FORMULATION AND EVALUATION OF HERBAL ANTIDANDRUFF GEL AND DETERMINATION OF THE EFFECT OF HERBAL ADJUVANTS ON THE ENHANCEMENT OF ANTIDANDRUFF ACTIVITY

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In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

BRANCH – I PHARMACEUTICS

Submitted by

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CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF HERBAL ANTIDANDRUFF GEL AND DETERMINATION OF THE EFFECT OF HERBAL ADJUVANTS ON THE ENHANCEMENT OF ANTIDANDRUFF ACTIVITY"** submitted by **Mrs. S. PRIYA DHARSHINI [Reg. No: 261710202]** for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by her in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences, Tiruchirappalli during the academic year 2018 - 2019 under my direct guidance and supervision.

Place: Tiruchirappalli

Date: (**Dr. K. Reeta Vijaya Rani)**

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ABBREVATION

AEECO	Aqueous Extract of *Emblica officinalis*
AECL	Aqueous Extract of *Citrus limonum*
ADME	Absorption, Distribution, Metabolism, Excretion
CBD	Convention on Biological Diversity
CM	Complementary Medicine
FTIR	Fourier Transform Infra Red Spectroscopy
GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
ICH	International Council for Harmonisation
IP	Indian Pharmacopoeia
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxy Propyl Methyl Cellulose
MS	Mass Spectroscopy
NCL	National chemical Laboratory
NMR	Nuclear Magnetic Resonance
PEG	Poly Ethylene Glycol
PhEur	European Pharmacopoeia
SDA	Sabouards Dextrose Media
TLC	Thin Layer Chromatography
TM	Traditional Medicine
USPNF	United States of Pharmacopoeia
WHO	World Health Organisation
UV	Ultra violet
cp	Centipoises
g	Gram
mg	Milligram
ml	milli litre
mpa	Mega pascal pressure
µg	Microgram
nm	Nanometer
λ max	Absorption maxima
SD	Standard deviation

1. INTRODUCTION

Many countries have their own traditional or indigenous forms of healing which are firmly rooted in their culture and history. Traditional Medicine (TM) is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Some forms of TM such as Ayurveda, traditional Chinese medicine and Unani medicine are popular nationally, as well as being used worldwide. At the same time, the terms "complementary medicine" (CM) or "alternative medicine" refer to a broad set of health care practices that are not part of that country‟s own tradition or conventional medicine and are not fully integrated into the dominant health-care system. They are used interchangeably with traditional medicine in some countries, some forms of CM such as anthroposophic medicine, chiropractic, homeopathy, naturopathy and osteopathy are also in extensive use.[1]

Traditional herbal medicines and their formulations have been generally used for the thousands of years in developing and urbanized countries because TM are naturally occurring, plant-derived substances with minimal or no side effects that have been used to treat illness within local or regional healing practices. Traditional herbal medicines are getting significant attention in global health debates.[2]

In recent history, the isolation of active compounds from the plant beginning with the isolation of morphine from opium in the early 19^{th} century. Drug discovery from medicinal plants led to the isolation of early drugs such as codeine, digitoxin, and quinine. Isolation and characterization of pharmacologicallyactive compounds from medicinal plants continue still. Nowadays, drug discovery techniques have been applied to the standardization of herbal medicines to elucidate analytical marker compounds.[3]

WHO classified herbal medicines into four different classes according to their origin, evolution and forms of current usage.

- ✓ Indigenous herbal medicines
- ✓ Herbal medicines in systems
- ✓ Modified herbal medicines
- ✓ Imported products with a herbal medicine base

Indigenous herbal medicines are those which historically used in a local community or region and are very well known through long usage by the local population in terms of its composition, treatment and dosage. It can be used freely by the local community or in the local region. However, if the medicines in this category enter the market or go away from the local community or region, they have to meet the requirements of safety and efficacy as per the national regulations for herbal medicines. Herbal medicines in systems have been used for a long time and are documented with their special theories and concepts and accepted by the countries. For example, Ayurveda, Unani and Siddha. Modified herbal medicines have been modified in shape or form including dose, dosage form, mode of administration, herbal medicinal ingredients, methods of preparation and medical indications. They have to meet the national regulatory requirements of safety and efficacy of herbal medicines. Imported products with herbal medicine base covers all imported herbal medicines including raw materials and products. Imported herbal medicines must be registered and marketed in the countries of origin. The safety and efficacy data have to be submitted to the national authority of the importing country and need to meet the requirements of safety and efficacy of regulation of herbal medicines in the recipient country.[4]

Drug Discovery and Development:

In ancient times, drug discovery of bioactive compounds from plants was time consuming and the process of isolation and identifying the chemical structures of bioactive compounds from an extract could take several months, or years. At the present time, new techniques like such as High-Performance Thin Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC) coupled to Mass Spectrometry (MS)/MS (Liquid Chromatography, LCMS), Higher Magnetic Field Strength Nuclear Magnetic Resonance (NMR) instruments and robotics have minimize time appreciably. With larger number of countries becoming the parties to the Convention on Biological Diversity (CBD), the process of accessing the basic lead resource, benefit sharing during the commercial phase, etc. became highly complex in many countries. These processes tend to impede the pace of discovery process at various phases irrespective of the concerns leading to such processes.[5]

Lead identification is the first step in a lengthy drug development process which is represented inFig.1. Lead optimization (involvingmedicinal and combinatorial

chemistry), lead development(including pharmacology, toxicology, pharmacokinetics, ADME [absorption, distribution, metabolism, and excretion], and drug delivery) and clinical trials all take a substantial length of time.[3]

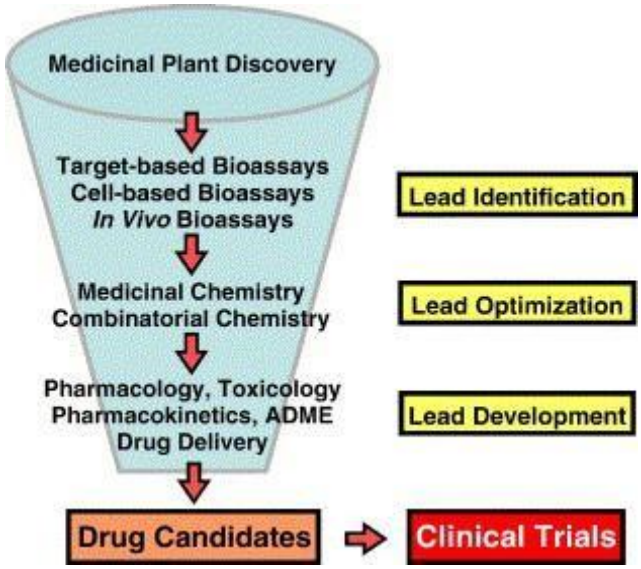


Fig. 1. Schematic of typical medicinal plant drug discovery and development

It has been estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for use.[6]

Standardization and regulatory measures of herbal medicinal products

Standardization of herbal medicines is quite difficult task because of the complication in their diverse secondary metabolites, with therapeutic actions that may depend largely on age, genetic factors and geographical location of the plant species. The variability in phytochemical contents amongst varieties of herbal products from the same plant species engenders profound differences in pharmacological activity. Also, the harvesting process and period, coupled with incidents of adulterations due to the presence of microorganisms and pesticides have profound impact on achieving uniform standards of herbal medicines worldwide. Improvement in the quality of herbal medicines could be achieved by deliberate implementation of Good Agricultural Practices (GAPs) at the point of cultivation of medicinal plants and Good Manufacturing Practices (GMPs) during the process of manufacture and packaging of finished herbal products, as well as post-marketing quality assurance surveillance.[7]

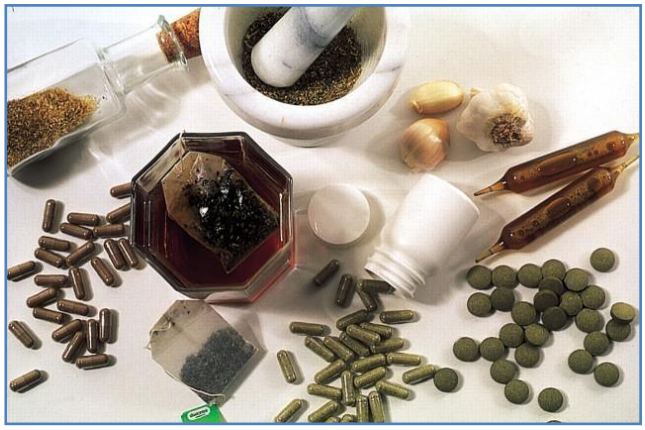


Fig. 2. Herbal remedies available in variety of formulation

Introduction to Hair

Hair is a unique character found on all mammals but not on other animals. In humans it is a special and cherished feature, especially in females, but its main functions are in protection of the skin from mechanical insults and to facilitate homeothermy for example, eyebrows and eyelashes stop things entering the eyes, while scalp hair prevents sunlight, cold, and physical damage to the head and neck. It also has a sensory function, increasing the perception of the skin surface for tactile stimuli and sub serves important roles in sexual and social communication, considering the psychological impact on quality of life seen in hair disorders, such as hirsutism, hair loss, etc[8]

Anatomy and Physiology of Hair

Hair is composed of cylindrical structures or shafts made up of tightly compacted cells that grow from small sac-like organs called follicles shown in **Fig. 3**. In man, the diameter of individual hair shafts may range from 15 to 120 mm depending upon the type of hair and the region of the body the follicle is located. Hair contains a family of sulphur -rich proteins called keratin (from the Greek word, ***keras,*** meaning horn). In the hair shaft, keratin forms long fibers which become bound together very tightly through the replacement of SH groups with S-S bonds and through chemical crosslinking with other proteins. The result is a very tough, highly stablestructure. The hair follicle serves as a reservoir for epithelial and melanocytestem cells and it is capable of being one of the few immune privileged sites of human body. Hair follicle development is related to the interactions between epithelial and mesenchymal cells.[9]

