1. INTRODUCTION

The practice of herbal medicine has existed since prehistoric times as the primary form of medicine. In this space age where the technology has very much advanced, herbal medicines still flourish and are finding exceptional acceptance in both the developing and the developed countries due to their natural origin and lesser side effects. Besides widespread use of botanicals as medicinal products in developing countries, such products are fast becoming a part of the integrative healthcare systems of the industrialized nations, known as complementary and alternative systems of medicine.

A number of herbal traditions have come to dominate the practice of alternative medicine. These include the western herbal tradition based on Greek, Roman and medieval sources, the essentially Ayurvedic tradition of India and the Chinese herbal medicine. The traditional Chinese medicine continues as a distinct branch of modern medical practice. The traditional herbal remedies as alternative medicine plays a significant role in South Africa also, where it forms a part of the culture and beliefs of the indigenous population and also features significantly in primary health care. Botanicals or phytomedicines have always been a major component of traditional systems of healing in developing countries, which have also been an integral part of their history and culture. In the ancient Indian system of medicine, Ayurveda and Siddha are such examples.¹

It was well known to the ancient world that plants are a rich source of a variety of chemicals with nutritive and therapeutics properties. Herbs belong to general botanicals of various types which are also often the aromatic plants used especially in medicine or as seasoning. Herbs may be used directly as teas or extracts and they may be used in the production of drugs. A drug or preparation made from a plant or plants and used for any of such purpose is better known as herbal drug.

Many of the pharmaceuticals currently available have a long history of use as herbal remedies including opium, aspirin, digitalis and quinine. While purification and
quantification of these plant extracts makes them more predictable and chemical processing can sometimes modify their effects in desirable ways, herbal remedies tend to have a more complex and subtle mix of chemicals, and can sometimes offer access to drugs or combinations of drugs, that the pharmaceutical industry has not yet exploited.

The widespread use of herbs in traditional medicine has also prompted demands that herbal remedies be regulated as drugs to ensure quality standards and to prove its scientific basis. Herbs hold promise not only for prevention but also for the treatment of various types of diseases. The drugs of natural origin constitute very important and valuable segments of modern medicine. Traditional medical practitioners and scientists are turning towards medicinal plants for curing ailments such as inflammation, rheumatoid arthritis, cancer, diabetes and many more because of the fact that they possess lesser side effects owing to their natural origin. These extracts are formulated into different formulations for ease of administration. The novel formulations are reported to have remarkable advantages over conventional formulations of plant actives and extracts which include enhancement of solubility, bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improved tissue macrophages distribution, sustained delivery, and protection from physical and chemical degradation. ²

1.1 Inflammation and use of herbal extracts in its treatment.
Inflammation is a defense reaction caused by tissue damage or injury, characterized by redness, heat, swelling and pain. The primary objective of inflammation is to localize and eradicate the irritant and repair the surrounding tissue. Inflammation aids disposal of microbes, toxins or foreign material at the site of injury, prevents their spread to other organs, and prepares the site for tissue repair. Thus it helps restore tissue homeostasis.³

1.1.1 Basic stages of inflammation:
   i. Vasodilatation and increased permeability of blood vessels.
   ii. Phagocyte migration
iii. Tissue repair

Signs of inflammation:
   i. Rubor – redness
   ii. Tumor – swelling
   iii. Calor – heat
   iv. Dolor – pain

1.1.2 Agents causing inflammation:
   i. Physical agents: like heat, cold, radiation
   ii. Chemical agents: like organic and inorganic poisons
   iii. Infective agents: like bacteria, viruses and their toxins
   iv. Immunological agents: like cell mediated and antibody reaction.

1.1.3 Types of inflammation:
   i. Acute inflammation: Acute inflammation is of short duration and represents the early body reactions. Accumulation of fluid and plasma at the affected site, intravascular activation of platelets and polymorph nuclear neutrophils as inflammatory cells are the basic stages in acute inflammation.

   ii. Chronic inflammation: Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occurs at the same site. Chronic inflammation can be caused by acute inflammation, recurrent attack of acute inflammation, chronic inflammation starting de nova.

1.1.4 Use of herbal extracts in inflammation
Acute and chronic inflammations are complex processes that can be induced by a variety of means. All the steroidal and non steroidal anti-inflammatory drugs currently available are probably polycomponent in that they are able to modulate more than one mediator or cellular event concerned with the inflammatory response. Flavonoids are a polyphenols subclass which are widely distributed in the plant kingdom, and are characterized by two or more aromatics rings, each bearing at least one aromatic hydroxyl group. Several mechanisms explaining the anti-inflammatory
activity of flavonoids have been described, including antioxidative and radical scavenging activities, regulation of cellular activities of inflammation-related cells, modulation of the activities of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase, lipoxygenase) and nitric oxide synthase, modulation of the production of other proinflammatory molecules, modulation of proinflammatory gene expression. Apart from this the flavonoids also bear a high antioxidant activity. They are scavengers of free radicals that cause oxidation. Similarly steroids obtained from plant extracts also show a promising anti-inflammatory activity. Many herbal extracts can exert their anti-inflammatory effect through a spectrum of different modes of action, herbal extracts containing curcumin, capscicin and some formulations of gels containing extracts of *Withania somnifera* and *Boswellia serrate* have been already reported to possess anti-inflammatory effect. 4,5,6,7

1.2 Wounds and herbal extracts in their treatment

1.2.1 Classification of Wounds

Wounds are classified as open wounds and closed wounds on the basis of underlying cause of wound creation and as acute and chronic wounds on the basis of physiology of wound healing.

i. Open Wound

Through an open wound blood escapes the body and bleeding is clearly visible. Open wound is further classified in various types according to the object that cause the wound.

- Incised wound: It is an injury with no tissue loss and minimal tissue damage. It is caused by a sharp object such as knife. Bleeding in such cases can be profuse, so immediate action should be taken.
- Abrasions or superficial wounds: It is caused by sliding fall onto a rough surface. During abrasion the topmost layer of the skin i.e. epidermis is scraped off that exposes nerve ending resulting in a painful injury. Blood loss similar to a burn can result from serious abrasions.
• Laceration wound or tears wounds: This is the nonsurgical injury in conjunction with some type of trauma, resulting in tissue injury and damage.

• Puncture wounds: They are caused by some object puncturing the skin, such as needle or nail. Chances of infection in them are common because dirt can enter into the depth of wound.

• Gunshot wounds: They are caused by a bullet or similar driving into or through the body.

• Penetration wounds: Penetration wounds are caused by an object such as a knife entering and coming out from the skin.

ii. Closed Wound

In closed wounds blood escapes the circulating system but remain in the body. It includes Contusion or bruises, heamatomas or blood tumor, Crush injury etc.

• Contusions or bruises: Bruises are caused by a blunt force trauma that damage tissue under the skin.

• Hematomas or blood tumor: They are caused by damage to a blood vessel that consequently causes blood to collect under the skin.

• Crush injury: Crush injury is caused when great or extreme amount of force is applied on the skin over long period of time.

Wounds can also be acute or chronic. Acute wound is a tissue injury that normally proceeds through an orderly and timely reparative process that results in sustained restoration of anatomic and functional integrity. Acute wounds are usually caused by cuts or surgical incisions and complete the wound healing process within the expected time frame. Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation. Chronic wounds either require a prolonged time to heal or recur frequently. Local infection, hypoxia, trauma, foreign bodies and systemic problems such as diabetes mellitus, malnutrition, immunodeficiency or medications are the most frequent causes of chronic wounds.
1.2.2 Factors Affecting Wound Healing: \(^1,8\)

- Improper diet
- Infection at the wound site
- Insufficient oxygen supply and tissue perfusion to the wound area
- Drugs
- Elderly age
- Diabetes and other disease conditions

Wound healing is a normal biological process in the human body. Many factors can adversely affect this process and lead to improper and impaired wound healing. A thorough understanding of these factors and their influence on wound healing is essential for better therapeutic option for wound treatment.

1.2.3 Phases of Wound Healing \(^8,9\)

- **Inflammatory phase:** The inflammatory phase starts immediately after the injury that usually lasts between 24 and 48 hours and may persist for up to 2 weeks. In some cases, the inflammatory phase launches the haemostatic mechanisms to immediately stop blood loss from the wound site. Clinically recognizable cardinal sign of inflammation, rubor, calor, tumor, dolor and function appear as the consequence. This phase is characterized by vasoconstriction and platelet aggregation to induce blood clotting and subsequently vasodilatation and phagocytosis to produce inflammation at the wound site.

- **Fibroblastic phase:** The second phase of wound healing is the fibroblastic phase that lasts up to 2 days to 3 weeks after the inflammatory phase. This phase comprises of three steps viz., granulation, contraction and epithelisation. In the granulation step fibroblasts form a bed of collagen and new capillaries are produced. Fibroblast produces a variety of substances essential for wound repair including glycosaminoglycans and collagen. Under the step of contraction wound edges pull together to reduce the defects.

- **Epithelization phase:** Epithelial cell migration is one of the vital processes of wound healing. The stem cells of epithelium must detach from the edges of the
wound and migrate into wound. Normally dermal basal cells adhere to each other and to the underline basal layer of the dermis. Following mobilization, epithelial cells begin to enlarge and migrate down and across the wound. Transected hair follicles also contribute to the number of migrating epithelial cells. Epithelial cell migrating across wound usually move along the basal lamina or fibrin deposits, this phenomenon is called contact guidance and is an important factor in epithelial migration. Epithelial migration is followed by increased mitosis of epithelium. Recent evidence suggests that a water soluble heat labile substance called chalone which is secreted at the wound site is responsible for regulation for mytosis.

- **Proliferative phase:** Proliferative Phase (2 days to 3 weeks) includes granulation stage: Fibroblasts lay bed of collagen fills defect and produces new capillaries. Contraction stage: Wound edges pull together to reduce defect. Epithelialization stage: Crosses moist surface cell travel about 3 cm from point of origin in all directions.

- **Contraction phase:** Wound contraction is caused by the action of differentiated fibroblasts (myofibroblasts) in the granulation tissue, which contain filaments of smooth muscle actin. Contraction of these fibroblasts makes the wound margins move towards the center of the wound. Remodeling phase: This phase lasts for 3 weeks to 2 years. New collagen is formed in this phase. Tissue tensile strength is increased due to intermolecular cross-linking of collagen via vitamin-C dependent hydroxylation. The scar flattens and scar tissues become 80% as strong as the original.

Wound is defined as the disruption of the cellular and anatomic continuity of a tissue. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissues. The process of wound healing consists of integrated cellular and biochemical events leading to re-establishment of structural and functional integrity with regain of strength in injured tissues. Naturally, the investigative curiosity to promote healing continues since ages. A lot of research has been envisaged to develop better healing agents and it has been a challenging task to generate them and keep pace with the problems encountered. Several drugs of plant, mineral and animal
origin are described in the Ayurveda for their wound healing properties. Inspite of recent advances in the basic mechanism of wound healing, knowledge of factors involved in the development and treatment of wounds and their prevention remains limited. Pathogenesis and failure to heal are two inseparable aspects in case of wound healing and this has guarded and intensified the use of herbal drugs as a wound healing agent. In the search of least toxic and most effective agent many medicinal plants have been tested for wound healing activity and there are also many marketed herbal preparations used in wound healing like Vicco turmeric antiseptic cream, Boro soft Antiseptic cream, Foot care by Himalaya Drug Company. Thus many herbal extracts having a considerable anti microbial activity and antioxidant potential can be used as wound healing agents. Plants and their extracts have immense potential for the management and treatment of wounds. The phytomedicines for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of traditional medicine before these could be recommended for healing of the wounds. Plants or chemical entities derived from plants need to be identified and formulated for the treatment and management of wounds. In this direction, a number of herbal products are being investigated at present. Various herbal products have been used in the management and treatment of wounds over the years.

1.3 Transdermal Drug Delivery
Transdermal drug delivery system is a therapeutic system of defined surface area that delivers a predetermined amount of drug to the surface of intact skin at a pre-programmed rate. These systems systemically provide drug at a predictable rate and maintain the rate for extended period of time, thus eliminating numerous problems associated with oral products such as, unpredictable or reduced bioavailability, enhanced first pass hepatic metabolism, relatively short residence time, dose dumping and dose inflexibility.
Also, transdermal drug delivery system provides continuous percutaneous administration of a drug at controlled rate which permits elimination of pulse entry into the systemic circulation, a phenomenon often associated with side effects. It also allows the option of rapidly terminating absorption of medication therapy, which needs to be interrupted.

Hence these systems can be designed to put in drugs, through intact skin at appropriate rates to maintain suitable plasma drug levels for therapeutic efficacy, without periodic fluctuations into plasma concentrations that would accompany toxicity or lack of efficacy.\textsuperscript{12}

The development of modern transdermal drug delivery system has its antecedents in ancient medical practice. The Egyptians applied ointments, ruminants, plasters and exotic injunctions to the skin till nineteenth century. Throughout first half of the twentieth century many advances were made with regards to understanding of topical drug delivery for local effect. The findings accumulated over the years practically revolutionized the old theory of an impermeable skin barrier and motivated a number of researchers to develop rate controlled drug delivery system for controlling the transdermal administration of drugs to accomplish the objective of systemic medication.

Hence in case of transdermal drug delivery system skin becomes the main route of administration with large surface area covering the body, layered anatomy and defined physicochemical properties.

\subsection{Anatomy of skin}\textsuperscript{13,14}

The skin is multilayered organ composed of many histological layers. It is generally described in terms of three tissue layers (Figure 1). Skin is an anatomical barrier between the body and its environment and contributes to 16-18\% of normal body weight.
i. Epidermis:

Figure 1. Histology of skin

Epidermis, the outermost skin layer comprises of stratified squamous epithelial cells. The epithelial cells are held together mainly by highly convoluted interlocking bridges, which are responsible for the unique integrity of the skin. Microscopic sections of the epidermis show two main parts: the stratum corneum and the stratum germinativum. The stratum corneum forms the outermost layer of the epidermis and consists of many layers of compacted flattened, dehydrated, keratinized cells in stratified layer. These cells loose their nuclei and are physiologically rather inactive. They are formed and continuously replenished by the slow upward migration of cells produced by basal cell layer of the stratum germinativum, which is regenerative layer of the epidermis. In the thicker part of the skin, the transition from the living cells of the germinativum to the zone of the dead, cornified cells of the stratum corneum is made prominent by three layers: the stratum spinosum (prickly layer), stratum granulosum (granular layer) and stratum lucidum (clear layer) Stratum corneum, also called as horny layer which consists of compacted, dead, keratinized cells with a density of 1.55. Because of the dense nature of stratum corneum, values of diffusion
coefficients in this tissue are very much smaller than any other skin tissue, which results in higher resistance and greater impenetrability. Hence, stratum corneum is responsible for the barrier function of the skin. It also behaves as the primary barrier to percutaneous absorption.

ii. Dermis:
The dermis (or corneum) essentially consists of 80% of protein in the matrix of mucopolysaccharides (ground substance). Protein including collagen and elastin fibres are oriented parallel to the epidermis. The dermis supports and interacts with the epidermis facilitating its conformation to the underlying muscles and bones. Blood vessels, lymphatic, nerves as well as the epidermal appendages such as the hair follicles, sebaceous glands and sweat glands are mainly contained and supported within the dermis. Beneath the dermis, the fibrous tissue opens out and merges with the fat-containing subcutaneous tissue.

iii. Subcutaneous fat layer
Subcutaneous fat layer serves as a cushion for the dermis and epidermis. It also provides a thermal barrier. Collagenous fibres form the dermis thread between the superficial skin layer and subcutaneous layer. Combined, these layers form the skin which is pierced at various places by two types of potential diffusion shunts: hair follicles and sweat glands. These skin appendages however, actually occupy only 0.1% of the human skin surface. This transappendageal route of percutaneous absorption, however, provides a very limited contribution to the transdermal permeation of most neutral molecules at a steady state, thus can be considered as a process of passive diffusion through the intact stratum corneum in the interfollicular region.\textsuperscript{15,16,17}

1.3.2 Skin as a site for transdermal drug administration
The skin is one of the most extensive and readily accessible organs of the human body. The skin of an average adult body covers a surface area of approximately two square meters and receives about one third of the blood circulating through the body. With a thickness of only a few millimeters (2.97 ± 0.28 mm), skin separates the
underlying blood circulation network from the outside environment and serves as a barrier against physical and chemical attacks. It acts as a thermostat in maintaining body temperature and shields the body from invasion by microorganisms. Skin is a well-known route of drug administration but its applications have earlier been restricted to local effect. Nowadays delivery of drugs or targeting of drugs to specific sites by topical application for systemic effects has been taken as a challenge by number of researchers. The delivery of drug via transdermal route has been recognized as one of the potential routes for both local and systemic delivery of drugs, due to several advantages. Topical delivery of bioactive substances is indeed a powerful strategy to reduce their systemic toxicity and at the same time restricts the therapeutic effect to specific tissues targeting to a specific site.

However, the major limitation of transdermal delivery of drugs is that the skin layers provide high resistance to the penetrant molecules. Consequently, many substances are topically and systemically ineffective when applied onto the skin, due to their complete failure to penetrate. Different strategies including the use of skin penetration enhancers, 18,19 iontophoresis 20,21 and sonophoresis 22 have been developed to minimize the skin’s barrier function.

1.3.3 Transdermal penetration of drugs: 23,24,25
Penetration of drugs through skin or percutaneous absorption is defined as the penetration of substances into the various layers of skin and permeation across the skin into systemic circulation.

The percutaneous absorption is a stepwise process and can be divided basically into three steps:

i. Penetration: This is the entry of a substance into a particular layer.
ii. Permeation: This is the penetration from one layer into another which is different both functionally and structurally from the first layer.
iii. Absorption: This is the uptake of a substance into the systemic circulation. Diffusion through stratum corneum is purely a passive process.
Epidermal diffusion is the first phase and clearance from the dermis is the second, the later being dependent on the effective blood flow.

**1.3.4 Routes of skin permeation:**

The structure of skin shows number of diffusional pores like hair follicles, sweat glands, intracellular spaces etc which help in the absorption. The routes are as follows:

i. Transcellular pathway / Transepidermal route:
The transcellular pathway, in which the drug travels through cells and across them, is the shortest and also the most likely path that it will follow. The drug travels all the way via intracellular and intercellular spaces. Both polar and nonpolar drugs diffuse by this route.

ii. Intercellular route:
Intercellular path involves the passage of drug molecules through the intercellular spaces. It avoids diffusion through cell contents.

iii. Transappendageal route:
This route transports the drug through sweat glands and hair follicles with their associated sebaceous glands. Structural data on the appendages of skin relevant to diffusion indicate that the fractional area of the skin covered by appendages over the most of the body is quite small (0.1%) and that the appendages do not constitute a completely open pore area but are filled with hair, keratin or sebum, or in case of sweat ducts with water. Hence it is of minor importance.

**1.3.5 Skin as permeability barrier:**
An important element in transdermal drug delivery system is the skin itself. It acts as permeability barrier against the transdermal absorption of various chemicals and biological agents.

Like all other epithelial systems of the body, the prime function of the skin is to keep water and other vital substances in the skin and restrict the entry of foreign substances.
The permeability barrier of skin is constituted of three major layers that include:

i. Stratum corneum (10 µm thick).
ii. Viable epidermis (100 µm thick).
iii. Papillary epidermis (100-200 µm thick).

Stratum corneum has less water content as compared to other skin components. It is 10 µm thick and is the most important barrier. It has been proved experimentally that the permeability of water and other substances was enhanced after the stratum corneum was stripped out layer by layer by employing a cellophane tape. The stratum corneum is a wall like structure with proteins and lipids. The lipid matrix, especially keratin-phospholipids complex of the stratum corneum, plays a significant role in determining the permeability of substances across the skin. Human stratum corneum lipids consist of ceramide and natural lipids such as, free sterols, free fatty acids, and triglycerides. The latter are of considerable importance to the desquamation process. Despite it is an evidence to show that stratum corneum lipids are capable of forming bilayers, suggesting that the intercellular space exists in the form of lamellar liquid crystals. These broad sheets which contain predominantly saturated lipids comprise the major epidermal barrier to water and water soluble penetrants. Once the dosage form is applied topically, the transdermal penetration can be visualized as a composite of a series of following steps as:

- Adsorption of penetrant molecule on to the surface layer of stratum corneum.
- Diffusion through stratum corneum and through viable epidermis.
- The final step involves passage through papillary dermis into the microcirculation. The viable tissue layers and the capillaries are relatively permeable and the peripheral circulation is sufficiently rapid. Hence permeation through stratum corneum acts as the rate-limiting step in the permeability of most of the substances.

Stratum corneum is composed of dead keratinized and metabolically inactive cells. These keratinized cells are closely packed together and the bulk of the stratum
corneum is mechanically coherent with the exception of the few desquamating layers. Hence it limits the permeation of substances. The intercellular spaces are not only narrow but also filled with the overlapping interdigitation of adjacent cells and cell walls. The ultra structure of the inner cellular keratin may also play a role in the process of transdermal permeation, particularly for the selective permeability of polar and non polar molecules. The inner cellular lipid sheet limits penetration of the water soluble agents.

1.4 **Mechanism and Kinetics of Transdermal Permeation:**

For a systemically active drug to reach a target tissue, it has to possess some physicochemical properties which facilitate the sorption of the drug through the skin and enter the microcirculation.

The rate of permeation, \( dq/dt \), across various layers of skin tissues can be expressed as,

\[
dq/dt = Ps( C_d - C_r )
\]

\( Ps \) is the overall permeability coefficient of the skin and is defined as:

\[
Ps = Ks \cdot Dss/ Hs
\]

where, \( Ks \) = Partition coefficient of the penetrant

\( Dss \) = Apparent diffusivity of the penetrant

\( Hs \) = Thickness of skin tissues

As \( Ks \), \( Dss \) and \( Hs \) are constants under given condition, the permeability coefficient \( Ps \) for a skin penetrant can be considered to be constant. From equation (1), it is clear only when \( C_d \gg C_r \), then the equation (1) becomes -

\[
dq/dt = Ps \cdot C_d
\]
Molecular penetration through various regions of the skin is limited by the diffusional resistance ($R_{\text{skin}}$) to permeation through the skin and has been described by Chein as,

$$R_{\text{skin}} = R_{\text{sc}} + R_{\text{e}} + R_{\text{pd}}$$

Where $R$ is the diffusional resistance and subscripts sc, e and pd refer to stratum corneum, epidermis and papillary layer of the dermis respectively.

For keeping $C_d$ constant, the drug should be released from the device at a rate ($R_r$) that is either constant or greater than the rate of skin uptake ($R_a$) i.e. $R_r > R_a$. Since $R_r$ is greater than $R_a$, the drug concentration on the skin surface ($C_d$) is maintained at a level equal to or greater than the equilibrium (or saturation solubility of the drug) in the stratum corneum ($C_s$) i.e. $C_d >> C_s$. Therefore, a maximum rate of skin permeation $\left(\frac{dq}{dt}\right)_m$ is obtained and is given by the equation –

$$\left(\frac{dq}{dt}\right)_m = Ps.C_s$$

From the above equation, it can be seen that the maximum rate of skin permeation depends on the skin permeability coefficient ($Ps$) and its equilibrium solubility in the stratum corneum ($C_s$). Thus skin permeation appears to be stratum corneum limited.

### 1.5 Advantages of transdermal drug delivery

1. It can avoid the variation in the absorption and metabolism associated with oral administration.
2. It can substitute for oral administration of medication when that route is unsuitable, as in instances of vomiting and/or diarrhea.
3. The systems are noninvasive, avoiding the risks and inconveniences of injection.
4. It can increase the bioavailability and efficacy of drugs since hepatic first-pass elimination is bypassed.
5. It can provide a simple therapeutic regime, leading to good patient compliance, which can be easily terminated by simple removal of the application from the surface of the skin.
vi. It can permit continuous zero order drug administration and the use of drugs with short biological half-lives.

vii. It can deliver the drug directly to the site of action.

1.6 Disadvantages of transdermal drug delivery

i. Only relatively potent drugs are suitable candidates for transdermal delivery due to the natural limits of drug entry imposed by the skin's impermeability.

ii. Some patients may develop contact dermatitis at the site of application due to one or more of the system components, necessitating discontinuation.

iii. Useful only for low dose drugs.

iv. Skin irritation or contact dermatitis due to drug, excipients and enhancers is another limitation.

v. Less dose accuracy

1.7 Conventional Formulations in Dermal and Transdermal Delivery

1.7.1 Semisolid Formulations

i. Ointment

They are greasy, semisolid preparations which are often anhydrous and which contain the medicament either dissolved or dispersed in vehicle.

Ointment bases can be classified as:

- Hydrocarbon bases

They are not absorbed by the skin, immiscible with water, sticky in nature and almost inert. Water absorption is low (only 5-10 %). Constituents of hydrocarbon bases include soft, liquid, hard paraffin. They all are obtained from petroleum.

- Absorption bases:

They are hydrophilic in nature and therefore can absorb considerable amount of water or aqueous solutions.

Absorption bases are divided into 2 classes:
Non-emulsified bases: They produce w/o emulsions, less occlusive but nevertheless, are good emollients. They consist of oil soluble medicament to penetrate the skin and easier to spread. e.g. wool fat (anhydrous lanolin), cholesterol, wool alcohols, bees wax etc.

Water in oil emulsions: They are capable of absorbing more water. e.g. hydrous wool fat (lanolin).

- Water miscible bases

They are hydrophilic in nature, easily washed from the skin and used to prepare o/w cream. e.g Emulsifying ointment BP (anionic), Cetrimide emulsifying ointment BP (Cationic), Cetomacrogol emulsifying ointment BP (Non-ionic).

- Water soluble bases:

Completely water soluble bases have been developed from the macrogols (Polyethylene glycols). They are free from greasiness, have satisfactory aging properties, compatibility with many dermatological medicaments and good absorption by the skin.

ii. Creams: 10

Creams are semisolid emulsion systems with opaque appearance, as contrasted with translucent ointment. Their consistency and rheological character depends on whether the emulsion is water in oil or oil in water type and on the nature of solids in the internal phase.

- Water in oil creams:

Water in oil emulsion develops wide range of consistencies depending on the components of the oil and aqueous phase and of the emulsifier blend. Oily creams contain w/o emulsifying agents which typically may be wool fat, wool alcohols, fatty acid esters of sorbitan or the salt of a fatty acid with a divalent metal such as calcium. w/o cream is preferred over an ointment, because the cream spreads more readily and
is less greasy. As water evaporates from the skin the process soothes the inflamed tissue.

E.g. zinc cream BP, oily cream BP

The beeswax-borax system is anomalous in that it produces both w/o and o/w creams without the aid of so called secondary emulsifiers. The factors influencing the type of emulsion formed include the phase ratio of oil in water, the proportion of beeswax which saponifies, the other constituents of the cream and the temperature of mixing.

- **Oil in water creams:**

Oil in water creams when rubbed into the skin disappear easily. To prepare o/w creams, mixed emulsifier or the surfactant/ fatty amphiphile used is usually a long chain alcohol like cetyl alcohol, stearyl alcohol. These emulsifiers are used, mainly because of their ability, not only to stabilize the emulsion but also to impart ‘body’ and so turn a fluid into a semisolid cream. The water soluble surfactant may be anionic (e.g. sodium lauryl sulphate), cationic (e.g. cetrimide) or non-ionic (e.g. cetomacrogol). The surfactants are strong emulsifiers but their resultant emulsions are fluid and they tend to be unstable. So combining a surfactant with fatty amphiphile in the correct ratio will produce o/w cream with superior stabilizing and thickening properties. E.g. Aqueous cream BP, Cetrimide cream BPC, Cetomacrogol cream BPC. o/w creams get diluted with water and are therefore washable and non-staining. On application to skin much of continuous phase evaporates and increases concentration of water soluble drug in the adhering film. The concentration gradient of drug across the stratum corneum should therefore increase and promote percutaneous absorption. They minimize drug precipitation and to promote drug bioavailability, the formulation must include a non-volatile water miscible cosolvent such as propylene glycol.

iii. **Gels:** 31,32,33

The term “gel” represents a physical state with properties intermediate between those of solids and liquids. However, it is often wrongly used to describe any fluid system
that exhibits some degree of rigidity. A gel consists of a polymer which swells in the presence of fluid and perhaps within its structure. The rigidity of the gel is determined by the amount of fluid it entraps. These gels are wet and soft and look like a solid material. These are capable of undergoing large deformation in their physical state i.e. from solid to liquid. The term gel originated in the late 1800’s as chemical attempted to classify semisolid substance according to their phenomenon – logical characteristics rather than their molecular compositions. At that time, analytical methods needed to determine chemical structure were lacking. Gels are swollen networks possessing both the cohesive properties of solids and the diffusive transport properties of liquids. Elastically they tend to be soft and somatically they are highly reactive. They are semisolids being either suspension of small organic particles or large organic molecules interpenetrated with liquid. It is the interaction between the units of colloidal phase, inorganic or organic, which sets up structural viscosity, immobilizing the liquid continuous phase. Thus, gels exhibit characteristics intermediate to liquids and solids. Gels are transparent to opaque semisolids containing a high ratio of solvent to gelling agent. When dispersed in an appropriate solvent, gelling agent merge or entangle to form three dimensional colloidal network structures. This network limits fluid flow by entrapment and immobilization of the solvent molecules. The network structure is also responsible for a gel resistant to deformation and therefore its viscoelastic properties. Gels are an excellent formulation for several routes of administration. They are useful as liquid formulations in oral, topical, vaginal, and rectal administration. Gels can be clear formulations when all of the particles completely dissolve in the dispersing medium. But this doesn't occur in all gels, and some are therefore turbid. Clear gels are preferred by patients. If the gel contains small discrete particles, the gel is called a two-phase system. If the gel does not appear to have discrete particles, it is called as a one-phase system. Two-phase systems are thixotropic, e.g., they are semisolid on standing but liquefy when shaken. If the particle size in a two-phase system is large, the gel is referred to as magma. Examples of two-phase systems include Aluminum Hydroxide Gel and Bentonite Magma. Single-phase systems contain linear or
branched polymer macromolecules that dissolve in water and have no apparent boundary with the dispensing medium. These macromolecules are classified as natural polymers like tragacanth), semi synthetic cellulose derivatives (i.e., methylcellulose), or synthetic polymers like Carbomers. Single-phase gels made from synthetic or natural macromolecules are called mucilages.

- **Classification:**

There are a variety of ways to classify gels:

- According to source of gelling agent:
  
  *Natural gels*
  *Synthetic gels*

- According to the liquid medium entrapped:
  
  *Hydrogel*
  *Organogels*

- According to their cross linkage:
  
  *Chemical gels*
  *Physical gels*

- According to the chemical nature of gelling agent:
  
  *Organic gels*
  *Inorganic gels*

Most natural gums such as acacia, carrageenan, and xanthan gum are anionic polysaccharides that yield natural gels. Number of cellulose derivatives have been synthesized and used as gellants (e.g. sodium carboxy methyl cellulose, hydroxyl ethyl cellulose, hydroxyl propyl cellulose etc) that yield synthetic gels. The nature of the solvent also determines whether the gel is a hydrogel (i.e. water based) or an organogel (i.e. with non-aqueous solvent). Thus, bentonite magma and gelatin gels are hydrogels and dispersions of metallic stearates in oil are examples of organogel. A hydrogel is a polymeric material that exhibits ability to swell in water and absorb a significant fraction of water (~2000 times the polymer weight) within its structure without dissolving in water. A wide variety of natural materials of both plant and
animal origin, materials prepared by modifying naturally occurring structures and synthetic polymeric materials form hydrogels. Organic gels typically contain polymers such as carborner, polyethylene glycol, etc. as gel formers. These are further subdivided according to the chemical nature of the dispersed organic molecules. In case of inorganic gels, the chemical nature of gelling agent is inorganic e.g. bentonite magma. Solid gels with low solvent concentration are known as xerogels. These are often produced by evaporation of the solvent, leaving the gel framework behind. e.g. gelatin, tragacanth ribbons and acacia tears.

- **Structure of gels:**

Gels should, in general, be considered to be composed of heterogeneous structures in different orders from a few angstroms to several micrometers (structure hierarchy). Clays, (e.g. bentonite or kaolin) possess lamellar structure that can be extensively hydrated. The flat surfaces of bentonite particles are negatively charged while the edges are positively charged. The attraction of face to edge of these colloidal lamellae creates a three dimensional network of particles throughout the liquid, immobilizing the solvent. In gels with good solvents, as in aqueous gels, the long chains of organic gel formers are extended. This is due to hydrogen bond formation between water and hydroxyl groups of the gelling agent. In a poor solvent, the gel molecules will be tightly coiled. The viscosity and structure of organic gels is thus dependent upon molecular entanglement by solvent molecules. In case of hydrogels, if the degree of hydration is low then intermolecular attractive forces like hydrogen bonding and van der Waals forces form weak secondary bonds between polymer strands. At a sufficiently high concentration, a continuous network of chains can be formed. These results in local regions of crystalline nature dispersed through a bed of randomly entangled polymer strands. The individual particles of the hydrophilic colloid may consist of either spherical or anisometric aggregates of small molecules, or single macromolecules. In linear macromolecules the network is comprised of entangled particles at the point of contact between which they may
either be relatively small or may consist of several molecules aligned in the crystalline order.

- **Common Gelling Agents**

There are many gelling agents. Some of the common ones are acacia, alginic acid, bentonite, carbopol (now known as carboxomers), carboxymethylcellulose, ethylcellulose, gelatin, hydroxyethylcellulose, hydroxypropyl cellulose, magnesium aluminum silicate (Veegum), methylcellulose, poloxamers (Pluronics), polyvinyl alcohol, sodium alginate, tragacanth, and xanthan gum.

iv. **Emulgels**

Emulgels are emulsions, either of the oil-in-water or water-in-oil type, which are gelled by mixing with a gelling agent. Both oil-in-water and water-in-oil emulsions are extensively used for their therapeutic properties and as vehicles to deliver various drugs to the skin. Emulsions possess a certain degree of elegance and are easily washed off whenever desired. They also have a high ability to penetrate the skin. In addition, the formulator can control the viscosity, appearance, and degree of greasiness of cosmetic or dermatological emulsions. Oil-in-water emulsions are most useful as water-washable drug bases and for general cosmetic purposes, while water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications. Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, nonstaining, compatible with several excipients, and water-soluble or miscible.

So emulgels have a high patient acceptability since they possess, previously mentioned advantages of both emulsions and gels. Therefore, they have been recently used as vehicles to deliver various drugs to the skin. In the market, 2 emulgels are available: Voltaren emulgel (Novartis Pharma, Basle, Switzerland), containing diclofenac diethylamine, and Miconaz-H emulgel (Medical Union Pharmaceuticals, Egypt), containing miconazole nitrate and hydrocortisone. A major limitation of gels is in the delivery of hydrophobic drugs. So to overcome this limitation an emulsion
based approach is being used so that even a hydrophobic therapeutic moiety can enjoy the unique properties of gels. When gels and emulsions are used in combined form the dosage form are referred as emulgel. In recent years, there has been great interest in the use of novel polymers. A unique aspect of dermatological pharmacology is the direct accessibility of the skin as a target organ for diagnosis and treatment. The combination of hydrophilic cornified cells in hydrophobic intercellular material provides a barrier to both hydrophilic and hydrophobic substances. Polymer can function as emulsifiers and thickeners because the gelling capacity of these compounds allows the formulation of stable emulsions and creams by decreasing surface and interfacial tension and at the same time increasing the viscosity of the aqueous phase. In fact, the presence of a gelling agent in the water phase converts a classical emulsion into an emulgel. These emulgel are having major advantages on novel vesicular systems as well as on conventional systems in various aspects. Various permeation enhancers can potentiate the effect, so emulgels can be used as better topical drug delivery systems over present systems. The use of emulgels can be extended in analgesics and antifungal drugs.

Important constituents of emulgel preparation

- **Aqueous Material:**
  This forms the aqueous phase of the emulsion. Commonly used agents are water, alcohols.

- **Oils:**
  These agents form the oily phase in the emulsion. For externally applied emulsions, mineral oils, either alone or combined with soft or hard paraffins, are widely used both as the vehicle for the drug and for their occlusive and sensory characteristics. Widely used oils in oral preparations are non biodegradable mineral and castor oils that provide a local laxative effect, and fish liver oils or various fixed oils of vegetable origin like arachis oil, cotton seed oil and maize oil.
• **Emulsifiers:**
  Emulsifying agents are used both to promote emulsification at the time of manufacture and to control stability during a shelf life that can vary from days for extemporaneously prepared emulsions to months or years for commercial preparations. Some examples are polyethylene glycol 40 stearate, sorbitan monooleates, polyoxyethylene sorbitan mono oleate, stearic acid and many others.

• **Gelling Agent:**
  These are the agents used to increase the consistency of any dosage form can also be used as thickening agent. Carbopol, HPMC are commonly used gelling agents.

• **Permeation Enhancers:**
  These are agents that partition into and interact with skin constituents to induce a temporary and reversible increase in skin permeability. Alcohol, Oleic acid, some essential oils can be added as penetration enhancers.

1.7.2 **Topical Polymeric Film** \(^{39,40}\)

• **Backing membrane**
  Backing membranes are flexible and they provide a good bond reservoir, prevent drug from leaving the dosage form through the top, and accept printing. It is impermeable substance that protects the product during use on the skin e.g. metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible polyurethane) with occlusive base plate (aluminium foil disc) etc.

• **Polymer or polymer matrix**
  Polymers are the backbone of a topical drug delivery system. Systems for topical delivery are fabricated as multi-layered polymeric laminates in which a drug reservoir or drug–polymer matrix is sandwiched between two polymeric layers: an outer impervious backing layer that prevents the loss of drug through the backing surface and an inner polymeric layer that functions as an adhesive and/or rate-controlling membrane. The polymers, which are most widely used are polyvinyl pyrrolidone,
polypropylene, polyvinyl carbonate, cellulose acetate copolymer, EVA polymer, polyethylene terephthalate, hydroxypropyl cellulose, and polyesters. Recently used polymers are Eudragit with different grades, Carbopol 974, 934, 940.

