the edges of wound. Once the complete epithelial tissue formed the further movement of cells stops and simultaneously formation of basement membrane will start.

iv. Remodeling Phase:

In this phase formation of totally new epithelium and final scar tissue takes place therefore it is consider as a last phase of wound healing. This phase takes 1 to 2 years or in some cases more than that to complete. The tensile strength of healed wound totally depends on deposition of collagen as it is not regular. Starting collagen deposition is generally irregular and new collagen matrix takes more time for cross linking. In the final stage of process formation of mature scar will takes place. Tensile strength of healed wound is more as it contain less number of blood cells and blood vessels (Okane, 2002).

3.2 Factor affecting wound healing

Many factors are responsible for improper and impaired wound healing. Detail knowledge of those factors and how they affect the process of wound healing is important at the time of development of newer and better therapeutic agents for the treatment of wounds (Kerstein, 2007).

➢ Local Factors:
  o Ischemia
  o Infection
  o Foreign bodies
  o Edema/ Elevated tissue pressure
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➢ Systemic Factors
  o Diabetes mellitus
  o Hyperthyroidism
  o Age
  o Fetal wound healing
  o Tissue perfusion
  o Major trauma and burns
  o Sepsis
  o Nutrition
  o Smoking
  o Corticosteroids
  o Chemotherapy etc.

Table 1: Dermal Molecules Influencing Wound Closure (Iakovos N. et al., 2006)

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Source</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen type I</td>
<td>Fibroblast</td>
<td>Dermis Supports, epidermal cell attachment and migration</td>
</tr>
<tr>
<td>Collagen type IV</td>
<td>Epidermal cells,</td>
<td>Supports epidermal cell attachment and spreading</td>
</tr>
<tr>
<td></td>
<td>fibroblasts</td>
<td></td>
</tr>
<tr>
<td>Collagen type V</td>
<td>Epidermal cells</td>
<td>Basement membrane Support</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Fibroblasts</td>
<td>wound surface with adhesion properties</td>
</tr>
</tbody>
</table>
4. TOPICAL DRUG DELIVERY SYSTEM

Application of any agents or drug directly to the skin at the site of infection or wound to deliver the drug in dermal layer as well as in underlying tissue is known as topical drug delivery system.

Topical drug product are made for external use only however topical dermatology product are use for localized action on either one or more layer of skin. Some medication for this topical product may unintentionally reach systemic circulation.

Topical drug delivery systems represent a class of such delivery strategies which are utilized to deliver the drug topically for treatment of local pathophysiological conditions. Topical delivery systems are mainly fabricated for ophthalmic, dermatological, otitic, vaginal or rectal uses. The topical formulations are applied on a skin in such a way that no remaining mass of formulation will left on the skin after its application. This can be achieved by formation of thin film of formulation on the skin by rubbing or without rubbing. (Tapash; 2005).

The skin act as a two way barrier which is essential for osmoregulation result into good health of individual. Osmoregulation can be achieved by preventing extra absorption or loss of water and electrolytes. Basically following are 3 main mechanisms by which absorption of topical drugs through skin takes place.

i) Transcellular: in this type of mechanism, topical drugs absorb into the skin by passing directly from one cell to another but they are not completely entered into the cells.

ii) Intercellular: in this type of mechanism, topical drugs absorb into the skin by passing through inside of the cells.

iii) Follicular: in this type of mechanism, topical drugs absorb into the skin by passing through the hair follicles.
Apart from above 3 mechanisms most of the topical drugs pass via corneocytes and lipid bilayer to viable layers of the skin (Cecv; 2008).

Factors affecting absorption of topical drug through the skin (Ayub et al.2007)

The detail study of various factors which affects the topical absorption of drug through the skin is an important aspect for the development of newer drug or formulation in the field of dermatology. Mainly there are 2 types of factors viz. Physiological factors which are related to the anatomical and physiological structure of skin and Physiochemical factors which are related to the physical and chemical nature of the topical drug. This factors are describe in detail as follows

1. Physiological Factors

- **Skin thickness**: it is an important factor as the thickness of the skin is not common in all parts of the body. The thickness of skin at the site of application of topical drugs should be taken in consideration as it affects the absorption.