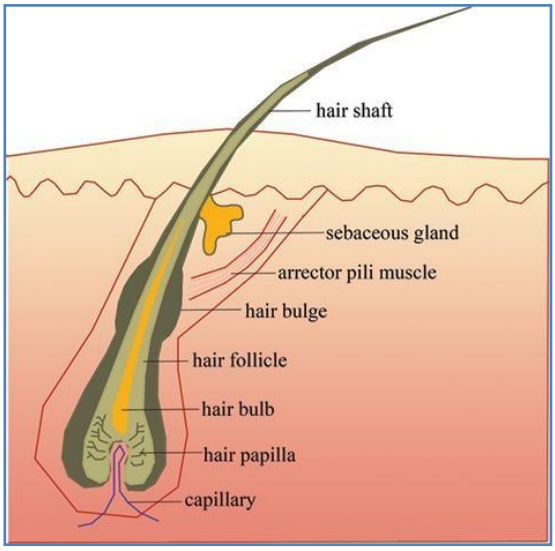


Fig. 3. Anatomy of Hair Follicle

Structure of the hair shaft

Each hair shaft consists of three distinct types of cells: an outer cuticle which surrounds a central cortex which, in turn, may contain a central medulla shown in **Fig. 4**.The cuticle of human hair consists of a single layer of elongated, overlapping individual cells. Each cuticle cell is approximately 0.5 to 1.0 mm thick. The function of the cuticle is to anchor the hair shaft in the follicle and to protect the interior fibers. However, the cuticle can be damaged or destroyed by chemicals, heat, light, or mechanical injury. As a result, the cuticle becomes less intact towards the distal end of the shaft and may become frayed and fall apart. The cortex forms the bulk of the hair shaft and is composed of long keratinized cells which are formed into long fibers approximately 100 lrn long. These fibers are held together by a special chemical cement. Between the cells of the cortex are very small air spaces called fusi. In the living portion of the hair root, these small spaces are filled with fluid but as the hair grows and dries out, air replaces the fluid. Pigment granules are also found in cortical cells and the type of pigment and alignment gives hair its color. Melanin (from the Greek word *melus*for black) is the principal pigment of hair, as well as the skin and eyes. Melanin is synthesized in specialized organelles called melano somes located within the hair bulb in small bodies called melanocytes. Here, melanin is made from the amino acid tyrosine through the action of the enzyme tyrosinase. The color of human hair, from black to white, is produced by different amounts, distribution and types of pigments. Medullar cells are initially loosely packed, but dehydrate and shrivel up to leave a series of vacuoles along the fiber axis. In general,

the number of medullar cells increases as the fiber diameter increases. Thus, fine animal hair contains only cuticle and cortex cells while thick animal hair, such as horse tail or mane or porcupine quills, contains a relatively large percentage of medullar cells. In human hair, medullar cells comprise only a small percentage of the mass and may be completely absent (such as in very line velus hairs), continuous along the center or discontinuous. In human beard hair the medulla is quite complex and, in some instances, a double medulla may be observed.

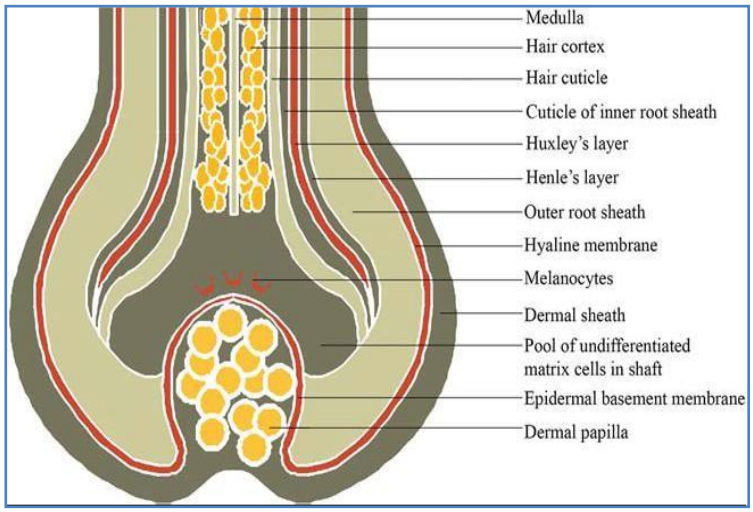


Fig.4.Structure of Proximal hair follicle

Chemical composition of hair

Hair is essentially a cross-linked, partially crystalline, oriented polymeric network which contains a number of functional chemical groups (e.g., acidic, basic, and peptide bonds) which have the potential of binding small molecules. Human hair (depending on its moisture content) consists of approximately 65-95% protein, 15-35% water, and l-9% lipids. The mineral content of hair is from 0.25 to 0.95% (on a dry weight basis). Both essential trace elements and heavy metals can be found in human hair. The lipid material found in hair is derived from sebum and the secretions of the apocrine gland and consists of free fatty acids, mono- di-, and triglycerides, wax esters, hydrocarbons, and alcohols. Human hair contains relatively large amounts of hydrocarbon side-chain amino acids (glycine), hydroxyl sidechain amino acids (threonine), primary amide side-chain amino acids (aspartic and glutamic acid), dibasic amino acids (lysine) as well as disulfide (cysteine) and phenolic amino acids (tyrosine).[10]

The Hair follicle

Hair follicles are embedded in the epidermal epithelium of the skin, approximately 3-4 mm below the skin‟s surface. Hair follicles are closely associated with two glands - the sebaceous gland and the apocrine gland. In most parts of the body, the hair follicle and the sebaceous gland are fused both anatomically and functionally into what is called a pilosebaceous unit. In the axillary and pubic areas, hair follicles are also associated with another type of sweat gland, the apocrine gland. The ducts of apocrine and sebaceous glands empty into the follicle. The ducts of eccrine sweat glands, on the other hand, are located near the follicles, but do not empty into the follicle[11].

The hair follicle can be divided functionally into three zones along the axis of the hair shaft. The innermost zone, in and around the bulb, is the site of biological synthesis of hair-cells. The next section, located directly above the bulb, is the site of keratinization (ketratogenous zone), where the hair undergoes hardening and solidification. The final zone is the region of permanent hair. Here the hair shaft consists of dehydrated, cornitied cells that have formed into tibrils fused by an intercellular binding material.

Hair growth begins in cells located in a germination center (called the matrix) located in the base of the follicle. As the cells divide, increase in volume, and elongate, they become larger and move up the follicle into the keratogenous zone. Here the cells synthesize pigment (melanin) and begin to „keratinize‟. Long fibers are formed through the cross-linking of the sulfhydryl groups in amino acids like cystine. Gradually, the hair cells die and decompose by eliminating the cell nucleus and releasing water. The cell contents coalesce into a dense, „horny‟ mass. This same process occurs in snails and in the outermost layer of skin and is also the process by which the hoofs and horns of animals are formed.

Secretary glands

Three glands are associated with the hair follicle - the apocrine, sebaceous, and sweat glands. Because their secretions bathe the hair shaft, these glands may be possible sources of trace elements in hair. It is possible that these secretions are also vehicles for the transfer of drugs into hair.[12]

Sebaceous glands

Sebaceous glands are present on the entire surface of the body except the palms, soles, and dorsum of the foot. They are located just below the surface of the skin and their ducts exit directly into the tunnel of the hair follicle. As a result, the permanently formed hair shaft is bathed in sebum just before it emerges from the skin. The exception is the beard follicle whose complimentary sebaceous gland has a duct that opens directly onto the surface of the skin. Sebaceous glands are largest and most numerous on the scalp, forehead, cheeks, and chin. The secretion of the sebaceous glands is a waxy substance called sebum. The quantity of sebaceous secretions is quite significant and gives unwashed hair its oily appearance. A lo-cm2 section of forehead skin will secrete 0.7-2.4 mg of lipid during a 4-h period. The composition of sebum varies somewhat depending upon the anatomical area from which it is collected; however, approximately one-third of the non-aqueous material is free fatty acids, one-third combined fatty acids, and one-third unsaponifiable material like squalene, cholesterol, and waxes.

a.Apocrine glands

In humans, apocrine glands are localized in the axilla, the external auditory meatus, the eyelids, and the perineal region. Apocrine glands are different from the type of sweat glands than are found on the scalp in that they secrete by the separation of cytoplasm from the secreting cells. Also, apocrine glands discharge directly into the hair follicle rather than onto the surface of the skin. Apocrine glands secrete an oily, colorless and odorless substance and little is known about its composition or physiological function except that body odor results from its bacterial decomposition.

b. Sweat glands

Eccrine sweat glands (also called merocrine sweat glands) are distributed overnearly the entire surface of the body. These glands originate in the dermal layer of the skin and their ducts exit near, but not connected to, the exit of the hair follicles. Each gland is a simple tubule that consists of a coiled segment located in the dermis and a straight duct that opens onto the epidermis. Eccrine sweat glands produce a fluid secretion that does not result from the removal of cytoplasm from the secreting cells. Both the quantity and concentration of eccrine sweat vary enormously dependingupon the individual and the environmental circumstances. Water, salts such as Na and K are the

principal ingredients of sweat along with urea, amino acids, lactate and pyruvate. Sweat also has been shown to be a vehicle for the excretion of a number of drugs.[13]

Physiology of the hair

A hair arises from the integrated activities of several keratinocyte layers in the hair follicle. The development of hair is a dynamic, cyclic process in which the duration of growth cycles is coordinated by many hormones and cytokines and depends not only on where the hair is growing but also on some other factors, such as the individual‟s age and stage of development, nutritional habits, or environmental alterations like day-length[14].Important players of this cycle are mainly cytokines (hormones), which are able to instruct the follicle to undergo appropriate changes, so that each hair can be in a different stage of growth cycle (**Fig. 5**) compared to the adjacent hairs..Hair follicles grow in repeated cycles, in which stages of rapid growth and hair shaft formation alternate with stages of apoptosis-driven hair follicle regression and relative hair follicle quiescence.[15]

In particular, the hair growth cycle can be divided into three distinct phases

1) Anagen or growth phase;
2) Catagen or transitional phase; and
3) Telogen or resting phase.

Anagen or growth phase

The anagen phase is an active growth phase, during which the hair follicle enlarges reaching its characteristic onion shape and a hair fiber is produced. It can be divided into six stages (I–VI). During anagen I–V (proanagen), hair progenitor cells proliferate, envelope the growing dermal papilla, grow downwards into the skin, and begin to differentiate into the hair shaft and IRS; then, the newly formed hair shaft begins to develop and the melanocytes located in the hair matrix show pigment producing activity; in anagen VI (metanagen), full restoration of the hair fiber-producing unit is realized, which is characterized by formation of the epithelial hair bulb surrounding the dermal papilla, located deep in the subcutaneous tissue, and the new hair shaft appears from the skin surface. This phase can last for several years in hair follicles.

Catagen or transitional phase

Following a period of active hair growth, the length of which varies with the type of hair, the hair follicle enters a short transitional phase called catagen. During this transition stage, cell division stops and the base of the hair shaft becomes fully keratinized and forms the dry, white node characteristic of a „club" hair. The bulb begins to degenerate and the follicle becomes considerably shorter.