- **Drug**

Topical route of administration cannot be employed for all types of drugs. It depends upon optimal physicochemical properties of the drug, its biological properties. In addition consideration of the pharmacokinetic and pharmacodynamic property of drug is necessary.

- The physicochemical properties are as follows:
  a. The drug should have a molecular weight less than approximately 1000 Daltons.
  b. The drug should have affinity for both lipophilic and hydrophilic phases.
  c. Extreme partitioning characteristic are not conducive to successful drug delivery via skin.
  d. The drug should have a low melting point less than 200°C.
  e. Since the skin has pH of 4.2 to 5.6. Solutions, which have this pH range, are used to avoid damage to the skin. However for a number of drugs, there may also be significant transdermal absorption at pH values at which the unionized form of the drug is predominant.

- The biological properties are as follows:
  a. The drug should be potent with a daily dose of the order of a few mg/day.
  b. The half life $t_{1/2}$ of the drug should be short.
  c. The drug should be non-irritating and non allergic
  d. Drugs which degrade in the GI tract or inactivated by hepatic first-pass effect are suitable candidates for transdermal delivery.

- **Permeation enhancers**

One challenge in designing topical drug delivery systems is to overcome the natural transport barrier of the skin. Oils and surfactants can easily penetrate the superficial layer of the skin.
- **Other Excipients**\(^{41,42}\)
  - **Adhesives**

The adhesion of all topical devices to the skin is an essential requirement and it has so far been accomplished using a pressure-sensitive polymeric adhesive. The adhesive system however, should possess the following characteristics-

a. It should not cause an irritation, sensitization or imbalance in the normal skin flora.

b. It should adhere to the skin strongly during the dosing and should be resistant to the normal routine disturbances such as bathing, clothing, abrasion and exercise.

c. It should be easily removable without leaving any un-washable remains.

d. It should have intimate contact with the skin.

- **Release liner**

Drug loaded matrix or reservoir is totally covered by a rate controlling membrane and these drug releasing surface is coated with an adhesive film, which is protected by a release liner. To use topical film device, the release liner is stripped from the adhesive layer and the device is placed on the body at the desired location e.g. Siliconized PVC / polypropylene / polyethylene terephthalate.
2. LITERATURE REVIEW

2.1 Mori Hardik et al (2013)\textsuperscript{43}

The present study was carried out to document, morphological, microscopical and histochemical features of the rhizome, of Tectaria species, a fern, following standard procedures. Macroscopic studies showed the rhizome of 10–20 cm in length and 3 cm in diameter, short creeping, up to 3 cm thick, densely scaly at the apex. Rhizome showed the presence of cuticle, parenchyma, meristele and vascular bundles. Powder microscopy of the dried rhizomes showed epidermal cells with tannin, starch grains, fragments of ramenta, and prismatic crystals.

2.2 Mamoon S et al (2013)\textsuperscript{44}

The objective of the study was to establish the fingerprint profile of Pisonea aculeata using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. HPTLC fingerprinting of chloroform extract of leaf revealed many different peaks. From this work it can be concluded that HPTLC fingerprint analysis of leaf extract of *Pisonea aculeata* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

2.3 Sushma G S et al (2013)\textsuperscript{45}

The aim of the present research work was to establish the fingerprint profile of *Ficus nervosa* using high performance thin layer chromatography (HPTLC) technique. Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. The results of preliminary phytochemical studies confirmed the presence of alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and
phenol. It can be concluded that HPTLC fingerprint analysis of leaf of *Ficus nervosa* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

2.4 Patel C et al (2013)\textsuperscript{46}

In the present review emulgels have been discussed as one of the novel drug delivery systems. It is mentioned that the emulgels have emerged as one of the most interesting topical delivery system as it has dual release control system i.e. gel and emulsion. When gel and emulsion are used in combined form the dosage form are referred as emulgel. Emulgels are having major advantages on novel vesicular systems as well as on conventional systems in various aspects. The emulgel for dermatological use has several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water-soluble, longer shelf life, bio-friendly, transparent & pleasing appearance. Various permeation enhancers can potentiate the effect, so emulgels can be used as better topical drug delivery systems over present systems. The use of emulgels can be extended in analgesics and antifungal drugs.

2.5 Rao M et al (2013)\textsuperscript{47}

The purpose of the present study was to develop and optimize the emulgel system for MTZ (Metronidazole), a poorly water soluble drug. The pseudoternary phase diagrams were developed for various microemulsion formulations composed of Capmul 908 P, Acconon MC8-2, and propylene glycol. The emulgel was optimized using a three-factor, two-level factorial design, the independent variables selected were Capmul 908 P, and surfactant mixture (Acconon MC8-2 and gelling agent), and the dependent variables (responses) were a cumulative amount of drug permeated across the dialysis membrane in 24 h ($Y_1$) and spreadability ($Y_2$). Mathematical equations and response surface plots were used to relate the dependent and independent variables. The regression equations were generated for responses $YY_1$ and $YY_2$. The statistical validity of the polynomials was established, and optimized formulation factors were selected. Validation of
the optimization study with 3 confirmatory runs indicated a high degree of prognostic ability of response surface methodology. Emulgel system of MTZ was developed and optimized using $2^3$ factorial design and could provide an effective treatment against topical infections.

### 2.6 Bhargava V V et al (2013)\(^{48}\)

The present work was carried out to determine the quanititative estimation of total Phenolic content and Flavonoids present in the roots of *Anogeissus latifolia* commonly known as Dhavdo in the local language and is supposed to have high quantities of flavonoids and Phenols. Methanolic and Aqueous extracts of the roots were made and were subjected to quantitative estimation of the phytoconstituents using the Aluminium Chloride and Folin Ciocalteau method.

### 2.7 Brindha P et al (2012)\(^{49}\)

In this research article with a view to aid the development of medicinal products of *Spathodea campanulata* macroscopic, microscopic properties, chemical and biochemical characteristics of *Spathodea campanulata* have been studied. The investigation revealed the presence of gray colored bark surface containing thick flakes of uneven shape having circular cushion shaped spots. Smooth, shining cream colored inner surface of the bark was observed. Periderm or Rhytidome, the outer zone consists of multiple dark wavy zones alternating with light bands in a successive manner. Inner zone has faint parallel vertical bands. Distinct features of this plant are the presence of collapsed phloem and noncollapsed phloem in outer zone and inner zone, respectively. Abundant calcium oxalate crystals of spindle and prismatic shape seen in phloem parenchyma cells. Elemental analysis, organic content, biochemical and phyto constituent analysis revealed the presence of iron at more than 56% while alkaloids and flavanoids such as are present in 1.5 and 2 %, respectively and starch as much as 1.36%.
2.8 Zahid Z et al (2012)

In this study optimization of extraction process of phytoconstituents and phytochemical investigations of the flowers of *Spathodea campanulata* have been carried out. Comparative preliminary phytochemical screening study of the flowers and bark of the plant, *Spathodea campanulata* have been summarized in the present article.

2.9 Johnson M et al (2012)

The aim of the present study was to explore the phytochemical constituents of the methanolic flower extracts of *Helictres isora* (H. isora), *Spathodea campanulata* (S. campanulata), *Antigonon leptopus* (A. leptopus) and *Thunbergia grandiflora* (T. grandiflora). The preliminary phytochemical screening was performed by Harborne method. The results of the phytochemical screening revealed that alkaloids, phenol, tannins, xanthoproteins, carboxylic acid, coumarins and carbohydrates presence in the methanolic extracts of *H. isora*. The methanolic extracts of *S. campanulata* displayed the presence of alkaloids, phenol, coumarins and carbohydrates. Similarly the phytoconstituents present in the other plants under study have also been reported.

2.10 Kowti R et al (2011)

The ethanol extract of leaf and flower of *Spathodea campanulata* was investigated for antimicrobial activity at 10 mg/ml concentrations by using Kirby-Bauer disc diffusion method against gram positive and gram negative organisms like *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas* sps, *Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus, Vibrio cholera*. After incubation for 24 hrs, the zone of inhibition was compared with standard antibiotics genatmycin and streptomycin (10 μg/ disc). From the dose dependent study it was observed that the ethanolic flower extract was more potent than leaf extract. Flavonoids and tannins present in the ethanolic extract may be responsible for the antimicrobial activity.
2.11 Sheikh M et al (2012) 53

The aim of the present study was to check the antimicrobial potential of eleven different aqueous leaf extracts on *Xanthomonas campestris*, *Agrobacterium rhizogenes* and *Aspergillus fumigatus* based on formation of the zone of inhibition (ZOI). *Prosopis juliflora* showed maximum and significant inhibitory effect on the growth of all the three pathogens. In case of *Xanthomonas campestris* the effect of this plant extract was almost equal to the strength of Streptomycin 10 mcg, (the metric system uses the term mcg to represent micrograms). Aqueous extracts of some plants formed low zone of inhibition. The findings revealed that some plant extracts were comparatively more effective against all the three pathogens *Xanthomonas campestris*, *Agrobacterium rhizogenes* and *Aspergillus fumigatus*.

2.12 Saeed N et al (2012) 54

The aim of this study was to screen various solvent extracts of whole plant of *Torilis leptophylla* to display potent antioxidant activity in vitro and in vivo, total phenolic and flavonoid contents in order to find possible sources for future novel antioxidants in food and pharmaceutical formulations. A detailed study was performed on the antioxidant activity of the methanol extract of whole plant of *Torilis leptophylla* (TLM) and its derived fractions {n-hexane (TLH), chloroform (TLC) ethyl acetate (TLE) n-butanol (TLB) and residual aqueous fraction (TLA)} by in vitro chemical analyses and carbon tetrachloride (CCl4) induced hepatic injuries (lipid peroxidation and glutathione contents) in male Sprague-Dawley rat. The total yield, total phenolic (TPC) and total flavonoid contents (TFC) of all the fractions were also determined. TLM was also subjected to preliminary phytochemical screening test for various constituents. Data from present results revealed that *Torilis leptophylla* act as an antioxidant agent due to its free radical scavenging and cytoprotective activity.

The study was done to establish the fingerprint profile of *Aloe barbadensis* by using High Performance Thin Layer Chromatography (HPTLC) technique. After optimization of solvent system by Thin Layer Chromatography, different fractions of aqueous extract of Aloe was collected by column chromatography and the fingerprinting was done by High Performance Thin Layer Chromatography (HPTLC). Solvent system of methanol:chloroform :: 1:1 was found appropriate for fractionation by column chromatography. Total seven fractions were collected and they were analysed by HPTLC. Distinct spectra of different constituents were obtained for each fraction. HPTLC fingerprinting of *Aloe barbadensis* Miller may be useful in characterization of different phytochemicals found in this species. It may also be useful in differentiating the species from the adulterant and act as a biochemical marker for this.


The leaves of the plant *Annona squamosa* were collected, powdered and extracted successively with different solvents. The extracts were subjected to preliminary phytochemical screening, which revealed the presence of alkaloids, flavonoids, carbohydrates, saponins, tannins, and steroids. The TLC and HPTLC techniques were used for qualitative determination of possible number of components in the various extracts. Solvent systems for all the extracts were optimized in order to get maximum separation on plate. Presence of various phytochemicals was confirmed by the use of different spraying reagents in this study.

2.15 Eugene I et al (2012)  

This study was conducted to evaluate the antibacterial and wound healing properties of methanolic extract of dried fresh leaves of *Gossypium barbadense*. The antibacterial properties of the extract were studied against five wound isolates (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, and Shigella sonnet*) using the Well diffusion method. The wound healing properties were carried out using the excision wound model on healthy albino rats. The results showed that methanolic extract of dried fresh *Gossypium*
barbadense leaves had a dose dependent activity against all the test organisms except Escherichia coli. The extract solvent, propylene glycol, used as a negative control, had no activity against any of the test organisms. Dettol® antiseptic, and Cicatrin® powder used for the positive control also had a dose dependent activity against all the test organisms except Escherichia coli and Pseudomonas aeruginosa. The concentrated Dettol® (i.e. the undiluted solution) however inhibited growth of all test organisms. Comparing all test substances, it was observed that after ten days of treatment of the rats that when the extract was applied at a concentration of 20mg/ml, there were about 91% healing of wound on the rats whereas about 80% healing of wound on the rats was noticed for Cicatrin® powder. The distilled water used as a negative control however produced only about 36% healing of wound on the rats. The distilled water treated group percentage healing of wounds was significant.

2.16 Negi A et al (2012) 58
This study was carried out to evaluate a noble herbal gel formulation containing extract from the leaves of Eupatorium adenophorum for its topical anti-inflammatory activity against carrageenan induced oedema. Gelling agent used in this study was 1% w/w concentration of Carbopol-934. The studies were conducted on Albino Wistar rats of either sex (150-200gm). Change in oedema volume of the rat hind paw was measured. The anti-inflammatory effect produced after topical administration of herbal gel formulation on Carrageenan-induced hind paw oedema exhibited a high degree of reproducibility. The initial physicochemical parameters of formulations i.e. pH, viscosity, spreadability, extrudability and stability were also examined. The pH of all the formulations was near about 6.8, which was in the normal pH range of the skin. It was concluded that the preparation was stable under normal storage conditions and did not produce any skin irritation, i.e., erythema and oedema for about a month, when applied over the skin.
2.17 Khullar R et al, (2012)\textsuperscript{59}

The objective of the study was to prepare emulgel of mafenamic acid, a NSAID, using Carbopol 940 as a gelling agent. Mentha oil and clove oil were used as penetration enhancers. The emulsion was prepared and it was incorporated in gel base. The formulations were evaluated for rheological studies, spreading coefficient studies, bioadhesion strength, skin irritation studies, \textit{in vitro} release, \textit{ex vivo} release studies, anti-inflammatory activity and analgesic activity. Formulation F2 and F4 showed comparable analgesic and anti-inflammatory activity when they were compared with marketed diclofenac sodium gel. So, it can be concluded from the work that topical emulgel of mafenamic acid possess an effective anti-inflammatory and analgesic activity.

2.18 Hendra R et al (2011)\textsuperscript{60}

In the present study all parts of the fruits of \textit{Phaleria macrocarpa} were extracted with methanol. The antioxidant activity of the extracts were characterized in various in vitro model systems such as FTC, TBA, DPPH radical, reducing power and NO radical. Anti-inflammatory assays were done by using NO production by macrophage RAW 264.7 cell lines induced by LPS/IFN-g and cytotoxic activities were determined by using several cancer cell lines and one normal cell line. The results showed that different parts (pericarp, mesocarp, and seed) of \textit{Phaleria macrocarpa} fruit contain various amount of total phenolic (59.2 ± 0.04, 60.5 ± 0.17, 47.7 ± 1.04 mg gallic acid equivalent/g DW) and flavonoid compounds (161.3 ± 1.58, 131.7 ± 1.66, 35.9 ± 2.47 mg rutin equivalent/g DW). Pericarp and mesocarp showed high antioxidant activities by using DPPH (71.97%, 62.41%), ferric reducing antioxidant power (92.35%, 78.78%) and NO scavenging activity (65.68%, 53.45%). Ferric thiocyanate and thiobarbituric acid tests showed appreciable antioxidant activity in the percentage hydroperoxides inhibitory activity from pericarp and mesocarp in the last day of the assay. Similarly, the pericarp and mesocarp inhibited inducible nitric oxide synthesis with values of 63.4 ± 1.4% and 69.5 ± 1.4% in macrophage RAW 264.7 cell lines induced by LPS/IFN-g indicating their notable anti-inflammatory potential. Cytotoxic
activities against HT-29, MCF-7, HeLa and Chang cell lines were observed in all parts. These results indicated the possible application of P. macrocarpa fruit as a source of bioactive compounds was potent as an antioxidant, anti-inflammatory and cytotoxic agents.

2.19 Parthasarthy G et al (2011)\textsuperscript{61}
In the present study an attempt was made to design the transdermal drug delivery system of Naproxen with Ethylcellulose and Hydroxy propyl methyl cellulose polymer in various concentrations. Transdermal films were fabricated by matrix technique with various polymer proportions using dibutylphthalate as plasticizer. These transdermal drug patches were characterized for their thickness, tensile strength, content uniformity, in-vitro release. The release profiles were found to be varied with various concentrations of Ethylcellulose Polymer. The sample of patches prepared with 2:8 and 8:2 ratios of Ethyl cellulose and Hydroxy propyl methyl cellulose shows highest and lowest in-vitro release of Naproxen respectively.

2.20 Panprung S(2011)\textsuperscript{62}
Panprung et al successfully prepared a wound dressing material from alginate, a natural polymer capable of forming into hydrogels, and asiaticoside (PAC), a substance from the plant \textit{Centella asiatica} which has commonly been used in traditional medicine to heal wounds. Various amounts of PAC (i.e., at 2.5, 5 and 10\%, based on the weight of alginate) were mixed with alginate in distilled water. The mixtures were later cast into films. Due to its insolubility in water, PAC existed in the films as discrete entities. The release of PAC from the PAC-loaded alginate “immersed” films was achieved by both the swelling and the erosion of the alginate matrix in the phosphate buffer solution (PBS) that contained methanol at about 10\% (v/v). The potential for use of both the neat and the PAC loaded alginate “immersed” films as wound dressings was assessed by indirect cytotoxicity evaluation and direct cell culture, using normal human dermal fibroblasts (NHDF). The results showed that these materials were non-toxic to the skin cells. Almost all of the asiaticoside-loaded PAC could be released from the
PAC-loaded alginate “immersed” films (i.e., greater than or equal to 92% on average) into the releasing medium of phosphate buffer solution (PBS) and 10% (v/v) of methanol within 24 hours.

2.21 Vijaya Bhanu P et al (2011)\textsuperscript{63}
In this paper they have studied Diclofenac diethylamine is a Non-Steroidal Anti-Inflammatory drug, used in the treatment of inflammation and degenerative disorder of the musculoskeletal system. The conventional diclofenac emulgel formulation contains isopropyl alcohol to increase solubility of diclofenac diethylamine, it is highly flammable may cause eye and cutaneous irritation. Prolonged skin contact with isopropyl alcohol may cause eczema and sensitivity. The working hypothesis of this study was that to develop diclofenac emulgel without isopropyl alcohol and match the in vitro and ex vivo permeability of optimized formulation with the conventional formulation.

2.22 Shahin M et al (2011)\textsuperscript{64}
This study describes the formulation of different stable plain o/w emulsions containing several oils (jojoba oil, liquid paraffin and isopropyl myristate) with variable oil contents (20%, 30% and 40% w/w ) together with several surfactant blends (Span 60, Span 83, Span 80, Myrj 53, Brij 35 and Tween 80). In the first place, the required hydrophilic lipophilic balance (RHLB) for jojoba oil was determined based on three different methods including the assessment of the degree of creaming after centrifugation and after shelf storage for 28 days at room temperature and the turbidimetric method. While the RHLB of liquid paraffin and isopropyl myristate were taken from the literatures. As such RHLB for jojoba was found to be 12.50. On the other hand, the proper non ionic surfactant type was selected by the use of two methods, namely: the degree of creaming after 28 days shelf storage at room temperature and the turbidimetric method. Results revealed that a blend of span 60 and brij 35 gave the most stable emulsion for all oils used. Finally, the most suitable emulsifier concentration for each oil type and level was determined using the turbidimetric method. Nine formulae were obtained that could used for variety of purposes.
2.23 Kulkarni P A et al (2010) 65

This study was aimed to evaluate a new herbal preparation containing extract from leaves of *Vitex negundo* for its topical anti-inflammatory activity against carrageenan induced edema, formalin test, anti-nociceptive effect. Gelling agent used in this study was 1% w/w concentration of carbopol-940 in the formulation. The studies were conducted on wistar rats of either sex (160-180 g). The change in oedema volume of the rat hind paw was measured. From the study it was observed that the 1% herbal formulation also potentiated the anti-inflammatory and anti-nociceptive effect topically.

2.24 Stankovic M (2010) 66

In this study, *in vitro* antioxidant activity, total phenolic content and concentration of flavonoids of five different extracts, from the whole herb of *Marrubium peregrinum* L. (Lamiaceae) were determined using spectrophotometric methods. Antioxidant activity of extracts was expressed as percentage of DPPH radicals inhibition and IC50 values (μg/ml). Values in percentage ranged from 27.26 to 89.78%. The total phenolic content ranged from 27.26 to 89.78 mg/g of dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations varied from 18.72 to 54.77 mg/g, expressed as rutin equivalents. Methanolic extract of *M. peregrinum* showed the highest phenolic and flavonoid concentration and strong antioxidant activity. The significant linear correlation was confirmed between the values for the total phenolic content and antioxidant activity of plant extracts. The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The *M. peregrinum* can be regarded as promising candidates for natural plant sources of antioxidants with high value.


In the present investigation, topical gel of meloxicam (MLX) was formulated using N-methyl pyrrolidone (NMP) as a solubilizer and Carbopol Ultrez 10® as a gelling polymer. MLX gel was evaluated with respect to different physicochemical parameters such as pH, viscosity and spreadability. Irritation potential of MLX gel
was studied on rabbits. Permeation of MLX gel was studied using freshly excised rat skin as a membrane. Anti-inflammatory activity of MLX gel was studied in rats and compared with the commercial formulation of piroxicam (Pirox® gel, 0.5% m/m). Accelerated stability studies were carried out for MLX gel for 6 months according to ICH guidelines. MLX gel was devoid of any skin irritation in rabbits. After 12 h, cumulative permeation of MLX through excised rat skin was 3.0 ± 1.2 mg cm-2 with the corresponding flux value of 0.24 ± 0.09 mg cm-2 h-1. MLX gel exhibited significantly higher anti-inflammatory activity in rats compared to Pirox® gel. Physicochemically stable and non-irritant MLX gel was formulated which could deliver significant amounts of active substance across the skin in vitro and in vivo to elicit the anti-inflammatory activity.

2.26 Dash S H et al (2010) 68
In this paper the mathematical models used to determine the kinetics of drug release from drug delivery systems are reviewed. It is discussed that the quantitative analysis of the values obtained in dissolution/release rates is easier when mathematical formulae are used to describe the process. Finally it was concluded that the mathematical modeling can ultimately help to optimize the design of a therapeutic device to yield information on the efficacy of various release models.

2.27 Kuehl K H (2010) 69
Kerry et al studied the efficacy and tolerability of the diclofenac epolamine topical patch 1.3% in patients with acute pain due to soft tissue injuries. Based on data from clinical studies and postmarketing experience, significant pain relief in patients with soft tissue injuries, with good tolerability was reported.

2.28 Lopez C et al (2009) 70
This study focuses on developing a kanamycin-based auxiliary system intended to be used in the treatment of mycetoma caused by Actinomadura madurae. Transdermal patches (with two different formulations: one with free kanamycin [K] and the other one with kanamycin adsorbed in silica [K-SG]) and an emulgel
were developed. Both patches were prepared by the casting-evaporation technique. To characterize them, differential scanning calorimetry, bioadhesion, post-moisture detachment, strength and rupture distance, gas exchange, water uptake, and dissolution studies were carried out. The emulgel (containing 0.57% of kanamycin) was prepared from an oil-in-water emulsion, which was then incorporated to a gel. The patches with the best characteristics contained 22.9% of silica and 14.6% of kanamycin. Dissolution studies indicated that 8.8% of kanamycin released from K and 3.2% from K-SG at 24h. The emulgel containing 0.57% of kanamycin showed good technological characteristics for its application to the skin (viscosity, 44.9 +/- 1.4 poises; pH, 6.9 +/- 0.4; and penetrability, 52.7 +/- 5.1). The optimal patches were those containing 15.9% of freely dispersed kanamycin (K) and 14.6% of kanamycin adsorbed in silica (K-SG), which corresponds to the batch 2-0.8. The assessments performed on both pharmaceutical forms (patches and emulgel) showed that they have the adequate technological characteristics for being used as an auxiliary in the treatment of actinomycetoma caused by *A. madurae*.

2.29 Suruse P B et al (2012)\(^71\)

The present investigation was aimed to formulate anti-inflammatory transdermal pad by incorporating herbal extracts. The incorporation of herbal drugs such as boswellic acid (*Boswellia serrata* Rox.), shivlingi extract (*Bryonia laciniosa* Linn.), guggul extract (*Commiphora mukul* Hook.) and isolated compounds from raladhupa namely CS1 and CS2 (*Canarium strictum* Rox.) were envisaged. The drugs were selected on the basis of their synergistic action in suppressing inflammation. The anti-inflammatory transdermal pads were evaluated for their physical properties like thickness of film, moisture absorption and diffusion studies across shaved rat skin. The qualitative drug release of each constituents of formulation on TLC plate indicated that drug release had occurred at a constant rate. The skin irritation study on albino rabbit skin showed that the formulation does not produce any irritation.
2.30 Sasikumar J M et al (2009)\textsuperscript{72}
In this study root extracts of \textit{Pandanus odoratissimus L} were examined for their contents of phenolics and flavonoids as well as for their in vitro antioxidant activities using 1, 1-diphenyl-2- picryl hydrazyl (DPPH) radical quenching assay and reducing power. The methanolic root extract yielded 56.24 ± 0.02 mg/g GAE of phenolic content and 81.25 ± 0.046 mg/g CE of flavonoid content. Higher antioxidant potential was observed in both DPPH scavenging assay (EC = 48.3 50 ± 0.002 μg mL⁻¹) and reducing capacity (OD at 1000 μg mL⁻¹ = 0.787) by the methanolic root extract than by the aqueous extract. A positive correlation was found between phenolics and flavonoid contents and antioxidant properties of the extracts.

2.31 Ilodigwe E E et al (2009)\textsuperscript{73}
In this study analgesic and anti-inflammatory potentials of the ethanol leaf extract of \textit{Spathodea campanulata}, a Nigerian traditional medicinal plant was studied using cold, thermal and chemical-induced pain models, and carrageenan-induced acute inflammation in rats. The acute toxicity and the phytochemical constituents of the extract were also determined. The results showed that the extract (250-1000 mg/kg) significantly (P<0.05) and dose-dependently prolonged the pain reaction times in hot-plate and tail flick pain models, and reduced acetic acid –induced writhing. The extract demonstrated significant anti-inflammatory activity against acute inflammation induced by carrageenan. The estimated LD50 of the extract was 4500 mg/kg. Phytochemical analysis revealed the presence of tannins, saponins, anthraquinone glycosides and flavonoids. These findings indicate that the leaf extract of \textit{Spathodea campanulata} has both analgesic and anti-inflammatory properties and could be beneficial in alleviating painful inflammatory conditions.

2.32 Zhao L et al (2007)\textsuperscript{74}
This study was carried to explore the inner anti-inflammatory mechanism on petroleum ether extract from \textit{Melilotus suaveolens Ledeb}. Inflammatory cellular model was founded by intervention of lipopolysaccharide (LPS) on RAW264.7
cell line. Secretion of TNF-a, IL-1b, IL-6, NO and IL-10 in supernatant, mRNA expression of TNF-a, COX-2, iNOS and HO-1, protein expression of COX-2 and HO-1, activation of NFkB and ingredients in the extract were assayed. The extract could not only reduce production of pro-inflammatory mediators by blocking NF-kB activation but promote release of anti-inflammatory mediator HO-1 significantly. The only active ingredient in the extract was coumarin and the concentration of coumarin in each 1 g extract was 0.27822 mg. Compared to Dexamethasone, the extract not only had similar effects on antagonizing proinflammatory mediators and cytokines but has effects on promoting production of anti-inflammatory

2.33 Ghogari A M et al (2006)\textsuperscript{75}

The objective of the present work was to evaluate the free radical scavenging activity of methanolic extract of \textit{Aspidium cicutarium} rhizome. Preliminary phytochemical analysis, estimation of total phenolic content and total tannin content was carried out as a part of phytochemical evaluation of \textit{Aspidium cicutarium} rhizome. Free radical scavenging activity was studied using two in vitro models viz, DPPH assay and superoxide scavenging assay. Preliminary phytochemical screening showed the presence of phenols, tannins, flavonoids and sterols. Total phenolics and total tannin content was found to be 17.35\% (w/w) and 7.98 \% (w/w) respectively. Methanolic extract of \textit{Aspidium cicutarium} rhizome showed concentration dependent free radical scavenging activity comparable to that of the positive controls pyrogallol and ascorbic acid in the respective models. EC50 was found to be 30.63 \(\mu\)g/ml and 85.19 \(\mu\)g/ml in DPPH assay and superoxide scavenging assay respectively. The results indicate that methanolic extract of \textit{Aspidium cicutarium} rhizome exhibited good free radical scavenging activity.


3 AIMS AND OBJECTIVES
The primary aim of the present research work is to explore new alternatives for the treatment of conditions like inflammation and wounds which are commonly associated with many other diseased conditions like rheumatoid arthritis, pain and inflammation associated with cancer. The conventional treatment for these conditions comprises of oral medication which include the tablets of NSAIDs. However there are many side effects associated with these conventional NSAIDs.

In India Ayurveda has been practiced since many years and many medicinal plants are used in the form of their powders, extracts to treat many diseases and have claimed to have lesser side effects than the Allopathic medication available in the market to treat the same conditions. The aim of this work is to establish a relation between the activity shown by certain medicinal plants used already in the Ayurvedic system of medicine and the phytoconstituents which are present in the medicinal plants. Many phytoconstituents show a synergistic effect when used in the form of whole extract. The study also aims at making these valuable and useful constituents in a dosage form that will increase patient convenience and be stable and effective at the same time.

Oral route though most convenient and compliant with the patients, has disadvantages of drug metabolism and low bioavailability. Therefore the current work aims at transdermal delivery of drug, the site of action being the inflammation at a particular site on the skin or some layers beneath the skin.

The aim of the study is to make transdermal formulations of a few anti-inflammatory agents like extracts of leaves of *Spathodea campanulata* family Bignoniaceae and extracts of rhizomes of *Aspidium cicutarium* family Dyropteridaceae commonly known as kombadnakhi.

3.1 Objective of Study:
The broad objective of the present work is to develop a formulation made from extracts of medicinally important plants which have already been reported and widely used in Ayurveda. *Aspidium cicutarium (Nephrodium cicutarium)* also known very
well as Kukkutnakhi or kombadnakhi is used for treating conditions like tonsilitis, rheumatoid arthritis in the form of a traditional paste made by rubbing the rhizomes in water and applying the paste on the affected area according to Ayurvedic practitioners. The other medicinal plant under study *Spathodea campanulata* leaves has been reported to contain various flavonoids and phenolic substances which can be responsible for antioxidant and anti-inflammatory conditions. However these extracts have not been incorporated into stable formulations. So the objective of the present study is to evaluate the phytochemical properties of the extract and incorporate it into stable formulations. The main objective therefore was to find out and confirm the phytochemical constituents in the extracts of these plants and develop a formulation which can be topically applied and penetrate across the skin to treat inflammatory conditions.

Specific objectives include:

- Exhaustive literature survey.
- Selection of suitable solvent for extraction of rhizomes of *Aspidium cicutarium* and leaves of *Spathodea campanulata*.
- Qualitative & quantitative phytoprofiles of extracts by chemical test & TLC and HPTLC screening of extracts.
- Evaluation of antioxidant activity and acute toxicity studies of extracts.
- Cytotoxicity studies and anti-inflammatory studies on cell lines.
- Evaluation of anti-inflammatory and wound healing studies of extracts.
- Development of various formulations using these extracts
  - Films
  - Emulgels
- Evaluation of formulations.
• In vitro diffusion studies of the formulations

• In vivo studies for anti-inflammatory activity and wound healing properties.

• Accelerated stability studies.
4 PLAN OF WORK

- Phase I Literature survey (5 months)
  ✓ Exhaustive literature survey of the plants under study
  ✓ Literature survey of methodology of work.

- Phase II Procurement and authentication of both medicinal plants (1 month)
  ✓ Procurement of rhizomes of *Aspidium cicutarium*.
  ✓ Procurement of leaves of *Spathodea campanulata*.
  ✓ Authentication of both plants.

- Phase III Extraction with suitable solvents (3 months)
  ✓ Extraction of rhizomes and leaves using various solvents.

- Phase IV Phytochemical evaluation of extracts (6 months)
  ✓ Evaluation of extracts for pharmacognostic properties.
  ✓ Evaluation of phytoconstituents.
  ✓ Evaluation of extracts by TLC, HPTLC techniques.
  ✓ Evaluation on antimicrobial, antioxidant activity.

- Phase V Pharmacological evaluation of extracts (3 months)
  ✓ Evaluation of acute toxicity of extracts.
  ✓ Cell viability studies and anti-inflammatory study on cell line.
  ✓ Evaluation of topical anti-inflammatory activity.
  ✓ Evaluation of wound healing activity.

- Phase VI Preformulation studies on excipients and compatibility of excipients with extracts (3 months)
  ✓ Choice of polymers for film formulation
✓ Compatibility of extracts with polymers by FTIR.

- **Phase VII Formulation and evaluation of films and emulgel formulations (5 months)**
  ✓ Formulation of trial batches of film and factorial batches.
  ✓ Evaluation of properties of films.

- **Phase VIII Optimization of formulation (1 month)**
  ✓ Use of Design Expert software to optimize film and emulgel formulations.

- **Phase IX Evaluation of optimized formulation (6 months)**
  ✓ In vivo studies of optimized formulation.
  ✓ Stability studies and antioxidant studies of optimized formulation.

- **Phase X Compilation of data (4 months)**
5 MATERIALS AND METHODS

5.1 Plant Profile:

5.1.1 *Aspidium cicaturn*  
i. Plant description:43

Class: Filicopsida  
Order: Polypodiales  
Family: Dyropteridaceae  
Genus: Aspidium  
Species: cicaturn  

Common Names: Kombadnakhi, Kukkutnakhi fern species.

Parts of the plants used: Rhizomes, leaves

2a. Leaves of *Aspidium cicaturn*  
2b. Rhizomes of *Aspidium cicaturn*

Figure 2. Leaves and rhizomes of *Aspidium cicaturn*.

It is a pteridophyte fern. The fern is found throughout India from the plains up to 5000 feet height and in the tropics throughout the world. In folklore medicine, it is used in clinical conditions like tonsillitis, arthritis, mental disorders, rheumatism and obesity by Vaidyas of Maharashtra. Rhizome is short creeping, densely scaly at the apex; apex is acuminate. Stipes are scattered.75,76 Lamina ovate or broadly ovate,
about 30×40 cm; base cordate, bipinnate or bipinnatifid. Primary pinnae appear opposite to subopposite or alternate, about 8 cm apart, slightly ascending; secondary pinnae was mostly adnate and decurrent, about five pairs, alternate about 2 cm apart. Multicellular hair densely distributed all over the lamina except the abaxial side of the main rachis. Sori on the end of the veinlets arranged in two rows along the costules of the secondary pinnae or lobes of secondary pinnae, about 1.5 mm in diameter, compital; spores are reniform or planoconvex or spherical, pale brown with broad anasomosed winged perispore.

ii. Macroscopic characters of dried rhizome of *Aspidium cicutarium*

- **Shape:** Horizontal rhizome 15-30 cm in length (covered with stipe-bases, "fingers," which remain green several years and often constitute the greater bulk of the official drug; when peeled (deprived of stipes, roots) the rhizome itself is 7.5-15 cm. (3-6') long, 1-3 cm.
- **Thickness:** 5-7.5 cm. (2-3') thick
- **Surface:** scarred with remains of stipe-bases, or bearing several coarse longitudinal ridges and grooves
- **Colour:** Blackish brown
- **Fracture:** Short
- **Taste:** astringent, bitter, acrid

iii. Chemical constituents in fern species

It has been noted earlier that the chemical constituents present in fern species are derivatives of phloroglucinol and butyric acid. It has been observed that two or more molecules of simple monocyclic derivatives, such as aspidinol, filicinic acid and acylfilicinic acid may get condensed to give rise to bicyclic derivatives, for instance; albaspidin, flavaspidic acid and filic acid.

iv. Uses:

In the folklore medicines and in ancient Ayurveda the rhizomes of *Aspidium cicutarium* were reported to be used as vermifluge (anthelmintic) and as antifertility agent.
5.1.2 *Spathodea campanulata Beauvis*

i. **Plant description:**

Class: Dicotyledons
Order: Scrophulariales
Family: Bignoniaceae
Genus: Spathodea
Species: Campanulata

Common Names: African tulip tree, Flame-of-the-forest, RudraPalash,

Parts of the plants used: Flowers, leaves and barks

---

**Figure 3. Branch with flowers of Spathodea campanulata**

*Spathodea campanulata Beauvis* is a flowering plant belonging to the Bignoniaceae family. It is commonly known as the Fountain Tree, African tulip tree, Flame-of-the-forest, RudraPalash, Pichkari or Nandi Flam. It is a tree that grows between 7–25 m (23–82 ft) tall and is native to tropical Africa. Several phytochemical studies have been performed with different parts of *S. campanulata*, including stem barks, flowers, leaves, and fruits. Spathodic acid, steroids, saponins, ursolic acid, tomentosolic acid,
and pectic substances have ever been isolated from the stem bark. These are also employed in diuretic and anti-inflammatory treatments. Presence of anthocyanins in flowers of *Spathodea campanulata* have been reported in literature. The stem bark preparations are used to treat fungal skin diseases, herpes, stomach aches and diarrhea. Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts. The leaves are used against kidney diseases, urethral inflammations and as an antidote against animal poisons. *In vitro* antimalarial activity against *Plasmodium falciparum* has been reported. The leaves have been found to contain spathodol, caffeic acid, other phenolic acids and flavonoid.