- **Lipid content**: it affects the absorption of drugs by its solubility pattern. If lipid content of skin is more, lipophilic drugs easily get absorb but hydrophilic drug absorption restricted and vice-versa.

- **Density of hair follicle**: generally follicular absorption get affected by the density of hair follicle.

- **Density of sweat glands**: if the density of sweat gland is more, more sweating will takes place which results into more hydration of skin.
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- **Skin pH**: it is an important factor for absorption of drugs; pH of topical formulation should be compatible with skin pH for optimum activity.

- **Blood flow**: for good absorption of drugs the blood flow at the site of application should be optimum.

- **Hydration of skin**: it is an important factor for normal health of the skin, if the skin is hydrated hydrophilic drugs can easily absorb through skin.

- **Inflammation of skin**: in any type of an infection, skin became inflamed and it is differ from normal skin.

2. Physiochemical Factors

- **Partition coefficient**: every drug has its own partition coefficient which is depend on physical and chemical nature of the drugs.

- **Molecular weight**: it is an important parameter which directly related to the absorption of drugs. Molecular weight size of drug molecules should be such that it can easily pass through skin pore size.

- **Degree of ionization**: This factor depends on chemical nature of the drugs. Only unionized drugs can absorb well from the skin.

- **Effect of vehicles**: Selection of suitable vehicles for preparation of topical formulation is important. Vehicle can improve the absorbance capacity of certain drug molecule. (e.g. some vehicle increases penetration of the active drug.
molecules and improves its efficacy. Vehicle itself may show a cooling, drying, protective or emollient action).

Apart from above 2 factors which are directly related with the nature of drug molecules and pathological state of the skin. Following methods can be used to enhance the drug penetration and absorption from the skin.

- Chemical enhancement
- Physical enhancement
- Biochemical enhancement
- Supersaturation enhancement

Once the topical formulation have been prepared by considering above one or more factors. For choosing suitable formulation for its application following points should be consider.

- Topical formulation should be match with the type of lesions (e.g. in case of acute weepy dermatitis greasy ointments should be avoided).
- Topical formulation should be match with the site of application (e.g. for hairy areas gel or lotion can be preferred).
- Before application of any type of topical formulation it should be checked for its irritation or sensitization potential.
4.1 HYDROGEL

With recent research in field of novel drug delivery system for providing stable and economical drug delivery systems, the main focus is on hydrogels which are known for minimizing the problems related not only to conventional dosage forms but also of novel drug delivery systems. Hydrogel can be prepared by using varied size molecules. It can be suitably prepared by molecules as small as Non-steroidal anti-inflammatory drugs or as large as proteins and peptides. Hydrogel is biocompatible, convenient and stable drug delivery system. (Bajpai and Sonkusley, 2002).

Hydrogel is a hydrophilic mixture having the properties of both solid and liquid. It can be defined as matrix of cross linked polymer and has the capacity to hold water inside its porous design. The presence of water loving groups, viz. carboxyl, amino and hydroxyl groups, in the polymer chains is responsible for water holding capacity of the hydrogels. Apart from the synthetic polymers, the natural polymers also called as biopolymers can be used for the development of hydrogels. Such two biopolymers are Chitosan and Alginate which have been comprehensively studied and used for the years. Hydrogels can be used as a regenerative medicine as it has capability to absorb large quantity of water without dissolving in it. (Lin and Metters, 2006).

**Benefits of using hydrogel as controlled drug delivery system are as follows:**

1. Biocompatible and degraded products have no noxious effect.
2. Soft rubbery nature of hydrogel reduces mechanical irritation by in-vivo implant.
3. Low hydrogel water interfacial tension suitable for less protein adsorption and cell adhesion.
4. Release of drug can be synchronized by maintaining water swelling and cross-linking density of polymers.
5. It can be prepared by using large variety of drugs such as hydrophilic, hydrophobic and charged drugs.