Telogen or resting phase:

Following the transition stage, the hair follicle enters a resting or quiescent period in which the hair shaft stops growing completely and is retained in the upper portion of the follicular canal where it can be removed easily by pulling. The length of time the follicles are in their quiescent period depends upon the body area and the age of the individual. For scalp hair, the resting phase is relatively short, about 10 weeks; for the general body surface, it is about 2-6 years. The length of the quiescent period increases gradually with age. In man and the guinea pig, hair is said to grow in a mosaic pattern, that is, each follicle has its own growth cycle independent of others. In most other animals, including the laboratory rat, hair grows in waves in which the activity of the follicles is synchronized. When follicles go into their resting stage in synchrony and thus are easily removed by pulling, the result is called „shedding". The percentage of hairs in the growing phase compared with the percentage in the resting phase is quite different for hair from different parts of the body. In general, the longer the type of hair, the longer the growing phase. On the scalp of an adult human, approximately 15% of the hairs are in a resting stage and the remaining 85% are in their growing phase. These normal patterns can be complicated by disease states, nutritional deticiencies and even drugs. One striking example is the changes associated with pregnancy. During the second and third trimester, the percentage of scalp hair in the growing phase increases from 85 to 90%. After delivery, the normal ratio of growing to resting hairs is slowly re-established, but this results in an increased hair loss at about 3 months after delivery.

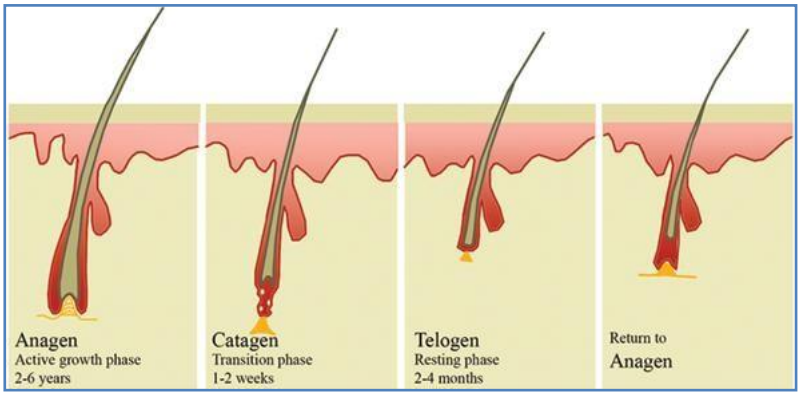


Fig. 5.The Hair Cycle

Dandruff

Dandruff is a very common non-contagious hair problem, nearly affecting person irrespective of age. Medically it is defined as pityriasis simplex capitis – shedding of dead skin from the scalp. It may be – dry or grease. Dry dandruff appears silvery and white while greasy flakes appear pale yellowish and may have an unpleasant smell[16].Historically there have been multiple other descriptive names reflecting the fungal cause of this condition, such as *pityriasis simplex* and *pityriasis capitis* (referring to *Pityrosporum*) and furfuracea (referring to *Malassezia furfur*)[17]. It is a common disorder which effects 5% of the global population. Dandruff affects the aesthetic value and causes the itching and keratinocytes play major role in the expressions and the generation of immunological reaction during dandruff formation. The severity of dandruff may fluctuate with season as a often worsen in winter. Dandruff is common scalp condition that producing the irritating white flakes and itchy scalp. Excessive drying of skin and over-activity of oil gland known as seborrhea[18].

Types of Dandruff

Dandruff can be classified as disorders of the sebaceous gland or skin scaling disorders.

Dandruff can be of two types, They are:

1. Oily dandruff
2. Dry dandruff

Oily dandruff (*Pityriasis Steatoides*)

On the scalp, waxy, greasy, yellowish, thick scales crusts are present. Beneath the crusts, the scalp is red or pale but dry. The hair may be dull and flat without shine. There

may be slightitching. If irritated eczematization complicates the condition to produce seborrhoeic dermatitis. Patients with *Pityriasis steatoides* usually develop thing and later loss of hair.

Dry dandruff (*Pityriasis sicca*)

The scales are fine, thin, furfuraceous, white or grayish and dry or only slightly greasy. The hair is dry and lusterless. There is mild to moderate itching. The scales fall freely on the shoulders. This type of dandruff is more common in winter than in summer. It signifies exaggeration of normal exfoliation of the horny layer of the epidermis. It usually affects people with dry integument and scalp. In nutritional disorders, scaliness of the scalp isexaggerated.[19]

Causes of dandruff

One explanation for dandruff is that the fungus *Pityrosporumovale*, which is naturally present on the scalp and other parts of the skin. Typically, this fungus causes no damage. However, with the weather changes, hormonal, and stress, the scalp will produce more oil, causing the fungus *P. ovale*to proliferate. With the proliferation of the fungus, itchiness of the scalp skin cells and also the loss of hair follicles and so-called dandruff will come. The exact mechanism of dandruff formation is now believed to be the result of the formation of enzymes called lipases. The *Malassezia fungus* break down sebum to oleic acid by using these enzymes (**Fig. 6**). The oleic acid then penetrates the top layer of skin and causes increased skin cell turnover in susceptible people. This, in turn, causes dandruff flakes and sometimes itching and redness.[20]

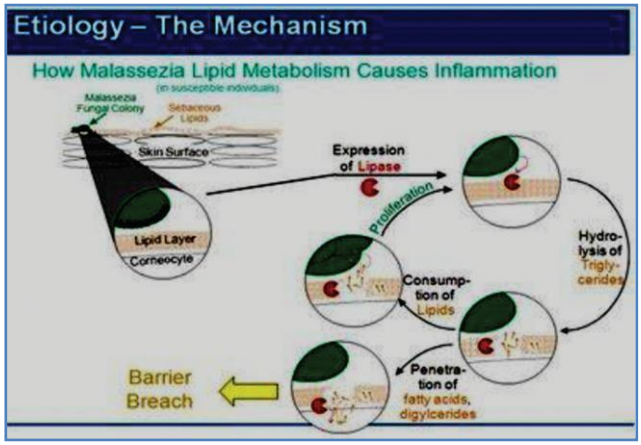


Fig.6.Etiology of dandruff formation[21]

Other reasons of dandruff are excess androgenic hormone, excessive sebaceous secretion. Keratinization of the scalp tissue may be accelerated by physical irritation, such as scratching with the nails, chemical irritation from drugs, photosensitivity, *tinea capitis*, xerotic eczema and vitamin B or zinc deficiency or however the result of poor personal hygiene or use of dirty comb.

Dandruff has been shown to be the result of 3 required factors.

- ✓ Skin oil commonly referred to as sebum or sebaceous secretions.
- ✓ The metabolic byproduct of skin micro-organisms (most specifically *Malassezia* yeast, a lipophillic fungus).
- ✓ Individual susceptibility against presence of *Malassezia* species.

Candida albicans is one of the major causes for dandruff together with the fungus. There may be some bacterial infestation on scalp wound by nail scratching.

Signs and Symptoms of Dandruff

- ✓ Presence of fragments
- ✓ Itching of the scalp
- ✓ Redness around the scalp.
- ✓ Intense itching of the scalp where you will notice dead skin flaking off.
- ✓ Patches of skin scaling and turning red. The area of the skin that appears red are scalp, forehead, hairline, forehead, creases of nose and ears, eyebrows, mid-section of your back**,**
  armpit, groin, breastbone, ear canals, beard areas**,** or eyelids**.**
- ✓ If you have dark skin (the areas affected appear lighter than the rest).[23]

Mechanism of action

Malassezia organism can be found on the skin in 75-90% healthy people. *Malassezia Furfur* is a lipophillic, saprophytic, budding, unipolar, dimorphic, gram positive double walled, oval to round yeast. Colonization by *M. furfur* begins soon after birth, the peak presence of yeast occurs in late adolescence and early adult life. *Pityriasis ovale*is present on 90-100% of surface of healthy skin, *pityrosoprum follicullities* is a most common in those aged 13-45 years. *Malassezia* yeast requires free fatty acid for survival. Usually found in *stratum corneum* and in pilarfolliculi. The yeast hydrolyzes

triglycerides into free fatty acids and creates long chain and medium chain fatty acids from free fatty acids. The result is a cell mediated response and activation of the alternative complement path way, which leads to inflammation. *C. albicans* is also a major cause of dandruff together with bacterial infestation on scalp. More than 7 species of *Malassezia* has been reported i.e. *M. globa, M. sympodialis, M. furfur, M. obtuse, M. sloofiae, M. restricta, M. pachydermatis.*[24]

Herbs as Antidandruff

Dandruff is an overall scalp disorder/disease. The treatment of dandruff includes application of topical, antifungal or other products. Since recurrence occurs commonly prophylaxis using products for skin and hair to maintain good healthy skin of scalp and hairs. Herbs are compatible with both human skin and hair. Unlike chemical based products, herbs are completely safe, extremely effective and have almost no side effects due to their compatibility with human body.

Herbal drugs or their formulations are viable alternative to synthetic drugs. During the past few decades, there has been a dramatic increase in the use of natural products in cosmetics. The awareness and need for cosmetics with herbs is on the rise, primarily because it is believed that these products are safe and free from side-effects.[25]

GELS

The term gel represents a physical state with properties intermediate between those of solid and liquids. It is recommended that the term should be restricted to those systems that satisfy the following criteria, which are similar to suggested byHerman.[26]

1. They are coherent colloidal system of at least two components (the gelling agent and a fluid component).
2. They exhibit mechanical properties characteristic of the solid state.
3. Each component is continuous throughout the system.

The term “gels” is broad, encompassing semisolid of a wide range of characteristics from fairly rigid gelatin slabs, to suspensions of colloidal clays, to certain greases. A gel can be looked upon as being composed of two interpenetrating phase (the gelling agent and a fluid component).

Gels are semisolid, being either suspensions of small inorganic particle or large organic molecule interpenetrated with liquid. In the first case, the inorganic particles, such as bentonite, form a three-dimensional “house of cards” structure throughout the gel. This is a true two-phase system, as the inorganic particles are not soluble but merely dispersed throughout the continuous phase.

Large organic molecules tend to exist in solution as randomly coiled flexible chains. These molecules, either natural or synthetic polymers, tends to entangle with each other because of there random motion. It is interaction between the units of the colloidal phase, inorganic or organic, that sets up the “structural viscosity” immobilizing the liquid continuous phase. Thus gels exhibit characteristics intermediate to those of liquid and solids[27].