**ii. Botanical Description:**

*Spathodea campanulata* is medium sized, reaching a height of 10-35 m, deciduous, with a round, heavy crown of dense, dark foliage, sometimes somewhat flattened; young bark pale, grey-brown and smooth but turns grey-black, scaly and cracked vertically and horizontally with age. The opposite imparipinnate leaves are extipulate. Each leaf consists of 5-7 pairs of opposite leaflets and a terminal one. The leaflets are oblong elliptic, about 1 cm long and 0.5 cm broad, entire, broadly acuminate, unequal at the base, dark green on top and light green on the underside; there are glandular swellings at the base of the lamina (usually a pair); the midrib and nerves are yellow, raised and very slightly pubescent; the venation is reticulate; the short, thick petiole is about 0.7 cm long; there are conspicuous lenticels on the rachis; rachis base is swollen. Flowers large, red, hermaphrodite, orange inside; calyx green, about 1 cm long and split on the posterior side, ribbed and tomentellous; petals 5, each about 1.5 cm long; stamens 4 with orange filaments; style extruding with a 2-lipped stigma; flower buds curved and contain a red sap. A yellow flowered variety has been reported. Fruit upstanding, dark brown, cigar-shaped, woody pod, 15-25 cm long and split on the ground into 2 boat-shaped valves, releasing many flat winged seeds; 1-4 pods usually develop from 1 flower cluster; seeds thin, flat and surrounded by a filmy wing.
iii. **Ecology**\(^1\):
It grows naturally in Africa in secondary forests in the high forest zone and in deciduous, transition, and savannah forests.

iv. **Biophysical Limits:**
Altitude: 0-2000 m, Mean annual temperature: 27-30 deg. C, Mean annual rainfall: 1300-2000 mm. Soil type: The African tulip tree develops best in fertile, deep, well-drained loams. Soil texture may range from loamy sands to clays, pH is between 4.5-8, and soil drainage may vary from poor to excessive.

v. **Documented Species Distribution:**
Exotic range Angola, Ethiopia, Ghana, Kenya, Sudan, Tanzania, Uganda, Zambia
Native range Colombia, Costa Rica, Cuba, India, Jamaica, Puerto Rico, Sri Lanka, Zanzibar

vi. **Products:**
Food: The seeds are edible and used in many parts of Africa.
Timber: In its original habitat, the soft, light brownish-white wood is used for carving and making drums.
Poison: The hard central portion of the fruit is used to kill animals.
Medicine: The bark has laxative and antiseptic properties, and the seeds, flowers and roots are used as medicine. The bark is chewed and sprayed over swollen cheeks. The bark may also be boiled in water used for bathing newly born babies to heal body rashes.\(^1\)
5.2 Excipient Profile

5.2.1 Eudragit RL 100 \( ^{82} \)

i. **Structure**

\[
\text{[Chemical Structure Image]}
\]

ii. **CAS number:** 33434–24–1

iii. **Formula:** \( C_5H_8O_2 \)

iv. **Molecular Weight (Avg):** 1,50,000

v. **Characters:**

Colourless, clear to cloudy granules with a faint amine-like odour.

vi. **Solubility:**

1 g of the substances dissolves in 7 g aqueous methanol, ethanol and isopropyl alcohol (containing approx. 3 % water), as well as in acetone, ethyl acetate and methylene chloride to give clear to cloudy solutions. The substances are practically insoluble in petroleum ether, 1 N sodium hydroxide and water.

vii. **Stability:**

Minimum stability dates are given on the product labels and batch-related certificates of analysis.

viii. **Typical Properties:**

- Index of Refraction: 1.4
- Molar Refractivity: \( 26.556 \text{ cm}^3 \)
- Molar Volume: \( 109.446 \text{ cm}^3 \)
- Polarizability: \( 10.528 \times 10^{-24} \text{ cm}^3 \)
- Surface Tension: 23.5100002288818 dyne/cm
- Density: 0.915 g/cm\(^3\)
- **Flash Point:** 10 °C
- **Enthalpy of Vaporization:** 33.96 kJ/mol
- **Boiling Point:** 100.299 °C at 760 mmHg
- **Vapour Pressure:** 36.889993896484 mmHg at 25°C

**ix. Storage:**
Store at controlled room temperature (USP, General Notices). Protect against moisture. Any storage between 8°C and 25°C fulfils this requirement. EUDRAGIT® RL 100 tends to form lumps at warm temperatures. This has no influence on the quality. The lumps are easily broken up again.

**x. Application:**
Controlled release organic coatings for tablets and matrix formulations.

### 5.2.2 Ethyl Cellulose

---

**i. Structure**

![Structure of Ethyl Cellulose](image)

**ii. CAS Number:** [9004-57-3]

**iii. Empirical Formula:**
Ethyl cellulose with complete ethoxyl substitution (DS = 3) is C_{12}H_{23}O_{6} (C_{12}H_{22}O_{5})_{n}C_{12}H_{23}O_{5} where \( n \) can vary to provide a wide variety of molecular weights. Ethyl cellulose, an ethyl ether of cellulose, is a long-chain polymer of β-anhydroglucose units joined together by acetal linkages.

**iv. Molecular Weight:** variable

**v. Functional Category:**
Coating agent, flavoring fixative, tablet binder, tablet filler, viscosity-increasing agent.

vi. **Description:**
Ethyl cellulose is a tasteless, free-flowing, and white to light tan-colored powder.

vii. **Typical Properties:**
- Density (bulk): 0.4 g/cm\(^3\)
- Glass transition temperature: 129–133°C
- Auto ignition temperature: 698
- Viscosity: 40-52 cps
- Water vapour transmission: 890 g/m\(^2\)/24hrs, 3-mil. Film

viii. **Moisture content:**
Ethyl cellulose absorbs very little water from humid air or during immersion, and that small amount evaporates readily.

ix. **Solubility:**
Ethyl cellulose is practically insoluble in glycerin, propylene glycol, and water. Ethyl cellulose that contains less than 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures of aromatic hydrocarbons with ethanol (95%). Ethyl cellulose that contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.

tax. **Storage:**
Ethyl cellulose should be stored at a temperature not exceeding 32°C (90°F) in a dry area away from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

xi. **Incompatibilities:**
Incompatible with paraffin wax and microcrystalline wax.

xii. **Applications in Pharmaceutical Formulation or Technology:**
The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation; for
example, where granules are coated with ethyl cellulose to inhibit oxidation. Modified-release tablet formulations may also be produced using ethyl cellulose as a matrix former. In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions, or gels, provided an appropriate solvent is used. Ethyl cellulose has been studied as a stabilizer for emulsions.

5.2.3 Carboxymethylcellulose Sodium

i. Structure

![Structure of Carboxymethylcellulose Sodium]

ii. CAS Number: [9004-32-4]

iii. Synonyms
Akucell; Aqualon CMC; Aquasorb; Blanose; Carbose D; carmellosum natricum; Cel-O-Brandt; cellulose gum; Cethylose; CMC sodium.

iv. Empirical Formula:
The USP 32 describes carboxymethylcellulose sodium as the sodium salt of a polycarboxymethyl ether of cellulose.

v. Molecular Weight: 262.196689 But variable.

vi. Functional Category
Coating agent; stabilizing agent; suspending agent; tablet and capsule disintegrant; tablet binder; viscosity-increasing agent; water-absorbing agent.

vii. Description
Carboxymethylcellulose sodium occurs as a white to almost white, odorless, tasteless, granular powder. It is hygroscopic after drying.

viii. **Typical Properties:**
Melting Point: Approx 2278 °C

Viscosity: 1500-3000 cp

pH: 6-8.5

Loss on drying: Not more than 12% after drying (105°C, to constant weight)

ix. **Moisture content:**
Typically contains less than 10% water. However, carboxymethylcellulose sodium is hygroscopic and absorbs significant amounts of water at temperatures up to 37°C at relative humidities of about 80%.

x. **Solubility:**
Practically insoluble in acetone, ethanol (95%), ether, and toluene. Easily dispersed in water at all temperatures

xi. **Stability and Storage Conditions:**
Carboxymethylcellulose sodium is a stable, though hygroscopic material. Under high-humidity conditions, carboxymethylcellulose sodium can absorb a large quantity (>50%) of water. In tablets, this has been associated with a decrease in tablet hardness and an increase in disintegration time.

xii. **Incompatibilities**
Carboxymethylcellulose sodium is incompatible with strongly acidic solutions and with the soluble salts of iron and some other metals, such as aluminum, mercury, and zinc. It is also incompatible with xanthan gum. Precipitation may occur at pH < 2, and also when it is mixed with ethanol (95%).

xiii. **Safety**
It is generally regarded as a nontoxic and nonirritant material. However, oral consumption of large amounts of carboxymethylcellulose sodium can have a laxative
effect; therapeutically, 4–10 g in daily divided doses of the medium- and high-viscosity grades of carboxymethylcellulose sodium have been used as bulk laxatives.

xiv. **Application:**
Used in detergents, Toothpastes, emulsions, viscosity modifiers, disintegrant for tablet.

5.2.4 **Propylene Glycol**

i. **Structure:**

![Propylene Glycol Structure](image)

ii. **Chemical name:** 1,2-Propanediol

iii. **CAS Number:** 57-55-6

iv. **Synonyms:**
1,2-Dihydroxypropane; E1520; 2-hydroxypropanol; methyl ethylene glycol; methyl glycol; propane-1,2-diol.

v. **Formula:** C₃H₈O₂

vi. **Molecular Weight:** 76.09

vii. **Functional category:**
Antimicrobial preservative; disinfectant; humectant; plasticizer; solvent; stabilizer for vitamins; water-miscible cosolvent.

viii. **Description:**
Propylene glycol is a clear, colorless, viscous, practically odorless liquid with a sweet, slightly acrid taste resembling that of glycerin.

ix. **Solubility:**
Miscible with acetone, chloroform, ethanol (95%), glycerin, and water; soluble at 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils, but will dissolve some essential oils.
x. Typical properties:
- Autoignition temperature 371°C
- Boiling point 188°C
- Density 1.038 g/cm³ at 20°C
- Flammability Upper limit, 12.6% v/v in air; lower limit, 2.6% v/v in air.
- Flash point: 99°C (open cup)
- Heat of combustion: 1803.3 kJ/mol (431.0 kcal/mol)
- Heat of vaporization: 705.4 J/g (168.6 cal/g) at b.p.
- Melting point: −59°C
- Osmolarity: 2.0% v/v aqueous solution is iso-osmotic with serum.
- Refractive index: \( n^2_0 \) = 1.4324
- Specific rotation: \([\alpha]^2_0\): −15.0° (neat) for \((R)\)-form; +15.8° (neat) for \((S)\)-form.
- Specific heat: 2.47 J/g (0.590 cal/g) at 20°C
- Surface tension: 40.1 mN/m (40.1 dynes/cm) at 25°C
- Vapor density: (relative): 2.62 (air = 1)
- Vapor pressure: 9.33 Pa (0.07 mmHg) at 20°C
- Viscosity (dynamic): 58.1 mPa s (58.1 cP) at 20°C

xi. Stability:
At cool temperatures, propylene glycol is stable in a well-closed container, but at high temperatures, in the open, it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid.

xii. Storage:
Propylene glycol is hygroscopic and should be stored in a well-closed container, protected from light, in a cool, dry place.

xiii. Incompatibilities:
Propylene glycol is incompatible with oxidizing reagents such as potassium permanganate.
xiv. **Safety:**
Propylene glycol is used in a wide variety of pharmaceutical formulations and is generally regarded as a relatively nontoxic material. In topical preparations, propylene glycol is regarded as minimally irritant, although it is more irritant than glycerin. Some local irritation is produced upon application to mucous membranes or when it is used under occlusive conditions.

xv. **Applications of Propylene glycol:**
Propylene glycol has been widely used as a solvent, extractant, and preservative in a variety of parenteral and nonparenteral pharmaceutical formulations. Propylene glycol is also used in cosmetics and in the food industry as a carrier for emulsifiers and as a vehicle for flavors in preference to ethanol, since its lack of volatility provides a more uniform flavor.

5.2.5 **Glycerin**

i. **Structure:**

ii. **CAS Number:** 56-81-5

iii. **Synonyms:** 1,2,3-propanetriol; trihydroxypropane glycerol.

iv. **Formula:** \( \text{C}_3\text{H}_8\text{O}_3 \)

v. **Molecular Weight:** 92.09

vi. **Functional Category:**
Antimicrobial preservative, emollient, humectant, plasticizer, solvent, sweetening agent and tonicity agent. In topical pharmaceutical formulations and cosmetics, glycerin is used primarily for its humectant and emollient properties. In parenteral formulations, glycerin is used mainly as a solvent. In oral solutions, glycerin is used as a solvent, sweetening agent, antimicrobial preservative and viscosity-increasing agent. It is also used as a plasticizer and in film coatings. Glycerin is additionally used in topical formulations such as creams and emulsions. Glycerin is used as a
plasticizer of gelatin in the production of soft-gelatin capsules and gelatin suppositories. Glycerin is employed as a therapeutic agent in a variety of clinical applications and is also used as a food additive.

vii. Description:

Glycerin is a clear, colorless, odorless, viscous, hygroscopic liquid; it has a sweet taste, approximately 0.6 times as sweet as sucrose.

viii. Typical Properties:

- Boiling point: 290°C (with decomposition)
- Density: 1.2656 g/cm³ at 15°C; 1.2636 g/cm³ at 20°C; 1.2620 g/cm³ at 25°C.
- Flash point: 176°C
- Hygroscopicity: Hygroscopic
- Melting point: 17.8°C
- Osmolarity: A 2.6% v/v aqueous solution is iso osmotic with serum.
- Solubility: Soluble in water, methanol and ethanol.

ix. Stability and Storage Conditions

Glycerin is hygroscopic. Pure glycerin is not prone to oxidation by the atmosphere under ordinary storage conditions but it decomposes on heating, with the evolution of toxic acrolein. Mixtures of glycerin with water, ethanol (95%), and propylene glycol are chemically stable. Glycerin may crystallize if stored at low temperatures; the crystals do not melt until warmed to 20°C. Glycerin should be stored in an airtight container, in a cool, dry place.

5.2.6 Polyethylene Glycol

i. Structural Formula:

![Structural Formula](image)

ii. CAS Number: [25322-68-3]

iii. Chemical name: α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)

iv. Synonyms: PEG, polyoxyethylene glycol.

v. Empirical Formula:

\[
\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_m\text{CH}_2\text{OH}
\]

Alternatively, the general formula \(\text{H(OCH}_2\text{CH}_2)_n\text{OH}\) may be used to represent polyethylene glycol, where \(n\) is a number \(m\) in the previous formula + 1.

vi. Description:

Polyethylene glycol is an addition polymer of ethylene oxide and water. Polyethylene glycol grades 200–600 are liquids; grades 1000 and above are solids at ambient temperatures.

Liquid grades (PEG 200–600) occur as clear, colorless or slightly yellow-colored, viscous liquids. They have a slight but characteristic odor and a bitter, slightly burning taste. PEG 400 can occur as a solid at ambient temperatures. Solid grades (PEG>1000) are white or off-white in color, and range in consistency from pastes to waxy flakes. They have a faint, sweet odor. Grades of PEG 6000 and above are available as free-flowing milled powders.

vii. Functional Category: Carrier solvent, excipient

viii. Typical Properties:
• Flash Point : 390 °F
• Refractive index : 1.4630
• Density :1.1254
• Freezing range : 4-8°C

ix. Toxicity And Safety:
Polyethylene glycols are chemically stable in air and in solution, although grades with a molecular weight less than 2000 are hygroscopic. Polyethylene glycols do not support microbial growth, and they do not become rancid.

Polyethylene glycols should be stored in well-closed containers in a cool, dry place. Stainless steel, aluminum, glass, or lined steel containers are preferred for the storage of liquid grades.

x. Applications in pharmaceutical formulation or technology
• PEGs are widely used in a variety of pharmaceutical formulations including parenteral, topical, ophthalmic, oral and rectal preparations.
• Polyethylene glycols are stable, hydrophilic substances that are essentially nonirritant to skin. Although they do not readily penetrate the skin, PEGs are water soluble and as such are easily removed from skin by washing; they are therefore useful as ointment base.
• Aqueous polyethylene glycol solutions can be used either as suspending agents or to adjust the viscosity and consistency of other suspending vehicles.
• Liquid polyethylene glycols are used as water miscible solvents for the contents of soft gelatin capsules.
• In concentration up to approximately 30% v/v, PEG 300 and PEG 400 have been used as the vehicle for parenteral dosage forms.
• Polyethylene glycols can also be used to enhance solubility or dissolution characteristics of poorly soluble compounds by making solid dispersions.
5.2.7 **Hydroxy Propyl Methylcellulose**\(^{82}\)

i. **Structure:**

![Structure Diagram]

\[ R = H, -CH_3 \text{ or } -\text{CH}_2\text{CH(OH)CH}_3 \ n: \text{Polymerization degree} \]

ii. **CAS Number:** 9004-65

iii. **Synonyms:**
Benecel MHPC; E464; hydroxypropyl methylcellulose; HPMC; Methocel; methylcellulose propylene glycol ether; methyl hydroxypropylcellulose; Metolose; Tylopur.

iv. **Functional Category:**
Coating agent; film-former; rate-controlling polymer for sustained release; stabilizing agent; suspending agent; tablet binder; viscosity-increasing agent.

v. **Description:**
Hypromellose is an odorless and tasteless, white or creamy-white fibrous or granular powder.

vi. **Typical Properties:**
- Acidity/alkalinity: \( \text{pH} = 5.5 \text{ to } 8.0 \) for a 1% w/w aqueous solution.
- Ash: 1.5-3.0%, depending upon the grade and viscosity.
- Auto ignition temperature: 360°C
- Density (bulk): 0.341 g/cm\(^3\)
- Density (tapped): 0.557 g/cm\(^3\)
- Density (true): 1.326 g/cm\(^3\)
- Melting point: Browns at 190.200°C; chars at 225.230°C

vii. **Solubility:**
Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol / methanol and dichloromethane, and mixtures of water and alcohol.

viii. **Specific gravity:** 1.26

ix. **Moisture content:**

Hypromellose absorbs moisture from the atmosphere; the amount of water absorbed depends upon the initial moisture content and the temperature and relative humidity of the surrounding air.

x. **Viscosity (dynamic):**

Typical viscosity values for 2% (w/v) aqueous solutions of Methocel nominal viscosity (mPa s), methocel K4M Premium 4000.

xi. **Stability and Storage Conditions:**

Hypromellose powder is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3.11. Hypromellose undergoes a reversible sol.gel transformation upon heating and cooling, respectively. The gel point is 50.90°C, depending upon the grade and concentration of material. Aqueous solutions are comparatively enzyme-resistant, providing good viscosity stability during long-term storage. However, aqueous solutions are liable to microbial spoilage and should be preserved with an antimicrobial preservative aqueous solutions may also be sterilized by autoclaving; the coagulated polymer must be redispersed on cooling by shaking. Hypromellose powder should be stored in a well-closed container, in a cool, dry place.

xii. **Incompatibilities:**

Hypromellose is incompatible with some oxidizing agents. Since it is nonionic, hypromellose will not complex with metallic salts or ionic organics to form insoluble precipitates.

xiii. **Safety:**

Hypromellose is widely used as an excipient in oral and topical pharmaceutical formulations. It is also used extensively in cosmetics and food products.
Hypromellose is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption have a laxative effect.

xiv. **Handling Precautions:**
Hypromellose dust may be irritant to the eyes and eye protection is recommended. Excessive dust generation should be avoided to minimize the risks of explosion. Hypromellose is combustible.

xv. **Related Substances:**
Hydroxyethyl cellulose; hydroxyethylmethyl cellulose; hydroxypropyl cellulose; hypromellose phthalate; methyl cellulose.

xvi. **Applications in Pharmaceutical Formulation or Technology:**
Hypromellose is widely used in oral, ophthalmic and topical pharmaceutical formulations. In oral products, hypromellose is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended-release tablet formulations. Depending upon the viscosity grade, concentrations of 2.20% w/w are used for film forming solutions to film-coat tablets. Lower-viscosity grades are used in aqueous film coating solutions, while higher-viscosity grades are used with organic solvents. Hypromellose is also used as a suspending and thickening agent in topical formulations. As a protective colloid, it can prevent droplets and particles from coalescing or agglomerating, thus inhibiting the formation of sediments. It is also widely used in cosmetics and food products.

### 5.2.8 Rice bran oil

i. **CAS number:** 68553-81-1

ii. **Composition:**
Rice Bran Oil is 81.3-84.3% triglycerides, 2-3% diglycerides, 5-6% monoglycerides, 2-3% free fatty acids, 0.3% waxes, 0.8% glycolipids, 1.6% phospholipids, 4% unsaponifiables.

iii. **Functional Category:**
Emollient, oleaginous vehicle; antioxidant, sunscreen agent, antimicrobial

iv. **Description:**
Rice bran oil is clear, pale yellow-colored oil

v. **Typical Properties:**
   - Iodine Value: 99-108
   - Saponification Value: 180-190
   - Smoke point: 232°C
   - Fire point: 352 °C
   - Refractive index: 1.470-1.473
   - Unsaponifiable matter: 3.50%
   - Relative density: 0.912 - 0.920

vi. **Storage:**

Can be stored at room temperature.

vii. **Uses:**

Used as a cooking oil. Has been reported to possess antifungal activity.

---

5.2.9 **Polyoxyethylene Sorbitan Fatty Acid Esters (Tween 80)**

i. **Structure**

![Structure diagram](image)

ii. **CAS Numbers:** 9005-65-6

iii. **Synonyms:** Polysorbatum 80; Tween 80
iv. **Formula:** $C_{64}H_{124}O_{26}$
v. **Molecular Weight:** 604.8128
vi. **Functional Category:**
Dispersing agent; emulsifying agent; nonionic surfactant; solubilizing agent; suspending agent; wetting agent.
vii. **Description:**

viii. **Typical Properties:**
- Boiling point: $>100^\circ C$
- Density: 1.08 g/ml at 20°C
- Vapour Pressure: $<1$ mm Hg
- Refractive Index: $n/20 \ 1.473$
- Flash point: $>230^\circ F$

ix. **Storage:** Store at room temperature.

x. **Applications in Pharmaceutical formulation:**
Polysorbates containing 20 units of oxyethylene are hydrophilic nonionic surfactants that are used widely as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They may also be used as solubilizing agents for a variety of substances including essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions.

---

**5.2.10 Sorbitan Fatty Acid Esters (Span 80)**

i. **Structure**
ii. **CAS Numbers:** 1338-43-8

iii. **Synonyms:**
Ablunol S-80; Arlacel 80; Armotan MO; Capmul O; Crill 4; Crill 50; Dehymuls SMO; Drewmulse SMO; Drewsorb 80K; E494; GlycomulO; Hodag SMO; Lamesorb SMO; LiposorbO; Montane 80; Nikkol SO-10; Nissan Nonion OP-80R. Sorbitan monooleate.

iv. **Molecular Formula:** C_{64}H_{124}O_{26}.

v. **Molecular weight:** 428.60

vi. **Functional Category:**
Dispersing agent; emulsifying agent; nonionic surfactant; solubilizing agent; suspending agent; wetting agent.

vii. **Description:** Non ionic surfactant liquid

viii. **Typical Properties:**
- Acid value: > 8
- Hydroxyl value: 190–215
- Iodine value: 62–76
- Peroxide value: >10.0
- Saponification value: 145–160
- Residue on ignition: > 0.5%
- Flash point: >149ºC
- Density (g/cm3): 1.01
- HLB value: 4.3
- Hydroxyl value: 193–209
- Pour point (0C): -12 º C
- Saponification value: 149–160
- Viscosity at 250C (mPa.s): 970–1080
- Water content (%): 0.5

ix. **Solubility:**
Sorbitan esters are generally soluble or dispersible in oils; they are also soluble in most organic solvents. In water, although insoluble, they are generally dispersible.

x. Stability and Storage Conditions:
Gradual soap formation occurs with strong acids or bases; sorbitan esters are stable in weak acids or bases. Sorbitan esters should be stored in a well-closed container in a cool, dry place.

xi. Safety:
Sorbitan esters are widely used in cosmetics, food products, and oral and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials.

xii. Applications in Pharmaceutical formulation:
Sorbitan esters are widely used in cosmetics, food products, and pharmaceutical formulations as lipophilic nonionic surfactants. They are mainly used in pharmaceutical formulations as emulsifying agents in the preparation of creams, emulsions, and ointments for topical application.

5.2.11 Carbomer 934

i. Structure

ii. CAS number: 9007-16-3

iii. Synonyms:
Acrypol; Acritamer; acrylic acid polymer; carbomera; Carbopol; carboxy polymethylene; polyacrylic acid; carboxyvinyl polymer; Pemulen; Tego Carbomer.

iv. Composition:
Carbomers are synthetic high-molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 52% and 68% of carboxylic acid (COOH) groups calculated on the dry basis.

v. Molecular Weight:
The molecular weight of carbomer is theoretically estimated at 7×105 to 4×109.

vi. Functional Category:
Bioadhesive material; controlled-release agent; emulsifying agent; emulsion stabilizer; rheology modifier; stabilizing agent; suspending agent; tablet binder.

vii. Description:
Carbomers are white-colored, ‘fluffy’, acidic, hygroscopic powders with a characteristic slight odor.

viii. Typical Properties:

- Acidity/alkalinity: pH = 2.5–4.0 for a 0.2% w/v aqueous dispersion
- Density (bulk): 0.2 g/cm3 (powder); 0.4 g/cm3 (granular)
- Density (tapped): 0.3 g/cm3 (powder); 0.4 g/cm3 (granular)
- Dissociation constant pKa: 6.0-0.5
- Glass transition temperature: 100–105°C
- Moisture content: 2% w/w
- Specific gravity: 1.41

ix. Particle size distribution:
Primary particles average about 0.2 mm in diameter. The flocculated powder particles average 2–7 mm in diameter and cannot be broken down into the primary particles. A granular carbomer has a particle size in the range 150–425 mm.

x. Solubility:
Swellable in water and glycerin and, after neutralization, in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels.

**xi. Viscosity (Dynamic):**

Carbomers disperse in water to form acidic colloidal solutions of low viscosity these solutions when neutralized, produce highly viscous gels. Neutralization expands long chain of carbomers by charge repulsion to produce an ‘entangled gel’ network. Under acid conditions a small proportion of –COOH group on polymer chain dissociates to promote a flexible coil. Base addition dissociates more groups and electrostatic repulsion between charged regions extends the molecule, making it more rigid and so augmenting the gel. Neutralized aqueous gels are more viscous at pH 6-11. Examples of neutralizing agents are strong bases (sodium hydroxide), amines (triethanolamine), weak inorganic amines (ammonium hydroxide) etc. 1 gm of carbomer is neutralized by approximately 0.4 gm of triethanolamine.

**xii. Stability and Storage Conditions:**

Carbomers are stable, hygroscopic materials. These may be heated at temperatures below 104 °C for upto 2 hours without affecting their thickening efficiency. However, exposure to excessive temperature can result in discoloration and decreased stability. Exposure to light causes oxidation that is reflected in a decrease in dispersion viscosity.

**xiii. Incompatibilities:**

Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes.
5.2.12 Triethanolamine

i. Structure

![Formula](attachment:formula.png)

ii. CAS Number: 102-71-6

iii. Synonyms: TEA; Tealan; triethylolamine; trihydroxytriethylamine; tris (hydroxyethyl)amine; trolaminum.

iv. Empirical Formula: C₆H₁₅NO₃

v. Molecular Weight: 149.19

vi. Functional Category: Alkalizing agent; emulsifying agent.

vii. Description: Triethanolamine is a clear, colorless to pale yellow-colored viscous liquid having a slight ammoniacal odor.

viii. Typical Properties:
- Surface tension: 48.9 mN/m (48.9 dynes/cm) at 25°C
- Viscosity (dynamic): 590 mPa s (590 cP) at 30°C
- Acidity/alkalinity: pH = 10.5 (0.1 N solution)
- Boiling point: 335°C
- Flash point: 208°C
- Freezing point: 21.6°C
- Hygroscopicity: very hygroscopic
- Melting point: 20–21°C
- Moisture content: 0.09%

ix. Stability and Storage Conditions:
Triethanolamine may turn brown on exposure to air and light. The 85% grade of triethanolamine tends to stratify below 15°C; homogeneity can be restored by
warming and mixing before use. Triethanolamine should be stored in an airtight container protected from light, in a cool, dry place.

x. Incompatibilities:
Triethanolamine is a tertiary amine that contains hydroxy groups; it is capable of undergoing reactions typical of tertiary amines and alcohols. Triethanolamine will react with mineral acids to form crystalline salts and esters. With the higher fatty acids, triethanolamine forms salts that are soluble in water and have characteristics of soaps. Triethanolamine will also react with copper to form complex salts. Discoloration and precipitation can take place in the presence of heavy metal salts.

xi. Safety:
Triethanolamine is used primarily as an emulsifying agent in a variety of topical pharmaceutical preparations. Although generally regarded as a nontoxic material, triethanolamine may cause hypersensitivity or be irritant to the skin when present in formulated products.

xii. Applications in Pharmaceutical formulation:
Triethanolamine is widely used in topical pharmaceutical formulations primarily in the formation of emulsions. Triethanolamine is also used in salt formation for injectable solutions and in topical analgesic preparations. It is also used in sun-screen preparation.

5.2.13 Propyl paraben

i. Structure:

![Propyl paraben structure](image)

ii. CAS Number: 94-13-3

iii. Synonyms: Aseptoform P; CoSept P; E216; 4-hydroxybenzoic acid

iv. Formula: C_{10}H_{12}O_{3}
v. **Molecular Weight:** 180.20

vi. **Functional Category:** Antimicrobial preservative.

vii. **Applications in Pharmaceutical formulation:**
Propyl paraben is widely used as an antimicrobial preservative in cosmetics, food products, and pharmaceutical formulations. It may be used alone, in combination with other paraben esters, or with other antimicrobial agents. It is one of the most frequently used preservatives in cosmetics.

Propylparaben occurs as a white, crystalline, odorless, and tasteless powder.

viii. **Typical Properties:**
- Melting Point: 98 °C
- Boiling point: 295°C
- Density(true): 1.288 g/cm3
- Dissociation constant pKa: 8.4 at 22°C

ix. **Stability and Storage Conditions:**
Aqueous propylparaben solutions at pH 3–6 can be sterilized by autoclaving, without decomposition. At pH 3–6, aqueous solutions are stable (less than 10% decomposition) for up to about 4 years at room temperature, while solutions at pH 8 or above are subject to rapid hydrolysis (10% or more after about 60 days at room temperature). Propylparaben should be stored in a well-closed container in a cool, dry place.

x. **Safety:**
Propylparaben and other parabens are widely used as antimicrobial preservatives in cosmetics, food products, and oral and topical pharmaceutical formulations.
5.2.14 Methyl paraben

i. **Structure:**

![Structure of Methyl paraben](image)

ii. **Synonyms:**
Aseptoform M; CoSept M; E218; 4-hydroxybenzoic acid methyl ester; metagin;
Methyl Chemosept; methylis parahydroxybenzoas; methyl p-hydroxybenzoate;
Methyl Parasept; Nipagin M; Solbrol M; Tegosept M; Uniphen P-23.

iii. **CAS Number:** 99-76-3

iv. **Formula:** C₈H₈O₃

v. **Molecular Weight:** 152.15

vi. **Functional Category:** Antimicrobial preservative.

vii. **Typical Properties:**
- Melting point: 125-128°C
- Boiling point: 280 °C
- Density(true): 1.352g/cm³
- Dissociation constant pKa: 8.4 at 22 °C

viii. **Storage:** Can be stored at room temperature in dry place away from moisture.

ix. **Applications in Pharmaceutical formulation:**
Methyl paraben is widely used as an antimicrobial preservative in cosmetics, food
products, and pharmaceutical formulations.
5.2.15 **Oleic acid**

![Chemical Structure of Oleic Acid](image)

i. **CAS Number:** 112-80-1

ii. **Formula:** $\text{CH}_3\text{(CH}_2)_7\text{CH}=\text{CH(\text{CH}_2)_7\text{COOH}}$

iii. **Molecular Weight:** 282.46

iv. **Typical Properties:**
   - Vapour pressure: 1mm Hg
   - Refractive index: 1.459
   - Boiling point: 194-195° C
   - Melting point: 13-14° C
   - Density: 0.89g/ml
   - Storage temperature: -20° C

v. **Solubility:**
   Oleic acid is practically insoluble in water; soluble in alcohol, benzene, chloroform, ether, fixed and volatile oils.

vi. **Storage:**
    The product should be kept well closed and protected from light. The neat liquid on exposure to air, especially when impure, can cause oxidation and the oleic acid can acquire a yellow to brown color and a rancid odor.

vii. **Applications:**
   Used in pharmaceutical preparations as penetration enhancers in various dosage forms.
5.3 Methodology

5.3.1 Equipments and softwares used in the study.

i. List of Equipments and Instruments used in the study.

Table 1. List of equipments and instruments used in the study

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Name of the equipment</th>
<th>Name of manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UV spectrophotometer</td>
<td>Jasco</td>
</tr>
<tr>
<td>2.</td>
<td>FTIR</td>
<td>Jasco</td>
</tr>
<tr>
<td>3.</td>
<td>Tensile /Adhesive strength apparatus</td>
<td>Ubique</td>
</tr>
<tr>
<td>4.</td>
<td>Bionocular Microscope</td>
<td>Motic B3 Professional Series</td>
</tr>
<tr>
<td>5.</td>
<td>pH meter</td>
<td>Systronics</td>
</tr>
<tr>
<td>6.</td>
<td>Incubator</td>
<td>Labindia</td>
</tr>
<tr>
<td>7.</td>
<td>Autoclave</td>
<td>Labindia</td>
</tr>
<tr>
<td>8.</td>
<td>Franz Diffusion cell.</td>
<td>Fabricated</td>
</tr>
<tr>
<td>9.</td>
<td>Viscometer</td>
<td>Brookefield</td>
</tr>
<tr>
<td>10.</td>
<td>Ultra Turrex homogenizer</td>
<td>IKA 25</td>
</tr>
<tr>
<td>11.</td>
<td>Electronic Balance</td>
<td>Mettler Toledo</td>
</tr>
<tr>
<td>12.</td>
<td>Micrometer screw guage</td>
<td>J B Scientific</td>
</tr>
<tr>
<td>13.</td>
<td>Stability chamber</td>
<td>Thermolab</td>
</tr>
</tbody>
</table>

Table 2. List of softwares used in the study

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Software used in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PCP Disso</td>
</tr>
<tr>
<td>2.</td>
<td>Design Expert Software</td>
</tr>
<tr>
<td>3.</td>
<td>Graph Pad prism</td>
</tr>
</tbody>
</table>

5.3.2 Authentication of plants.

i. Authentication of *Aspidium cicutarium* rhizomes.

The plant specimens for the proposed study were purchased from Ayurvedic Bhandar located in Bibvewadi, Pune, Maharashtra, India. The dried rhizomes of *Aspidium cicutarium* were authenticated by Dr. Upadhyay Senior Scientist at Botany Department at Agharkar Research Institute, Pune, India. A voucher specimen was stored in our laboratory.
ii. **Authentication of *Spathodea campanulata* leaves.**

The plant specimens for the proposed study were collected from local *Spathodea campanulata* trees growing in Pune and were subjected to authentication to Botanical Survey of India. The specimen was authenticated by Dr. J Jayanthi from Botanical Survey of India. A voucher Specimen was deposited.

5.3.3 **Pharmacognostic Evaluation:**

i. **Macroscopic and Microscopic evaluation:**

The macroscopic and microscopic features were studied according to the standard methods. For macroscopy, the colour, odour, taste, size, shape, apex was studied. Microscopical studies were done by preparing a thin section of the specimen. The sections were cleared, stained with Phloroglucinol and Hydrochloric acid and mounted in glycerine and observed under microscope using 10X lens. For powder characteristics, the same staining reagent was used.

ii. **Determination of Physical constants.**

- **Determination of foreign organic matter:**

1 gm of air dried coarsely powdered drug was spread in a thin layer. The sample was inspected with the unaided eye. The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined from the weight of the drug taken.

- **Determination of Loss on Drying:**

Accurately weighed glass stoppered, shallow weighing bottle was dried and 2g of sample was transferred to the bottle and covered, the weight was taken and sample was distributed evenly and poured to a depth not exceeding 10 mm. Then loaded bottle was kept in oven and stopper was removed. The sample was dried to constant weight. After drying it was collected to room temperature in a desiccator. Loss on drying in terms % w/w was calculated.
• **Total Ash:**

Accurately weighed 2g of the air-dried crude drug was taken in a tared silica dish and incinerated at a temperature not exceeding 450°C until free from carbon, cooled in a desiccator and weight was taken. The process was repeated till constant weight was obtained. The percentage of ash was calculated with reference to air-dried drug.

• **Water-Soluble Ash:**

The ash, obtained as per the method described above was boiled for 5 minutes with 25 ml of water, filtered, and the insoluble matter in a Gooch crucible, was collected and washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C and weight was taken. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

• **Acid-Insoluble Ash:**

The ash obtained as per method described above was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, filtered, and the insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited, and cooled in a desiccator and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

• **Determination of water-soluble extractive value:**

5 gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs.
• **Determination of alcohol-soluble extractive value:**

5 gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs. Same process was repeated for methanol soluble extractive value. The solvent ethanol was replaced by methanol.

• **Determination of chloroform soluble extractive value:**

5 grams of air-dried drug was macerated with 100 ml of chloroform in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of Chloroform. 25 ml of filtrate was evaporated to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage chloroform soluble extractive was calculated with reference to the air-dried drug.

5.3.4 **Preparation of extracts**

i. **Methanolic and aqueous extract of rhizomes of *Aspidium cicutarium***

50 gm of powdered rhizomes of *Aspidium cicutarium* were taken and defatted with Petroleum ether (60-80°C). The defatted rhizomes were further extracted using Methanol to get methanolic extract using a Soxhlet apparatus. The liquid extract was subjected to rotary vacuum evaporator to evaporate excess solvent and dried extract was obtained.

For aqueous extract distilled water was used to carry out extraction. Defatted powdered rhizomes were boiled with distilled water and liquid extract was further concentrated to get dried extract.

ii. **Methanolic and aqueous extract of leaves of *Spathodea campanulata***
Similarly for *Spathodea campanulata* dried leaves were first powdered coarsely in a grinder and defatted with Petroleum ether (60-80°C). 100 g of defatted powder was packed in a Soxhlet apparatus and extracted with methanol. Similarly for aqueous extract 100 g of dried powdered drug was defatted using Petroleum ether (60-80°C) and then extracted with distilled water. The extraction was carried out until the extractive becomes colorless. The methanolic extract was filtered and evaporated under reduced pressure using Rotary vacuum Evaporator. The aqueous extract was filtered, evaporated and dried.

5.3.5 **Preliminary phytochemical investigation**

The extracts were concentrated and subjected to phytochemical screening using standard procedures. The compounds were analysed for alkaloids, glycosides, flavonoids, tannins, proteins, carbohydrates, amino acids and steroid.

i. **Test for Carbohydrates:**

- **Molisch test:** Two ml of extract solution was treated with few drops of 15 percent ethanolic α- naphthol solution in a test tube and 2 ml of concentrated Sulphuric acid was added carefully along the side of tubes. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

ii. **Test for reducing sugars:**

- **Benedict’s test:** To 2 ml of Benedict’s reagent, 1 ml of extract solution was added, warmed, and allowed to stand. Formation of red precipitate indicates presence of sugars.

- **Fehling’s test:** 5 ml of extract solution was mixed with 5 ml Fehling’s solution (equal mixture of Fehling’s solution A and B) and boiled. Development of brick red precipitate indicates the presence of reducing sugars.

iii. **Test for monosaccharides:**

- **Barfoed’s test:** Equal volume of Barfoed’s reagent and test solution was mixed and heated for 1–2 min. in boiling water bath and cooled. Red precipitate indicates presence of monosaccharides.
v. **Test for Proteins:**
- **Biuret test:** The extract was treated with 1 ml of 10 percent sodium hydroxide solution and heated. A drop of 0.7 percent copper sulphate solution was added to the above mixture. The formation of purple violet colour indicates the presence of proteins.
- **Million’s test:** The extract was treated with 2 ml of Million’s reagent. Formation of white precipitate indicates the presence of proteins and amino acids.

vi. **Test for amino acids:**
- **Ninhydrin test:** The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Formation of purple colour indicates the presence of amino acids.

vii. **Test for Steroids:**
- **Salkowski test:** 1 ml of concentrated Sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.
- **Liebermann – Burchard reaction:** 2 ml of extracts were mixed with chloroform. Add 1 – 2 ml acetic anhydride and two drops of concentrated Sulphuric acid from the side of the test tube. First red, then blue and finally green colour suggested presence of steroid.

viii. **Test for Cardiac Glycosides:**
- **Test for deoxysugars (Keller-Killiani test):** To 2 ml of extract, glacial acetic acid, one drop 5 % Ferric chloride and conc. Sulphuric acid was added. Presence of cardiac glycosides was indicated by formation of reddish brown colour at junction of the two liquid layers and upper layer appeared bluish green.
- **Legal’s test (Test for cardenoloids):** To the extract 1 ml pyridine and 1 ml sodium nitroprusside was added. Appearance of Pink to red colour indicated presence of cardiac glycosides.
ix. **Test for Anthraquinone Glycosides:**

- **Borntrager’s test:** To 3 ml extract, dil. H$_2$SO$_4$ was added and the mixture was boiled and filtered. To cold filtrate, equal volume benzene or chloroform was added. The mixture was shaken. The organic layer was separated and ammonia was added to it. Ammonical layer turned pink or red.

- **Modified Borntrager's test:** To 5 ml extract 5 ml, 5 % FeCl$_3$ and 5 ml dil. HCl was added. It was heated by boiling in a water bath. It was cooled and benzene or any organic solvent was added and shaken well. Organic layer was separated and equal volume dilute ammonia was added to it. Appearance of pinkish red colour showed presence of glycosides.

x. **Test for Alkaloids:** To the extract, add dilute HCL. Shake well and filter. With filtrate perform following tests.

- **Dragendorff’s test:** To 2-3 ml filtrate, few drops Dragendorff’s reagent were added. Orange brown precipitate indicate presence of alkaloids.

- **Mayer’s test:** To 2-3 ml filtrate few drops of Mayer’s reagent was added to give precipitate.

- **Hager’s test:** 2-3 ml. filtrate with few drops Hager’s reagent gives yellow ppt.

- **Wagner’s test:** 2-3 ml. filtrates with few drops Wagner’s reagent gives reddish brown ppt.

xi. **Test for Tannins and Phenolic compounds:**

- **Ferric Chloride test:** 5 ml of extract solution was allowed to react with 1 ml of 5 % ferric chloride solution. Greenish black colouration indicates the presence of tannins.

- **Lead acetate test:** 5 ml of extract solution was allowed to react with 1 ml of 10 percent aqueous lead acetate solution. Development of yellow coloured precipitate indicates the presence of tannins.

xii. **Test for Flavonoids:**

- **Lead acetate test:** To the extract few drops of 10 percent lead acetate are added Development of yellow coloured precipitate confirms the presence of flavonoids.
• **Sodium Hydroxide test:** To the extract increasing amount of Sodium Hydroxide was added gives yellow colour, which disappeared after addition of acid.

• **Shinoda test (Magnesium Hydrochloride reduction test):** To the test solution add few fragments of Magnesium turning and add cone. Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

5.3.6 **Determination of Total Flavonoid Content**\(^90,92\)

An aluminium chloride colorimetric method was used for flavonoids determination. The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids, in presence of alkaline medium(NaOH, NaNO\(_2\)) to give color intensity as per concentration of flavonoid present in that, which can be detected spectrophotometrically. The standard curve was prepared using concentration range of catechin (100-3600\(\mu\)g/ml) in methanol. 0.3 ml of aluminium chloride 10\% and 3 ml of NaNO\(_2\) (5\% w/v) were added to 1 ml of sample and kept for 5 minutes. To this 2 ml NaOH (1 M) was added and it was kept for 5 minutes and distilled water was used to make the final volume upto 10 ml. The mixture was allowed to stand at room temperature for 15 min and the absorbance of the reaction mixture was measured at 510 nm. The total flavonoid contents were calculated as catechin equivalent.

5.3.7 **Determination of Total Phenolic Content**\(^54\)

Folin–Ciocalteau colorimetric method was used to determine the total phenolic content of the methanolic and aqueous extract. The Folin–Ciocalteau reagent (FCR) is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. However, this reagent does not only measure total phenols and will react with any reducing substance. The reagent therefore measures the total reducing...
capacity of a sample, not just the level of phenolic compounds. Folin & Ciocalteu’s phenol reagent does not contain phenol. Rather, the reagent will react with phenols and nonphenolic reducing substances to form chromogens/phenolates that can be detected spectrophotometrically. The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valency. Addition of Folin-Ciocalteau’s phenol reagent generates chromogens that give increasing absorbance between 550 nm and 750 nm the phenolates are only present in alkaline solution but the reagent and products are alkali unstable. Hence a moderate alkalinity and a high reagent concentration are used in the procedure below.

Gallic acid was used as the standard and the standard curve was plotted using concentration range of 10 -100µg/ml. 1ml of sample was taken, to which 5mL of Folin-Ciocalteau reagent and 4mL of 7.5% Na$_2$CO$_3$ were added, then the volume was made up to 10 ml with methanol : water (6:4). After 1 hour of incubation at room temperature, the absorbance was measured at 740 nm against reagent blank. The results were expressed as gallic acid equivalents.

### 5.3.8 Antioxidant activity of extracts

Antioxidant activity of both methanolic and aqueous extracts was determined using DPPH assay. DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) is stable nitrogen centered free radical, with deep violet color gives absorption within 515-528 nm range. Assay is based on ability of DPPH radical to react with H$_2$ donor species mainly phenols. Upon receiving proton from extract constituent, the colour of DPPH solution fades and changes to yellow. Percentage DPPH scavenging activity increases as the concentration of phenols increases. 3.3mg of DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was dissolved in 100ml Methanol. Ascorbic acid stock solution of concentration 100µg/ml was prepared in distilled water and further dilutions were made to obtain 10-80µg/ml of ascorbic acid. Extract was weighed and dilutions were made using distilled water for aqueous extract and methanol for methanolic extract to yield concentrations ranging from 10 -80 µg/ml. 1 ml extract solution was mixed with 1 ml
DPPH in Methanolic solution and vortexed thoroughly. The resulting mixture was kept in dark for 30 minutes and absorbance was noted at 517 nm. % inhibition was measured using the formula.

\[
\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}.
\]

The concentration of Ascorbic acid that shows 50% inhibition was noted. The control absorbance was noted using DPPH in methanol.

5.3.9 In vitro anti-inflammatory activity

The invitro anti-inflammatory activity was determined by protein denaturation assay using bovine serum albumin as the protein. Proteins are required for structural integrity of our body. Denaturation is phenomenon that involves transformation of well defined, folded structure of protein formed under physiological condition, to an unfolded state under non-physiological condition. When we heat bovine albumin (protein) solution, it leads to denaturation which increases solubility of the protein upon cooling its solubility decreases hence turbidity increases which can be measured spectrophotometrically. Denaturation of tissue proteins is one of the well-documented causes of inflammatory diseases. Production of auto antigens may be due to denaturation of proteins in vivo. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. Bovine albumin was weighed and 1% solution was made by dissolving initially in Dimethylformamide then diluted with phosphate buffer pH 7.4. Similarly extracts were weighed and diluted to get concentration range of 100- 800 μg/ml of extract solution in phosphate buffer for all the extracts. 1 ml of extract solution was mixed with 1 ml of albumin solution. The mixture was incubated for 15 minutes at 27º C. It was then kept for 10 min at 60 º C and then cooled and spectrophotometric determinations were made at 660nm. % Inhibition was calculated by the formula.

\[
(Absorbance \text{ of Control} - \text{Absorbance of Test}) \div \text{Absorbance of Control} \times 100.
\]

Results were compared with Diclofenac sodium as standard.
5.3.10 Antimicrobial activity\textsuperscript{52, 95, 96}
Cup plate method was used to determine the antimicrobial activity of extracts. Beef extract, Peptone and Sodium chloride was dissolved into Distilled Water in 500ml conical flask. It was gently heated for homogeneous solution. Agar powder was added at the end. Conical flask was covered with cotton plug. Nutrient agar was sterilized in autoclave at 15-20 psig, 121°C, for 30 minutes. Petri plates, pipette was wrapped in to paper and sterilized in to hot air oven at 160°C for 30 minutes. Spreader and cork borer was sterilized by ethanol. Nutrient agar was aseptically poured into Petri plates. 2-3 drops of standard culture were added on nutrient agar. It was spread on nutrient agar in Petri plate with spreader. Wells were made in Petri plate with cork borer. Extract solution was added into well. Petri plates were labeled and incubated for 24hrs.at 37° C in incubator. Zone of Incubation was measured on the zone reader. Ampicillin (10µg/ disc) was used as standard.

5.3.11 Thin Layer Chromatographic studies\textsuperscript{97, 98}
i. Steps involved in performing TLC of extracts:

- **Precoated TLC plate:** (silica gel- G\textsubscript{60}F\textsubscript{254})
- **Activation of TLC plate:**
  Heating in oven for 30 min. at 105°C activated TLC plate.
- **Sample application:** Dipping the capillary into the solution to be examined and applied the sample by capillary touched to the thin layer plate at a point about 2 cm from the bottom. The spot was air dried.
- **Chamber saturation:** The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid and allowed to staurate about 30 min.
- **Chromatogram development:** After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. The solvent was allowed to run around 10-15 cm on the
silica plate. TLC chamber was saturated with mobile phase Ethyl acetate : benzene (95:5) for detection of steroidal compounds. Spots of methanolic extract solution in methanol for both the plants under study were applied on the TLC plates. The plates were developed using Vanillin in sulphuric acid and also observed under UV light at the wavelength of 254 and 366 nm. The R_f value was calculated. Similarly for detection of Flavonoids the mobile phase selected was Ethyl Acetate: Formic acid : Glacial Acetic acid: Water (100:1.1:1.1:2.6). The detection was done after spraying the plates with anisaldehyde in sulphuric acid and also under UV light 254nm and 366 nm. R_f values were calculated.

- **Visualization:**

  Plates were removed and were examined visually, under UV and suitable visualizing agent (Vanillin-sulphuric acid, Anisaldehyde in sulphuric acid) after that R_f was calculated by following formula

  \[
  R_f = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}.
  \]

**5.3.12 High Performance Thin Layer Chromatographic Studies**

HPTLC studies were carried out following the method of Harborne and Wagner et al.

i. **Sample Preparation**

Each extract residue was re-dissolved in 1ml of chromatographic grade methanol and water which was used for sample application on pre-coated silica gel 60 F 254 aluminium sheets.

ii. **Developing Solvent System**

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent n hexane: Ethyl Acetate in the ratio of 7:3 for methanolic extract of *Aspidium cicutarium*. The solvent system selected for the aqueous extract was Ethyl acetate: n Butanol in the ratio of 6:4.

iii. **Sample Application**
Application of bands of each extract was carried out (4mm in length and 1µl in concentration) using spray technique. Samples were applied in duplicate on pre-coated silica gel 60 F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

iv. Development of Chromatogram
After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with and n hexane: Ethyl Acetate in the ratio of 7:3 for methanolic extract and ethyl acetate: n Butanol in the ratio of 6:4 for aqueous extract.

v. Detection of Spots
The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 200 nm for methanolic extract and 220 nm after spraying with anisaldehyde with sulphuric acid for aqueous extract. The Rf values and finger print data were recorded by WIN CATS software.

5.3.13 Spectrophotometric studies

i. Preparation of Phosphate buffer 7.2
Monobasic 0.2 M Phosphate buffer was prepared. 50 ml was placed in volumetric flask and 34.7 ml Sodium hydroxide (0.2M) was added to it. Water was added to make 200 ml.

ii. For Methanolic extract of Rhizomes of Aspidium cicutarium
Extract was weighed and a solution of concentration 100 mg/ml was prepared in Phosphate buffer pH 7.2. Spectra was run on UV spectrophotometer. Extract was dissolved in Phosphate Buffer pH 7.2 (USP) to get concentration of 100mg/ml and then various dilutions were made to obtain concentrations in the range of (10-100 µg/ml) and absorbances were noted.
iii. **Preparation of Phosphate Buffer 6.8**

Monobasic 0.2 M Phosphate buffer was prepared. 50 ml was placed in volumetric flask and 22.4 ml Sodium hydroxide (0.2M) was added to it. Water was added to make 200 ml.

iv. **For Methanolic extract of leaves of *Spathodea campanulata*.**

Extract was weighed and a solution of concentration 100 mg/ml was prepared in Phosphate buffer pH 6.8. Spectra was run on UV spectrophotometer. Various concentrations were made of the solution to obtain concentration range of 10 -100 μg/ml and calibration curve was plotted.

5.3.14 **Analytical characterization of markers**

Markers for *Aspidium cicutarium* extract. It has been noted earlier that the chemical constituents present in fern species are derivatives of phloroglucinol and butyric acid. It has been observed that two or more molecules of simple monocyclic derivatives, such as: phloroglucinol, aspidinol, filicinic acid and acylfilicinic acid may get condensed to give rise to bicyclic derivatives, for instance; albaspidin, flavaspidic acid and filicic acid. Hence Phloroglucinol was considered as a marker for the flavonoids and polyphenols present on the methanolic extract. Phloroglucinol was analysed for its physicochemical properties.

i. **Solubility and Melting point**

   The marker was analysed for characteristics like solubility, melting point.

ii. **UV absorption spectra of Phloroglucinol.**

   0.1g of Phloroglucinol was dissolved in 100 ml Phosphate buffer pH 7.2 to obtain a solution of 1000 μg/ml. It was further diluted to get a solution of 10 μg/ml. The solution was subjected to UV spectrophotometer and λmax was determined.

iii. **FTIR Spectra of Phloroglucinol**

   Sample preparation was done by mixing pre dried IR grade KBr with the extract by trituration and the spectra was scanned over a frequency range 4000 – 400 cm⁻¹.
Markers for *Spathodea campanulata* extract

From the literature survey done so far it has been noted that leaf and bark extract of *Spathodea campanulata* has shown presence of several polyphenolic derivatives, steroidal compounds, flavonoids as well as tannins. A few phytoconstituents which were already found in this extract are Kaempferol, Ajugol, Spathodin and Spathodol. The marker selected for the present study is Kaempferol.

i. **Solubility and Melting point**

The characteristics like solubility, melting point was noted from the literature.

ii. **UV absorption spectra of Kaempferol**

0.1g of Kaempferol was dissolved in 100 ml phosphate buffer pH 6.8 to obtain a solution of 1000 µg/ml. It was further diluted to get a solution of 10 µg/ml. The solution was subjected to UV spectrophotometer and λmax was determined.

iii. **FTIR Spectra of Kaempferol**

Sample preparation was done by mixing pre dried IR grade KBr with the extract by trituration and the spectra were scanned over a frequency range 4000 – 400 cm⁻¹.

5.3.15 **Acute Toxicity studies of extracts**

The experimental protocols were subjected to scrutiny of Institutional Animal Ethics Committee. The experimental clearance was given in letter vide ACP/IAEC/11-12/12-05. OECD guidelines 423 were followed for determination of LD₅₀ of the extracts.

i. **Animals used**

Swiss Albino mice (weighing 23-25 g) both male and female were used for the study.

ii. **Housing and feeding conditions**

The temperature in the experimental animal room was maintained at 22°C (+ 3°C). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim was to maintain 50-60%. Lighting was artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets were used with an unlimited supply of drinking water. Animals were
iii. Preparation of animals

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions.

iv. Procedure for Aspidium cicutarium extract

Sixty rats were divided into six groups of ten per group after six hour fasting period. The rats in group 1 received normal saline (10 ml/kg oral) while the animals in groups 2-6 received oral doses of the extract (200, 400, 800, 1200, 1500 mg/kg). The animals were observed for obvious toxic symptoms, and mortality in each group was determined twenty four hours after administration. The median lethal dose of the extract (LD_{50}) was estimated using probit analysis.

v. Procedure for Spathodea campanulata extract

Forty rats were divided into four groups of ten per group after six hour fasting period. The rats in group 1 received normal saline (10 ml/kg oral) while the animals in groups 2-4 received oral doses of the extract (2000, 4000, 5000 mg/kg). The animals were observed for obvious toxic symptoms, and mortality in each group was determined twenty four hours after administration. The median lethal dose of the extract (LD_{50}) was estimated using probit analysis.

5.3.16 Studies on cell line

i. Cell culture and treatment

Mouse macrophage RAW 264.7 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37°C in 5% CO_{2}/95% air. Cells in 96 well plates (2×10^4 cells/well) were treated with *Aspidium cicutarium* methanolic
extract dissolved in phosphate buffer pH 7.2. (10, 25, 50, 100 μg/mL) for 2 h, and then incubated with LPS (1 μg/mL) for 20 h.

ii. Cell viability

Cell viability was assessed using a modified 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells (2x10⁴ cells/well) were seeded in a 96 well plate and treated with ACME. Following treatment, 100 μL of MTT solution (5 mg/mL in phosphate buffered saline) was added to each well and further incubated for 4 hours at 37°C. Subsequently, 100 μL of dimethyl sulfoxide (DMSO) was added to each well to dissolve any deposited formazan. The optical density (OD) of each well was measured at 540 nm with a microplate reader.

iii. Antiinflammatory activity on RAW 264.7 cell line.⁷⁴,¹⁰⁶

Generally considering, macrophages play an important role in inflammatory diseases by producing cytokines, interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and other inflammatory mediators. Twenty-four hours prior to LPS treatment, the cells were inoculated into 96 micro-well plates. Twenty-four hours later, when the cells were observed for adherence at the bottom of the well, cell supernatants were disposed and 10 ng/ml LPS with prepared exact solution were added into the well. The stimulation and intervention lasted for different hours and the supernatants and the cells were harvested for ELISA. Cells stimulated by LPS without any intervention were observed as blank control. Inhibitory effects of 0.5mg/ml and 1 mg/ml extract on the cytokine TNF-α, IL-1β and IL-6 production from LPS treated RAW 264.7 cells were determined by sandwich ELISA. After stimulation and intervention on RAW 264.7 cells for 48 hours, supernatants were harvested and assayed for TNF-α, IL-1β and IL-6 by respective ELISA kits. The procedure obeyed instructions from related kits.

Set 1: TNF-α
Set 2: IL 6
Set 3 IL1 β
Each set had 4 groups numbered 1 to 4.
Group 1: Control,
Group 2: LPS
Group 3: ACME 0.5 mg/ml + LPS
Group 4: ACME 1mg/ml+ LPS.

After incubating for 48 hours, with intermittent washing of unbound antibodies, the solutions were subjected to measurement of optical density and inhibition of inflammatory cytokinines was measured. Calibration curve for TNF-α, IL6 and IL1 β expression was plotted using concentration of the mediator against its optical density. The straight line equation was used to obtain values of x. these were plotted against the treatment given to the 4 different groups.

5.3.17 Invivo topical anti-inflammatory studies
The mouse ear oedema was produced by topical application of croton oil (CO) and carried out according to the method described previously by Tubaro et al. 1985. Croton oil was dissolved in a 5% acetone solution (v/v) and 10 μl were applied with an automatic pipette to both anterior and posterior surfaces of the right ear of the mouse. The left ear (control) received the vehicle (acetone 80, olive oil 20, v/v). Groups of mice (n = 10) received topically the graded doses of ACME, ACAE, SCAE or SCME (50, 100 and 200μl ), vehicle 50 μl (0.5% sodium CMC) and dexamethasone (3 μl), 1 hour before CO application. The solution of the test drug was made at a concentration of 100mg/ml for each extract. Then 50, 100 and 200μl of each extract was administered topically on the ears to each group of animal. Inflammation was allowed to develop for 5 hours, after which the animals were killed by cervical dislocation, and a section (6 mm diameter) of the central portion of both ears was punched and weighed. The swelling induced by CO was assessed in terms of the increase in the weight of the right ear punch biopsy over that of the left ear. Inhibition percentages were calculated by comparison with the control group that only received the CO application but none of the treatments.
Statistical Analysis: The groups were analyzed by one way ANOVA followed by Dunnet’s test. * p<0.05, **p<0.01, ***p<0.001

Animal grouping: Each group (n=6)

Table 3. Treatments received in topical anti-inflammatory studies

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>µl/ear</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80% Acetone + 20% Olive oil</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Dexamethasone</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>ACME</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>ACME</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>ACME</td>
<td>200</td>
</tr>
<tr>
<td>6</td>
<td>ACAE</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>ACAE</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>ACAE</td>
<td>200</td>
</tr>
<tr>
<td>9</td>
<td>SCME</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>SCME</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>SCME</td>
<td>200</td>
</tr>
<tr>
<td>12</td>
<td>SCAE</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>SCAE</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>SCAE</td>
<td>200</td>
</tr>
</tbody>
</table>

5.3.18 Wound healing activity and histopathology

The albino rats weighing 150-200 gms were selected for the study. The animals were randomly separated into four groups. Each group had six rats.

i. Incision wound model

Animals were anesthetized prior to and during creation of the wounds, with 1 mL of intravenous ketamine hydrochloride (10 mg/kg). The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back. After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed. Extracts were topically applied to the wound once a day. The sutures were removed on 8th post wound day and the application of the extract was continued. The wound breaking strength was measured on the 10th day evening after the last application.
ii. Histological studies of wounds

For histological studies, pieces of granulation tissues from incision wound model were fixed in 10% neutral formalin solution for 24 h and dehydrated with a sequence of ethanol-xylene series of solutions. The materials were filtered and embedded with paraffin (40–60°C). Microtome sections were taken at 10 μ thickness. The sections were processed in alcohol-xylene series and stained with hematoxylin-eosin dye. The histological changes were observed under a microscope. Photographs were taken of each slide.

iii. Excision wound model

Animals were anesthetized prior to and during creation of the wounds, with 1 mL of intravenous ketamine hydrochloride (10 mg/kg). An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area of 500 mm² and 2 mm depth was created along the markings using toothed forceps, scalpel and pointed scissors. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The entire wound was left open. All surgical procedures were performed under aseptic conditions. The wound closure rate was assessed by tracing the wound after 5th, 10th and 15th wounding day using transparent paper and a permanent marker. The wound areas recorded were measured using graph paper. The percentage of wound healing was calculated of original wound size for each animal of group on predetermined days post-wounding for final analysis of results. Changes in wound area were calculated, giving an indication of the rate of wound contraction. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound. The grouping of animals and dosing is given in results and discussion.
5.3.19 Preformulation of Films\textsuperscript{109,110}

i. Selection of Polymers\textsuperscript{110,111}

Initially three polymers were selected Sodium CMC (Carboxy Methyl Cellulose)-
medium viscosity, Ethyl-Cellulose 7cps, Eudragit RL-100. Each polymer was used separately for the formulation of the film. The selection of the polymers was based upon the proper formulation of topical polymeric film. For the preliminary screening of the polymer the work was based on Schroeder et al.

Initially the evaluation system was based on three criteria: self-adhesive, cosmetically attractiveness and drying time. The self-adhesive property of the film forming polymer is required to be high enough so as to enable an application to be applied directly, which would ensure most convenient for the patient. The formed film is required to be non-sticky from the back so as to avoid adhesion to the clothes of the patient or other physical contact. Hence a backing membrane was developed of PVA. Considering the fact that many patients complain about the high visibility of transdermal patches which is considered cosmetically unattractive the polymeric film is supposed to be transparent. Drying time was taken into consideration. The formulation should take minimum time to form the film. In addition to this, the delivery system is required to show certain permanence on the skin and nail in order to be able to provide a continuous drug supply over a prolonged period of time. Solvent casting method was used for the preparation of the topical film. Each polymer was used separately to formulate the film.

ii. Development of simple score/rating system.\textsuperscript{112}

For a first assessment of the suitability of film forming polymer, the obtained formulations were evaluated according to a rating system for three characteristics. The stickiness of the outer surface was tested by pressing cotton wool on the dry film under low pressure. Depending on the quantity of cotton fibers that were retained by the film the stickiness was rated high (dense accumulation of fibers on the film), medium (thin fiber layer on the film) or low (occasional or no adherence of fibers). The cosmetical attractiveness of the films was assessed by visual examination of the dry films. Transparent films possess high attractiveness as they were almost invisible. Opaque were considered less attractive as they exhibited an decreased visibility.
Whitish films displayed only a low attractiveness. Drying time was accessed on the time taken for the drying of the film. The selected polymer will be further developed and optimize for the proper formulation of the film and all the evaluation will be carried out.

iii. **DSC Studies:**
A differential scanning calorimetry (JADE DSC, Perkin Elmer, USA) was used to study the thermal analysis of drug-excipient compatibility. Firstly, binary mixtures of extract and excipients (in 1:1 mass/mass ratio) was made. The drug-excipient mixture was scanned in the temperature range of 50-220°C under an atmosphere of nitrogen. The obtained thermograms were observed for any type of interaction.

iv. **FTIR studies for extracts:**
The IR spectra were recorded using FTIR spectrophotometer (Jasco-450 plus, Japan) with diffuse reflectance principle. Sample preparation involved mixing the sample with KBr, triturating in glass mortar and finally placing in the sample holder. The mixtures were prepared by triturating the drug with excipient and stored for 24 hrs at room temperature in tightly closed vial. The spectra were scanned over a frequency range 4000 – 400 cm⁻¹.

v. **Formulation of Eudragit, Ethyl cellulose films**
In a 50ml beaker 15ml ethanol was taken followed by propylene glycol, glycerol and PEG 400 with proper stirring. A clear solution was thus obtained. Extracts were then added in this clear solution, the drug dissolved again giving a clear solution. Polymer was then further added in this solution which get solubilize. The obtained solution was then added in the petri-dish already containing the backing membrane and kept in the open air for drying.

vi. **Formulation of CMC films.**
- In a 50 ml beaker 10ml methanol, glycerol and PEG 400 was taken extracts were then added to this solution with proper stirring. A clear solution was thus obtained.
• In a second 50ml beaker 15 ml water was taken and the polymer was dissolved in it.

• Both the solution was then mixed together, and then added in the petri-dish already containing the backing membrane and kept in the open air for drying.

vii. Formulation of the films with Eudragit and HPMC was done and 4 trial batches were made. Films were cated on petri dishes with aluminium foils on the plate. They were dried and removed from the base plate.\textsuperscript{114,115}

\textbf{5.3.20 Formulation of films}

\textbf{Table 4. Trial batches of films.}

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of ingredient</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Extract</td>
<td>1g</td>
<td>1g</td>
<td>1g</td>
<td>1g</td>
</tr>
<tr>
<td>2.</td>
<td>Eudragit RL 100</td>
<td>350 mg</td>
<td>400 mg</td>
<td>450 mg</td>
<td>500 mg</td>
</tr>
<tr>
<td>3</td>
<td>HPMC K 4M</td>
<td>10 mg</td>
<td>15 mg</td>
<td>20 mg</td>
<td>25 mg</td>
</tr>
<tr>
<td>4.</td>
<td>Dibutyl Phthalate</td>
<td>0.2 ml</td>
<td>0.1 ml</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>5.</td>
<td>PEG 400</td>
<td>-------</td>
<td>-------</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>6.</td>
<td>Ethanol</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
</tbody>
</table>

i. \textbf{3² Full factorial Experimental Design Layout For Films.}\textsuperscript{116}

Independent variable:

\[ X_1 = \text{Concentration of Eudragit RL 100} \]

\[ X_2 = \text{Concentration of PEG 400 (ml)} \]

Dependent variable:

\[ Y_1 = \text{In-vitro Drug Release (\%)} \]

\[ Y_2 = \text{Adhesive Strength (g/20mm)} \]
Table 5. Factorial batches of film formulations

5a. Coded levels translated in actual units.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1</td>
</tr>
<tr>
<td>X 1</td>
<td>-1</td>
</tr>
<tr>
<td>Concentration of</td>
<td>450mg</td>
</tr>
<tr>
<td>Eudragit RL 100</td>
<td></td>
</tr>
<tr>
<td>X 2</td>
<td></td>
</tr>
<tr>
<td>Concentration of PEG</td>
<td>0.1 ml</td>
</tr>
</tbody>
</table>

Independent variable:

X1 = Concentration of Eudragit RL 100

X2 = Concentration of PEG 400 (ml)

Dependent variable:

Y1 = In-vitro Drug Release (%)

Y2 = Adhesive Strength (g/20mm)
5b. Factorial design layout.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Batch No</th>
<th>X1</th>
<th>X2</th>
<th>Conc of Film forming agent (in mg)</th>
<th>Conc of PEG (in ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>0</td>
<td>0</td>
<td>500</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>0</td>
<td>-1</td>
<td>500</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>0</td>
<td>+1</td>
<td>500</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>-1</td>
<td>0</td>
<td>450</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>-1</td>
<td>-1</td>
<td>450</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>-1</td>
<td>+1</td>
<td>450</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>+1</td>
<td>0</td>
<td>550</td>
<td>0.15</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>+1</td>
<td>_1</td>
<td>550</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>550</td>
<td>0.2</td>
</tr>
</tbody>
</table>

5c. Actual factorial design batches of *Aspidium cicutarium* methanolic extracts films

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of ingredient</th>
<th>AF1</th>
<th>AF2</th>
<th>AF3</th>
<th>AF4</th>
<th>AF5</th>
<th>AF6</th>
<th>AF7</th>
<th>AF8</th>
<th>AF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Extract (g)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Eudragit RL 100(mg)</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>450</td>
<td>450</td>
<td>450</td>
<td>550</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>3.</td>
<td>HPMC 4M(mg)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>PEG 400 (ml)</td>
<td>0.15</td>
<td>0.1</td>
<td>0.2</td>
<td>0.15</td>
<td>0.2</td>
<td>0.15</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Oleic acid(ml)</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Ethanol</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
</tbody>
</table>
5d. Actual factorial design batches of *Spathodea campanulata* methanolic extract films

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of ingredient</th>
<th>SF1</th>
<th>SF2</th>
<th>SF3</th>
<th>SF4</th>
<th>SF5</th>
<th>SF6</th>
<th>SF7</th>
<th>SF8</th>
<th>SF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Extract (g)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Eudragit RL 100(mg)</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>450</td>
<td>450</td>
<td>550</td>
<td>550</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>3.</td>
<td>HPMC K 4M(mg)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>PEG 400 (ml)</td>
<td>0.15</td>
<td>0.1</td>
<td>0.2</td>
<td>0.15</td>
<td>0.1</td>
<td>0.15</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>5.</td>
<td>Oleic acid(ml)</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
<td>0.075</td>
</tr>
<tr>
<td>6.</td>
<td>Ethanol q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
</tbody>
</table>

ii. **Evaluation Parameters**

a. **Physical Appearance**

The physical appearance of the film was observed. The colour, appearance, presence of any clogging/precipitation and texture of the film was evaluated.

b. **Thickness**

Thickness of film was measured by micrometer screw guage at three different places and mean value was calculated.

c. **Weight Variation**

Weight variation was studied by individually weighing 3 selected circular films of area from each preparation. Such determination was performed for each formulation and mean value was calculated.

d. **Moisture uptake study**

A film of area size 2cm x 2cm was put in a desiccators containing silica gel for 24 h and weighed (Ws), the films were transferred to another desiccators containing saturated NaCl solution (relative humidity 75%) at 25 °C. After equilibrium was attained, the films were taken out and weighed (Wm). Moisture uptake capacity was calculated according to the following equation:
Moisture uptake capacity (%) = $\frac{W_m - W_s}{W_s} \times 100$

**e. Drug content**

For drug content determination a piece of 1 cm$^2$ was cut from the film and added to a beaker containing 100 mL methanol. It was then stirred for 4-5 hrs with the help of a magnetic stirrer. The solution was then analyzed for drug content at respective detection wavelength.

**f. Folding Endurance**

The test was carried out to determine the brittleness of the films. The films were folded at the same point for several times until they broke. The number of times the folding was required was counted.

**g. Tensile Strength**

Tensile strength of the film was determined with the help of Ubique’s Digital Tensile Strength Tester, Model-UTT-P. A film of size 20mm x 40mm was mounted on the tensile tester with the help of the clamps. The tensile strength was calculated as follow.

\[
\text{Tensile Strength} = \frac{\text{Load taken to break the wire in Newton’s (N)}}{\text{Cross-Sectional area in mm}^2}
\]

**h. Adhesive Test**

The prepared patches were cut into strips 20mm wide and 40mm in length and conditioned for 24 h at 23±2 °C and 50±5%RH. The samples were applied to an adherent plate made of stainless steel, smoothened with a 4.5 pound roller five times; the lower end of attached sample was tied with thread. The stainless steel plate on which the sample was adhered was vertically fixed on the lower clamp of the Ubique’s Digital Tensile Strength Tester, Model-UTT-P and the thread was tied to the upper clamp and pulled from the substrate at a 180 °C angle at a rate of 300 mm/min. The stainless steel plate on which the sample was adhered was fixed on the
lower clamp of the Ubique’s Digital Tensile Strength Tester, Model-UTT-P and the adhesive test was determined. The peel adhesive test was calculated in g/20mm.

i. **In vitro drug release study**\(^\text{120,121,122,123,124}\)**

In vitro drug release studies were performed by using a Franz diffusion cell with a receptor compartment capacity of 25ml Phosphate buffer, pH 7.2 for *Aspidium cicutarium* films and Phosphate buffer pH 6.8 for *Spathodea campanulata* films. Cellophane dialysis membrane previously soaked for 24 h in respective buffer solutions. The cellophane membrane having pore size 2.4 nm was used for the determination of drug release from the topical films. The contents of the receptor compartment were thermostatically adjusted to 37±0.5 °C and stirred at 50 rpm with the help of magnetic stirrer. Topical films were applied to a donor compartment. Samples (2 ml) were withdrawn at 1, 2, 3, 4, 6, 8 hours. An equal volume of fresh Phosphate buffer was immediately added to the receptor cell after each sampling. The concentration of drugs was spectrophotometrically determined at 281 nm for *Aspidium cicutarium* films and 235 nm for *Spathodea campanulata* films respectively.

---

### 5.3.21 Formulation of Emulgels

i. **Preformulation studies for emulgels of extracts.**

a. **Determination of the Required hydrophilic lipophilic balance (RHLB) of o/w emulsion**: \(^\text{124}\)**

   Tween 80 and span 80 were selected for preparation of o/w emulsion on the basis of:
   
   - Right chemical type:
     
     A blend of polyoxyethylene sorbitan oleate ester (tween 80) with its unsaturated lipophilic oleate (span 80) have an unsaturated chain which "attract" oils having unsaturated bonds. Emulsifiers which "attracts" the oil will be more effective. Rice bran oil contains higher % of unsaturated fatty acids.
   
   - The HLB of emulsifiers:
The most stable emulsion systems usually consist of blends of two or more emulsifiers, one portion having lipophilic tendencies, the other hydrophilic.

HLB of span 80 = 4.3

HLB of tween 80 = 15

By blending two emulsifiers, the exact HLB ranging from 4.3 to 15 as calculated. The amount of each emulsifier added was calculated according to the HLB Computagrapb. HLB values of the Span group are marked along the left edge; those of the tween group along the right edge; and percentage of tween is shown from 0 to 100 across the bottom. Ruler line drawn from the HLB value of one emulsifiers to the HLB value of the other; then a horizontal line was drawn for the required HLB value. By drawing a perpendicular line through the intersection of two previous lines, the percentage of tween and span was calculated.

b. Determination of % of emulsifier concentration.