Classification

Gels are classified according to the following ways:

I. According to source of gelling agent:

a. Natural gels

b. Synthetic gels

II. According to the liquid medium entrapped:

a. Hydro gels

b. Organo gels

III. According to their cross linkage:

a. Chemical gels

b. Physical gels

IV. According to the chemical nature of gelling agent:

a. Organic gels

b. Inorganic gel

Most natural gums such as acacia, carrageenan, and xanthan gum, are anionic polysaccharide that yields natural gels. Number of cellulose derivatives have been synthesized and used as gallants (e.g. sodium carboxymethylcellulose, hydroxyl

ethyl cellulose, Carbapol, hydroxyl propyl cellulose etc.) that yield synthetic gels. The nature of the solvent also determines whether the gel is a hydro gel (i.e. water based) or an organo gel (i.e. with non-aqueous solvent).

Thus, bentonite magma and gelatin gel are hydro gels and dispersions of metallic stearates in oil are examples of organo gels. A hydro gel 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 material are hydro gels [27]

Organic gel typically contains polymers such as carbomer, 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 xero gels. These are often produced by evaporation of the solvent, leaving the gel framework behind. E.g. gelatin, tragacanth.

Structure of Gels

The rigidity of a gel arises from the presence of a network formed by the interlinking of particles gelling agent. The nature of the particles and the type of force that is responsible for the linkages, which determines the structure of the network and the properties of gel. The individual particles of hydrophilic colloid may consist of either spherical or anisometric aggregates of small molecules, or single macro- molecules. Inlinearmacromoleculesthenetworkiscomprisedofentangledmolecules,the point of contact between which may either be relatively small or consist of several molecules aligned in a crystalline order,

The cross-linking of macromolecules by primary valency bonds provides a further mechanism for the formation of gel network. This behaviour is exhibited by silicic acid gel, which consists of a three- dimensional network of Si-O bonds.

The force of attraction responsible for the linkage between gelling agent particles

may range from strong primary valencies, as in silicic acid gels, to weaker hydrogen bonds and Vander Waals forces. The weaker nature of these latter forces is indicated by the fact that a slight increase in temperature often causes liquefaction of gel.

Systems that exhibit this type of transition, such as agar and gelatin gels, are termed thermal gels. In addition, the transition from gel state to a colloidal dispersion may in some case be brought about by mechanical agitation. Systems, such as bentonite and aluminum hydroxide gels, that exhibit this type of transition are termed thixotropic gels.

Properties of Gels[26]

Gels should posses the following properties:

- ✓ Ideally, the gelling agent for pharmaceutical or cosmetic use should be inert, safe, and should not react with other formulation components.
- ✓ The gelling agent included in the preparation should produce a reasonable solid-like nature during storage that can be easily broken when subjected to shear forces generated by shaking the bottle, squeezing the tube, or during topical application.
- ✓ The gel should exhibit little viscosity change under the temperature variations of normal use and storage.
- ✓ It should posses suitable anti-microbial to prevent from microbial attack.
- ✓ The topical gel should not be tacky.
- ✓ The ophthalmic gel should be sterile.
- ✓ It should be economical.

Characteristics of Gels[26]

A. Swelling:

When a gelling agent is kept in contact with a liquid that solvates it, then an appreciable amount of liquid is taken up by the agent and the volume increases. This process is referred to as swelling. This phenomenon occurs as the solvent penetrates the matrix. Gel- gel interactions are replaced by gel- solvent interactions. The degree of swelling depends on the number of linkages between individual molecules of gelling agent and on the strength of these linkages.

A. Syneresis:

Many gels often contract spontaneously on standing and exude some fluid medium. This effect is known as syneresis. The degree to which syneresis occurs, increases as the concentration of gelling agent decreases. The occurrence of syneresis indicates that the original gel was thermodynamically unstable. The mechanism of contraction has been related to the relaxation of elastic stress developed during the setting of the gels. As these stresses are relieved, the interstitial space available for the solvent is reduced, forcing the liquid out.

B. Ageing:

Colloidal systems usually exhibit slow spontaneous aggregation. This process is referred to as ageing. In gels, ageing results in gradual formation of a denser network of the gelling agent. Theimer suggests that this process is similar to the original gelling process and continues after the initial gelation, since fluid medium is lost from the newly formed gel.

C. Structure

The rigidity of a gel arises from the presence of a network formed by the interlinking of particles of the gelling agents. The nature of the particle and the type of force that is responsible for the linkages determine the structure of the network and the properties of thegel.

D. Rheology

Solutions of the gelling agents and dispersion of flocculated solid are pseudo plastic i.e. exhibiting Non-Newtonian flow behavior, characterized by a decrease in viscosity with increase in shear rate. The tenuous structure of inorganic particles dispersed in water is disrupted by applied shear stress due to breaking down of inter particulate association, exhibiting a greater tendency to flow. Similarly, form acromolecules the applied shear stress aligns the molecules in the direction of stress, straightening them out and lessening the resistance to flow.

Gel forming compounds [28,29]

A number of polymers are used to provide the structural network that is the essence of a gel system. These include,

1. **Natural gums:** Alginates, carragenan, tragacanth, pectin, xanthan, gum, etc.
2. **Carbomers:** Carbopol 934, carbopol 940 and carbopol941.
3. **Cellulose derivatives:** Methyl cellulose, sodium carboxy methyl cellulose, hydroxy ethyl cellulose, hydroxy propyl cellulose and hydroxy propyl methylcellulose.
4. **Polyethylenes:** PEG 200 to PEG8000.
5. **Colloidally dispersed solids**: Microcrystalline silica, montmorrillonite clays, colloidalcellulose.
6. **Surfactants:** Non-ionicsurfactants.
7. **Other gellants:** Bees wax, carnauba wax, cetyl esters wax, PEGs, etc.

METHODS OF PREPARATION OF GELS

1. Temperatureeffect

The solubility of most lypophillic colloids – e.g., Gelatin, agar is reduced on lowering of temperature, so that, cooling a concentrated hot solution will produce a gel. In contrast to this some materials such as the cellulose owe their water solubility to hydrogen bonding with water. Raising the temperature of these solutions disrupts the hydrogen bonding and the reduced solubility will cause gelation.

Flocculation with salts andnon-solvents

Gelation is produced by adding just sufficient precipitant to produce the gel state, but insufficient to bring about complete precipitation. It is necessary to ensure rapid mixing to avoid local high concentrations of precipitant. Solutions of ethyl cellulose, polystyrene, etc., in benzene can be gelled by rapid mixing with suitable amounts of a non-solvent such as petroleum ether. The addition of salts to hydrophobic solutions to bring about coagulation and gelation is rarely observed. However, the addition of suitable proportion of salts to moderately hydrophilic solutions, such as aluminum hydroxide and bentonite produces gels. As a general rule, the addition of about half of the amount of

electrolyte needed for complete precipitation is adequate. The gels formed have frequently thixotropic behavior.

Chemical reaction

In the preparation of gels by precipitation from solution e.g. Aluminum hydroxide gels prepared by interaction in aqueous solution of an aluminum salts and sodium carbonate, an increased concentration of reactants will produce a gel structures. Silica gel is another example and is produced by the interaction of sodium silicate and acids in aqueous solution.

Kinetic of drug release from topical gels [28,29]

It is generally understood that the release of drug from topical gels can be considered as mass transport phenomenon involving diffusion of drug molecule from a region of higher concentration in the dosage form to a region of lower concentration in the surrounding environment. An attempt to model drug release from topical gels has been reported and in the treatment of their data, it was assumed that the drug release was confined to any of the order, such as zero order or first order process. One indication of the mechanism can be obtained using a plot of log of cumulative percentage of drug remaining in the gel base against time.

A first order release would be linear as predicted by the following equation.

$$\text{Log } C = \text{Log} C_0 - \frac{Kt}{1\ 2.303}$$

Where,

C - Amount of drug left in the gel base

C_0 - Initial amount of drug in the gel base

K - First order rate constant time

t - Time

An attempt was also made to know the mechanism of drug release, the data was plotted according to Higuchi‟s equation as cumulative % release v/s. Sq. root of time.

$$Q = k\sqrt{t}$$

Where,

Q - Amount of drug release at time, t

K - Slope.

t - Time.(Hours).

Uses of Gels[27]

In the pharmaceutical and cosmetic industry, gel may be enumerated to have the following uses

1. As delivery systems for orally administered drugs.
2. To deliver topical drug applied directly to the skin, mucus membrane or the Eye.
3. As long acting forms of drug injected intramuscularly.
4. As binders in tablet granulation, protective colloids in suspensions, thickeners in oral liquid, and suppository bases.
5. In cosmetics like shampoos, fragrance products, dentifrices, skin and hair care preparations.
6. The bulk property of swelling is of particular interest for “swelling implants,” which can be implanted in a small dehydrated state via a small incision and which then swell to fill a body cavity and/or to exert a controlled pressure.

2. LITERATURE REVIEW

Literature Survey of *Emblica officinalis*

- **Md. Sahab Uddin *et al.*, (2016)**[30] examined the effect of ethanolic extracts of *Phyllanthus emblica*(EEPE) ripe (EEPEr) and EEPE unripe (EEPEu) fruits on cognitive functions, brain antioxidant enzymes, and acetyl cholinesterase (AChE) activity in Swiss albino male rats for 12 days. Learning and memory enhancing activity of EEPE fruit was examined by using passive avoidance test and rewarded alternation test. Antioxidant potentiality was evaluated by measuring the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GSH-Px), glutathione reductase, reduced glutathione (GSH), glutathione-S-transferase, and the contents of thiobarbituric acid reactive substances (TBARS) in entire brain tissue homogenates. AChE activity was determined using colorimetric method. The study showed that EEPE fruit possesses an excellent source for natural cognitive enhancer which could be developed in the treatment of AD and other neurodegenerative diseases

- **Harpreet Singh Grover *et al.*, (2015)**[31] reviewed the therapeutic effects of Amla in medicine and dentistry showed that it has its beneficial role in cancer, diabetes, liver treatment, heart trouble, ulcer, anaemia, and various other diseases. Similarly, it has the application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive, and gastroprotective. In addition, it is useful in memory enhancing, ophthalmic disorders, and lowering cholesterol level

- **Sankaran mirunalini*et al.*, (2013)**[32] reviewed the phytoconstituents of Amla and reported that it becomes a notable fruit for its rich amount of vitamin C, polyphenols such as tannins, gallic acid, ellagic acid, flavonoids like quercetin and rutin

- **M. Krishnaveni*et al.*, (2011)**[33]reviewed *Phyllanthus emblica*(Amla) possesses a vast ethnomedical history and represented a phytochemical reservoir of heuristic medicinal value. The fruit is rich in quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components including

terpenoids, alkaloids, flavonoids, and tannins have been shown to posses' useful biological activities. Many pharmacological studies have demonstrated the ability of the fruit shows antioxidant, anticarcinogenic, antitumour, antigenotoxic, antiinflammatory activities, supporting its traditional uses. This review focused the interest on phytochemistry, traditional uses, cancer chemopreventive activity of *Phyllanthus emblica*both *in vivo* and *in vitro*