A set of six o/w emulsions each of 100 ml and containing 10% v/v rice bran oil were prepared as previously mentioned. The emulsifiers (span 80 and tween 80) were mixed in different ratios to cover an HLB range from 4.3 to 15 in the prepared set of six emulsions. A second set of emulsions was then prepared using the same blend of emulsifiers but at closer ratio between the most two stable emulsions obtained from the first set. To determine the most stable emulsion, the prepared emulsions were subjected to evaluation using several methods, regarding their stability. Accurate quantity of rice bran oil (10% v/v) was mixed with Span 80. It was heated to 75°C, so a clear solution was formed. Measured quantity of Tween 80 was taken and dissolved with distilled water, heated up to 75°C. Oil phase was mixed dropwise in water phase with stirred by T 25 digital Ultra-Turrax®. Span 80 and tween 80 were mixed in a ratio giving a blend with the indicated RHLB. The emulsifiers were examined at different total blend concentrations (3%, 4% and 5%) for o/w emulsions. To determine the most stable emulsion, the prepared emulsions were subjected to evaluation using several methods, regarding their stability.
surfactant chemical type to be used with the O/W emulsion was the mixture of span 80 and tween 80. The prepared emulsion was examined visually for physical appearance and phase separation. Amongst all HLB ranges, the RHLB of oil/water was determined experimentally using span 80 and tween 80 as emulsifying blends and it was around 10.7. For O/W emulsions containing either 10% v/v oily phase, a surfactant blend composed of 3 % v/v span 80 and tween 80 in a ratio of 40:60 would be appropriate to obtain a stable emulsion. These various HLB valued emulsions were subjected to measurement of droplet size. The HLB value that gave smallest droplet size was chosen. Various concentrations of this blend were then observed for stability.

ii. **Formulation and Evaluation of trial batches of emulgels.**

From the preformulation studies the concentrations of Surfactant and cosurfactant was fixed. The emulsions were gelled using Carbopol 934 as gelling agent. 4 trial batches of emulgel formulations were made and evaluated for spreading coefficient and viscosity.

**Table 6. Trial Batches of emulgel.**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Ingredients (in %)</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Extract</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>Rice Bran oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>Tween 80</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>4.</td>
<td>Span 80</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>5.</td>
<td>Propylene glycol</td>
<td>2.5</td>
<td>5</td>
<td>7.5</td>
<td>10</td>
</tr>
<tr>
<td>6.</td>
<td>Oleic acid</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>7.</td>
<td>Carbopol 934</td>
<td>1</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>8.</td>
<td>Water( upto)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

iii. **Formulation of factorial batches**

A two-factor, three-level factorial experimental design was used to optimize the formulation variables as the response surface methodology requires 9 experiments.
Table 7.  Factorial Batches of emulgel formulations

7a. Coded levels translated in actual quantities.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1</td>
</tr>
<tr>
<td>X 1</td>
<td>Concentration of gelling agent</td>
</tr>
<tr>
<td>X 2</td>
<td>Concentration of propylene glycol</td>
</tr>
</tbody>
</table>

Independent Variables:

X1 = Concentration of gelling agent (Carbopol 934).

X2 = Concentration of propylene glycol.

Dependent Variables:

Y1 = Spreading Coefficient (gm.cm/sec).

Y2 = % Cumulative drug release at 8 hrs.

7b. Factorial design layout.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Coded values</th>
<th>Actual values</th>
<th>Concentration of Gelling agent in %</th>
<th>Concentration of Propylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>F2</td>
<td>0</td>
<td>-1</td>
<td>2</td>
<td>3.5</td>
</tr>
<tr>
<td>F3</td>
<td>0</td>
<td>+1</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>F4</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>F5</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>3.5</td>
</tr>
<tr>
<td>F6</td>
<td>-1</td>
<td>+1</td>
<td>1</td>
<td>7.5</td>
</tr>
<tr>
<td>F7</td>
<td>+1</td>
<td>0</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>F8</td>
<td>+1</td>
<td>-1</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>F9</td>
<td>+1</td>
<td>+1</td>
<td>2.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>
7c. Actual Factorial design batches of *Aspidium cicutarium* methanolic extract emulgels.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>AE1</th>
<th>AE2</th>
<th>AE3</th>
<th>AE4</th>
<th>AE5</th>
<th>AE6</th>
<th>AE7</th>
<th>AE8</th>
<th>AE9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Carbopol 934</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Rice Bran Oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Span 80</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>5</td>
<td>3.5</td>
<td>7.5</td>
<td>5</td>
<td>3.5</td>
<td>7.5</td>
<td>5</td>
<td>3.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Water (q.s.)</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
</tbody>
</table>

7d. Actual factorial design batches of *Spathodea campanulata* methanolic extract emulgels

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>SE1</th>
<th>SE2</th>
<th>SE3</th>
<th>SE4</th>
<th>SE5</th>
<th>SE6</th>
<th>SE7</th>
<th>SE8</th>
<th>SE9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Carbopol 934</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Rice Bran Oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Span 80</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>5</td>
<td>3.5</td>
<td>7.5</td>
<td>5</td>
<td>3.5</td>
<td>7.5</td>
<td>5</td>
<td>3.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Water (q.s.)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
</tbody>
</table>
iv. Evaluation of Emulgels.\textsuperscript{128,129,130}

a. Appearance:
The prepared emulgel formulations were inspected visually for their color, homogeneity, and consistency.

b. Measurement of pH:
\( \text{pH} \) of all formulations was determined by using \( \text{pH} \) meter. The \( \text{pH} \) meter was calibrated before each use with standard \( \text{pH} \) 4, 7 and 10 buffer solutions. 1 gm of formulation was stirred in distilled water till forms a uniform suspension. The volume was made upto 100 ml and \( \text{pH} \) of the dispersion was measured using \( \text{pH} \) meter.

c. Spreading Coefficient:
One of the requirement of the emulgel is that it should possess good spreadability. It is term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value.

Spreading coefficient was determined by apparatus consists of a wooden block, which is attached to a pulley at one end (Figure 20). Spreading coefficient was measured on the basis of ‘Slip’ and ‘Drag’ characteristics of emulgel. A ground glass slide was fixed on the wooden block. An excess of emulgel (about 1 gm) under study was placed on this ground slide. The emulgel preparation was then sandwiched between this slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 100 gm was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the two slides. The weight was removed, and the top slide was subjected to a pull obtained by attaching 50 g weight over the pulley. The time (in sec) required by the top slide to travel a marked distance marked previously(6.5 cm) was noted and expressed as spreadability. A shorter interval indicates better spreading coefficient. It is calculated by using the formula

\[
S = \frac{M \cdot L}{T}
\]

Where \( M = \) Weight tied to upper slide
\( L = \) length of glass slides
\( T = \) time taken to separate the slides
d. **Viscosity Measurement:**
The viscosity of different emulgel formulations was determined at 25°C using a Brookfield viscometer (Brookfield DV-E viscometer). The emulgel were rotated at 10 (min.) and 100 (max.) rotation per minute. The readings were noted as the viscosity of Emulgel.

e. **Drug content:**
1 gm of emulgel samples were weighed and dissolved in phosphate buffer pH 7.2 for ACME emulgels and 6.8 for SCME emulgels. They were filtered and necessary dilutions were made and then the drug content was determined spectrophotometrically.

f. **In vitro Drug Release study:**
The in vitro drug release studies of the emulgel were carried out in Franz Diffusion cell using Dialysis membrane (Himedia laboratories Pvt Ltd, flat width: 42.44 mm; inflated diameter: 25.4 mm). The membrane was soaked in phosphate buffer for overnight before experiment. The membrane was positioned between the two cell halves of a glass chamber. The two compartments were held together with a clamp. The receiver compartment contained phosphate buffer and in the upper donor compartment 1 gm of formulation was spread evenly on the membrane. The receptor phase phosphate buffer was continuously stirred with help of magnetic stirrer and was maintained at temperature of 37 ± 10°C during the experiments. One ml of the sample was withdrawn from the receiver compartment at every hour for 8 hours and the same amount of fresh buffer solution was added to maintain the sink condition in receiver compartment. Care was taken to ensure that no air bubbles were lodged underneath the diffusion membrane during the experiments. The samples were diluted upto 10 ml with buffer and were analyzed spectrophotometrically at a suitable wavelength. Percentage of extract released from each sample was determined by referring a previously prepared standard curve. This experiment was carried out for a period of 8 hours.
g. **Kinetics of drug release:**

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to different models using PCP Disso V2.08.
- Zero order (cumulative % drug release v/s. time).
- First order (log cumulative % drug retained v/s. time).
- Higuchi model (cumulative % drug retained v/s. Square root of time).
- Peppas model (log cumulative % drug release v/s. log time)

h. **Statistical Analysis and Optimization Model Validation:**

Statistical validation of the polynomial equations generated by Design Expert was established on the basis of ANOVA provision in the software. The models were evaluated in terms of statistically significant coefficients and $R^2$ values. The optimized formulations were selected on the basis of desirability based on acceptance criteria according to Design Expert software. Various 3D response surface graphs were provided by the Design Expert software. The optimized formulation factors were evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with the predicted values.

5.4 Evaluation of optimized film formulations.

5.4.1 **In vivo anti-inflammatory studies**

The carrageenan-induced paw edema method was used for anti-inflammatory evaluation. Wistar albino rats were divided into 4 groups (n =6 ). The first (negative control) and second (positive control) groups were treated with the gel base and Volini gel dosage form respectively. Animals of the third and fourth groups were treated with the extract emulgel. All treatments were applied to the planter surface of the left hind paw of rats by gentle rubbing of 0.5 g with the index finger. After one hour, a subplantar injection of 0.1 mL of 1% carrageenan in normal saline was injected into the treated paw of all rats. The volumes of the injected paws were measured in mL using a plethysmometer immediately before and 3 h following carrageenan injection. The percentage of anti-inflammatory activity was calculated using the following equation:
where, $V$ is the paw volume 4 hours after carrageenan injection and $V_i$ is Initial paw volume.

Groups of animals:

**Animals Required – Rats**

- **Species**: Wistar Albino rats.
- **Age/weight/Size**: 150-200gm
- **Gender**: Female/Male
- **Numbers to be used**: 24 females/Male
- **Number of days each animal will be housed**: 10 days

**5.4.2 Wound healing activity**
The optimized formulations were subjected to excision wound model studies and the contraction in wound after 15 days of application of emulgels formulations was studied. Due to occlusive effect of films on wounds the activity was not studied for film formulations.

**5.4.3 Antioxidant activity.**
Optimized formulations of Emulgels were coded as OAE for Emulgel of *Aspidium cicutarium* methanolic extract and OSE for *Spathodea campanulata* methanolic extract. The formulations were dissolved in phosphate buffer 7.2 and 6.8 respectively for OAE and OSE. Filtered through Whatmann filter paper and then suitably diluted to prepare concentrations ranging from 10 µg/ml to 100 µg/ml. The antioxidant property was determined using the DPPH assay method as explained previously.

**5.4.4 Stability studies.**
Stability studies on the optimized films of ACME and SCME were carried out as per ICH guidelines. The films were stored in an aluminum foil and subjected to elevated temperature and humidity conditions of 40 ± 2°C and 75 ± 5% RH respectively. Samples were withdrawn at the end of 0, 30, 60 and 90, 120,150 and 180 days and evaluated for adhesive strength and tensile strength.
Stability studies were also performed on emulgels of ACME and SCME. They were subjected to Freeze-thaw and thermal cycling test:

The optimized emulgels of both ACME and SCME were exposed to different temperature conditions in a cyclic pattern that simulate the changes likely to be encountered during its use or in distribution process. During the course of study the selected formulations were subjected to refrigerated temperature (2 - 8° C) for two days, followed by 40° C for two days, one cycle. Such types of three cycles in twelve days were completed and major changes were observed. The selected gels were also subjected to the following condition of temperature and relative humidity during stability studies.

40°C ± 2°C at 75 ± 5% RH

Formulations were evaluated for various parameters after every month for six months. The parameters of the emulgels studied were pH, appearance and spreading coefficient.
6 RESULTS AND DISCUSSIONS:

6.1 Plant Profile

6.1.1 Pharmacognostic evaluation
i. Macroscopy and microscopy
a. Rhizome measure 15-30 cm in length and are covered with stipe-bases, "fingers," The length of each rhizome was 7.5-15 cm. (3-6') long 1-3 cm was its width.
b. Cork: A wide cork of thin-walled, rectangular cells arranged in radial rows, cork cambium 1-3 layered is seen. It appeared dark brown in colour.
c. Secondary cortex: Thin-walled, hexagonal parenchymatous cells showing presence of brownish matter was seen in the secondary cortex. Presence of vascular bundles was seen in abundance.
d. Calcium oxalate crystals: Prismatic and cluster crystals of calcium oxalate were seen.

4a. Cork layer
4b. Parenchymatous cells
4c. Vascular bundle.

Figure 4. Tranverse section of rhizomes of *Aspidium cicutarium*
ii. Studies of powdered dried rhizome
a. Dark brown colour was seen in the powdered sample.
b. Lignified fibres having length of 921.7μm and width of 235.8μm were seen.
c. Non Lignified fibres having length of 500μm were seen.
d. Brownish matter and calcium oxalate crystals were seen in the powdered sample.

![Image of lignified fibres](image1)

5a. Lignified fibres

![Image of non-lignified fibres](image2)

5b. Non Lignified fibres

![Image of calcium oxalate crystals](image3)

5c. Calcium oxalate crystals

Figure 5. Microscopic studies of powdered rhizomes of *Aspidium cicutarium*

iii. Microscopic Characters of leaves of Spathodea
a. Covering trichomes: Uniseriate, multicellular, warty covering thricomes blunt at apex were seen. They were approximately 115 to 220 μm long.

![Image of covering trichomes](image4)

b. Glandular trichomes: Glandular trichomes having unicellular stalk were seen. They had 2 to 4 celled head and are 23 to 35 μm in length.

![Image of glandular trichomes](image5)

c. Colenchyma: Ventral side showed presence of multilayered, thick walled parenchyma

d. Xylem Vessel: It showed annular to spiral thickening and was lignified.
e. Phloem: Cells were nonlignified present at dorsal surface

f. Lamina: Dorsiventral lamina was seen.

g. Midrib: Midrib almost triangular with pronounced projection on dorsal side was seen.

iv. **Surface preparation and powder characteristics**

a. Stomata and covering trichome were prominent in the leaf preparation.

b. Two subsidiary cells at right angle to the stomata were seen.

c. Covering trichome 2 to 4 cell long, bent, thick walled, pointed and uniseriate was seen.

d. Trichome was prominently seen in the powdered leaf.

**Figure 6. Microscopic studies of leaves of *Spathodea campanulata***
v. **Evaluation of Physical constants:**  
The powdered rhizomes were subjected to evaluation of physical constants. The studies were carried in triplicate and the results are mentioned below.

**Table 8. Determination of physical constants.**

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Evaluation Parameter</th>
<th>Mean + Std Dev (n=3) for <em>Aspidium cicutarium</em></th>
<th>Mean + Std Dev (n=3) for <em>Spathodea campanulata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Foreign Matter</td>
<td>1.06 ±0.0551</td>
<td>1.050±0.050</td>
</tr>
<tr>
<td>2</td>
<td>Moisture Content</td>
<td>10.55 ±0.05</td>
<td>10.567±0.252</td>
</tr>
<tr>
<td>3</td>
<td>Total Ash Value</td>
<td>13.73±0.2082</td>
<td>31.367±0.473</td>
</tr>
<tr>
<td>4</td>
<td>Water Soluble Ash Value</td>
<td>3.66±0.1914</td>
<td>6.580±0.363</td>
</tr>
<tr>
<td>5</td>
<td>Acid Insoluble Ash Value</td>
<td>12.16±0.2026</td>
<td>4.467±0.451</td>
</tr>
<tr>
<td>6</td>
<td>Water Soluble Extractive Value</td>
<td>5.69±0.1077</td>
<td>3.73 ± 0.351</td>
</tr>
<tr>
<td>7</td>
<td>Chloroform Soluble Extractive Value</td>
<td>0.11±0.0115</td>
<td>0.130±0.026</td>
</tr>
<tr>
<td>8</td>
<td>Methanol Soluble Extractive Value</td>
<td>6.47±0.1528</td>
<td>2.093± 3.297</td>
</tr>
<tr>
<td>9</td>
<td>Ethanol Soluble Extractive Value</td>
<td>5.23±0.0577</td>
<td>2.03± 0.6429</td>
</tr>
</tbody>
</table>

As seen in the above table the proximate analysis satisfactory result with respect to foreign matter, moisture content, Ash value and Extractive values was seen. The water soluble extractive value was found to be 5.69% and methanolic extractive value of 6.47% was observed for *Aspidium cicutarium*. The higher methanolic extractive suggests that the methanolic extract can be used for further phytochemical investigation.

The proximate analysis for *Spathodea campanulata* showed satisfactory results with respect to foreign matter, moisture content, ash value and extractive values. The water soluble extractive value 3.73% and methanolic extractive value 2.09%. was obtained from the studies. From this it can be concluded that extraction can be carried by using methanol and water.

**6.1.2 Extraction**

The powdered rhizomes of *Aspidium cicutarium* and the leaves of *Spathodea campanulata* were extracted with three different solvents. The properties of extracts of both the plants are given below. All the xtracts of *Aspidium cicutarium* yielded
crystalline extracts. While the extract of Spathodea campanulata yielded slightly sticky green coloured extracts.

**Table 9. Properties of extracts and phytochemical investigation**

9a. Properties of extracts

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Name of Plant</th>
<th>Type</th>
<th>Colour of extract</th>
<th>Appearance</th>
<th>% Yield</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspidium cicutarium</td>
<td>Aqueous</td>
<td>Reddish brown</td>
<td>Crystalline</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td>Ethanolic</td>
<td>Reddish brown</td>
<td>Crystalline</td>
<td>8.3</td>
<td>5-6</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>Methanolic</td>
<td>Reddish brown</td>
<td>Crystalline</td>
<td>14.4</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>Spathodea campanulata</td>
<td>Aqueous</td>
<td>Dark green</td>
<td>Sticky</td>
<td>8.3</td>
<td>5-6</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>Ethanolic</td>
<td>Dark greenish brown</td>
<td>Crystalline</td>
<td>9.4</td>
<td>5-6</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>Methanolic</td>
<td>Dark greenish brown</td>
<td>Slightly sticky</td>
<td>10.4</td>
<td>5-6</td>
</tr>
</tbody>
</table>

6.1.3 **Phytochemical investigation**

Presence of sugars, carbohydrates, tannins, steroids, flavonoids and saponins can be confirmed in the methanolic and aqueous extracts of rhizomes of *Aspidium cicutarium*. The results are reported in Table. 9b. The methanolic extract showed presence of alkaloids while the alkaloids were completely absent in the aqueous extracts of *Aspidium cicutarium*. Steroids were also seen in methanolic extract while the aqueous extract did not show positive result for the presence of steroids. Flavonoids and carbohydrates were present in both the extracts. Presence of tannins and saponins was seen in both the extracts. The presence of flavonoids and steroids can be related to the use of this medicinal plant to treat inflammatory conditions in traditional medicine system Ayurveda.
9b. Phytochemical screening of methanolic and aqueous extracts of *Aspidium cicutarium*.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Tests/ Reagents</th>
<th>Methanolic Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s reagent</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s reagent</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hager’s reagent</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish’s reagent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Barfoed’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sodium Hydroxide test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermann-Burchard test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Salkowski reaction</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Libermann’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Potassium Dichromate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dilute Potassium Permanganate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>Keller-Killiani test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Legal’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone Glycosides</td>
<td>Borntrager’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin Glycosides</td>
<td>Foam Test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Heamolytic test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehlings test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>Biuret’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Millon’s test</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
9c. Phytochemical screening of methanolic and aqueous extracts of *Spathodea campanulata*

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Tests/ Reagents</th>
<th>Methanolic Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s reagent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s reagent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hager’s reagent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish’s reagent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Barfoed’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Sodium Hydroxide test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>Libermann-Burchard test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Salkowski reaction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Libermann’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Potassium Dichromate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dilute Permanganate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>Keller-Killiani test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Legal’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinone Glycosides.</td>
<td>Borntrager’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponin Glycosides</td>
<td>Foam Test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hemolytic test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehlings test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Benedict’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>Biuret’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Milon’s test</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
The *Spathodea campanulata* extracts showed presence of carbohydrates, steroids, flavonoids and phenolic compounds in both the extracts, however alkaloids were absent in both the extracts.

### 6.1.4 Determination of Total Flavonoid Content

For determining the flavonoid content the standard chosen was Catechin. Calibration curve of Catechin was plotted.

**Calibration curve of Catechin**

![Calibration curve of Catechin](image)

**Figure 7. Calibration curve of Catechin**

**Table 10. Total flavonoid content of extracts**

<table>
<thead>
<tr>
<th>Type of Extract</th>
<th>Absorbance at 510 nm (n=2)</th>
<th>Concentration (equivalent to Catechin (mg/ml))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic <em>Aspidium cicutarium</em></td>
<td>0.1501± 0.00236</td>
<td>1.27mg/ml</td>
</tr>
<tr>
<td>Aqueous <em>Aspidium cicutarium</em></td>
<td>0.0910±0.00245</td>
<td>0.591mg/ml</td>
</tr>
<tr>
<td>Methanolic <em>Spathodea campanulata</em></td>
<td>0.2644±0.0045</td>
<td>2.4mg/ml</td>
</tr>
<tr>
<td>Aqueous <em>Spathodea campanulata</em></td>
<td>0.1256±0.0056</td>
<td>0.245mg/ml</td>
</tr>
</tbody>
</table>

The flavonoid content was carried out according to the Aluminium chloride method and shows high concentration of Flavonoid was seen in methanolic extract of *Aspidium cicutarium* and hence these extracts can be tested further for the anti
oxidant and anti-inflammatory activity as this biological activity can be attributed to the presence of high flavonoid content in the drug. The flavonoid content of methanolic extract of *Spathodea campanulata* was much higher than the aqueous extract using Catechin as standard flavonoid. This indicates that the methanolic extract can be further investigated for antioxidant activity.

### 6.1.5 Determination of Total Phenolic content

The standard for the phenolic content was Gallic acid. Various concentrations were made to plot a calibration curve.

![Calibration curve of Gallic acid](image)

**Figure 8. Calibration curve of Gallic acid.**

**Table 11. Total phenolic content of extracts**

<table>
<thead>
<tr>
<th>Type of Extract</th>
<th>Absorbance at 740 nm</th>
<th>Concentration equivalent to Gallic acid (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic <em>Aspidium cicutarium</em></td>
<td>0.3168 ±0.00424</td>
<td>103.64</td>
</tr>
<tr>
<td>Aqueous <em>Aspidium cicutarium</em></td>
<td>0.1610± 0.00422</td>
<td>47.64</td>
</tr>
<tr>
<td>Methanolic <em>Spathodea campanulata</em></td>
<td>0.0769±0.00212</td>
<td>18</td>
</tr>
<tr>
<td>Aqueous <em>Spathodea campanulata</em></td>
<td>0.0844±0.00155</td>
<td>20.64</td>
</tr>
</tbody>
</table>
The phenolic constituents and phenolic derivatives are responsible for defense mechanism of medicinal plants from the fungi and bacteria present in soil. A high concentration of phenols was obtained in the extracts of rhizomes so it can be further subjected to evaluation of antioxidant activity. Higher phenolic content was observed in the aqueous extract of *Spathodea campanulata* than its methanolic extract. This result is similar to the higher extractive value obtained for water soluble extractive in the preliminary studies.

### 6.1.6 Antioxidant activity of extracts

The antioxidant activity was carried out using DPPH assay.

i. **Antioxidant activity of extracts of *Aspidium cicutarium***

The DPPH assay was carried out to compare the antioxidant activity of methanolic and aqueous extracts of *Aspidium cicutarium*. The reading for control was found to be 0.6575. Various concentrations of Ascorbic acid were prepared and similar treatment was given. The results are shown in Table 12. The 50% inhibitory concentration (IC$_{50}$) was shown to be 15 µg/ml.

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>% Inhibition by Ascorbic acid</th>
<th>Inhibition by Methanolic extract (n=3)</th>
<th>Inhibition by Aqueous extract (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>31.53± 0.21</td>
<td>24.35±0.3</td>
<td>25±0.1</td>
</tr>
<tr>
<td>15</td>
<td>51.5±1.2</td>
<td>49.04±0.73</td>
<td>28.92±0.37</td>
</tr>
<tr>
<td>20</td>
<td>56.78±1.3</td>
<td>54.32±0.7</td>
<td>45.58±0.65</td>
</tr>
<tr>
<td>40</td>
<td>57.8±0.65</td>
<td>78.7±0.26</td>
<td>51.32±0.97</td>
</tr>
<tr>
<td>60</td>
<td>81.01±0.89</td>
<td>86.42±0.49</td>
<td>76.51±0.26</td>
</tr>
<tr>
<td>80</td>
<td>92.16±0.91</td>
<td>96.3±0.30</td>
<td>77.50±0.24</td>
</tr>
<tr>
<td>100</td>
<td>98.47±0.14</td>
<td>97.68±0.28</td>
<td>80.51±0.07</td>
</tr>
</tbody>
</table>
Figure 9. Antioxidant assay of extracts of rhizomes of *Aspidium cicutarium*.

From the inhibitory concentrations obtained the concentration that gives 50% inhibition of free radicals is considered as IC$_{50}$. From the values in the table the IC$_{50}$ for the methanolic extract of rhizomes of *Aspidium cikutarium* was 15 µg/ml and the IC$_{50}$ for the aqueous extract was 40 µg/ml. Methanolic extracts have IC$_{50}$ close to Ascorbic acid.

ii. **Antioxidant Activity of extracts of *Spathodea campanulata*$_{140,141,142}$**

From the table it is seen that at 40 (µg/ml) concentration the aqueous extract of *Spathodea campanulata* showed 50% inhibition. While the methanolic extract showed IC$_{50}$ at 80(µg/ml). Better antioxidant activity was seen in aqueous extract. This can be attributed to an antioxidant principle soluble in water present in the aqueous extract. DPPH Assay of methanolic and aqueous extract of leaves of *Spathodea campanulata* can be seen in the table below.
Antioxidant activity of extracts of leaves of *Spathodea campanulata*

Table 13. **Antioxidant activity of extracts of leaves of Spathodea campanulata**

<table>
<thead>
<tr>
<th>Concentration in (µg/ml)</th>
<th>% Inhibition by Ascorbic acid</th>
<th>% Inhibition by Methanolic Extract</th>
<th>% Inhibition by Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>31.53± 0.21</td>
<td>35.73±0.214</td>
<td>34.20±0.721</td>
</tr>
<tr>
<td>15</td>
<td>51.5±1.2</td>
<td>38.38±0.34</td>
<td>39.32±0.32</td>
</tr>
<tr>
<td>20</td>
<td>56.78±1.3</td>
<td>41.80±0.267</td>
<td>41.52±0.285</td>
</tr>
<tr>
<td>40</td>
<td>57.8±0.65</td>
<td>43.47±0.155</td>
<td>55.38±0.329</td>
</tr>
<tr>
<td>60</td>
<td>81.01±0.89</td>
<td>45.60±0.359</td>
<td>61.62±0.429</td>
</tr>
<tr>
<td>80</td>
<td>92.16±0.91</td>
<td>51.73±0.666</td>
<td>68.33±0.812</td>
</tr>
<tr>
<td>100</td>
<td>98.47±0.14</td>
<td>63.77±0.252</td>
<td>75.25±0.095</td>
</tr>
</tbody>
</table>

**Figure 10.** Antioxidant activity of extracts of leaves of *Spathodea campanulata*

6.1.7 **In vitro anti-inflammatory activity:** 94, 95

90 % inhibition was shown by standard drug Diclofenac sodium at the concentration of 50 µg/ml. Similar treatment was given to extracts.
Table 14. Protein denaturation assay of extracts of rhizomes of *Aspidium cicutarium*

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Absorbance at 660 nm of methanolic extract</th>
<th>% Inhibition (n=3) by methanolic extract</th>
<th>Absorbance at 660 nm of aqueous extract</th>
<th>% Inhibition (n=3) by aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.69</td>
<td>30.65±1.2</td>
<td>0.73</td>
<td>26.13±0.010</td>
</tr>
<tr>
<td>200</td>
<td>0.45</td>
<td>54.77±1.05</td>
<td>0.52</td>
<td>47.03±0.8</td>
</tr>
<tr>
<td>400</td>
<td>0.33</td>
<td>66.83±0.9</td>
<td>0.42</td>
<td>57.08±0.7</td>
</tr>
<tr>
<td>600</td>
<td>0.25</td>
<td>74.87±1.03</td>
<td>0.25</td>
<td>74.27±0.7</td>
</tr>
<tr>
<td>800</td>
<td>0.11</td>
<td>88.94±1.2</td>
<td>0.2</td>
<td>79.29±0.8</td>
</tr>
</tbody>
</table>

The methanolic extract of *Aspidium cicutarium* showed dose dependent inhibition of protein denaturation. In concentrations as high as 800 µg/ml the % inhibition was found to be 88.94 %. Inhibition of protein denaturation up to 79% at concentrations 800 µg/ml were found in aqueous extracts of *Aspidium cicutarium*.

Table 15. Protein denaturation assay of extracts of leaves *Spathodea campanulata*

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Absorbance at 660 nm of methanolic extract</th>
<th>% Inhibition (n=3) by methanolic extract</th>
<th>Absorbance at 660 nm of aqueous extract</th>
<th>% Inhibition (n=3) by aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.62</td>
<td>37.65±1.5</td>
<td>0.82</td>
<td>17.11±0.9</td>
</tr>
<tr>
<td>200</td>
<td>0.6</td>
<td>39.89±1.2</td>
<td>0.72</td>
<td>27.60±1.2</td>
</tr>
<tr>
<td>400</td>
<td>0.57</td>
<td>42.68±2.2</td>
<td>0.7</td>
<td>29.41±2.6</td>
</tr>
<tr>
<td>600</td>
<td>0.54</td>
<td>45.92±2.13</td>
<td>0.59</td>
<td>40.30±1.87</td>
</tr>
<tr>
<td>800</td>
<td>0.38</td>
<td>62.01±2.9</td>
<td>0.52</td>
<td>47.53±1.64</td>
</tr>
</tbody>
</table>

The % inhibition of protein denaturation was found to be lesser in both the extracts of *Spathodea campanulata* as compared to the extracts of *Aspidium cicutarium*. This can also be related to the Total Flavonoid content and Total Phenolic content results of both the plants.
6.1.8 **Antimicrobial Activity.**

i. **Antimicrobial activity of methanolic extract of *Aspidium cicutarium* and *Spathodea campanulata***

Ampicillin (10 µg/ml) was used as standard and showed zone of inhibition 32 mm±0.14 for *Pseudomonas aeroginosa* and 34 mm±0.18 for *Staphylococcus aureus*.

Zone of inhibition of methanol : 5mm

**Table 16. Antimicrobial studies of extracts against various microorganisms.**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Concentration in (µg/ml)</th>
<th>Zone of Inhibition for ACME in mm</th>
<th>Zone of Inhibition for SCME in mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>P. aeroginosa</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1.</td>
<td>2000</td>
<td>10.40±0.529</td>
<td>5.67±0.4</td>
</tr>
<tr>
<td>2.</td>
<td>4000</td>
<td>12.00±0.5</td>
<td>10±0.75</td>
</tr>
<tr>
<td>3.</td>
<td>6000</td>
<td>14.53±1.2</td>
<td>8.43±0.59</td>
</tr>
<tr>
<td>4.</td>
<td>8000</td>
<td>18.43±0.5</td>
<td>12.77±1.56</td>
</tr>
</tbody>
</table>
The antimicrobial activity of both the extracts was obtained against both gram positive and negative microorganisms. The presence of phenolic compounds in the extracts of both the plants can be considered responsible for antimicrobial activity.

6.1.9 Thin Layer Chromatography

i. TLC for Steroids of Methanolic extract of Aspidium cicutarium

The TLC plates were studied under various UV wavelengths and also by spraying agents. The following tables show the Rf values for various spots seen on the TLC plate. The presence of multiple spots on the TLC plates signify the presence of many different steroidal compounds.
12 a. Rf value at 366nm    12b. Rf value by spraying with anisaldehyde in sulphuric acid.

Figure 12. TLC images for determination of steroids in methanolic extracts of *Aspidium cicutarium*

Table 17. Detection of steroids in extracts of rhizomes of *Aspidium cicutarium*

<table>
<thead>
<tr>
<th>Number of spots</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; Values at UV 366 nm</th>
<th>Colour of spot</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; Values by spraying with anisaldehyde in sulphuric acid.</th>
<th>Colour of spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.025</td>
<td>Yellow</td>
<td>0.062</td>
<td>Green</td>
</tr>
<tr>
<td>2</td>
<td>0.062</td>
<td>Yellow</td>
<td>0.12</td>
<td>Violet</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>Fluorescent green</td>
<td>0.62</td>
<td>Violet</td>
</tr>
<tr>
<td>4</td>
<td>----------</td>
<td>----------</td>
<td>0.86</td>
<td>Violet</td>
</tr>
<tr>
<td>5</td>
<td>----------</td>
<td>----------</td>
<td>0.9</td>
<td>Dark violet</td>
</tr>
</tbody>
</table>

ii. TLC for flavonoids of methanolic extract of *Aspidium cicutarium*

Mobile Phase used in the studies was Ethyl Acetate: Formic acid : Glacial Acetic acid: Water (100:1.1:1.1:2.6) From the images of the TLC plate it can be concluded that many spots at different R<sub>f</sub> Values indicate presence of flavonoids. The spots showing
$R_f$ value of 0.5 can be compared to the $R_f$ value of Rutin which a flavonoid. Thus these results indicate the need of further HPTLC screening for the extract.

**Figure 13.** TLC images for detection of flavonoids in methanolic extract of rhizomes of *Aspidium cicutarium*

**Table 18.** Detection of flavonoids in extracts of *Aspidium cicutarium*

<table>
<thead>
<tr>
<th>Number of spots</th>
<th>$R_f$ Values at UV 254nm</th>
<th>Colour of spot</th>
<th>$R_f$ Values at UV 366nm</th>
<th>Colour of spot</th>
<th>$R_f$ Values at visible light</th>
<th>Colour of spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.32</td>
<td>Brown</td>
<td>0.32</td>
<td>Fluorescent green</td>
<td>0.06</td>
<td>Yellow</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>Brown</td>
<td>------</td>
<td>------</td>
<td>0.32</td>
<td>Yellow</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>Brown</td>
<td>------</td>
<td>------</td>
<td>0.32</td>
<td>Yellow</td>
</tr>
</tbody>
</table>
iii. TLC for steroids of methanolic extract of *Spathodea campanulata*

Three different spots at various R_f values can be seen in determining the steroids. It can be concluded that the extract contains a range of steroidal compounds.

iv. TLC studies for flavonoids of *Spathodea campanulata*

Presence of spots is evident on the TLC plate image for the detection of flavonoids. On spraying the TLC plate with Vanillin in sulphuric acid many spots were seen. So the presence of flavonoids can be confirmed. The extracts can be subjected to HPTLC screening.

![TLC images](image1.png)

14 a

14 b

14a. R_f Values of steroids by spraying with anisaldehyde in sulphuric acid.
14 b. R_f Values of flavonoids by spraying with vanillin in sulphuric acid.

Figure 14. TLC images for detection of steroids and flavonoids in methanolic extracts of *Spathodea campanulata*
Table 19. Detection of steroids and flavonoids in extract of *Spathodea campanulata*

<table>
<thead>
<tr>
<th>Number of spots</th>
<th>( R_f ) Values for steroids by spraying with anisaldehyde in sulphuric acid.</th>
<th>Colour of spots</th>
<th>( R_f ) Values for flavonoids by spraying with vanillin in sulphuric acid.</th>
<th>Colour of spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.025</td>
<td>Brown</td>
<td>0.32</td>
<td>Brown</td>
</tr>
<tr>
<td>2</td>
<td>0.062</td>
<td>Brown</td>
<td>0.5</td>
<td>Brown</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>Brown</td>
<td>0.6</td>
<td>Brown</td>
</tr>
<tr>
<td>4</td>
<td>------------------</td>
<td>---------------</td>
<td>0.8</td>
<td>Blue violet</td>
</tr>
<tr>
<td>5</td>
<td>------------------</td>
<td>---------------</td>
<td>0.82</td>
<td>Yellow brown</td>
</tr>
<tr>
<td>6</td>
<td>------------------</td>
<td>---------------</td>
<td>0.86</td>
<td>Violet</td>
</tr>
</tbody>
</table>

6.1.10 High Performance Thin Layer Chromatographic Studies\(^{148,149}\)

i. **HPTLC studies of methanolic extract of *Aspidium cicutarium***
   The mobile phase selected for the HPTLC studies of methanolic extract was n hexane: Ethyl Acetate in the ratio of (7:3). The resolution was obtained at 200 nm. The chromatogram shows 7 peaks at \( R_f \) values 0.01, 0.11, 0.41, 0.81, 1.11, 1.311.41 at 200 nm.

ii. **HPTLC studies of aqueous extract of *Aspidium cicutarium***.
   The mobile phase for the aqueous extract of *Aspidium cicutarium* was ethyl acetate: n Butanol at the ratio (6:4). The resolution was obtained at 220 nm. The Figure shows 06 peaks. The peaks at Rf value 1.01 shows the maximum concentration with an area under the curve of 200.8 peaks were obtained having Rf values 0.11, 0.31, 0.41, 0.61, 0.81, 1.01, 1.21, 1.41.

iii. **HPTLC studies of methanolic extract of *Spathodea campanulata***
   The resolution was obtained at 450 nm. Mobile Phase was n Hexane : Ethyl acetate (7:3). Resolution was obtained into 10 peaks. The volume of injection for the present study was kept 10 µl. The peaks had Rf values of 0.31, 0.51, 0.61, 0.71, 0.81, 1.01, 1.21, 1.31, 1.41, 1.61. Same extract was tried with sample volume 20 µl keeping the mobile phase same. Resolution was obtained into 10 peaks with
maximum at peak 6 at the Rf value of 0.91 which was very similar to the results obtained when the sample volume was 10µl. Mobile Phase: n Hexane : Ethyl acetate (7:3)

iv. HPTLC studies of aqueous extract of *Spathodea campanulata*

For the aqueous extracts 10 µl was injected first but there was no elution obtained.