Literature Survey of *Citrus limonum*

- **Junab Ali *et al.*, (2017)**[34]evaluated the antimicrobial activity of methanolic extract from the peel of the fruit of *Citrus Limon* (Family-Rutaceae) in conjugation with phytochemical analysis. The methanolic extract of the peel of *Citrus Limon* contains the high presence of phytochemicals such as alkaloids, saponin, flavonoids, carbohydrates, glycosides and citric acids and tannins. The antimicrobial activity of the methanolic extract of the plant showed significant result against all the test organisms 2 bacterial strains among one is Gram-positive (*Staphylococcus aureus*) and other is Gram-negative (*Escherichia coli*) and 1 fungal strains (Candida *albicans)* using agar well diffusion method when compared with the standards (Fluconazole - 10 µl) which was used as positive control and DMSO (10%) as the negative control

- **Sheila John *et al.*, (2017)**[35]determined the antioxidant and antimicrobial potential of lemon peel as it is a rich source of molasses, pectin, limonene and other secondary metabolites. The antioxidant activity of lemon peel was determined using DPPH assay, FRAP and phosphomolybdenum assay while the antibacterial activity was tested against four bacterial strains. The result indicated that acetone extract of lemon peel exhibited greater potential to scavenge free radicals and reducing power that increased with increase in concentration. Methanol extract of lemon peel showed greater antimicrobial activity thereby indicating the effectiveness of lemon peel as a potent antimicrobial agent

- **Amit Pandey *et al.*, (2011)**[36] found out the antimicrobial activity of ethanolic, methanolic, ethyl acetate & hot water extract of lemon fruit parts like peels & seeds. Antimicrobial analysis was done by using agar well diffusion method against bacterial and fungal pathogens. Methanolic extract of lemon peels exhibited the maximum zone of inhibition against *Pseudomonas aeruginosa*

whereas hot water extract of lemon peels exhibited least zone of inhibition. Ethanolic extract of lemon seeds showed maximum zone of inhibition against *Pseudomonas aeruginosa* whereas hot water extract showed least zone of inhibition. MIC value was determined by using micro broth dilution method. The least concentration was obtained 2.4 mg/ml for ethanolic and hot water extracts of lemon peels against *S. aureus*. The MBC value also determined and phytochemical analysis showed the presence of tannins, glycosides, reducing sugars and flavonoids

- **M. Viuda-Martos*et al.*,(2008)**[37] studied the effect of the essential oils of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata*L.), grapefruit (*Citrus paradisi*L.) and orange (*Citrus sinensis*L.) on the growth of moulds commonly associated with food spoilage: *Aspergillusniger*, *Aspergillus flavus*, *Penicillium chrysogenum*and *Penicillium verrucosum*, using the agar dilution method. All the oils showed antifungal activity against all the moulds. Orange essential oil was the most effective against *Aspergillus niger*, mandarin essential oil was most effective at reducing the growth of *Aspergillus flavus*while grapefruit was the best inhibitor of the moulds *Penicilliumchrysogenum*and *Penicilliumverrucosum.* Citrus essential oils could be considered suitable alternatives to chemical additives for use in the food industry

2. **3. Literature Survey of *Allium sativum***

- **Abdulaziz Bashir Kutawa*et al.*, (2018)**[38] determined the antifungal activity of aqueous and ethanolic garlic extract on some selected fungi namely, Fusarium spp and Rhizopus spp. The diameter of zones of inhibition for the ethanolic extract ranged between 4.1-14.3 mm, while that of aqueous extract ranged between 2.4-10.4 mm. The MIC for the ethanolic extract was 2.5 mg/ml and 5.0 mg/ml for Fusarium spp and Rhizopus spp respectively. While for aqueous extract there was no effect on both tested organisms. It can be concluded that garlic extract showed antifungal activity against the test organism. Moreover, the ethanolic extract showed inhibitory activity among the tested fungi

- **J. P. Burian*et al.*, (2017)**[39] evaluated the antifungal potential of garlic with *S. schenckii* through minimum inhibitory concentration test and colony-forming

units. The results showed that garlic offers antifungal potential with *S. schenckii*. Mice that consumed garlic responded more effectively to fight against the infection

- **Neeta Rai *et al.*,(2013)**[40]formulated herbal antidandruff shampoo containing garlic loaded solid lipid nanoparticles by using garlic as a antifungal agent. The ALL -SLNs were formulated by hot homogenization method and evaluated by using different parameter. The zeta potential, particle size and polydispesity index ,SEM and drug release were performed for ALL-SLNs. Result showed that ALL-SLNs containing Tween 80 (1ml) and soy lecithin (10ml) formulations showed the excellent zeta potential, particle size entrapment efficiency, SEM study revealed irregular surfaces with pores. The in vitro release upto 90% and %EE was found to be 30-50% .Then herbal antidandruff shampoo were formulated by using mixing process and evaluated. Result of antidandruff shampoo containing different concentration of CMC and EDTA were excellent appearance, viscosity, pH, foam ability, spreadability and in vitro release. The pH and viscosity of herbal antidandruff shampoo were found to be 3.7-7.2 and 1010cps -1700 cps. The spreadability was found to be by different method and result was found to be 46.4% and 53.1%. Foamability was found to be 157ml. The in vitro drug release profile was 79.26 to 80.4%. Thus it is more effective for the treatment of dandruff on scalp and hair with no side effect

2. 4. Literature Survey of *Zingiber officinalis*

- **Tuanwei Chen *et al.*, (2018)**[41] evaluated the antifungal activities of ginger oleoresin (GO) against *Pestalotiopsismicrospora (P. microspora)* and illuminated the underlying action mechanisms. The *in vitro* assay indicated that GO exhibited strong antifungal activity against mycelial growth of *P. microspora*, and with 50%-inhibition concentration (EC50) and 90%-inhibition concentration (EC90) at 2.04 µL GO and 8.87 µL GO per mL propylene glycol, respectively, while the minimal inhibitory concentration (MIC) and minimal fungicidal concentration were at 10 µL GO and 30 µL GO per mL propylene glycol, respectively. *In vivo* assay confirmed that GO treatments remarkably suppressed disease development in *P. microspore* inoculated-Chinese olive fruit. These results demonstrated that

GO can be used as a promising antifungal agent to inhibit the growth of pathogenic fungi in Chinese olives

- **Pratibha Rawal *et al.*, (2016)[42]** determined the antimicrobial activity of dried ginger powder, using paper disc diffusion assay, by using chloroform, ethanol, acetone and petroleum ether solvents, against *Fusarium oxysporumf. sp. lycopersici*. The study showed the potent antimicrobial activity of the ginger extract against the pathogen by all tested solvents. Chloroform extract of ginger at its 750 mg/ml concentration showed highest zone of inhibition as 25.75 mm against tested pathogen

- **Supreetha.S*et al.*,(2011)[43]**assessed the effect of ethanolic extract of ginger on *candida albicans in vitro*. The antifungal activity of the agent was tested in the following dilution range - 1g, 2g, 4g of shuntichoorna (ginger powder) in 99.9% ethanol. Ginger paste at room temperature showed inhibition zone better than ethanol alone, but cold ethanolic ginger extract showed the maximum inhibition zone at 24 hrs. The study showed that the ethanolic extract of ginger powder has pronounced inhibitory activities against *Candida albicans*. It can be concluded that although ethanol in itself has antifungal activity, ethanolic extract of ginger has a synergistic activity

2. 5. Literature Survey of Antidandruff formulation

- **Revansiddappa M, *et al.*,(2018)[44]**prepared herbal shampoo with Ritha fruits, Liquorice stolons, Bengal gram seeds, Brahmi leaves, Greengram seeds were collected from Ayurvedic store and remaining like Banana roots, Pomegranate seeds, Hibiscus leaves, Marigold flowers, and Lemon fruits with all ingredient extract and formulated different formulations and stability tested with marketed Dove shampoo. Formulation four was found to be the best formulation based on the evaluation parameters and stability studies. When investigation data were assessed, formulation four of anti-dandruff herbal shampoo contains all good characters of an ideal shampoo and it was found to be harmless, more effective and economical compared to synthetic Dove anti dandruff shampoo, it was quite evident that development of stable, effective anti-dandruff herbal shampoo which may be commercially replacing the existing synthetic shampoo is quite possible

- **M. Narshana*et al.*,(2017)**[45] reported *Malassezia furfur* is the main cause of dandruff and investigated other causes of dandruff which include microbial and non - microbial factors and also highlighted the various treatment options and newer formulations. It concluded that Novel delivery systems have been successfully used for pharmaceutical formulations and they can prove to be a promising delivery system for scalp treatment too

- **Elakkiya T *et al.*, (2014)**[46] studied anti-dandruff activity of the ethanolic and chloroform extracts (100, 200, 300, 400, 500µl) of fruit peel such as Lemon and apple peel. It concluded that compared to chloroform extract the ethanolic extractexhibit better antifungal activity. This fruit may be applicable instead ofsynthetic antidandruff shampoo

3. AIM AND OBJECTIVE

Aim

To formulate and evaluate Herbal Antidandruff Gel and to determine the effect of Herbal Adjuvants on the enhancement of Antidandruff activity.

Objectives

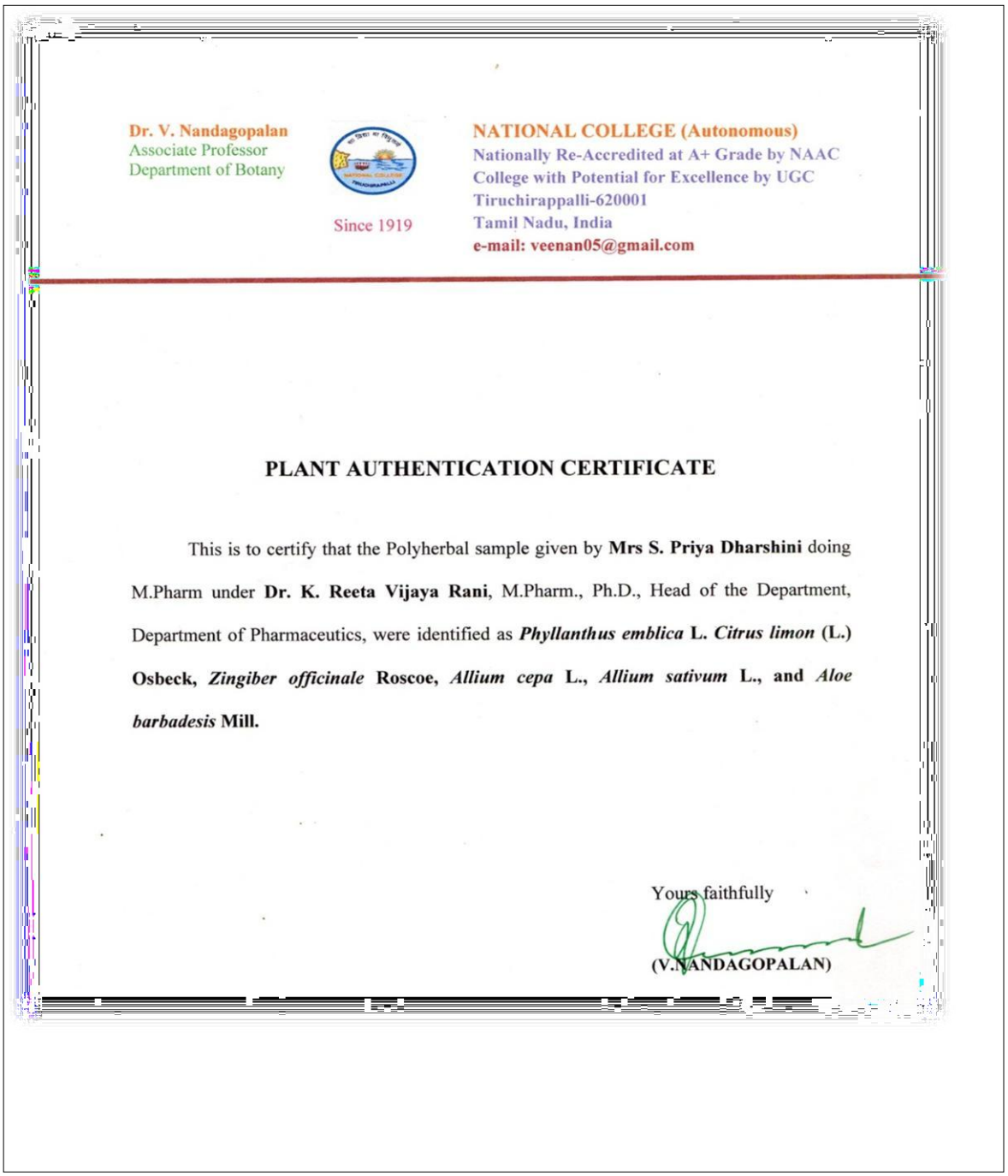
The significance of Pharmaceutical Research and Development is on the creation of therapeutic, prophylactic and diagnostic substances with specific functions and minimum side effects in particular of being tools for modern medicine satisfying these conditions. The objectives of the present investigations are :

- To explore the ethnopharmacology of the plant formulation for the selected herbals
- To assess the effect of adjuvants on enhancement of antidandruff activity of *Emblica officinalis* L, *Citrus Limon*L
- To strengthen and promote the rational use of erbal formulations
- To formulate herbal anti-dandruff gel which is effective in terms of safety & efficacy and treating the dandruff condition better than the chemical based anti-dandruff gel (Producing side effects such as allergy, hair fall, drug resistant, etc.,)

4. PLAN OF THE WORK

- Literature Survey
- Selection of Herbs
- Collection and Authentication of selected Herbs
- Extraction
- Phytochemical screening
- Identification of selected Herbs
    - HPTLC
    - UV
    - FTIR
- Trial formulations of Herbal Antidandruff Gel
- Physicochemical evaluations
- Selection of Best formulations
- *In vitro* diffusion studies&Release Kinetics
- Screening of Antimicrobial activity
- Skin irritation
- *Ex vivo* studiesstudy
- Stability studies of Herbal Antidandruff Gel (as per ICH guidelines)
- Results and Discussion
- Summary and Conclusion

PLANT AUTHENTICATION CERTIFICATE



Dr. V. Nandagopalan
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PLANT AUTHENTICATION CERTIFICATE

This is to certify that the Polyherbal sample given by **Mrs S. Priya Dharshini** doing M.Pharm under **Dr. K. Reeta Vijaya Rani**, M.Pharm., Ph.D., Head of the Department, Department of Pharmaceutics, were identified as ***Phyllanthus emblica* L. *Citrus limon* (L.) Osbeck, *Zingiber officinale* Roscoe, *Allium cepa* L., *Allium sativum* L., and *Aloe barbadesis* Mill.**

Yours faithfully

(V.NANDAGOPALAN)

Fig. 7. Authentication of Plants

5. PLANT DESCRIPTION[47,48,49]

***Emblica officinalis*Gaertn (Amla)**

Fig. 8. Amla Fruit

Taxonomical Classification

Kingdom - Plantae

Division - Angiospermae

Class - Dicotyledonae

Order - Geraniales

Family - Euphorbiaceae

Genus - Emblica

Species - officinalis. Gaertn

Vernacular Names

Tamil - Toppi, Nellikai

English - Emblic myrobalan, Indian gooseberry

Hindi - Amla, Aoula

Kannada - Nellikai

Malayalam - Nellikai

Sanskrit - Dhatri-phala, Amraphalam, Amalakai,Sriphalam

Telugu - Nelli

Bengali - Amalakai

Description

- A deciduous tree with small leaves which are very closely set in pinnate fashion
- Flowers are pale green with small dense cluster. Male and female flowers borne on the same tree
- Fruits are fleshy, roundish, rather indistinctly marked with six lobes, pale green or yellowish in colour, 1.5 to 2.5 cm in diameter

Parts used

Dried fruit, the nut or seed, leaves, root, bark and flowers. Ripe fruits used generally fresh, dry also used.

Chemical Constituents

Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin Phyllantine, Phyllembein, Phyllantidine, Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine, Pectin, Ascorbic acid Quercetin, Kaempferol.

Uses

- Immuno stimulant
- The fresh or dried fruit is used as drug for many infections
- The fruit poultice is used to stop bleeding from cuts
- The fruit powder (about 30g) is used as a coolant and laxative
- Fruits are good liver tonic
- The fruit juice is useful in indigestion, jaundice, anaemia, heart complaints
- Dried fruits are useful in diarrhoea and dysentery

- Seeds are administered against asthma, stomach disorder and bronchitis
- It is one of the ingredients of triphala and chyavanprash

***Citrus limonum*. Risso (Lemon)**

Fig. 9. Lemon Fruit

Taxonomical

Classification Kingdom - Plantae Division - Magnoliophyta Class - Magnoliopsida

Order - Sapindales

Family - Rutaceae

Genus - Citrus

Species - limonum. Risso

Vernacular Names

Tamil - Elimichcham

English - Lemon

Hindi - Jambira, Pahadi- nimbu, Paharikaghju

Kannada	-	Dodda nimbi hannu
Malayalam	-	Cerunarakam
Sanskrit	-	Limpaka, Mahajambiram, Nimbaka
Telugu	-	Peddanimba
Bengali	-	Karna-nelu, Gora- nelu, Pahari- nimbu

Description

- It is an evergreen shrub upto 5m tall with fragrant flowers. Fruits are loose skinned, pale yellow in colour, oblong with terminal nipple, very sour in taste
- Flowers are arranged singly or in short, sparsely flowered racemes, hermaphrodite or functionally male
- The petals are suffused with purple on the outer surface. There are 25 to 40 stamens in coherent groups
- The fruit is yellow when ripped and grows to 6.5 to 12.5cm. It is 8 to 10 locular, oblong or ovoid with a broad, low mamilli from projection at the apex
- The rind is somewhat rough to almost smooth
- The pulp is acidic
- Citrus limonum is a small tree, growing only 3 to 6 m tall with twigs that are angular when young and soon become rounded and glabrous with stout axillary spines
- The leaves are pale green, broadly elliptical acute and serrate or crenate

Parts used

The medicinal parts are the juice, peel and oil of the fruit.

Chemical Constituents:

Citric acid, Sugars, Citral, Citronellal, Limonene, α – terpineol, Geranyl acetate, Linalyl, Flavone glycoside, potash, gum, Pinene, Geraniol

Uses:

- Rind is principally employed as a flavouring agent

- NimbaTailam applied is of special use in leprotic ulcers
- Lemon oil mixed with glycerine is applied to the eruption of acne, to the pruritus of the vulva and scrotum to sunburns etc
- Lemon oils applied to check post partum haemorrhage and is highly prized in medicine as a flavouring agent
- In rheumatic affections such as plerodynia, sciatica, lumbago, pain in the hip-joints etc.. Lemon juice and gun powder is applied topically for scabies
- Fresh lemon juice is recommended to be taken in the evening for the relief of dyspepsia with vomiting and bilious headaches
- Juice of the leaves or the water resulting from the boiling together of the stems and leaves is given with ghee for three consecutive days colic and constipation.

***Allium sativum* Linn (Garlic)**

Fig. 10. Garlic Cloves

Taxonomical

Classification Kingdom -

Plantae Division -

Magnoliophyta Class -

Liliopsida

Order - Liliales

Family - Liliaceae

Genus - Allium

Species - sativum Linn

\

Vernacular Names

Tamil - Vellapundu, Ullipoondu, Vellaipundu

English - Garlic

Hindi - Lasan

Kannada - Belluli

Malayalam - Velluli

Sanskrit - Lasuna, Ugragandha, Bhutagan, Mahusdha,

Telugu - Velluli, Tellagadda

Bengali - Rasun

Description

- The plant consists of a clusters of long flowers where the floral axis terminates in a single flower and contains few florets (small flowers or buds). There are numerous 1cm deciduous bulbs capable of producing new plants which shed simultaneously
- The flowers usually remain in bud form and often do not produce any seed
- The petals are reddish or greenish- white and longer than the stamens
- The anther of the middle stamens are spread at the base and have fan- shaped tips
- The garlic bulb is usually a compound bulb and the secondary bulb are oval in shapeThe bulb skin colour is either silky white or green

Parts used

Whole fresh ulb, the dried bulb and the oil of garlic.