Mobile Phase was Ethyl acetate: N Butanol (6:4) Aqueous extracts of *Spathodea campanulata* showed good resolution at sample volume 20 µl at 450nm. All four peaks obtained after the injection of 20µl sample were obtained at 0.81,1.21,1.41,1.51 and had areas of 120 for first two peaks and 100 and 80 for the rest two.

![HPTLC of *Aspidium cicutarium* methanolic extract](image)

15a HPTLC of *Aspidium cicutarium* methanolic extract
15b HPTLC of *Aspidium cicutarium* aqueous extract

15c HPTLC of *Spathodea campanulata* methanolic extract (10 µl)
The presence of multiple peaks of considerable intensity prove that the extracts for both the plants under study have varied compositions of flavonoids and steroids which have eluted out in the presence of the typical mobile phases.
6.1.11 Spectrophotometric studies

i. Determination of $\lambda_{\text{max}}$ for Methanolic extract of *Aspidium cicutarium*
   The UV spectrum of solution of *Aspidium cicutarium* (10µg/ml) was scanned between 200-400 nm regions on UV spectrophotometer. $\lambda_{\text{max}}$ of *Aspidium cicutarium* in Phosphate buffer pH 7.2 was found to be 281 nm.

ii. Calibration curve of Methanolic extract of *Aspidium cicutarium*
   Calibration curve was plotted for various concentrations of methanolic extract from 10µg/ml to 60 µg/ml.

![Graph of UV absorption](image)

16a $\lambda_{\text{max}}$ of *Aspidium cicutarium* methanolic extract

16 b Calibration curve of *Aspidium cicutarium* methanolic extract

Figure 16. UV Absorption studies of methanolic extract of *Aspidium cicutarium*
iii. Determination of $\lambda_{\text{max}}$ for Methanolic extract of *Spathodea campanulata*

The UV spectrum of solution of *Spathodea campanulata* (10µg/ml) was scanned between 200-400 nm regions on UV spectrophotometer. $\lambda_{\text{max}}$ of *Spathodea Campanulata* in PBS 6.8 was found to be 235 nm.

iv. Calibration curve of methanolic extract of Spathodea campanulata.

Various concentrations ranging from 1-7 µg/ml were made and calibration curve was plotted. The $r^2$ was 0.9953.

![Graph showing UV spectrum of Spathodea campanulata methanolic extract](image)

17 a. $\Lambda_{\text{max}}$ of *Spathodea campanulata* methanolic extract
17 b. Calibration curve of *Spathodea campanulata* methanolic extract

Figure 17. UV absorption studies of methanolic extract of leaves of *Spathodea campanulata*

6.1.12 **Analytical characterization of markers.**

i. Analytical profile of Phloroglucinol (marker for ACME)

a. Chemical structure

![Chemical structure of Phloroglucinol](image)

b. CAS: 108-73-6

c. Empirical Formula: C₆H₆O₃

d. Molecular weight: 126.11

e. Melting Point: 215 - 220° C

f. Solubility: Soluble in ethanol, diethyl ether, methanol, acetone.

g. UV absorption spectra of Phloroglucinol.

Calibration curve of methanolic extract of *Spathodea campanulata*
The $\lambda_{\text{max}}$ was obtained at 273 nm for Phloroglucinol which was selected as a flavonoid marker for *Aspidium cicutarium* methanolic extract.

![Graph showing calibration curve of Phloroglucinol](image)

18a $\lambda_{\text{max}}$ of Phloroglucinol

**Calibration curve of Phloroglucinol**

$y = 0.1086x + 0.03$

$R^2 = 0.9908$

![Graph showing calibration curve of Phloroglucinol](image)

18b. Calibration curve of Phloroglucinol

**Figure 18. Spectroscopy of marker of *Aspidium cicutarium*.**
h. IR Spectra

The IR Spectra of Phloroglucinol showed presence of aromatic rings and prominent –OH functional groups.

![FTIR spectra of Phloroglucinol](image)

**Figure 19. FTIR spectra of Phloroglucinol**

**Table 20. Interpretation of IR Spectra for Phloroglucinol**

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3330</td>
<td>OH stretch</td>
</tr>
<tr>
<td>2920</td>
<td>CH stretch</td>
</tr>
<tr>
<td>1660</td>
<td>C = O</td>
</tr>
<tr>
<td>1620</td>
<td>C = C</td>
</tr>
<tr>
<td>1600</td>
<td>Aromatic Structure</td>
</tr>
<tr>
<td>1510</td>
<td>C = C</td>
</tr>
<tr>
<td>1360</td>
<td>C - O – C</td>
</tr>
<tr>
<td>1295</td>
<td>C - O – C</td>
</tr>
<tr>
<td>1200</td>
<td>C - O – C</td>
</tr>
<tr>
<td>1060</td>
<td>C – O – C</td>
</tr>
<tr>
<td>810</td>
<td>Substituted Aromatics</td>
</tr>
</tbody>
</table>

The similarity between the analytical profile of Phloroglucinol and ACME showed the presence of phenolic compounds and flavonoids in the extract.
ii. Analytical profile of Kaempferol

a. Structure of Kaempferol

![Structure of Kaempferol](image)

b. CAS: 520-18-3M

c. Molecular Formula: \( C_{15}H_{10}O_6 \)
d. Molecular weight: 286.2

e. Solubility: ethanol, methanol and DMSO

f. UV absorption spectra of Kaempferol: The UV absorption spectra of Kaempferol reveals two wavelengths 257nm and 353 nm which show maximum absorption. Since the wavelength 257 nm was nearer to the \( \lambda_{\text{max}} \) of Spathodea campanulata the calibration curve of Kaempferol was plotted at this wavelength

![UV absorption spectra of Kaempferol](image)

\[ 20a \ \lambda_{\text{max}} \text{ of kaempferol} \]
20b. Calibration curve of Kaempferol

Figure 20. Spectroscopy of marker of *Spathodea campanulata*: Kaempferol

\[ y = 0.0944x - 0.0348 \]
\[ R^2 = 0.9727 \]

g. FTIR spectra of Kaempferol

Figure 21. FTIR spectra of Kaempferol.
Table 21. Interpretation of IR Spectra of Kaempferol

<table>
<thead>
<tr>
<th>Frequency (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3330</td>
<td>OH stretch</td>
</tr>
<tr>
<td>2920</td>
<td>CH stretch</td>
</tr>
<tr>
<td>1660</td>
<td>C = O</td>
</tr>
<tr>
<td>1620</td>
<td>C = C</td>
</tr>
<tr>
<td>1600</td>
<td>Aromatic Structure</td>
</tr>
<tr>
<td>1510</td>
<td>C = C</td>
</tr>
<tr>
<td>1360</td>
<td>C - O – C</td>
</tr>
<tr>
<td>1295</td>
<td>C - O – C</td>
</tr>
<tr>
<td>1200</td>
<td>C - O – C</td>
</tr>
<tr>
<td>1060</td>
<td>C – O – C</td>
</tr>
<tr>
<td>810</td>
<td>Substituted Aromatics</td>
</tr>
</tbody>
</table>

Presence of hydroxyl groups and aromatic structures were revealed in the IR spectra of Kaempferol. When this IR spectrum was compared to the IR spectra of *Spathodea campanulata* similarities were observed in the presence of functional groups.

iii. Analytical profile of βsitosterol

a. Structure

![βsitosterol structure](image)

b. Synonym: α-Dihydrofucosterol, 22,23-Dihydrostigmasterol, 24β-Ethylcholesterol, 5- Stigmasten-3β-ol

c. CAS Number: 83-46-5

d. Empirical Formula: C$_{29}$H$_{50}$O
e. **Molecular weight**: 414.17
f. **Colour**: Yellow
g. **Odour**: Characteristic
h. **Taste**: Tasteless
i. **Melting point**: 31°C
j. **Solubility**: Soluble in Ethanol and Methanol and in alkaline solvents.
k. **UV Spectroscopy**: The $\lambda_{\text{max}}$ was obtained at 242 nm. Various concentrations were made in methanol and calibration curve was plotted.

![UV Spectroscopy of \( \beta \) Sitosterol](image)

22a $\lambda_{\text{max}}$ of \( \beta \) Sitosterol

![Calibration curve of \( \beta \) Sitosterol](image)

22b Calibration curve of \( \beta \)-Sitosterol

*Figure 22. Spectroscopy of marker of Spathodea campanulata: \( \beta \)Sitosterol*
1. IR Spectrum of β-Sitosterol

![IR Spectrum of β-Sitosterol](image)

Figure 23. FTIR Spectra of β sitosterol

<table>
<thead>
<tr>
<th>Frequency(cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3373.6cm⁻¹</td>
<td>O-H stretching</td>
</tr>
<tr>
<td>2940.7 cm⁻¹and 2867.9cm⁻¹</td>
<td>aliphatic C-H stretching</td>
</tr>
<tr>
<td>1641.6cm⁻¹</td>
<td>C=C absorption peak</td>
</tr>
<tr>
<td>1457.3cm⁻¹</td>
<td>CH2</td>
</tr>
<tr>
<td>1381.6cm⁻¹</td>
<td>OH def</td>
</tr>
<tr>
<td>1038.7cm⁻¹</td>
<td>Cycloalkane</td>
</tr>
</tbody>
</table>

On comparison of the results with SCME there was found to be a striking resemblance in results of β- sitosterol and SCME suggesting presence of steroids. In the literature already studies have reported the presence of Ajugol as a steroidal compound present in *Spathodea campanulata*. β- sitosterol can be compared to similar steroidal compound.
6.1.13 **Acute toxicity studies.**
The acute toxicity studies were conducted using the OECD guidelines. The LD$_{50}$ for *Aspidium cicutarium* methanolic extract was found to be 3000 mg/kg. The LD$_{50}$ for methanolic extract of *Spathodea campanulata* was found to be 4000mg/kg.

6.1.14 **Cell line studies.**

i. **MTT assay.**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Concentration of extract</th>
<th>% Cell Viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1mg/ml</td>
<td>89.47±1.2</td>
</tr>
<tr>
<td>2</td>
<td>0.1mg/ml</td>
<td>106.39±1.56</td>
</tr>
<tr>
<td>3</td>
<td>0.01mg/ml</td>
<td>87.68±2.1</td>
</tr>
<tr>
<td>4</td>
<td>1 μg/ml</td>
<td>110.26±2.65</td>
</tr>
<tr>
<td>5</td>
<td>0.1 μg/ml</td>
<td>110.14±1.45</td>
</tr>
<tr>
<td>6</td>
<td>0.01 μg/ml</td>
<td>106.39±2.15</td>
</tr>
<tr>
<td>7</td>
<td>1ng/ml</td>
<td>90.26±1.4</td>
</tr>
</tbody>
</table>

Cytotoxic effects of the extract were evaluated using the MTT assay. The concentrations were scanned for cell viability after the induction of inflammation by LPS in the cell line RAW 264.7. The concentrations as high as 1 mg/ml also show 89% of cell viability. Thus it can be concluded that the extract is safe to be used in larger concentrations.
ii. Anti-inflammatory responses of ACME extract on RAW 264.7 cell lines.\textsuperscript{104}

Set 1: TNF $\alpha$

The straight line equation obtained with the pcg/ml concentration ranges of TNF $\alpha$ mediator was $y=0.0001 \times + c$. The four groups were selected and they received respective treatments and gave various optical densities for the supernatents. Using this equation and putting the optical density values for the four groups the concentrations in pcg/ml were obtained and plotted against the groups under study.
Table 24. Concentrations of inflammatory mediators after treatment with methanolic extracts of rhizomes of *Aspidium cicutarium*

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration of TNF α Pcg/ml (n=3)</th>
<th>Concentration of IL 6 Pcg/ml (n=3)</th>
<th>Concentration of IL1 β (pcg/ml) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LPS</td>
<td>2841±7.8</td>
<td>860.85±5.6</td>
<td>773.75±10.2</td>
</tr>
<tr>
<td>ACME 0.5mg/ml</td>
<td>2728.5±5.9</td>
<td>312.5±7.5</td>
<td>426.25±14.3</td>
</tr>
<tr>
<td>ACME 1mg/ml</td>
<td>1318.5±4.6</td>
<td>111.85±4.5</td>
<td>199.75±10.9</td>
</tr>
</tbody>
</table>

25a. Comparative inhibition of production of TNF α in groups.

25b. Comparative inhibition of production of IL6 in groups.
**Figure 25. Anti-inflammatory activity of *Aspidium cicutarium* methanolic extract in cell line**

From the above graph the comparative inhibition of the expression of TNF α in the 4 different treatment groups was seen. While the control showed 0% inhibition. The LPS showed high optical density. The ACME extract showed dose dependent inhibition. This can be seen by the reduction in optical density suggesting reduction in the expression of TNF α. The dose 1mg/ml showed almost 50% reduction in 48 hours. The ACME in concentration of 0.5 mg/ml showed 63% suppression of IL6 mediator while 1mg/ml showed inhibition of 89%. It can be concluded that *Aspidium cicutarium* methanolic extract has good inhibitory action against mediators of inflammation especially against the expression of IL6.

From the results in Set 3 it can be seen that the methanolic extract of *Aspidium cicutarium* inhibited the expression of IL 1 β mediator in dose dependent manner. Excellent results were seen in concentration 1mg/ml of extract.

From the cell line studies carried on murine macrophages RAW 264.7 in which inflammation was induced by LPS, it can be seen that *Aspidium cicutarium* showed inhibition of the cytokinin mediators of inflammation. The cytokine mediator IL 6 was suppressed considerably amongst the three mediators under study. Since IL6 is a pro-inflammatory cytokine that produces multifunctional effects. Deregulated IL-6
production and signaling are associated with chronic inflammatory diseases, autoimmunity and cancer. On this basis, inhibition of IL-6 production, its receptors or the signaling pathways are strategies currently being widely pursued to develop novel therapies for a wide range of diseases. This potential of the extract can be used to establish a definite pathway for anti-inflammatory action of the extract.

6.1.15 In vivo topical anti-inflammatory studies. 150,151

Figure 26. Topical anti-inflammatory studies of extracts
Table 25.  Topical anti-inflammatory activity of extracts.

<table>
<thead>
<tr>
<th>Type of extract</th>
<th>Concentration (μl/ear)</th>
<th>% inhibition</th>
<th>Std dev(n=6)</th>
<th>Std error</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACME</td>
<td>50</td>
<td>20.8</td>
<td>2.111</td>
<td>2.373</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>46.93</td>
<td>9.528</td>
<td>0.5451</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>68.47</td>
<td>6.334</td>
<td>1.636</td>
</tr>
<tr>
<td>ACAE</td>
<td>50</td>
<td>5.8</td>
<td>3.052</td>
<td>0.788</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.933</td>
<td>1.163</td>
<td>0.3003</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>17.67</td>
<td>0.488</td>
<td>0.126</td>
</tr>
<tr>
<td>SCME</td>
<td>50</td>
<td>33.07</td>
<td>10.1</td>
<td>2.609</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>64.07</td>
<td>8.771</td>
<td>2.265</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>68.93</td>
<td>6.33</td>
<td>1.634</td>
</tr>
<tr>
<td>SCAE</td>
<td>50</td>
<td>6.857</td>
<td>3.183</td>
<td>0.8507</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>17.67</td>
<td>0.488</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>29.4</td>
<td>5.138</td>
<td>1.327</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>3</td>
<td>78.07</td>
<td>9.192</td>
<td>2.573</td>
</tr>
</tbody>
</table>

Statistical Analysis: The groups were analyzed by one way ANOVA followed by Dunnet’s test. * p<0.05, **p<0.01, ***p<0.001

Topical anti-inflammatory activity of aqueous and methanolic extracts of both *Aspidium cicutarium* and *Spathodea campanulata* was carried out using croton oil induced mouse ear edema method. Dexamethasone was used as standard drug as it is well known for its topical anti-inflammatory activity. The 3 doses chosen were 50, 100 and 200 μl / ear of the mouse. According to the % inhibition results of the various extracts the % inhibition of the edema was negligible in the aqueous extracts. But the methanolic extracts of *Aspidium cicutarium* showed dose dependent anti-inflammatory activity. As the dose of extract increased the % inhibition also increased and at 200 μl/ear concentration the % inhibition went as high as 68.47 % which is very close to the results obtained by Dexamethasone. Similarly the methanolic extract of *Spathodea campanulata* also showed dose dependent inhibition. The 200μl/ ear dose showed very high anti-inflammatory activity. Thus it could be concluded that the methanolic extracts of *Aspidium cicutarium* and *Spathodea campanulata* can be incorporated into anti-inflammatory formulations.
6.1.16 **In vivo wound healing activity of extracts.**

i. **Incision wound model for wound healing activity.**
The histological profiles of granulation tissue of control animals showed more macrophages and less collagenation. The sections of granulation tissue of extract-treated animals showed the sign of tissue repair with increased collagen formation. Breaking strength of the wound measured after 10\textsuperscript{th} day is shown in following table.

**Table 26. Breaking strength of wounds after application of extracts.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Breaking strength in gm (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>321±3.15</td>
</tr>
<tr>
<td>II</td>
<td>Standard 0.2% w/w Nitrofurazone ointment</td>
<td>487±2.14</td>
</tr>
<tr>
<td>III</td>
<td>ACME</td>
<td>416±3.98</td>
</tr>
<tr>
<td>IV</td>
<td>SCME</td>
<td>384±4.17</td>
</tr>
</tbody>
</table>

ii. **Histopathology study of wounds.**

**Table 27. Histopathological findings of wound healing activity of extracts**

<table>
<thead>
<tr>
<th>Images</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image_url" alt="Image" /></td>
<td>VEHICLE CONTROL: (100 x) Lesser or no tissue repair was evident. collagenous mass indicated by arrow head was very less.</td>
</tr>
<tr>
<td>Image</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
</tr>
</tbody>
</table>
| ![Vehicle Control](image1) | VEHICLE CONTROL  
Vascular changes and angiogenesis (red arrow), neutrophilic infiltration (blue arrow), edema and fibroblasts (white arrow), necrosis (orange arrow) very less collagenous mass (arrow head) can be seen in the photograph. |
| ![ACME H&E stain 100X](image2) | ACME H&E stain 100X  
Vascular changes and angiogenesis (red arrow), neutrophilic infiltration (blue arrow), edema and fibroblasts (white arrow), necrosis (orange arrow) collagenous mass (arrow head) can be seen. |
| ![ACME H&E stain 400X](image3) | ACME H&E stain 400X:  
Vascular changes and angiogenesis (red arrow), neutrophilic infiltration (blue arrow), edema and fibroblasts (white arrow), necrosis (orange arrow) collagenous mass (arrow head) can be seen. |
| ![SCME H&E stain 100X](image4) | SCME: H&E stain 100X  
Vascular changes and angiogenesis (red arrow), neutrophilic infiltration (blue arrow), edema and fibroblasts (white arrow), necrosis (orange arrow) collagenous mass (arrow head) can be seen. |
From the pictures taken from the slides it was evident that since wound healing process is a natural process the vehicle control group also shows some positive changes that the wound healing process has began. The standard that was 0.2% Nitrofurazone ointment showed the best results. It showed beginning of remodelling phase after the proliferation phase. Enough collagenous mass was seen which is shown by white arrows. The vascular changes also suggested angiogenesis at some parts which is a part of proliferative phase. The two extracts under study coded as
ACME (methanolic extract of *Aspidium cicutarium*) and SCME (methanolic extract of *Spathodea campanulata*) were also applied on the incision wounds. The slides for both the extracts show almost same response however the ACME showed better wound healing and can be incorporated into a formulation for wound healing. This extract can also be further tested for infected wound healing.

iii. **Excision Wound Model**

The Excision wound model studies were also performed on the rats. They showed exactly similar results as the histopathological findings. The wound closure after 15 days was maximum in methanolic extract of *Aspidium cicutarium*. In case of methanolic extract of *Spathodea campanulata* the closure was lesser than the ACME. The wound healing activity of both the extracts were near the values of wound contraction showed by the standard 0.2% Nitrofurazone ointment. The activity can be attributed to the presence of phenols and flavonoids and the anti microbial activity possessed by the extracts which can be seen in the previous study.

<table>
<thead>
<tr>
<th>Treatment Given</th>
<th>Average Contraction Size of Wound in mm²</th>
<th>% Contraction after 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>5th day</td>
</tr>
<tr>
<td>Control</td>
<td>499±1.9</td>
<td>496±3.2</td>
</tr>
<tr>
<td>Standard</td>
<td>500±4.1</td>
<td>359.1±2.7</td>
</tr>
<tr>
<td>ACME</td>
<td>502.9±3.2</td>
<td>387.0±4.5</td>
</tr>
<tr>
<td>SCME</td>
<td>503.8±4.1</td>
<td>414.5±1.9</td>
</tr>
</tbody>
</table>

(The groups were analyzed by one way ANOVA followed by Dunnet’s test. ***p<0.001, ****p<0.0001)
The standard preparation was 0.2% Nitrofurazone ointment and it showed 67.66% wound contraction. ACME had good results it showed 34.86% contraction while SCME showed 24.64% of wound contraction. The low wound healing potential can be attributed to the lesser release rate from the soft paraffin base in which the extract was mixed.

### 6.2 Formulation of films

**6.2.1 Preformulation studies of films**

As suggested by Schroeder et al a scoring system was developed and the polymers were selected.

#### Table 29. Scoring system for films of various polymers.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Self-Adhesiveness</th>
<th>Cosmetical Attractiveness</th>
<th>Drying Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit RL 100 and HPMC K4M</td>
<td>High</td>
<td>High</td>
<td>&lt;6hrs</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>Medium</td>
<td>Medium</td>
<td>&gt;8hrs</td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td>Low</td>
<td>Low</td>
<td>&gt;8hrs</td>
</tr>
</tbody>
</table>

From the scoring system prepared for the films it is obvious that the film made from a mixture of Eudragit RL 100 and HPMC K4 M show clear transparent films. The films
casted by Ethylcellulose had flaky and rough appearance. While the Sodium CMC film was tacky. So the polymers chosen for further study would be HPMC K4M and Eudragit R L 100. The polymers were subjected further to study compatibility with the extracts.

6.2.2 Compatibility studies

i. DSC of Methanolic extract of Aspidium cicutarium

![Graph](image1.png)

28a. DSC of ACME.

![Graph](image2.png)

28b. DSC of ACME + Eudragit RL 100 and HPMC K4M
DSC is a very important tool in carrying out excipient drug compatibility studies. The above thermograms show the DSC for the methanolic extract of *Aspidium cicutarium*. While figure no 28b showed the DSC of mixture of the polymers and the extract in a 1:1 proportion. Peak obtained initially at the beginning of the study was obvious even in the thermogram of the polymer and extract mixture. Also a trough seen at 2 minutes interval also appeared in the DSC of Mixture. From the above thermograms it can be concluded that the excipients chosen for the film are compatible with the extract. The
images obtained from DSC studies suggested that there is no major interaction involved between the excipients and the extract SCME and so the films of this mixture can be casted and evaluated further.

6.2.3 Formulation and evaluation of films

The film formulations containing the extracts were subjected to preliminary studies like texture and appearance, % flatness, weight, adhesive strength, tensile strength and drug content.

i. Studies on trial Batches of films of ACME

Preliminary evaluation included texture and appearance of the films. The ACME films appeared reddish in colour. They showed good adhesive strength and tensile strength. They were satisfactorily flat without any imperfections. The moisture uptake was satisfactory.

Table 30. Evaluation of trial batches of methanolic extracts of rhizomes of *Aspidium cicutarium*

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Thickness mm</th>
<th>Adhesive Strength (g/20mm)</th>
<th>Tensile strength (N/mm2)</th>
<th>% flatness</th>
<th>% moisture uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF1</td>
<td>0.23</td>
<td>20.5</td>
<td>500</td>
<td>99.45±0.5</td>
<td>2.4</td>
</tr>
<tr>
<td>AF2</td>
<td>0.25</td>
<td>21.4</td>
<td>528</td>
<td>99.24±0.3</td>
<td>3.1</td>
</tr>
<tr>
<td>AF3</td>
<td>0.21</td>
<td>19.7</td>
<td>493</td>
<td>99.18±0.6</td>
<td>3.1</td>
</tr>
<tr>
<td>AF4</td>
<td>0.22</td>
<td>20.3</td>
<td>493</td>
<td>99.33±0.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

ii. Formulation and Evaluation of factorial batches of ACME films (AF1 to AF9)

![Figure 29. Films of *Aspidium cicutarium* methanolic extract](image-url)
9 batches of films were made according to the factorial design and subjected to evaluation.

Table 31. Evaluation of film batches AF1 to AF9

31a. Evaluation of thickness, weight and folding endurance from SF1 to SF9

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Thickness (mm)</th>
<th>Weight (g)</th>
<th>Folding Endurance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF1</td>
<td>0.23±0.5</td>
<td>0.115±0.12</td>
<td>139±0.25</td>
</tr>
<tr>
<td>AF2</td>
<td>0.25±0.8</td>
<td>0.114±0.11</td>
<td>145±1.2</td>
</tr>
<tr>
<td>AF3</td>
<td>0.21±0.5</td>
<td>0.115±0.09</td>
<td>150±0.14</td>
</tr>
<tr>
<td>AF4</td>
<td>0.22±0.3</td>
<td>0.111±0.11</td>
<td>143±0.5</td>
</tr>
<tr>
<td>AF5</td>
<td>0.25±0.2</td>
<td>0.112±0.13</td>
<td>138±0.14</td>
</tr>
<tr>
<td>AF6</td>
<td>0.23±0.4</td>
<td>0.112±0.14</td>
<td>157±0.5</td>
</tr>
<tr>
<td>AF7</td>
<td>0.25±0.3</td>
<td>0.118±0.14</td>
<td>134±0.25</td>
</tr>
<tr>
<td>AF8</td>
<td>0.23±0.2</td>
<td>0.119±0.11</td>
<td>140±0.25</td>
</tr>
<tr>
<td>AF9</td>
<td>0.22±0.5</td>
<td>0.118±0.13</td>
<td>149±0.5</td>
</tr>
</tbody>
</table>

31b. Evaluation of Tensile and adhesive strength, moisture uptake and drug content from SF1 to SF9

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Tensile Strength(N/mm²)</th>
<th>Adhesive Strength (g/20mm)</th>
<th>%moisture uptake</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF1</td>
<td>20.67±0.55</td>
<td>531±0.33</td>
<td>2.60±0.62</td>
<td>82.27±2.61</td>
</tr>
<tr>
<td>AF2</td>
<td>21.2±2.6</td>
<td>498±0.67</td>
<td>3.33±0.42</td>
<td>82.17±0.91</td>
</tr>
<tr>
<td>AF3</td>
<td>21.36±0.90</td>
<td>525±0.70</td>
<td>3.53±0.55</td>
<td>79.40±0.82</td>
</tr>
<tr>
<td>AF4</td>
<td>19.33±0.72</td>
<td>499±0.70</td>
<td>2.63±0.57</td>
<td>80.80±0.46</td>
</tr>
<tr>
<td>AF5</td>
<td>17.6±1.21</td>
<td>503±0.54</td>
<td>2.37±0.55</td>
<td>86.60±2.67</td>
</tr>
<tr>
<td>AF6</td>
<td>19.13±1.06</td>
<td>498±0.23</td>
<td>2.33±0.31</td>
<td>87.33±1.1</td>
</tr>
<tr>
<td>AF7</td>
<td>21.5±1.25</td>
<td>535±0.28</td>
<td>3.13±0.12</td>
<td>86.93±1.55</td>
</tr>
<tr>
<td>AF8</td>
<td>19.66±1.48</td>
<td>535±0.28</td>
<td>2.23±0.32</td>
<td>85.83±0.4</td>
</tr>
<tr>
<td>AF9</td>
<td>20.9±1.44</td>
<td>523±0.25</td>
<td>3.27±0.31</td>
<td>85.63±1.44</td>
</tr>
</tbody>
</table>
The films were evaluated initially for some preliminary physical properties like appearance, colour and thickness.

a. Appearance:

The films appeared reddish brown in colour and were uniformly thick without any imperfections.

b. Thickness:

Thickness of the films was measured by micrometer screw guage at three different places and mean was calculated.

c. Folding Endurance:

Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme condition of folding. Folding endurance was determined by repeatedly folding the film at the same place. The number of times the film could be folded at the same place without breaking is folding endurance. Highest folding endurance was shown by AF6.

d. Tensile Strength:

The Tensile strength of the films was good. The highest value for tensile strength was shown by AF7 suggesting that the film was the strongest, this high tensile strength of the film can be attributed to the high amount of film forming agent and lesser amount of PEG 400 present in the film.

e. Adhesive Strength:

The adhesive strength of the film is also an important parameter that determines the ability of the film to adhere to the skin for the period of treatment time without undergoing any major changes. From the results of the adhesive strength it can be concluded that the batch coded as AF1 which contain an optimum amount of film forming agent and also an optimum amount of PEG 400 had the highest adhesive
strength. The batch AF7 which had highest tensile strength also showed high adhesive strength.

f. **Drug Content:**

All the 9 batches showed satisfactory drug content ranging from 79.4% to 87.33%.

g. **% Moisture uptake.**

The % moisture uptake of all the films F1 to F9 was between the range of 2% to 3%. The batches AF3 and AF9 which have higher % of PEG 400 show higher moisture uptake.

h. **% Cumulative release of the extract from the films of ACME**

The release studies were carried out in the Franz diffusion cell. The studies lasted for 8 hours and the following values for % release were obtained.
Table 32. % cumulative release from film batches AF1 to AF9

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>AF1</th>
<th>AF2</th>
<th>AF3</th>
<th>AF4</th>
<th>AF5</th>
<th>AF6</th>
<th>AF7</th>
<th>AF8</th>
<th>AF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.2± 0.69</td>
<td>12.5± 1.3</td>
<td>18.2± 0.66</td>
<td>15.3± 0.18</td>
<td>19.8± 0.36</td>
<td>17.4± 0.33</td>
<td>15.4± 1.2</td>
<td>18.5± 0.36</td>
<td>19.4± 0.86</td>
</tr>
<tr>
<td>2</td>
<td>35.1± 1.2</td>
<td>32.2± 0.68</td>
<td>31.4± 0.33</td>
<td>34.2± 1.9</td>
<td>36.1± 0.18</td>
<td>22.5± 1.65</td>
<td>25.4± 1.22</td>
<td>34.8± 1.25</td>
<td>34.6± 0.32</td>
</tr>
<tr>
<td>3</td>
<td>40.1± 1.2</td>
<td>41.2± 1.2</td>
<td>43.1± 1.8</td>
<td>42.6± 1.7</td>
<td>41.1± 0.25</td>
<td>32.4± 0.65</td>
<td>32.1± 2.1</td>
<td>38.4± 1.4</td>
<td>38.5± 2.3</td>
</tr>
<tr>
<td>4</td>
<td>43.2± 1.3</td>
<td>41.9± 1.5</td>
<td>47.1± 1.9</td>
<td>46.5± 0.24</td>
<td>45.3± 0.29</td>
<td>39.8± 0.14</td>
<td>35.6± 1.1</td>
<td>40.1± 1.8</td>
<td>42.1± 1.9</td>
</tr>
<tr>
<td>5</td>
<td>46.5± 1.5</td>
<td>45.9± 0.59</td>
<td>50.2± 0.24</td>
<td>49.7± 0.64</td>
<td>48.7± 0.25</td>
<td>45.6± 1.6</td>
<td>39.8± 2.3</td>
<td>45.8± 1.9</td>
<td>46.3± 2.5</td>
</tr>
<tr>
<td>6</td>
<td>47.1± 1.6</td>
<td>47.1± 0.41</td>
<td>56.4± 0.36</td>
<td>52.1± 0.25</td>
<td>51.6± 0.36</td>
<td>50.4± 1.8</td>
<td>44.8± 1.6</td>
<td>46.8± 0.15</td>
<td>50.1± 0.21</td>
</tr>
<tr>
<td>7</td>
<td>49.8± 2.6</td>
<td>49.5± 0.35</td>
<td>59.8± 0.52</td>
<td>54.9± 1.24</td>
<td>55.4± 0.33</td>
<td>56.8± 2.9</td>
<td>51.7± 0.33</td>
<td>49.2± 0.99</td>
<td>54.2± 0.52</td>
</tr>
<tr>
<td>8</td>
<td>69.8± 1.3</td>
<td>51.3± 0.21</td>
<td>65.2± 0.25</td>
<td>66.4± 1.84</td>
<td>58.3± 0.41</td>
<td>68.2± 0.2</td>
<td>52.6± 0.41</td>
<td>55.2± 0.36</td>
<td>63.5± 0.23</td>
</tr>
</tbody>
</table>
30a. Plot of % release vs time from film batches AF1 to AF5

30b. Plot of % release vs time from film batches AF6 to AF9

Figure 30. % Cumulative release from films
iii. Optimization of Films of ACME using Design Expert software

Table 33. Actual Experimental Design of film batches AF1 to AF9

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Conc of Film forming agent (in mg)</th>
<th>Conc of PEG (in ml)</th>
<th>Response 1 Adhesive strength (g/20mm)</th>
<th>Response 2 % release</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF1</td>
<td>500</td>
<td>0.15</td>
<td>531±0.33</td>
<td>69.8±1.3</td>
</tr>
<tr>
<td>AF2</td>
<td>500</td>
<td>0.1</td>
<td>498±0.67</td>
<td>51.3±0.21</td>
</tr>
<tr>
<td>AF3</td>
<td>500</td>
<td>0.2</td>
<td>525±0.70</td>
<td>65.2±0.24</td>
</tr>
<tr>
<td>AF4</td>
<td>450</td>
<td>0.15</td>
<td>525±0.70</td>
<td>66.4±1.85</td>
</tr>
<tr>
<td>AF5</td>
<td>450</td>
<td>0.1</td>
<td>536±0.21</td>
<td>58.3±0.41</td>
</tr>
<tr>
<td>AF6</td>
<td>450</td>
<td>0.2</td>
<td>535±0.28</td>
<td>55.2±0.36</td>
</tr>
<tr>
<td>AF7</td>
<td>550</td>
<td>0.15</td>
<td>536±0.21</td>
<td>52.6±0.41</td>
</tr>
<tr>
<td>AF8</td>
<td>550</td>
<td>0.1</td>
<td>535±0.28</td>
<td>63.5±0.23</td>
</tr>
<tr>
<td>AF9</td>
<td>550</td>
<td>0.2</td>
<td>523±0.25</td>
<td></td>
</tr>
</tbody>
</table>

The responses to be evaluated by the experimental design were Adhesive strength of the film and the % release of the extract from the film. The above table shows the design which gives actual values of the two factors Film forming agent and the plasticizer which are expected to have an impact on the release and adhesive strength of the films.
a. Design Expert software version 8.0.7.1 results for films of ACME.

31a. 3D plot for adhesive strength of film batches AF1 to AF9

31b. Plot of predicted Vs Actual adhesive strength film batches AF1 to AF9.
31c. 3D plot of % release film batches AF1 to AF9

31d. Plot of predicted vs actual values of % release from film batches AF1 to AF9

Figure 31. Design expert studies of *Aspidium cicutarium* methanolic extract films
The adhesive strength of all the nine factorial batches was subjected to design expert software version 8.0.7.1. The 3D plot was obtained. The values ranged from 498 to 536 g/20mm. The predicted value of the adhesive strength is 516 g/20mm. However out of the 9 batches most of the formulations did not fall in the range they are either above or below the predicted result. The adhesive strength of batch AF4 which is 499 g/20mm and batch AF3 which has adhesive strength of 525 g/20mm can be considered for optimization. The Design Expert software also provided with a plot of predicted vs actual values of the batches for their adhesive strength. It can be seen from the plot that most of the points are lying away from the straight line. However since the adhesive strength of batch AF4 and AF3 is near the predicted value they can be considered for optimization.

The 3D plots for the % release from the films of ACME can be seen in the above figure. The lowest value for release is 51.3 % and the highest value is 69.8 %. The plot indicates that almost 3 points lie below the predicted value while three lie above it. The predicted value according to the design software expert results was 61.666 %. There are two batches whose % release is closer to this. Batch AF3 has medium quantity of film forming agent and it showed % release of 65.2%. This batch had higher quantity of PEG 400 which contributes to its release. So the batch AF3 can be considered as optimized batch. The design expert software version 8.7.0.1 also provided with the plot of predicted Vs actual values of % release data for all the 9 batches. Most of the points are scattered away from the straight line. Batch AF7 and batch AF3 showed % release near the straight line and can be considered for the optimized batch.

The results of ANOVA suggested a linear model for both the responses selected in the model which were adhesive strength of the film and % release from the film. ANOVA results showed that the model is significant for both the responses. For adhesive strength the F value obtained was 6.11 which indicated that the model is significant and there are only 3.57 % chances that the model so significant could be due to noise. The F values of both the dependent variables concentration of film
forming agent as well as concentration of PEG were also significant. It can be hence concluded that both the factors are important in affecting the adhesive strength of the films. Similarly for % release studies the F value was 5.2 which also indicated that the model is significant. For the ANOVA of % release also a linear equation is given which is suggestive of significance of both the ingredients in the formulation and their contribution to the performance of the film.

b. Predicted values of responses according to Design Expert Software.