Chemical Constituents

Ajoene, Allicin, Alliin, Allixin, γ- Glutamyl- S- 2- Propenyl cysteine, Diallyl disulfide, Methyl allyl disulfide, S- allyl- cysteine, 1,2 - Vinyldiithin

Uses

- Hot stimulant, carminative, emmenagogueand antirheumatic
- Externally the bulb is used as resolvent
- Oil is a powerful antiseptic
- Internally garlic is beneficial in several forms of atomic dyspepsia
- It is a flavouring agent
- In medicine it is used on a decotion in the form of tincture, juice,syrup or poultice
- It is boiled in milk and administered as an anthelmintic and expectorant
- Importance of garlic for the treatment of hypertension, diabetes, helminthiasis, chronic colitis and gastritis, angina pectoris, bacterial and fungal infections, amoebiasis, arteriosclerosis, rheumatoid arthritis and cancer were reported
- Garlic is a stimulant and cures digestive disorder, lungs and respiratory tract diseases
- A paste made from it can relieve pain cause by the sting of scorpion

***Zingiberofficinale.*Roscoe (Ginger)**

Fig. 11. Ginger Rhizome

Taxonomical

Classification Kingdom -

Plantae Division -

Magnoliophyta Class -

Liliopsida

Order - Zingiberales

Family - Zingiberaceae

Genus - Zingiber

Species - officinale Roscoe

Vernacular Names

Tamil - Shukhu, Chukku(dried) ,Inji (fresh)

English - Ginger

Hindi - Adrak

Kannada - Vona (dried), Shunti, Hashi- Shunti

Malayalam - Chukka

Sanskrit - Srangavera, Sringa- beram (dried) – suntan, Nagara ,

Nagaram, Visoushada, Maha-oushdam, Mahaushada

(fresh) – Ardhrakam, varam, katu- patram, Mihijam

Telugu - Sonti (dried), Allam (fresh)

Bengali - Sonti (dried), Adrak, Ada, Adi

Description

- The flower scape grows directly from the root and terminates in a long, curved spike
- A white or yellow flower grows from each spike

- Ginger is a creeping perennial on a thick tuberous rhizome which spreads underground
- In the first year a green,erect,reed-like stem about 60cm high grows from this rhizome
- The plant has narrow lanceolate to linear- lanceolate leaves 15 to 30 cm long which die off each year
- The fracture is short and fibrous
- The odour and taste are characteristic, aromatic and pungent

Parts used

Scraped and dried rhizome as well as the green ones. Root also a medicinal part.

Chemical Constituents

Zingiberene, β- bisabolene, α- farnesene, β- sesquiphellandrene, α- curcumene, Gingerol, Paradols, Shogaol, Gingerdiols, Diarylheptanoids, Carbohydrates, Lipids, Nicotinic acid

Uses:

- Aromatic, carminative, stimulant to gastro intestinal tract and stomachic also sialogogue and digestive. Externally a local stimulant and rubefacient
- Ginger is extremely valuable in dyspepsia, flatulence, colic, vomiting, spams and other painful affections of the stomach and the bowels unattended by fever, for cold,cough, asthma and indigestion
- Ginger has antiemetic effect, anti- inflammatory effect.

***Aloe barbadensis*Mill (Aloe vera)**

Fig.12. Aloe vera Leaves

Taxonomical Classification

Kingdom - Plantae

Division - Magnoliophyta

Class - Liliopsida

Order - Liliales

Family - Aloaceae

Genus - Aloe

Species - barbadensis Mill

Vernacular Names

Tamil - Kattalai, Kumari

English - Indian alces

Hindi - Ghikanvar

Kannada - Kathaligida

Malayalam - Kattavala

Sanskrit - Ghrita Kumari

Telugu - Ghrit- kumara, Musabbar

Description

- The inflorescence is forked once or twice and is 60 to 90 cm high. The raceme is dense,cylindrical and narrow towards the top. The terminal raceme is upto 40 cm high while the lower ones are somewhat shorter. The bracts are almost white and the flowers are yellow, orange or red and are 3cm long
- The lily- like succulent- leafed rosette shrub either does not have a stem or has a 25 cm stem. The lanceolate leaf is thick and fleshy, 40 to 50 cm long and 6 to 7 cm wide at the base. The upper surface is concave, gray-green often with a reddish tinge which sometimes appears in patches in the young plants
- The leaf margin has a pale pink edge and 2mm long pale teeth

Parts used

Expressed and dried juice of leaves and pulp

Chemical Constituents

Barbaloin, Isobarbaloin, Anthranol, Anthracenes, galactogalacturan, arabinogalactan, Xylon, Cellulose, aloe emodin, Emodin, Chrysophanic acid, Ethereal oil, Resistannol, Aloetic acid, Cholesterol

Uses

- Aloes are used as laxative, Antibacterial agent, Antiviral agents, Anti neoplastic agent
- Topically it has analgesic and anti- inflammatory effects
- Aloe is a favourite remedy for intestinal worms in children
- Used in general debility, cough, dyspnoea, asthma, consumptiom, piles, epilepsy, colic and tympanitis

5. EXCIPIENT PROFILE[50]:

Carbopol

Carbopol, a synthetic high molecular weight, non-linear polymer of acrylic acid cross-linked with polyalkenyl polyether with average molecular weight 3x106 Daltons. It contains not less than 56% and not more than 68% of carboxylic acid (-COOH) groups.

- **Synonym**

  Acritamer, acrylic acid polymer carboxy vinylpolymer.

- **Nonproprietarynames**

BP:Carbomer

USP:Carbomer

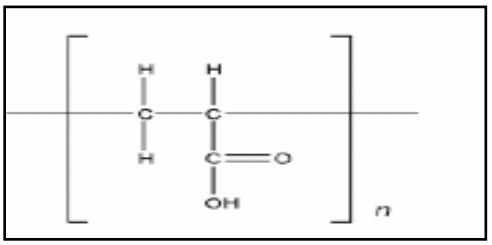
- **Chemical name**

  Carboxyl polymethylene, carbomer 910, 934, 934P, 940, 941, 971P and974P

- **Empirical formula**

  ($C_3H_4O_2)_x$ (-C_3H_5-sucrose)$_y$

- **Structure**



- **Category**

Bioadhesive, emulsifying, suspending & viscosityenhancingagent,tablet binder and release-modifyingagent

- **Description**

White, fluffy, acidic, hygroscopic powder with a slight Characteristic odour

- **Solubility**

After neutralization with alkali hydroxides or amines, solublein water, in ethanol (96%) and inglycerol

- **pH**

  2.5-3.0 (1% aqueoussolution)

- **Glass transition temp**

  100–105°C

- **Melting point**

  Decomposition occurs within 30 minutes at 260°C

- **Specific gravity**

1.41

- **Viscosity**

Carbomers disappears in water to form acidic colloidal solutions of low viscosity which when neutralized produce highly viscous gels. 29,400 to 39,400 cps at 25^{o}C (0.5% neutralized aqueous solution)

- **Stability and storage**

Carbomers are stable, though hygroscopoic materials and can be heated at temperatures below 104^{0} for up to 2 hours without affecting their thickening efficiency

- **Applications**

It is used as thickening, emulsifying and gelling agent. It is used as a tablet binder and matrix forming agent in sustained-release formulations affording zero- to near-zero-order release. It is used as the bioadhesive component in mucoadhesive ointments, gels and tablets

- **Safety**

Carbomers are regarded as non toxic and non-irritant

Triethanolamine

- **Synonyms**

Trihydroxytriethylamine; Tris(hydroxyethyl)amine

- **Nonproprietary Names**

BP : Triethanolamine

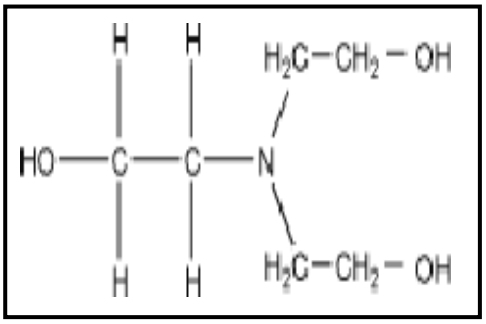
PhEur : Trolaminum

USPNF: Trolamine

- **Chemical Names**

2,2',2.-Nitrilotriethanol

- **StructuralFormula**



- **Empirical Formula**

$C_6H_{15}NO_3$

- **Molecular Weight**

  149.19

- **Functional Category**

  Alkalizing agent; emulsifying agent

- **Description**

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

- **Typical Properties**

Acidity/alkalinity	:	pH = 10.5 (0.1 Nsolution)
Boiling point	:	335°C
Flash point	:	208°C
Freezing point	:	21.6°C
Hygroscopicity	:	veryhygroscopic.
Melting point	:	20–21°C

- **Solubility**

Triethanolamine is miscible in acetone, carbon tetrachloride, methanol and water

- **Viscosity**

590 mPa (590 cP) at 30°C

- **Stability and Storage Conditions**

Triethanolamine may turn brown on exposure to air and light. Triethanolamine should be stored in an airtight container protected from light, in a cool, dry place

- **Incompatibilities**

Triethanolamine is capable of undergoing reactions typical of tertiary amines and alcohols. Triethanolamine will react with mineral acids to form crystalline salts and esters

- **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

- **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 preparations

Glycerin

- **Synonyms**

  Croderol: E422; glycerine; Glycon G- 100; Kemstrene; OptimPricerine; ,2,3-propanetriol; trihydroxypropane glycerol

- **Nonproprietary Names**

  BP: Glycerol

  PhEur: Glycerolum

- **Chemical Name**

  Propane 1,2,3- triol

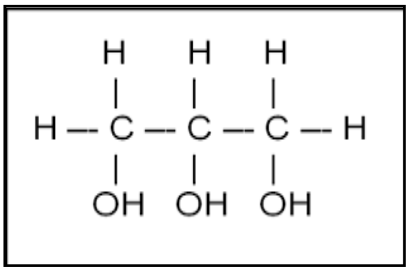
- **Empirical Formula**

  $C_3H_8O_3$

- **Molecular Weight**

  92.09

- **StructuralFormula**



- **Description**

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

- **Functional Category**

  Antimicrobial preservative, emollient, humectant, plasticizer, solvent, sweetening agent and tonicity agent

- **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

- **Application**

- Glycerin is used in a wide variety of pharmaceutical formulations including oral,ophthalmic, topical and parenteral preparationsIt is used as Viscosity enhancer and cosolvent
- 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 used in topical formulations such as creams and emulsions
- **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

Polyethylene Glycol

- **Synonyms**

PEG, polyoxyethylene glycol.

- **Nonproprietary Names**

BP	:	Macrogols
JP	:	Macrogol 400

Macrogol 1500

Macrogol 4000

Macrogol 6000

Macrogol 20000

PhEur : Macrogola

USP- NF : Polyethylene Glycol

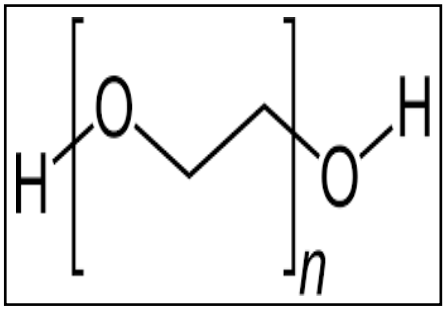
- **Chemical Name**

α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)

- **Empirical Formula**

$HOCH_2(CH_2OCH_2)_mCH_2OH$ where *m* represents the average number of oxyethylene groups. Alternatively, the general formula $H(OCH_2CH_2)_nOH$ may be used to represent polyethylene glycol, where *n* is a number *m* in the previous formula + 1.