Table 34. Predicted and Actual Values for film batches AF1 to AF9

<table>
<thead>
<tr>
<th>Response</th>
<th>Predicted Value</th>
<th>Actual value (AF3)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive Strength (g)</td>
<td>516.44</td>
<td>525</td>
<td>6.3</td>
</tr>
<tr>
<td>% release</td>
<td>61.1667</td>
<td>65.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

The predicted values for both the responses were given by the software. When values closer to the predicted values were compared, the batch AF3 showed the least standard deviation within the predicted and actual values. The batch AF3 has adhesive strength of 525 g/25 mm and % release from this batch was 65.2 % having low values of standard deviation. Thus it can be concluded that batch AF3 is optimized batch for ACME films. This batch will be coded as OAF and subjected to invivo anti-inflammatory studies and stability studies.
iv. Preliminary Evaluation of trial batches of films of methanolic extract of leaves of *Spathodea campanulata*.¹⁵¹

Table 35. Evaluation of trial batches of methanolic extract of leaves of *Spathodea campanulata*

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Thickness (mm)</th>
<th>Tensile strength (N/mm²)</th>
<th>Adhesive Strength (g/25mm)</th>
<th>% flatness</th>
<th>% moisture uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>0.22</td>
<td>19.8</td>
<td>497</td>
<td>99.27±0.4</td>
<td>3</td>
</tr>
<tr>
<td>SF2</td>
<td>0.22</td>
<td>20.2</td>
<td>492</td>
<td>99.36±0.3</td>
<td>2</td>
</tr>
<tr>
<td>SF3</td>
<td>0.24</td>
<td>21.3</td>
<td>520</td>
<td>99.58±0.5</td>
<td>2</td>
</tr>
<tr>
<td>SF4</td>
<td>0.25</td>
<td>21.7</td>
<td>514</td>
<td>99.20±0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Preliminary evaluation included texture and appearance of the films. The SCME containing films appeared greenish in colour. They showed good adhesive strength and tensile strength. They were satisfactorily flat without any imperfections. The moisture uptake was good enough. They were further subjected to release studies. The extra stickiness can be attributed to the sticky nature of the extract.

v. Formulation and Evaluation of Factorial Design Batches of films of SCME(SF1 to SF9)

All the 9 batches of SCME films were subjected to evaluation of characteristics.

![Figure 32. Films of *Spathodea campanulata* methanolic extract](image-url)
Table 36. Evaluation of film batches from SF1 to SF9

36 a. Evaluation of thickness, weight and folding endurance from SF1 to SF9

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Thickness (mm)</th>
<th>Weight (g)</th>
<th>Folding endurance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>0.22±0.21</td>
<td>0.114±0.11</td>
<td>135±0.12</td>
</tr>
<tr>
<td>SF2</td>
<td>0.22±0.14</td>
<td>0.113±0.14</td>
<td>145±0.09</td>
</tr>
<tr>
<td>SF3</td>
<td>0.24±0.15</td>
<td>0.114±0.08</td>
<td>143±0.13</td>
</tr>
<tr>
<td>SF4</td>
<td>0.25±0.32</td>
<td>0.110±0.098</td>
<td>150±0.098</td>
</tr>
<tr>
<td>SF5</td>
<td>0.22±0.16</td>
<td>0.109±0.11</td>
<td>146±0.089</td>
</tr>
<tr>
<td>SF6</td>
<td>0.23±0.5</td>
<td>0.112±0.13</td>
<td>147±0.14</td>
</tr>
<tr>
<td>SF7</td>
<td>0.24±0.6</td>
<td>0.118±0.13</td>
<td>139±0.12</td>
</tr>
<tr>
<td>SF8</td>
<td>0.23±0.25</td>
<td>0.118±0.12</td>
<td>145±0.11</td>
</tr>
<tr>
<td>SF9</td>
<td>0.24±0.24</td>
<td>0.119±0.14</td>
<td>152±0.18</td>
</tr>
</tbody>
</table>

36b. Evaluation of Tensile and Adhesive strength, moisture uptake and drug content from SF1 To SF9

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Tensile Strength(N/mm²)</th>
<th>Adhesive strength</th>
<th>% Moisture uptake</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>22.8±0.13</td>
<td>530±0.24</td>
<td>3.5±0.21</td>
<td>80.21±1.5</td>
</tr>
<tr>
<td>SF2</td>
<td>20.9±0.31</td>
<td>497±0.32</td>
<td>2±0.25</td>
<td>81.25±1.08</td>
</tr>
<tr>
<td>SF3</td>
<td>21.9±0.091</td>
<td>526±0.14</td>
<td>2±0.21</td>
<td>85.9±1.9</td>
</tr>
<tr>
<td>SF4</td>
<td>19.5±0.14</td>
<td>498±0.18</td>
<td>3±0.14</td>
<td>89.6±2.1</td>
</tr>
<tr>
<td>SF5</td>
<td>19.3±0.98</td>
<td>504±0.16</td>
<td>2.5±0.32</td>
<td>84.5±1.2</td>
</tr>
<tr>
<td>SF6</td>
<td>19.4±0.34</td>
<td>496±0.14</td>
<td>3±0.51</td>
<td>80.21±1.9</td>
</tr>
<tr>
<td>SF7</td>
<td>23.6±0.99</td>
<td>535±0.33</td>
<td>3±0.61</td>
<td>80.32±1.3</td>
</tr>
<tr>
<td>SF8</td>
<td>23.4±0.51</td>
<td>539±0.66</td>
<td>2±1.2</td>
<td>81.46±1.4</td>
</tr>
<tr>
<td>SF9</td>
<td>24.2±0.76</td>
<td>525±0.33</td>
<td>3.5±1.2</td>
<td>82.9±1.8</td>
</tr>
</tbody>
</table>

a. Appearance:
The films appeared greenish yellow in colour and were uniformly thick without any imperfections.

b. Thickness:
Thickess of the films was measured by micrometer screw guage at three different places and mean was calculated.
c. **Folding Endurance:**

Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme condition of folding. Folding endurance was determined by repeatedly folding the film at the same place. The number of times the film could be folded at the same place without breaking was folding endurance. Highest folding endurance was seen in SF9.

d. **Tensile Strength:**

The Tensile strength of the films was good. The batch SF7 had the highest value for tensile strength suggesting that the film was the strongest, this high tensile strength of the film can be attributed to the high amount of film forming agent and lesser amount of PEG 400 present in the film.

e. **Adhesive Strength:**

The adhesive strength of the film is also an important parameter that determines the ability of the film to adhere to the skin for the period of treatment time without undergoing any major changes. From the results of the adhesive strength it can be concluded that the batch coded as SF1 which contain an optimum amount of film forming agent and also an optimum amount of PEG 400 shows the highest adhesive strength. The batch SF7 which had highest tensile strength also showed high adhesive strength followed by SF3.

f. **Drug Content:**

All the 9 batches showed satisfactory drug content ranging from 80.21 % to 89.6 %.

g. **% Moisture uptake.**

The % moisture uptake of all the films was between the range of 2% to 3.5 %. The batch AF3 and SF9 which has higher % of PEG 400 showed higher moisture uptake.
h. % Cumulative release studies from Films.

All 9 batches were subjected to release studies in phosphate buffer pH 6.8 as receiving medium in the diffusion cell. The studies lasted for 8 hours and the following results were obtained.

Table 37. % Cumulative release from film batches SF1 to SF9

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>SF1</th>
<th>SF2</th>
<th>SF3</th>
<th>SF4</th>
<th>SF5</th>
<th>SF6</th>
<th>SF7</th>
<th>SF8</th>
<th>SF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.5±1.23</td>
<td>12±2.1</td>
<td>18.2±1.66</td>
<td>16.4±4.1</td>
<td>17.8±2.3</td>
<td>17.8±1.4</td>
<td>19.5±2.3</td>
<td>18.5±1.7</td>
<td>19.5±0.25</td>
</tr>
<tr>
<td>2</td>
<td>20.3±1.52</td>
<td>15.6±1.3</td>
<td>22.8±1.58</td>
<td>23.5±2.9</td>
<td>28.5±1.5</td>
<td>22.3±5.2</td>
<td>25.3±2.4</td>
<td>25.2±2.4</td>
<td>24.2±0.29</td>
</tr>
<tr>
<td>3</td>
<td>34.3±1.48</td>
<td>21.3±1.5</td>
<td>31.5±1.4</td>
<td>33.2±3.2</td>
<td>32.5±2.4</td>
<td>32.6±4.3</td>
<td>35.3±2.9</td>
<td>30.2±2.1</td>
<td>33.6±0.48</td>
</tr>
<tr>
<td>4</td>
<td>46.5±2.3</td>
<td>25.9±1.9</td>
<td>43.8±1.23</td>
<td>41.6±1.2</td>
<td>38.4±2.9</td>
<td>41.2±3.2</td>
<td>40.7±2.1</td>
<td>35.2±2.8</td>
<td>41.2±0.16</td>
</tr>
<tr>
<td>5</td>
<td>53.7±5.1</td>
<td>30.2±2.3</td>
<td>46.3±4.3</td>
<td>44.5±1.5</td>
<td>43.9±2.8</td>
<td>47.1±3.6</td>
<td>43.6±1.8</td>
<td>41.3±3.9</td>
<td>45.5±0.54</td>
</tr>
<tr>
<td>6</td>
<td>54.5±1.4</td>
<td>36.7±2.4</td>
<td>48.5±2.6</td>
<td>46.9±1.4</td>
<td>47.1±1.5</td>
<td>50.1±5.2</td>
<td>45.7±1.5</td>
<td>44.1±3.4</td>
<td>51.9±0.78</td>
</tr>
<tr>
<td>7</td>
<td>57.3±2.1</td>
<td>40.8±1.7</td>
<td>51.2±4.3</td>
<td>49.2±1.9</td>
<td>49.2±5.3</td>
<td>54.8±1.8</td>
<td>47.2±1.9</td>
<td>46.7±0.46</td>
<td>56.7±0.56</td>
</tr>
<tr>
<td>8</td>
<td>66.5±2.4</td>
<td>52.6±1.9</td>
<td>68.5±4.1</td>
<td>58.4±2.3</td>
<td>56.2±5.2</td>
<td>69.5±1.4</td>
<td>57.9±1.8</td>
<td>50.2±0.12</td>
<td>66.8±0.19</td>
</tr>
</tbody>
</table>
Figure 33. % Cumulative release from film batches SF1 to SF9

From the table and graph of % release of all 9 batches it can be seen that the batch SF6 shows the highest release. However the adhesive strength of this batch is less
as compared to the batches. Therefore the results should be subjected to Design expert software for optimization.

vi. Optimization of Films of SCME using Design Expert software

Table 38. Actual Experimental Design of film batches from SF1 to SF9

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Conc of Film forming agent (in mg)</th>
<th>Conc of PEG (in ml)</th>
<th>Response 1 Adhesive strength (g/20mm)</th>
<th>Response 2 % release</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>500</td>
<td>0.15</td>
<td>530±0.24</td>
<td>66.5±2.4</td>
</tr>
<tr>
<td>SF2</td>
<td>500</td>
<td>0.1</td>
<td>497±0.32</td>
<td>52.6±1.9</td>
</tr>
<tr>
<td>SF3</td>
<td>500</td>
<td>0.2</td>
<td>526±0.14</td>
<td>68.5±4.1</td>
</tr>
<tr>
<td>SF4</td>
<td>450</td>
<td>0.15</td>
<td>498±0.18</td>
<td>58.4±2.3</td>
</tr>
<tr>
<td>SF5</td>
<td>450</td>
<td>0.1</td>
<td>504±0.16</td>
<td>56.2±5.2</td>
</tr>
<tr>
<td>SF6</td>
<td>450</td>
<td>0.2</td>
<td>496±0.14</td>
<td>69.5±1.4</td>
</tr>
<tr>
<td>SF7</td>
<td>550</td>
<td>0.15</td>
<td>535±0.33</td>
<td>57.9±1.8</td>
</tr>
<tr>
<td>SF8</td>
<td>550</td>
<td>0.1</td>
<td>539±0.66</td>
<td>50.2±0.12</td>
</tr>
<tr>
<td>SF9</td>
<td>550</td>
<td>0.2</td>
<td>525±0.33</td>
<td>66.8±0.19</td>
</tr>
</tbody>
</table>

a. Design Expert software version 8.0.7.1 results for films of SCME

When subjected to Design expert software studies the 3D plots, plots of actual vs predicted values were obtained. ANOVA was done for each of the responses to find out the significance of the selected model for the studies and to know the contributions of the independent variables on the performance of the formulation.
34a. 3D Plot of adhesive strength of film batches SF1 to SF9

34b. Plot of predicted vs actual for adhesive strength of film batches SF1 to SF9
34c. 3D Plot of % release from film batches SF1 to SF9

34d. Plot of predicted vs actual % release from film batches SF1 to SF9

Figure 34. Design Expert studies of *Spathodea campanulata* methanolic extract films
The adhesive strength of all the nine factorial batches was subjected to design software expert version 8.0.7.1. The 3D plot was obtained. The values ranged from 500 to 533 g/20 mm. The predicted value of the adhesive strength is 516 g/20 mm. Most of the formulations out of the 9 batches of factorial design were either above or below the predicted result. So considering the adhesive strength as one of the responses to optimize the formulation it can be concluded that the batch SF1 which has medium level of film forming agent seems to be the optimized batch according to the design software results. The plot of Predicted values of Adhesive strength vs the actual values of adhesive strength was obtained using the design expert software. The values for all the nine batches appeared to be scattered around the straight line. The batch coded as SF7 has the adhesive strength nearer to the line. The Batch SF1 has adhesive strength 530 g/20mm which is close to straight line. So that batch can be considered as optimized.

The 3D plot for % release from the SCME film formulations can be seen in the above figure. The release is highest in batch SF6 which is also supportive of the theory that more the amount of plasticizer higher will be the release. However the design expert software suggests that such high release value lies above the predicted % release. There are a few points which were below the predicted value. The predicted value is 60.733. The batch SF1 show % release value of 66.5 which also a higher value than 60.73. Since both the responses have to be taken into consideration and since this batch has higher % release and also a good adhesive strength the interpretation suggested SF1 as optimized batch. The plot of predicted values vs Actual % release was obtained from the Design expert software. Three points lie very close to the line while there were 3 points which actually lie on the line. The % release value 66.5 shown by batch SF1 was considered as the optimized batch according to this study.

b. ANOVA of SCME Films.
ANOVA results for the SCME films were given by the Design expert software. For the adhesive strength. The model suggested by ANOVA was linear model. The F value for the model was 6.54 which indicates that the model F value so high is significant and there are only 3% chances the model F value so large could be due to
noise. The p \(>\) f value is 0.031 which is lesser than 0.05 so the model is significant. The terms in the linear equation are significant indicating that both the factors chosen are significant. The standard deviation between the predicted \( R^2 \) and actual \( R^2 \) was also less. For the % release the ANOVA results suggested linear model. The p \(>\) F value is 0.002 which was less than 0.05 and so the the model was significant. The model F value for this is 19.97 which suggested that the model was significant and there are only 0.2 % chances that the model F value could be due to noise. Thus both the factors were significant.

**c. Predicted values of responses according to Design Expert.**

Following table shows the result for predicted vs actual values of both the adhesive strength and the % release. From these values SF1 appeared to be the optimized batch. The batch SF1 is the batch having medium quantities of film forming agent and medium quantity of plasticizer. Thus the optimum quantities of both the factors contribute to the release and the adhesive strength of the films of SCME. The optimized batch was hence coded as OSF. This batch was further subjected to anti-inflammatory studies and stability studies.

<table>
<thead>
<tr>
<th></th>
<th>Predicted Value</th>
<th>Actual value (SF1)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive Strength (g/20mm)</td>
<td>516</td>
<td>530</td>
<td>9.89</td>
</tr>
<tr>
<td>% release</td>
<td>60.733</td>
<td>66.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**6.3 Studies on optimized film formulations**

**6.3.1 In vivo anti-inflammatory studies.**

i. **Effect of film formulation of extracts on rat paw edema.**

The extracts had shown good topical anti-inflammatory response. For the formulations rat paw edema method was used to study the anti-inflammatory activity.
Table 40. Antiinflammatory activity of optimized films.

<table>
<thead>
<tr>
<th>Group</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>% anti-inflammatory activity at the end of 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.6±0.12</td>
<td>3.5±0.15</td>
<td>3.6±0.32</td>
<td>3.9±0.14</td>
<td>------</td>
</tr>
<tr>
<td>Standard</td>
<td>2.1±0.1*</td>
<td>1.9±0.3**</td>
<td>1.5±0.21***</td>
<td>1.1±0.1***</td>
<td>71.79</td>
</tr>
<tr>
<td>OAF</td>
<td>2.4±0.21</td>
<td>2.3±0.36 *</td>
<td>2.1±0.5**</td>
<td>1.8±0.5**</td>
<td>53.84</td>
</tr>
<tr>
<td>OSF</td>
<td>2.5±0.21</td>
<td>2.3±0.24*</td>
<td>2.1±0.18*</td>
<td>2.0±0.16**</td>
<td>48.71</td>
</tr>
</tbody>
</table>

(Results subjected to two way ANOVA followed by Dunnets test. * p<0.05 ** p<0.01, ***p<0.001)

The optimized formulations of film of both the extracts were subjected to this study. It can be seen that for the first hour the edema increased in case of both formulations. However in case of standard Volini gel the edema decreased in the first hour as compared to the control group. The OAF films and OSF films showed a decrease in edema from second hour. At the end of 4 hours the edema was noted and % inhibition
of edema was calculated. The optimized batch of OAF showed 53% anti-inflammatory activity. While the optimized batch OSF showed 48.71% inhibition. Both the results are less than the standard gel formulation. This may be due to lesser penetration of the extract from the film into the rat paw. The results were subjected to two way ANOVA followed by Dunnet’s test. The results were nonsignificant at first hour as compared to the control. However after 2nd hour the results showed significance for standard at p<0.01 and both the films showed value at significance level p<0.05. In the third and final hour the standard as compared to control showed significance at p<0.001. While the film formulations were significant at p<0.01 as compared to control. Hence it can be concluded that the films possessed good anti-inflammatory activity.

6.3.2 **Antioxidant activity of optimized film formulations**

Table 41. Antioxidant activity of optimized films.

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Standard Ascorbic Solution</th>
<th>Inhibition by OAF (n=3)</th>
<th>Inhibition by OSF(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>31.53</td>
<td>21.35±0.3</td>
<td>19.21±0.1</td>
</tr>
<tr>
<td>15</td>
<td>51.50</td>
<td>33.19±0.73</td>
<td>25.92±0.23</td>
</tr>
<tr>
<td>20</td>
<td>57.80</td>
<td>51.32±0.65</td>
<td>31.58±0.54</td>
</tr>
<tr>
<td>40</td>
<td>81.01</td>
<td>60.3±0.26</td>
<td>45.32±0.27</td>
</tr>
<tr>
<td>60</td>
<td>92.16</td>
<td>75.4±0.49</td>
<td>50.51±0.46</td>
</tr>
<tr>
<td>80</td>
<td>98.47</td>
<td>85.12±0.30</td>
<td>75.50±0.34</td>
</tr>
</tbody>
</table>
Antioxidant activity of optimized films.

The extracts of both the plants had shown antioxidant activity closer to the standard Ascorbic acid. Out of the two films under study the film of methanolic extract of *Aspidium cicutarium* had higher antioxidant activity. The optimized film formulations when subjected to the antioxidant activity showed good results. The potential for free radical scavenging activity was thus maintained in the film formulations.

6.3.3 Accelerated stability studies

The ICH guidelines were referred for the conditions to be maintained for the accelerated studies. Studies were carried for 6 months. After every month the film formulations were reviewed for appearance and moisture uptake and also for adhesive strength of the film. The following results were obtained.

Accelerated stability studies for OAF and OSF gave the following results
Table 42. Accelerated stability studies of optimized films

<table>
<thead>
<tr>
<th>Period in months</th>
<th>OAF</th>
<th>OSF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% moisture uptake</td>
<td>% moisture uptake</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Both the optimized films maintained their characteristics when subjected to accelerated stability studies. They maintained the adhesive strength and moisture uptake levels. The films did not become tacky or slimy and thus had the original structural strength as well as flexibility. From the results obtained it can be concluded that the optimized films remained stable during the studies and showed good results.

6.4 Formulation of emulgels

6.4.1 Preformulation studies

i. HLB values of Blend of emulsifiers.

The values of HLB obtained with various blends of the emulsifier using a computagraph can be seen in the table.
Table 43. HLB values of various emulsifier blends.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>% Emulsifier blend</th>
<th>HLB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Span 80</td>
<td>Tween 80</td>
</tr>
<tr>
<td>T1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>T3</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>T4</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>T5</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>T6</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>T7</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>T8</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>T9</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>T10</td>
<td>30</td>
<td>70</td>
</tr>
</tbody>
</table>

From the broad range of emulsifier combination a wide range of HLB values ranging from 4.3 to 12.8 were obtained. A narrow range of HLB was selected and combinations were made and their HLB was determined using computagraph.

ii. Measurement of droplet size:

Droplet size of prepared emulsions was observed by using Motic digital microscope. The emulsions of narrow range of HLB values were subjected to evaluation of droplet size to ensure the stability.

Table 44. Droplet size of emulsions of narrow range of HLB.

<table>
<thead>
<tr>
<th>HLB</th>
<th>Appearance</th>
<th>Colour</th>
<th>Droplet Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.6</td>
<td>Turbid</td>
<td>White</td>
<td>55-60μm</td>
</tr>
<tr>
<td>10.2</td>
<td>Turbid</td>
<td>White</td>
<td>40-50 μm</td>
</tr>
<tr>
<td>10.7</td>
<td>Turbid</td>
<td>White</td>
<td>30-40 μm</td>
</tr>
<tr>
<td>11.2</td>
<td>Turbid</td>
<td>White</td>
<td>45-50 μm</td>
</tr>
<tr>
<td>11.8</td>
<td>Turbid</td>
<td>White</td>
<td>50-60μm</td>
</tr>
</tbody>
</table>

From the pictures obtained from Motic trinocular microscope it was obvious that the HLB of 10.7 yielded emulsions of smallest droplet size. So the ratio of Span 80: Tween 80 was chosen to be 40:60 for all the formulations. Also the concentration of
this emulsion blend was tried for 3%, 4% and 5%. The 5% blend showed maximum stability. The emulsions prepared using this blend were also subjected to various stability studies and it was observed that the ratio of emulsifiers showing the HLB 10.7 gives stable formulations.

The concentration of this emulsifier blend was 5% with the oil phase of 10%v/v.

6.4.2 Compatibility studies\textsuperscript{154, 155}

The compatibility studies included the FTIR studies of extracts alone and with the blend of Rice bran oil and Carbopol 934.

37a FTIR spectra of \textit{Aspidium cicutarium} methanolic extract.

37b FTIR of ACME+Rice Bran Oil+Carbopol 934
From FTIR spectra visible interactions were not seen between the extracts and the excipients which could lead to the vanishing or diminishing of any major peak. From this it was concluded that there is no incompatibility seen. Therefore extracts and excipient were compatible with each other and can be used in the formulation. So emulgels were formulated with rice bran oil and gelled using Carbopol 934.

6.4.3 Formulation and evaluation of emulgels

i. Evaluation of trial batches

4 trial batches were formulated and evaluated for viscosity and spreading coefficient. The results are depicted in the table below. The trial batch 1 and 2 have very high spreading coefficient which can be attributed to concentrations of Carbopol and propylene glycol. Hence for factorial batches the concentration of Carbopol 934
was chosen between 1-2 %. Similarly the % of propylene glycol was also selected in a narrow range.

Table 45. Evaluation of trial batches of emulgels

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>ACME Emulgels</th>
<th>SCME Emulgels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viscosity (Pa.s)</td>
<td>Spreading coefficient g.cm/sec</td>
</tr>
<tr>
<td>1</td>
<td>3.1</td>
<td>28.5</td>
</tr>
<tr>
<td>2</td>
<td>2.83</td>
<td>26.4</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>23.5</td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>20.1</td>
</tr>
</tbody>
</table>

Table 46. Evaluation of emulgel batches from AE1 to AE9

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulations</th>
<th>pH</th>
<th>Viscosity (Pa.s)</th>
<th>Spreading coefficient (gm.cm/s)</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AE1</td>
<td>7.4 ± 1.21</td>
<td>3.67 ± 1.08</td>
<td>25.4±1.02</td>
<td>89.2±1.21</td>
</tr>
<tr>
<td>2</td>
<td>AE2</td>
<td>7.2 ±0.82</td>
<td>3.38 ±1.32</td>
<td>21.5±1.06</td>
<td>89.4±1.25</td>
</tr>
<tr>
<td>3</td>
<td>AE3</td>
<td>6.9 ±1.11</td>
<td>2.83± 0.98</td>
<td>27.4±1.1</td>
<td>90.1±1.09</td>
</tr>
<tr>
<td>4</td>
<td>AE4</td>
<td>7.3 ±1.08</td>
<td>3.59±0.96</td>
<td>28.5±1.08</td>
<td>89.2±1.24</td>
</tr>
<tr>
<td>5</td>
<td>AE5</td>
<td>6.7 ± 0.98</td>
<td>2.54±0.95</td>
<td>20.1±1.25</td>
<td>85.5±0.98</td>
</tr>
<tr>
<td>6</td>
<td>AE6</td>
<td>7.3 ±0.99</td>
<td>2.96± 1.04</td>
<td>29.6±0.99</td>
<td>86.5±0.85</td>
</tr>
<tr>
<td>7</td>
<td>AE7</td>
<td>6.8 ±1.21</td>
<td>3.58± 0.99</td>
<td>22.5±1.65</td>
<td>91.4±2.3</td>
</tr>
<tr>
<td>8</td>
<td>AE8</td>
<td>6.8 ±0.99</td>
<td>3.65±1.67</td>
<td>19.56±1.2</td>
<td>89.2±1.2</td>
</tr>
<tr>
<td>9</td>
<td>AE9</td>
<td>6.7±1.46</td>
<td>3.36± 1.2</td>
<td>21.2±1.4</td>
<td>88.4±1.4</td>
</tr>
</tbody>
</table>
a. **Measurement of pH:**
The pH values of all formulations were found in the range 6.8 to 7.4. Hence all the formulations were in the normal pH range of the skin and would not produce any skin irritation.

b. **Measurements of viscosity:**
The values range from 2.54 to 3.67 Pa.s. The emulgels being the combination of emulsions and gels show moderate viscosities and hence better acceptability. Rheological behavior of the emulgels indicated that the systems were shear thinning in nature showing decrease in viscosity at the increasing shear rates. As the shear stress is increased, the normally disarranged molecules of the gelling material are caused to align their long axes in the direction of flow. Such orientation reduces the internal resistance of the material and hence decreases the viscosity. An increase in the concentration of Carbopol 934 was expected to show increase in viscosity.

c. **Spreading Coefficient:**
The spreading coefficient of the formulations is a characteristic derived from its more basic property i.e. viscosity. The greater the viscosity the longer will be the time taken for spreading. The emulgels are expected to spread easily on the skin areas when applied. It also depends on the polymer in formulation, possessing typical physicochemical properties which create surface tension between slide and product. It indicates that the emulgel is easily spreadable by small amount of shear. It shows that the formulation shows higher spreading coefficient as compared to other formulations. Good spreading coefficient amongst all batches was seen in AE6.

d. **% Drug Content**
All 9 batches of factorial design showed satisfactory values for % drug content.

e. **In vitro Drug Release study:**
The higher drug release was observed with formulations AE9 as is obvious from the release graphs. However since spreadability is also one of the important factors taken into consideration AE6 can be said to be a better formulation than the rest since the
release rates are not much different from AE9. The higher release values can be attributed to the lower concentration levels of Carbopol 934 and higher levels of Propylene glycol. However the viscosity of this batch is very less so the factorial batches can be optimized in a better way by subjecting them to Design expert Software.

Table 47.  % Cumulative release from emulgel batches AE1 to AE9

<table>
<thead>
<tr>
<th>Time (hour s)</th>
<th>% Cumulative drug release (mean ± SD, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AE1</td>
</tr>
<tr>
<td>1</td>
<td>32.11 ±1.28</td>
</tr>
<tr>
<td>2</td>
<td>43.48 ±2.31</td>
</tr>
<tr>
<td>3</td>
<td>53.84 ±2.8</td>
</tr>
<tr>
<td>4</td>
<td>67.10 ±2.7</td>
</tr>
<tr>
<td>5</td>
<td>69.53 ±1.8</td>
</tr>
<tr>
<td>6</td>
<td>74.33 ±3.9</td>
</tr>
<tr>
<td>7</td>
<td>80.62 ±3.4</td>
</tr>
<tr>
<td>8</td>
<td>87.4 ±1.2</td>
</tr>
</tbody>
</table>
38a. Plot of % release vs time from emulgels batches AE1 to AE5

38b. Plot of % release vs time from emulgels batches AE6 to AE9

Figure 38. % Cumulative drug release from emulgel batches AE1 to AE9
iii. Statistical Analysis and Optimization Model Validation:
A two-factor, three-level full factorial experimental design was used to optimize the formulation variables. The independent variables and the responses for all 9 experimental runs are given in the table below.

Table 48. Actual Experimental Design of emulgel batches AE1 to AE9

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Conc of Gelling agent in %</th>
<th>Conc of PG %</th>
<th>Spreading Coefficient (g.cm/sec)</th>
<th>% Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE1</td>
<td>2</td>
<td>5</td>
<td>25.4±1.02</td>
<td>87.4±1.1</td>
</tr>
<tr>
<td>AE2</td>
<td>2</td>
<td>3.5</td>
<td>21.5±1.06</td>
<td>84.5±2.1</td>
</tr>
<tr>
<td>AE3</td>
<td>2</td>
<td>7.5</td>
<td>27.4±1.1</td>
<td>85.6±2.9</td>
</tr>
<tr>
<td>AE4</td>
<td>1</td>
<td>5</td>
<td>28.5±1.08</td>
<td>80.52±3.7</td>
</tr>
<tr>
<td>AE5</td>
<td>1</td>
<td>3.5</td>
<td>20.1±1.25</td>
<td>72.8±1.9</td>
</tr>
<tr>
<td>AE6</td>
<td>1</td>
<td>7.5</td>
<td>29.6±0.99</td>
<td>88.65±1.69</td>
</tr>
<tr>
<td>AE7</td>
<td>2.5</td>
<td>5</td>
<td>22.5±1.65</td>
<td>82.65±1.78</td>
</tr>
<tr>
<td>AE8</td>
<td>2.5</td>
<td>3.5</td>
<td>19.56±1.2</td>
<td>68.5±2.36</td>
</tr>
<tr>
<td>AE9</td>
<td>2.5</td>
<td>7.5</td>
<td>21.2±1.4</td>
<td>89.4±3.1</td>
</tr>
</tbody>
</table>

a. Design Expert software version 8.0.7.1 results for % Release for emulgels of ACME

39 a. 3D Plot of % Release from emulgel batches AE1 to AE9
39b. Plot of Predicted vs actual % release from emulgel batches AE1 to AE9

b. Design Expert software version 8.0.7.1 results for spreading coefficient for emulgels of ACME

39c. 3D Plot of Spreading coefficient of emulgel batches AE1 to AE9
39d. Plot of predicted vs actual spreading coefficient of emulgels AE1 to AE9.

**Figure 39. Design Expert studies of Aspidium cicutarium methanolic extract emulgels**

The Design Expert software provided the 3D plot of factors affecting the release of extract from the emulgels. The 3D plot shows points above and below the predicted value. The value of % release predicted by the software is 82.22%. The batch AE7 which has higher % of Carbopol 934 and medium % of propylene glycol showed release profiles near the predicted value. The % release from this batch is 82.62% which is near the predicted value. The plot of predicted vs actual % release of extract from the emulgel formulation was obtained from the Design expert software. Some points lie scattered and away from the straight line. However the % release from batch AE7 lies on the line and touches it. It may be concluded that as far as the % release is concerned, AE7 batch can be considered for optimized batch.

The 3D plot for the spreading coefficient was obtained from the Design expert software. The plots showed points lying above the predicted value and similarly some points below the predicted value. It can be concluded that since the predicted value of the Design expert software for spreading coefficient is 26.26.g.cm/sec the batch AE7 can be considered for optimization as it showed a value closer to the predicted. However the spreading coefficient of AE4 is 28.5g.cm/sec which also does not
deviate much from the predicted value and the release from this batch is also good. Hence this batch can be considered for optimization. The plot for predicted Vs actual values of spreading coefficients was obtained from the Design Expert software. It is evident from the plot that most of the points lie on the straight line of the graph. The predicted value of spreading coefficient as given by the Design expert software is 26.26 g cm/sec. The Batch AE 7 shows the value nearest to the predicted value and hence this batch can be considered as the optimized batch from the 9 formulations.

c. ANOVA Results for Emulgels of ACME
   The ANOVA studies for the spreading coefficient showed a high F value 27.55. There are only 1.04% chances that model F value this large could be as a result of noise. The p>F value is 0.0104 which less than 0.1. This shows that the model is significant. The model suggested by ANOVA is a quadratic model and all terms in this model are significant leading to a quadratic equation. This shows that both the terms as well as square of the terms are also contributing to the spreading coefficient of the emulgel formulation. So the selected factors carry significant effect on spreading coefficient. However for % release the the p>F value is 0.0772 so it is greater than 0.05 and hence non significant. The ANOVA results show that the term concentration of propylene glycol is significant term in % release.

d. Predicted values of responses according to Design Expert Software.

   Table 49. Predicted and Actual emulgel batches AE1 to AE9.

<table>
<thead>
<tr>
<th>Response</th>
<th>Predicted Value</th>
<th>Actual value (AE4)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spreading coefficient</td>
<td>26.26</td>
<td>28.5</td>
<td>1.58</td>
</tr>
<tr>
<td>% release</td>
<td>82.22</td>
<td>80.52</td>
<td>1.2</td>
</tr>
</tbody>
</table>

From all the above stated results and from the results obtained from the predicted and actual values of spreading coefficient and % release it can be concluded that the batch AE4 is the optimized batch for emulgel formulations. This batch has less quantity of Carbopol 934 which is the gelling agent and medium value of propylene glycol.
Propylene glycol affects both the spreadability and % release higher the value of propylene glycol greater will be the release. The medium quantity ensures that the spreading coefficient is also satisfactory alongwith the release. This optimized batch was further coded as OAE and subjected to wound healing, anti-inflammatory and stability studies.

iv. Formulation and Evaluation of factorial batches of emulgels of SCME\textsuperscript{157,158}

9 Batches according to the $3^2$ factorial design were made using the method specified in the methodology. They were subjected to evaluation. The emulgels appeared greenish creamy in appearance and were smooth.

v. Evaluation of SCME emulgels \textsuperscript{160}

Table 50. Evaluation of emulgel batches from SE1 to SE9

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulations</th>
<th>pH</th>
<th>Viscosity (Pa/s)</th>
<th>Spreading coefficient (gm.cm/s)</th>
<th>% Drug Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>SE1</td>
<td>7.1 ± 0.9</td>
<td>2.458±0.48</td>
<td>24.3±1.24</td>
<td>80.99±1.03</td>
</tr>
<tr>
<td>2.</td>
<td>SE2</td>
<td>6.7 ±1.2</td>
<td>3.236±0.52</td>
<td>20.9±1.48</td>
<td>82.35±1.25</td>
</tr>
<tr>
<td>3.</td>
<td>SE3</td>
<td>7.2 ±1.1</td>
<td>2.438±0.12</td>
<td>26.8±1.28</td>
<td>83.66±0.99</td>
</tr>
<tr>
<td>4.</td>
<td>SE4</td>
<td>7.4 ±1.08</td>
<td>2.794±0.36</td>
<td>27.3±0.99</td>
<td>81.24±1.08</td>
</tr>
<tr>
<td>5.</td>
<td>SE5</td>
<td>6.8 ± 0.98</td>
<td>3.235±0.15</td>
<td>19.2±0.98</td>
<td>87.21±1.23</td>
</tr>
<tr>
<td>6.</td>
<td>SE6</td>
<td>6.7 ± 0.99</td>
<td>2.756±0.25</td>
<td>28.2±1.08</td>
<td>80.21±0.99</td>
</tr>
<tr>
<td>7.</td>
<td>SE7</td>
<td>7.4 ±1.02</td>
<td>3.258±0.16</td>
<td>21.4±1.04</td>
<td>83.52±0.63</td>
</tr>
<tr>
<td>8.</td>
<td>SE8</td>
<td>6.9 ±1.02</td>
<td>3.540±0.31</td>
<td>18.63±0.99</td>
<td>84.12±1.25</td>
</tr>
<tr>
<td>9.</td>
<td>SE9</td>
<td>7.1±1.05</td>
<td>3.65±1.23</td>
<td>20.14±1.20</td>
<td>82.58±1.4</td>
</tr>
</tbody>
</table>
a. Appearance:
All the 9 batches of SCME emulgels were greenish viscous creamy preparations with a smooth and homogeneous appearance.

b. pH
All formulations fall in the pH range of 6.7 to 7.4 which is a safe pH for skin. So the pH values of all the formulations were found to be satisfactory.

c. Viscosity
The emulgels are expected to spread easily on the skin areas when applied. The greater the viscosity the longer will be the time taken for spreading. So low values of viscosity were expected. The emulgel batch of SE9 shows highest viscosity values this may be attributed to the highest quantity of gelling agent which is making the emulgel formulation viscous.

d. Spreading coefficient
The spreading coefficient depends on both the factors decided in the design, the gelling agent in the formulation and propylene glycol as well. These ingredients possess typical physicochemical properties which create surface tension between slide and product. The spreadability indicates that the Emulgel is easily spreadable by small amount of shear. It shows that the SE6 formulation shows higher spreading coefficient as compared to other formulations. The viscosity of this formulation was also moderate than the other formulations. Good spreading coefficient can be attributed to the high concentrations of Propylene glycol and lesser quantities of the gelling agent.

e. Drug Content
All the emulgel formulations showed drug content in the range of 80.2% to 87.2 % which was satisfactory.

f. In vitro release from the emulgels
In vitro release profiles of extract from its various emulgel formulations are represented in figures below. All the formulations show a good release pattern for 8
hrs of diffusion studies. The release from SE9 batch is the highest. However the Batch SE6 also showed almost same release and at the same time good spreadability also. The higher concentration of propylene glycol can be the reason of high release rates in the batches.

Table 51. % Cumulative release from emulgel batches SE1 to SE9

<table>
<thead>
<tr>
<th>Time In hours</th>
<th>% Cumulative drug release (mean ± SD, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SE1</td>
</tr>
<tr>
<td>1</td>
<td>31.31 ±1.08</td>
</tr>
<tr>
<td>2</td>
<td>46.48 ±1.12</td>
</tr>
<tr>
<td>3</td>
<td>56.04 ±0.98</td>
</tr>
<tr>
<td>4</td>
<td>63.10 ±0.80</td>
</tr>
<tr>
<td>5</td>
<td>68.53 ±0.63</td>
</tr>
<tr>
<td>6</td>
<td>74.33 ±0.59</td>
</tr>
<tr>
<td>7</td>
<td>80.62 ±1.36</td>
</tr>
<tr>
<td>8</td>
<td>85±0.99</td>
</tr>
</tbody>
</table>
vi. Statistical Analysis and Optimization Model Validation: \(^{47}\)

For estimation of quantitative effects of the different combination of factors and factor levels on % cumulative drug release at 8 hrs. and spreadability, the
experimental design was subjected to optimization by use of Design expert software. The software provided results of ANOVA applied to the results of % release and spreading coefficient, 3D surface plots of both the responses, Graph of Predicted Vs actual values for release. A two-factor, three-level full factorial experimental design was used to optimize the formulation variables.

Table 52. Actual Experimental Design for emulgel batches SE1 to SE9

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Conc of Gelling agent in %</th>
<th>Conc of PG %</th>
<th>Spreading Coefficient(gm.cm/s)</th>
<th>% Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE1</td>
<td>2</td>
<td>5</td>
<td>24.3±1.24</td>
<td>85±0.99</td>
</tr>
<tr>
<td>SE2</td>
<td>2</td>
<td>3.5</td>
<td>20.9±1.48</td>
<td>83.6±1.02</td>
</tr>
<tr>
<td>SE3</td>
<td>2</td>
<td>7.5</td>
<td>26.8±1.28</td>
<td>84.9±0.64</td>
</tr>
<tr>
<td>SE4</td>
<td>1</td>
<td>5</td>
<td>27.3±0.99</td>
<td>80.4±1.09</td>
</tr>
<tr>
<td>SE5</td>
<td>1</td>
<td>3.5</td>
<td>19.2±0.98</td>
<td>70.16±1.05</td>
</tr>
<tr>
<td>SE6</td>
<td>1</td>
<td>7.5</td>
<td>28.2±1.08</td>
<td>86.64±0.99</td>
</tr>
<tr>
<td>SE7</td>
<td>2.5</td>
<td>5</td>
<td>21.4±1.04</td>
<td>80.61±1.8</td>
</tr>
<tr>
<td>SE8</td>
<td>2.5</td>
<td>3.5</td>
<td>18.63±0.99</td>
<td>71.16±0.99</td>
</tr>
<tr>
<td>SE9</td>
<td>2.5</td>
<td>7.5</td>
<td>20.14±1.20</td>
<td>87.34±1.08</td>
</tr>
</tbody>
</table>
a. Design Expert software version 8.0.7.1 results for emulgels of SCME

41a. 3D Plot of % release from emulgel batches SE1 to SE9

41b. Plot of predicted vs actual % release from emulgel batches SE1 to SE9.
41c 3D plot of spreading coefficient of emulgel batches SE1 to SE9

41d. Plot of predicted vs actual spreading coefficient of emulgel batches SE1 to SE9

Figure 41. Design expert studies of *Spathodea campanulata* methanolic extract emulgels.
The 3D plot for % release of extract from the emulgel formulation can be seen in the above figure. The predicted release from the ANOVA is 81%. Batch SE4 and SE7 show % release results near the predicted point and hence those points do not lie above or below the plot. Since the other response considered for optimization studies was spreading coefficient, the batch SE4 can be considered as optimized as its spreading coefficient is high.

The Design expert software provided the results for plot of predicted vs actual values of SCME emulgels. Some points were scattered away from the straight line, while there are 4 points which were located near the line. Since the predicted value for % release is 81% batch SE7 and batch SE4 both show results very much close to the predicted value. However the batch SE4 which shows lower levels of the gelling agent and moderate levels of propylene glycol as well can be considered as optimized batch.

The 3D plot for spreading coefficient was obtained from the Design expert software. It shows a curved plot with 4 points above the predicted value and 3 below it. The predicted value of spreading coefficient for the given design is is 25.37 g.cm/sec. Batch SE1 which has its spreading coefficient 24.3 g.cm/sec appears to be closer to the predicted value. However the release of this batch deviates largely from the prediction. Hence batch SE4 which shows release values near to predicted value and also good values for spreading coefficient can be considered as optimized batch. Plot of predicted values of spreading coefficient vs actual values obtained of all the 9 batches of factorial design can be seen in the above figure. The point in green colour which lies above the line represents the spreading coefficient of batch SE1 which 24.3 g.cm/sec is closer to the predicted value.

b. ANOVA results for emulgels of SCME

The ANOVA for the spreading coefficient of the emulgels applied to the model showed that the F value of the model is 47.78 which suggested that the model is significant. That means there are only 0.4% chances that the model F value so large
could be due to noise. The p value > F is 0.0046 which shows that is lesser than 0.05. All the terms of the model also show p value > F lesser than 0.1 which indicates that the model selected for the studies is significant. The model suggested by the ANOVA is quadratic model and all terms in the equation are significant. Similarly for the release from the emulgel formulations the model suggested by ANOVA is a quadratic model with 5 degrees of freedom. The F value of this model is 17.82 which means that there are only 1.9% chance that the model so significant could be due to noise. The p > F value is 0.019 which also confirms the significance of the model. All the terms are significant and hence both the ingredients contribute towards the % release from the formulation.

c. Predicted values of responses according to Design Expert.

Table 53. Predicted and Actual Values for emulgel batches SE1 to SE9

<table>
<thead>
<tr>
<th>Response</th>
<th>Predicted Value</th>
<th>Actual value (SE1)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spreading coefficient(g.cm/sec)</td>
<td>25.4</td>
<td>24.3</td>
<td>0.77</td>
</tr>
<tr>
<td>% release</td>
<td>81.1</td>
<td>85.99</td>
<td>3.42</td>
</tr>
</tbody>
</table>

The model fit summary and rest of the results clearly showed that the batch SE1 has shown very good spreading coefficient. The high spreading coefficient also indicates that the application of the emulgel can be done on the affected part very easily. The formulation SE1 shows less quantity of Carbopol and Propylene glycol is medium level. These factors attribute to the good spreadability of this formulation. The 3D surface plots also show that the spreadability of batch SE1 lies slightly below the predicted value which according to the design software is 25.4. Batch SE1 was coded as OSE which is now the optimized batch for emulgels of SCME. This batch was subjected to kinetics treatment, wound healing activity and anti-inflammatory activity. It was also kept for stability studies.
d. **Model fitting for Optimized Batches of both the emulgels**

The best fit model obtained for optimized formulation was Korsmeyer-Peppas kinetics ($r^2 = 0.9981$) and the ‘$n$’ value in Korsmeyer-Peppas model describes the drug release mechanism. It is known that, when $n \leq 0.5$ Fickian diffusion is observed and the release rate is independent of $t$, while $0.5 < n < 1.0$ indicate anomalous (non-Fickian) transport and when $n=1$, the release is zero order. Here $n$ value was found to be 0.4605 which signified that release pattern of optimized batch followed the Fickian diffusion.

The best fit model obtained for optimized formulation was 1st order ($r^2 = 0.9912$) and the ‘$n$’ value in Korsmeyer-Peppas model describes the drug release mechanism. It is known that, when $n \leq 0.5$ Fickian diffusion is observed and the release rate independent on $t$, while $0.5 < n < 1.0$ indicate anomalous (non-Fickian) transport and when $n=1$, the release is zero order. Here $n$ value was found to be 0.4605 which signified that release pattern of optimized batch followed the Fickian diffusion.

**6.5 Evaluation of optimized emulgel formulations.**

6.5.1 **Skin irritation testing**

No redness, inflammation or Escher formation was observed during the observation period of 5 days. Oedema was not present after follow up of observations for 5 days, formulations did not indicate any manifestation of skin irritation such as redness of skin or inflammation at site of application (erythema). Thus it may be concluded that optimized formulations can be used safely.

6.5.2 **In vivo antiinflammatory studies for optimized batch**

The in vivo studies were done using carageenan induced rat paw edema method. The extracts showed good anti-inflammatory activity earlier. Their formulations were tested for the same.
Table 54. Anti-inflammatory activity of optimized emulgel formulations

<table>
<thead>
<tr>
<th>Group</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>% anti-inflammatory activity at the end of 4 hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat paw edema in mm.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.6±0.14</td>
<td>3.3±0.13</td>
<td>3.6±0.39</td>
<td>3.8±0.19</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>2.1±0.1*</td>
<td>1.6±0.3***</td>
<td>1.3±0.25***</td>
<td>1.1±0.15***</td>
<td>71.05</td>
</tr>
<tr>
<td>OAE</td>
<td>2.3±0.21</td>
<td>2.1±0.28**</td>
<td>2.1±0.4**</td>
<td>1.7±0.15**</td>
<td>55.26</td>
</tr>
<tr>
<td>OSE</td>
<td>2.4±0.17</td>
<td>2.3±0.26**</td>
<td>2.1±0.21**</td>
<td>2.0±0.19**</td>
<td>47.36</td>
</tr>
</tbody>
</table>

(subjected to two way ANOVA followed by Dunnet’s test. * p< 0.05, **p< 0.01, *** p<0.001)

Antiinflammatory Activity of optimized emulgels

![Antiinflammatory Activity of optimized emulgels](image-url)

Figure 42. Anti-inflammatory activity of optimized emulgels

(Two way ANOVA * p<0.01 ** p<0.05, *** p<0.001)

The optimized formulations of emulgels of both the extracts were subjected to this study. It can be seen that for the three hours the edema increased in case of both...
formualtions. However in case of standard volini gel the edema decreased in the first hour as compared to the control group. After the 3rd hour it could be seen that the rat paw volume started to decrease. At the end of 4 hours the % inhibition of the edema as compared to the standard emulgel application for the OAE emulgel was 55.26 while for the OSE emulgel it was 47.36. For both the formulations the results were significant only after 2nd hour as compared with the control when subjected to two way ANOVA followed by Dunnet’s test. So OAE formulation showed better anti-inflammatory activity as compared to the OSE. This is in accordance with the total flavonoid and total phenolic content results of *Aspidium cicutarium* methanolic extracts.

6.5.3 Wound healing activity of optimized emulgels 107,165,166

The optimized formulation OAE and OSE were subjected to excision model studies. The excision wound was made and the formulations were applied once the hemostasis was achieved. The wound contraction was noted. The following were the observations.

**Table 55. Wound healing activity of optimized emulgel formulations**

<table>
<thead>
<tr>
<th>Treatment Given</th>
<th>Average Contraction Size of Wound in mm²</th>
<th>% wound contraction after 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>5th day</td>
</tr>
<tr>
<td>Control</td>
<td>499±1.9</td>
<td>496±3.2</td>
</tr>
<tr>
<td>Standard</td>
<td>500±4.1</td>
<td>356.1±2.7</td>
</tr>
<tr>
<td>OAE</td>
<td>502.9±3.2</td>
<td>389.0±4.5</td>
</tr>
<tr>
<td>OSE</td>
<td>503.8±4.1</td>
<td>495.5±1.9</td>
</tr>
</tbody>
</table>

(One way ANOVA followed by Dunnet’s Test *** p<0.001 and **** p<0.0001 as compared to control.)
Figure 43. Wound healing activity of optimized emulgel formulations

Contraction of wound in mm after 8 weeks was subjected to one way ANOVA followed by Dunnet’s Test. The results show that the standard formulation of 0.2% Nitrofurazone ointment showed very good contraction of wound at significance level p< 0.0001. While the OAE formulation showed the results significant at p<0.001, for OSE there was lowest contraction seen it is significant at p< 0.001. The optimized emulgel formulations showed wound healing activity, however it was not as good as compared to the standard Nitrofurazone ointment. From the % contraction of wound of the optimized formulations it can be concluded that the emulgels of Methanolic extract of *Aspidium cicutarium* and *Spathodea campanulata* can be used as wound healing preparations. The OAE formulation shows 30% wound contraction while the OSE formulation shows 15 % wound contraction.

6.5.4 Antioxidant activity of optimized emulgel formulations.\(^ {167}\)

The optimized formulations were subjected to determination of antioxidant activity by DPPH assay. The extracts in both the formulations OAE and OSE maintain their antioxidant properties. The OAE formulation shows IC\(_{50}\) of 20µg/ml as compared to Ascorbic acid whose IC\(_{50}\) value was 15µg/ml. But for OSE the IC50 was 40µg/ml.
These results are similar to the results obtained for the extracts. The IC\textsubscript{50} of OAE is closer to Ascorbic acid because of the higher phenolic and flavonoid content of the extract.

Table 56. Antioxidant activity of optimized emulgel formulations.

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Standard Ascorbic Solution</th>
<th>Inhibition by OAE (n=3)</th>
<th>Inhibition by OSE(n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>31.53</td>
<td>22.35±0.3</td>
<td>15.21±0.1</td>
</tr>
<tr>
<td>15</td>
<td>51.50</td>
<td>32.19±0.73</td>
<td>23.92±0.23</td>
</tr>
<tr>
<td>20</td>
<td>57.80</td>
<td>51.32±0.65</td>
<td>26.58±0.54</td>
</tr>
<tr>
<td>40</td>
<td>81.01</td>
<td>62.3±0.26</td>
<td>35.32±0.27</td>
</tr>
<tr>
<td>60</td>
<td>92.16</td>
<td>70.4±0.49</td>
<td>52.51±0.46</td>
</tr>
<tr>
<td>80</td>
<td>98.47</td>
<td>82.12±0.30</td>
<td>63.50±0.34</td>
</tr>
</tbody>
</table>

Figure 44. Antioxidant activity of optimized emulgels.
6.5.5 Accelerated stability studies of emulgels formulations

The freeze and thaw cycles performed as mentioned in the methodology showed that the formulations were stable and also had a good spreading coefficient. After the freeze and thaw cycles the optimized formulations OAE and OSE were evaluated for appearance and spreading coefficient. Both the formulations do not show any signs of creaming or phase separation. They appear to be stable. The spreading coefficient has also not changed. So both the optimized formulations OAE and OSE can withstand the freeze and thaw cycles. The optimized formulations were subjected to accelerated stability studies and tested for pH and spreading coefficient.

Table 57. Accelerated Stability studies of optimized emulgel formulations

<table>
<thead>
<tr>
<th>Period (in days)</th>
<th>OAE</th>
<th>OSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Spreading coefficient (g.cm/sec)</td>
</tr>
<tr>
<td>15</td>
<td>7.2</td>
<td>28.2±1.03</td>
</tr>
<tr>
<td>30</td>
<td>7.2</td>
<td>28.1±1.23</td>
</tr>
<tr>
<td>60</td>
<td>7.3</td>
<td>28.2±0.99</td>
</tr>
<tr>
<td>120</td>
<td>7.2</td>
<td>28.2±0.87</td>
</tr>
<tr>
<td>150</td>
<td>7.2</td>
<td>28.3±0.21</td>
</tr>
<tr>
<td>180</td>
<td>7.3</td>
<td>28±0.12</td>
</tr>
</tbody>
</table>

The formulations were kept in stability chamber for 6 months at 40° C ± 2° C at 75 ± 5 % RH according ICH guidelines for climatic zones III and IV. Initially after 15 days and then after regular intervals of 1 month the samples were removed. The appearance of the sample was checked and the spreadability was calculated. The above table shows that the formulations were stable in appearance. There was no change in its homogenous appearances. The pH values also remained unchanged. There was no phase separation seen. The spreading coefficient of both the optimized formulations also showed that the formulations were stable.
7 SUMMARY AND CONCLUSION
The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners. Natural products are a source of synthetic and traditional herbal medicine. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential properties. The Indian traditional system of medicine described several drugs of plant, mineral, and animal origin. Scientists who are trying to develop newer drugs from natural resources are looking toward the Ayurveda. Since time immemorial, man has used various parts of plants in the treatment and prevention of many ailments. Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in the more complex form of crude extracts.

Skin, the largest organ in the body, provides a barrier against UV radiation, chemicals, microbes and physical pollutants. Challenges of this nature can contribute to skin ageing and inflammation which is characterised by oxidative damage. Multiple studies have revealed that the skin is very sensitive to reactive oxygen species. Since an increase in the formation of the hydrogen peroxide ($\text{H}_2\text{O}_2$) has been associated with inflamed and diseased tissues, many herbal extracts and natural products which prevent or reduce oxidative stress and have a very good antioxidant potential can be well suited candidate for anti-inflammatory activity. Similarly the same potential can be useful for wound healing activity. “Best practice requires the assessment of the whole patient, not just the hole in the patient. All possible contributing factors must be explored.” The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient. At the site of wound closure a flexible and fine scar with high tensile strength is desired. Understanding the healing process and nutritional influences on wound outcome is critical to successful management of wound patients.

In India Ayurveda happens to be one of the best alternative system of medicine. Since many herbal medicines can be efficaciously used for the treatment of different diseases and ailments with lesser side effects. The aim of present research work was
to explore two indigenous plants which find their mention in the traditional Ayurvedic treatment. With an objective to establish a scientific basis to the medicinal activity of these plants the rhizomes of *Aspidium cicutarium* also known as *Nephrodium cicutarium* and leaves of *Spathodea campanulata* were selected for the study.

After the procurement of the plant parts they were subjected to authentication. The macroscopic and microscopic characters were studied. Both the plants were then subjected to preliminary evaluation which included the determination of physical constants and preliminary phytochemical evaluation. The rhizomes of *Aspidium cicutarium* showed a good extractive values in methanol and water suggesting that the phytoconstituents would be more concentrated in its aqueous and methanolic extract. Hence the powdered rhizomes were defatted and subjected to methanolic and ethanolic extraction in Soxhlett. Reddish brown crystalline powder was obtained on drying. Similarly aqueous extraction was also done. For the leaves of *Spathodea campanulata* the determination of physical constants yielded a higher extractive value for aqueous extract than the methanolic and ethanolic. The dried powdered leaves were subjected to extraction after defatting. Methanolic and ethanolic extracts were obtained by extracting in soxhlett apparatus. The aqueous extract was also obtained. Methanolic extract appeared greenish brown and slightly sticky in nature while the aqueous extract was sticky. The methanolic and aqueous extracts of both the plants were subjected to phytochemical screening.

The methanolic extract of *Aspidium cicutarium* shows presence of alkaloids while the alkaloids were completely absent in the aqueous extracts of *Aspidium cicutarium*. Steroids were also seen in methanolic extract while the aqueous extract has not shown positive result for the presence of steroids. Flavonoids and carbohydrates were present in both the extracts. Presence of tannins and saponins was seen in both the extracts. The presence of flavonoids and steroids can be related to the use of this medicinal plant to treat inflammatory conditions in traditional medicine system Ayurveda.
Similarly the methanolic and aqueous extract of *Spathodea campanulata* were subjected to phytochemical screening. The methanolic and aqueous extracts of leaves of *Spathodea campanulata* showed presence of carbohydrates, tannins, steroids and flavonoids. The presence of steroids and flavonoids may contribute to the antibacterial and wound healing activities.

- Determination of total flavonoid content:

The determination of flavonoid content was carried out by the Aluminium chloride method and methanolic extract of *Aspidium cicutarium* shows high concentration of flavonoids and hence these extracts can be tested further for the anti oxidant and anti-inflammatory activity as this biological activity can be attributed to the presence of high flavonoid content in the drug. The flavonoid content of methanolic extract of *Spathodea campanulata* was much higher than the aqueous extract using catechin as standard flavonoid. This indicates that the methanolic extract can be further investigated for antioxidant activity.

- Determination of Total Phenolic content.

The method used gallic acid as standard and the results were calculated as equivalent of gallic acid. The rhizomes extract showed a high concentration of phenols and needed to be evaluated further for anti oxidant activity. The methanolic extract yielded a higher phenolic content as compared to the aqueous extract of *Aspidium cicutarium*. For *Spathodea campanulata* also similar studies were carried. However here the aqueous extract showed higher phenolic content than the methanolic extract. Plant polyphenols, produced either from phenylalanine or from its precursor shikkimic acid, are important dietary antioxidants because they possess an ideal structural chemistry for free radical scavenging activity. Thus these extracts need to be further evaluated for antioxidant potential.

- Determination of antioxidant activity.

The antioxidant activity was carried out using DPPH assay. The standard antioxidant used was Ascorbic acid. The IC$_{50}$ for Ascorbic acid was shown to be 15 µg/ml while the IC$_{50}$ for methanolic extract was 15 µg/ml and the IC$_{50}$ for the aqueous extract was
40 μg/ml. This result can be correlated to the total phenolic and flavonoid content of *Aspidium cicutarium* methanolic extract. The satisfactory values for Phenolic and flavonoid content lead to better antioxidant activity. For *Spathodea campanulata*, IC$_{50}$ of aqueous extract was 40 (μg/ml). While the methanolic extract shows IC$_{50}$ at 80(μg/ml). Better antioxidant activity was seen in aqueous extract. This can be attributed to an antioxidant principle soluble in water present in the aqueous extract. This result also can be related to the lesser phenolic content in methanolic extract than the aqueous extract of *Spathodea campanulata*.

- Invitro anti-inflammatory activity.

The albumin denaturation method was used for invitro anti-inflammatory activity of extracts. The methanolic extract of *Aspidium cicutarium* shows dose dependent inhibition of protein denaturation. In concentrations as high as 800 μg/ml the % inhibition was found to be 88.94 %.The aqueous extract also shows inhibition of protein denaturation upto 79% at concentrations 800 μg/ml. The % inhibition of protein denaturation was found to be lesser in both the extracts of *Spathodea campanulata* as compared to the extracts of *Aspidium cicutarium*. This can also be related to the total flavonoid content and total phenolic content results of both the plants.

- Antimicrobial activity

Cup plate method was used to measure the zone of inhibition of methanolic extracts of *Aspidium cicutarium* and *Spathodea campanulata* against gram positive and gram negative microorganisms and was compared against Ampicillin (100 ug/ml) standard. The extracts showed satisfactory results at a concentration range of 1000 to 8000 μg/ml of concentration.

- TLC studies

The main aim of the TLC studies was to confirm the presence of steroids and flavonoids in the extracts. The presence of multiple spots on the TLC plates for
methanolic extract of *Aspidium cicutarium* signify the presence of many different steroidal compounds. Similarly at various wavelengths the TLC plates for flavonoids were developed for the methanolic extract of *Aspidium cicutarium* and it showed presence of flavonoids. These results thus help to confirm the results obtained in phytochemical screening. For the methanolic extract of *Spathodea campanula* the TLC images show the spots at various Rf values. The TLC plate images for detection of steroids also show many spots on the developed TLC plate indicating presence of steroids.

- **HPTLC studies**

The aqueous and methanolic extract of *Aspidium cicutarium* and *Spathodea campanulata* were put for HPTLC screening. The HPTLC studies show that both the plant extracts show a number of peaks which correspond to the steroid and flavonoid markers. This proves that the extracts contain steroidal compounds and flavonoids. After the HPTLC screening the extracts were subjected to UV absorption studies and calibration curves were plotted.

- **FTIR and UV spectrophotometric studies**

The FTIR spectra were obtained for methanolic extracts of both the plants. From the FTIR studies presence of ring structural and functional groups such as phenolic can be confirmed. Extracts showed good solubility in Phosphate buffers and so their solutions were subjected to determination of λ max and calibration curves were plotted.

- **Analytical characterization of markers**

From the literature survey and the results of phytochemical investigations, markers were selected for methanolic extracts of both the plants. For ACME the flavonoids were present in high quantities. The flavonoid marker selected was Phloroglucinol. It was subjected to UV absorption and FTIR studies. The results were compared to ACME. The results suggested that the Flavonoids present in the extract are similar to phloroglucinol or are derivatives of it. Similarly for SCME, both the flavonoid and steroid markers were chosen. Kaempferol was marker for flavonoid and β-sitosterol was the marker for steroids. These were also subjected to UV absorption and FTIR
studies. On comparison of the results with SCME there was a striking resemblance in results of β-sitosterol and SCME suggesting presence of steroids. In the literature already studies have reported the presence of Ajugol as a steroidal compound present in *Spathodea campanulata*.

- **Acute toxicity studies**

OECD guidelines were followed to perform acute toxicity studies. The LD$_{50}$ for *Aspidium cicutarium* methanolic extract was found to be 3000 mg/kg. The LD$_{50}$ for methanolic extract of *Spathodea campanulata* was found to be 4000 mg/kg.

- **Cytotoxicity and anti-inflammatory studies**

The cytotoxicity studies were carried out for ACME using the MTT assay and the extract was found to be safe at concentration upto 2.5 mg/ml of extract. The anti-inflammatory studies were also carried on RAW 264.7 cell lines. Generally considering, macrophages play an important role in inflammatory diseases by producing cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and other inflammatory mediators, such as nitric oxide (NO) and prostaglandins. These cytokines and mediators from macrophages have been found in many inflammatory cells and tissues, along with increased expression of their mRNA, following exposure to immune stimulants, including bacterial endotoxin lipopolysaccharide (LPS).

Several pro-inflammatory mediators have been identified that mediate a critical role in inflammation. Among these gene products are TNF-α and members of its superfamily, IL-1β, IL-6, etc. The studies were carried out on macrophages RAW 264.7 by inducing inflammation by LPS. The methanolic extract of ACME showed a dose dependent inhibition of the expression of mediators of inflammation. The highest inhibition was seen in IL 6 production. Thus it can be concluded that the high phenolic content and high flavonoid content of the extract result in high anti-inflammatory activity at cellular level.

From the studies carried out on both the herbal extracts it can be concluded that both showed presence of flavonoids, phenols and steroids. They also had a very good
antioxidant activity, hence they were subjected to anti-inflammatory studies. Both the aqueous and methanolic extracts were subjected to these studies using Dexamethasone as a standard topical anti-inflammatory agent. The methanolic extracts of *Aspidium cicutarium* showed dose dependent anti-inflammatory activity. As the dose of extract increased the % inhibition also increased and at 200 μl/ear concentration the % inhibition went as high as 68.47 % which is very close to the results obtained by Dexamethasone. Similarly the methanolic extract of *Spathodea campanulata* also showed dose dependent inhibition. The 200μl/ear dose showed very high anti-inflammatory activity.

- Wound healing activity

The wound healing activity was evaluated using both the incision wound model and excision wound model. The methanolic extract of *Aspidium cicutarium* showed 34.84 % wound contraction at the end of 15 days and methanolic extract of *Spathodea campanulata* showed 24.64% wound contraction as compared to standard Nitrofurazone ointment. The histopathological slides also show good results for both the extracts.

- Preformulation studies for film formulation.

Various film forming polymers were chosen for the casting of films. Ethyl cellulose, Sodium CMC and Eudragit RL 100 + HPMC combination were tried. They were evaluated on the basis of a scoring system. The films made from Eudragit RL 100 and HPMC K4M were used for the film formation.

Compatibility studies of film forming polymers with methanolic extracts of both the plants was carried out using DSC technique. The results showed that the excipients selected were compatible with both the extracts.

Trial batches of films were made. They were evaluated for texture, appearance, thickness, weight of the film, adhesive strength, tensile strength, weight, % moisture uptake and folding endurance. From the results of the trial batches the factorial design of two factors and three levels $3^2$ was made. Film forming agent Eudragit RL 100 and plasticizer PEG 400 was taken as factors and varied over three levels to get 9 batches. Results were subjected to Design Expert software to optimize the formulation.
Conclusions were made on the basis of 3D surface response plots, plots of predicted vs actual values of the responses and ANOVA. From the results of the design expert software three different batches were considered from the nine factorial batches whose results were near to the predicted values of responses. However from these batches for the film formulations, the batch AF$_3$ was the optimized batch. This batch had medium levels of film forming agent and higher levels of plasticizer. The % release from the batch and its adhesive strength was close to the predicted values. This batch was then coded as OAF and subjected to anti-inflammatory studies, antioxidant studies and stability studies for 6 months according to the ICH guidelines for the climatic zone IV. Similarly trial batches of films containing *Spathodea campanulata* leaf extracts were made and evaluated for preliminary tests. The concentrations which showed better results were considered as factors in the factorial design and 9 batches of film formulation were casted. They were subjected to various evaluation parameters however % release and adhesive strength were considered as responses. Design Expert software was used to optimize the batch. 3D surface response plots, plots of predicted vs actual values of % release and adhesive strength were taken into consideration for optimizing the formulation. SF$_3$, SF$_1$ and SF$_7$ were the three formulations which showed values closer to the predicted values for % release and adhesive strength. Considering the results for both the responses it was concluded that optimized batch is SF$_1$. The batch SF$_1$ has medium concentration of film forming agent Eudragit RL 100 and low level of PEG 400. These factors and their concentration thus contribute to the adhesive strength and the release of extract from the film. Thus this batch was coded as OSF and subjected to anti-inflammatory studies, antioxidant activity and accelerated stability testing.

The in vivo anti-inflammatory studies were carried for the batches OAF and OSF using Volini gel as standard formulation using the rat paw carrageenan induced edema method. The optimized batch of OAF showed 53.84 % anti-inflammatory activity. While the optimized batch of OSF films showed 48.71% inhibition. Both the results are less than the standard gel formulation. This may be due to lesser penetration of the extract from the film into the rat paw. The activity can be attributed
to the higher phenolic and flavonoid content of the extracts. Use of other penetration enhancers can improve the penetration of extract to the affected areas of inflammation and give better anti-inflammatory results. The antioxidant activity was maintained by the extracts in the film formulations.

The ICH guidelines were referred for the conditions to be maintained for the accelerated studies. Studies were carried for 6 months. After every month the film formulation were reviewed for appearance and moisture uptake and also for adhesive strength of the film. Both the optimized films maintained their characteristics when subjected to accelerated stability studies. They maintained the adhesive strength and moisture uptake levels, did not become tacky or slimy and thus had the original structural strength as well as flexibility.

- Preformulation studies of emulgels

The other formulation was emulgel which have the advantages of creams and gels combined into the same formulation. The oil phase of the emulsion was constituted by Rice Bran oil. The emulsifiers chosen were non ionic emulsifiers Tween 80 and Span 80.

Preformulation and compatibility studies included FTIR studies for the extracts and combinations of extracts with the oil and gelling agent. The spectra showed compatibility amongst the selected ingredients.

It is a well known fact that emulsifiers used in combination generally yield stable emulsions. The major task was to find out the RHLB for the Rice Bran oil and then use the correct combination of the emulsifiers to make a stable emulsion. Use of Computagraph was made to obtain the RHLB which was somewhere between 4.3 to 12. The ratio of emulsifiers so obtained was 40: 60 for Span 80 :Tween 80 in the concentration range of 5%. Concentration of Rice Bran oil was 10%. Since the composition of emulsion was fixed already, the factorial design was mainly applied to the gelling part of the formulation. The factors selected were Carbopol 934 and propylene glycol. These were varied over three different levels of concentrations to
obtain a $3^2$ factorial design that yielded 9 batches. The responses selected for the evaluation of emulgel formulation were % release from the formulation and spreading coefficient.

- Evaluation of factorial batches of emulgels

All the formulations lie in the normal pH range and would not produce any skin irritation. The values of viscosity range from 2.54 to 3.67 Pa.s. The emulgels being the combination of emulsions and gels show moderate viscosities and hence better acceptability. Rheological behavior of the emulgels indicated that the systems were shear thinning in nature showing decrease in viscosity at the increasing shear rates. An increase in the concentration of Carbopol 934 was expected to show increase in viscosity.

Spreading coefficient depends on the polymer in formulation, possessing typical physicochemical properties which create surface tension between slide and product. It indicates that the Emulgel is easily spreadable by small amount of shear. It shows that the formulation shows higher spreading coefficient as compared to other formulations. Batch AE6 shows good spreading coefficient amongst all batches. The higher drug release was observed with formulations AE9 as is obvious from the release graphs. However since spreadability is also one of the important factor taken into consideration AE6 can be said to be a better formulation than the rest since the release rates are not much different from AE9. Results were subjected to optimization using Design Expert software. The design expert software results were obtained in the form of 3D response plots, plots of predicted vs actual values and ANOVA. From the Design expert results two batches seem to have values closer to predicted value for % release. The batch AE7 which has higher % of Carbopol 934 and medium % of propylene glycol shows release profiles near the predicted value. The % release from this batch is 82.62% which is near the predicted value. It can be concluded that since the predicted value of the Design expert software for spreading coefficient is 26.26.g.cm/sec the batch AE7 shows a value closer to the predicted. However the spreading coefficient of AE4 is 28.5 which also does not deviate much from the
predicted value and the release from this batch is also good. Hence this batch was considered as optimized batch.

Similarly 9 batches were made of SCME emulgels and evaluated for spreading coefficient, viscosity, % release. The release from SE9 batch is the highest. The results of the factorial batches were subjected to Design expert software. Batch SE1 and SE7 show % release results near the predicted point and hence those points do not lie above or below the plot. Since the other response considered for optimization studies was spreading coefficient, the batch SE1 can be considered as optimized as its spreading coefficient is high. The formulation SE1 shows less quantity of Carbopol 934 whereas the quantity of Propylene glycol is medium level. These factors attribute to the good spreadability of this formulation. The batch SE1 was coded as OSE and subjected to wound healing activity, anti-inflammatory studies, antioxidant activity and stability studies.

In vivo anti-inflammatory activity was studied using rat paw edema method. At the end of 4 hours the % inhibition of the edema as compared to the standard emulgel application for the OAE emulgel was 55.26% while for the OSE emulgel it was 47.36%. So the OAE emulgel showed better anti-inflammatory activity than the OSE formulation. Similarly wound healing activity also showed good results. Better results were shown by OAE formulation as compared to OSE. So the *Aspidium cicutarium* methanolic extract had good wound healing activity which can be contributed to polyphenols present in the extract. The optimized formulations were also subjected to antioxidant activity evaluation by DPPH assay and it was seen that the extracts maintained their antioxidant activity in emulgel formulations.

Accelerated stability studies were performed for 6 months by keeping the formulations at 40°C ± 2°C at 75 ± 5 % RH. The formulations were tested after every month for appearance to check if there was any phase separation or creaming. They were also evaluated for spreading coefficient and pH. Both the optimized emulgels OAE and OSE showed stability and also maintained the spreading coefficient values.
From all the above exhaustive study of extracts of *Aspidium cicutarium* rhizomes and *Spathodea campanulata* leaves it can be concluded that the herbal extracts of both the plants have potential to be used as anti-inflammatory, wound healing and antioxidant activities. Studies with other plants have shown that phytochemical constituents like tannins and flavonoids are known to promote wound healing process mainly due to their astringent and antimicrobial properties, which appears to be responsible for wound contraction and increased rate of epithelization. The antioxidant activity of both and especially of *Aspidium cicutarium* methanolic extract indicates that this extract can be used for all such activities that are related to the free radical scavenging activity. The emulgel formulation of this extract has very high anti-inflammatory and wound healing activity that shows that the transdermal formulations could be successfully made in a safe and efficacious way. The results indicate the importance of herbal formulation in treatment of disease conditions like inflammation, wound healing. The antioxidant potential can be further exploited in anticancer studies. The MTT assay results for methanolic extract of *Aspidium cicutarium* rhizomes also show the safety of the extract at cellular levels and can be further evaluated for anticancer studies. 

Thus the extracts obtained from these plants can be used in conditions such as inflammation, wound healing and all the problems that involve oxidative stress in a safe and efficacious way. Better and efficacious formulations can be further developed using the extracts. Similarly the formulations can be subjected to clinical evaluations. Cell line studies can be performed on the formulations. The herbal extracts have a scope in the future to replace the existing allopathic drugs with side effects so the patients get a safer alternative. Thus these studies are a small contribution to the health care system with an aim to make available safer and efficacious alternative to treatment of inflammatory and oxidative stress related conditions.

The definite pathway of anti-inflammatory action for *Aspidium cicutarium* extract can be a future research work carried ahead. Similarly the high inhibitory activity against IL6 also suggests that the extract can have anticancer activity and would also be useful in auto immune disorders. For *Spathodea campanulata* similar cell line studies can be carried in the future work and stable formulations can be formulated.
8 REFERENCES


55. Ojha N, Kumar A, HPTLC profile of aqueous extract of different chromatographic fractions of *Aloe barbadensis* Miller, Asian Pacific Journal of Tropical Disease 2012; S104-S108


76. Dudani S, Chandran M D, Ramachandra T V, Pteridophytes of western ghats in biodiversity documentation and taxonomyes A. Biju Kumar (Ed),2012, 343-351


98. Wagner H, Baldt S. Plant drug analysis; Berlin: Springer; 1996.


106. Lanan W S et al, Regulation of inflammatory cytokines in lipopolysaccharide-stimulated RAW 264.7 murine macrophage by 7-o-methyl-naringenin, Molecules 2012;17: 3574-3585


120. Arora P, Mukherjee P, Design, development, physiochemical, in vitro and in vivo evaluation of transdermal patches containing Diclofenac


126. The HLB system, a time saving guide to emulsifier selection, edited and reprinted from Chemmunique, ICI Americas INC, 10-17.