- **Structural Formula:**



- **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

- **Functional Category**

Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant.

- **Typical Properties**

Flash Point	:	390 °F
Refractive index	:	1.4630
Density	:	1.1254
Freezing range	:	4-8°C

- **Toxicity And Safety**

Polyethylene glycols are widely used in a variety of pharmaceutical formulations. Generally, they are regarded as nontoxic and nonirritant materials. Adverse reactions to polyethylene glycols have been reported, the greatest toxicity being with glycols of low molecular weight. However, the toxicity of glycols is relatively low

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

- **Application**

  - ✓ 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 300and PEG 400 has 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

Propyl Paraben

- **Synonyms**

Aseptoform P; CoSept P; E216; 4-hydroxybenzoic acid E216; 4-hydroxybenzoic acid propyl ester; Nipasol M; propagin; propyl p-hydroxybenzoate; Propyl parasept; Solbrol P; Uniphen P

- **Nonproprietary Names**

BP : Propyl hydroxybenzoate
JP : Propyl parahydroxybenzoate
PhEur : Propylisparahydroxybenzoas
USPNF: Propylparaben

- **Chemical Name**

Propyl 4-hydroxybenzoate

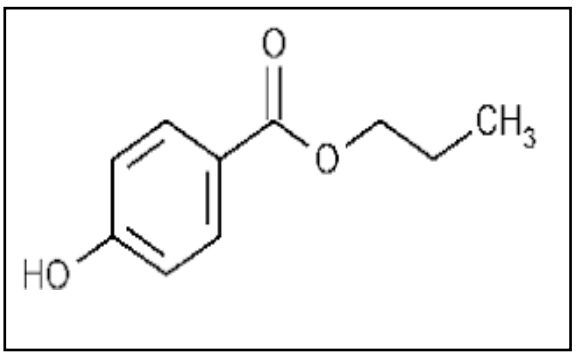
- **Empirical Formula**

$C_{10}H_{12}O_3$

- **Molecular Weight**

180.20

- **Structural Formula:**



- **Description**

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

- **Functional Category**

  Antimicrobial preservative.

- **Typical Properties**

Melting Point	:	98 °C
Boiling point	:	295°C
Density(true)	:	1.288 g/cm3
Dissociation constant pKa	:	8.4 at 22°C

- **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

- **Application:**

  - ✓ Propyl paraben is widely used as an antimicrobial preservative in cosmetics, food products, and oral and topical 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

6. MATERIALS AND METHODS

List of Chemicals

Table 1.List of Chemicals

S. No.	Chemical Name	Company Name
1	Carbopol 934	LOBA CHEMIE, Mumbai
2	Carbopol 940	LOBA CHEMIE, Mumbai
3	Triethanolamine	LOBA CHEMIE, Mumbai
4	Glycerine	Merk Limited, Mumbai
5	Polyethylene Glycol	Kemphasol, Mumbai
6	Propyl Paraben	National Chemicals, Maharastra
7	Aloe vera gel	Herbs and Crops Overseas, Gujarat

List of Equipments Table 2.

List of Equipments

S. No.	Name of Equipment	Name of Manufacturer	Purpose
1	Electronic balance	Precision Balance	Weighing purpose
2	Digital pH meter	Elico L 1120	Surface pH study
3	Magnetic Stirrer	ROTEK	Diffusion Studies
4	UV Spectrophotometer	Shimadzu 1700	Finding Absorption maxima
5	Environment test chamber	Heco	Stability studies
6	Brookfieldviscometer	Brookfield	Determine the viscosity of the gel
8	HPTLC	Camag	Identify and Quantify the active components

Collection of selected Herbs

Emblica officinalis. Gaertn, *Citrus limonum.*Risso, *Allium sativum.*Linn,and *Zingiber officinale.* Roscoewere collected from in and around Tiruchirappalli district, Tamilnadu. Collected herbs were authenticated by Botanist, Dept. of Botany, National College, Trichy.

Extraction of selected Herbs [51]

Preparation of aqueous extract of selected Herbs

Collected and selected parts of herbs such as*Emblica officinalis.* Gaertn, *Citrus limonum.*Risso, *Allium sativum.*Linn, and *Zingiber officinale.* Roscoe were washed with distilled water and grinded individually by simple grinding. Then the extract was filtered, centrifuged and used for further studies.

Phytochemical studies[52]

The aqueous extracts of *Emblica officinalis, Citrus limonum, Allium sativum,Zingiber officinalis* and *Aloe barbadensis* were subjected to the following preliminary phytochemical analysis

Table 3.Phytochemical Tests

S.No.	EXPERIMENT	OBSERVATION	INFERENCE
Test for Alkaloids			
1.	**Dragendroff's test:** The extract was treated with Dragendroff's reagent (potassium bismuth iodide solution)	Orange brown precipitate was formed	Presence of alkaloids
2.	**Mayer's reagents:** The extract was treated with Mayer's (potassium mercuric iodide solution) reagent	Precipitate formed	Presence of alkaloids
3.	**Wagner's reagent:** The extract was treated with wagner's reagent (iodide and potassium triiodide solution)	Reddish brown precipitate was formed	Presence of alkaloids

Test for Glycosides			
1.	**Brontragers test:** To the extract add dilute sulphuric acid and filtered. Filtrate was extract with little chloroform layer was separated out and add equal volume of dilute NH_3.	Red colour observed in ammoniacal layer	Presence of glycosides
Test for Saponin glycosides			
1.	**Foam test:** Shake the extract with water.	Foam was produced/formed	Presence of saponin glycosides.
Test for Tannins and Phenolic compounds			
1.	**Ferric chloride test:** To the aqueous extract few drops of ferric chloride solution were added	Dark black colour formed	Presence of tannins and phenolic compounds.
2.	**Bromine water test:** To the aqueous extract is treated with bromine water	Discoloration of bromine water	Presence of tannins and phenolic compounds
3.	**$KMnO_4$ test:** To the aqueous extract is treated with dilute $KMnO_4$	Discoloration of solution	Presence of tannins and phenolic compounds
Test for Reducing sugar			
1.	**Benedict's test:** 0.5ml of extract solution 1ml of water 5 to 8 drops of Fehlings solution was added.	No brick red precipitate	Absence of reducing sugars
Test for Amino acids			
1.	**Ninhydrin test:** The aqueous extract is heated with 5% ninhydrin solution on boiling water bath for 10 min.	No purple colour formed	Presence of amino acids

2.	The aqueous extract is treated with solution sodium hydroxide and lead acetate solution and boiled	No black precipitate is formed	Presence of amino acids
Test for Flavonoids			
1.	**Shinoda test:** To the extract add potassium hydroxide solution and then 10% ammonia.	Yellow colour precipitate formed	Presence of flavonoids
2.	To the extract, add few drops of Lead acetate solution	Yellow colour precipitate formed	Presence of flavonoids
Test for Terpenoids			
1.	1.4 g of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform and added concentrated solution of sulphuric acid	No red violet olour was obtained	Absence of terpenoids
Test for Steroids			
1.	**Libermann- Buchard Test:** To extract add chloroform solution a few drops of acetic anhydride and 1ml of con. H_2SO_4 were added through the side of the test tube and set aside for a while.	Brown ring was formed at the junction	Presence of steroids
2.	**Salkowski Yest:** To the extract add chloroform solution few drops of H_2SO_4 was added shaken and allowed to stand	Greenish fluorescence is formed	Presence of Steroids
3.	**Libermann's reaction :** Mix 3ml of extract with 3ml of acetic anhydride, heat and cool. Add few drops of Con. H_2SO_4	Blue colour was formed	Presence of Steroids

Preformulation Studies[53]

Preformulation testing is the first step in the rational development of dosage forms of drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The goal of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be man produced.

The following preformulation studies are carried out

- HPTLC
- Finding the absorption maxima
- Standard curve
- Drug-Excipients compatibility studies (FTIR)

HPTLC Analysis

High performance thin layer chromatography (HPTLC) is an important implement that can be used qualitatively as well as quantitatively for checking the purity and identification of crude drugs and also for quality control of finished products. However, recent reviews show that the thin layer chromatography (TLC) and HPTLC techniques can be used to solve many qualitative and quantitative analytical problems in a wide range of fields including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology, and environmental analysis.[54,55]

CAMAG HPTLC systemequipped with Linomat 5 applicator, TLC scanner3, reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS- 4 software were used. All the solvents used for HPTLC analysis were obtained from MERCK. A total of 100mg extract was dissolved in 5ml of ethanol and used for HPTLC analysis as test solution.

i) **For Aqueous extract of *Emblica officinalis***

Test solution	:Aqueous extract of *Emblica officinalis*
Stationary Phase	:Merck, TLC Plates Silica gel 60 F 254
Mobile Phase	:Toluene: Ethylene acetate: Formic acid (2:4:5:2 v/vv/v)

Tank :TTC 10 x 10

Saturation Time :20 min

ii)* For Aqueous extract of *Citrus limonum

Test solution :Aqueous extract of *Citrus limonum*

Stationary Phase :Merck, TLC Plates Silica gel 60 F 254

Mobile Phase :Ethylene acetate: Methanol: Water (7.5: 1: 5: 1) v/v/v

Tank :TTC 10 x 10

Saturation Time :20 min

Procedure

The samples (5µl, 10µl, 15µl,20µl) were spotted in the bands of width 8mm with a Camag microlitre syringe on pre- coated silica gel glass plate 60 F- 254. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapour) with respective mobile phase and the plate was developed up to 83 mm in the respective mobile phase.

Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber saturated with the mobile phase and the chromatoplate development with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The developed plate was dried by hot air to evaporate solvents from the plate.

The plate was photo- documented at UV 254nm and white light using photo documentation chamber. Finally, the plate in photo- documentation chamber and captured the images under white light, UV light at 254 nm. Densitometric Scanning was performed on Camag TLC scanner III and operated by Server Win Software (Version 2.5.18053.1).

Finding the absorption maxima (λ_{max})[56]

The absorption maxima were found for identification of active constituents. Ultra violet Visible spectrophotometry has been used to obtain specific information on the chromophoric part of the molecules. Organic molecules in solutions when exposed to light in the Visible/ ultraviolet region of the spectrum absorb light of particular wavelength depending on the type of electronic transition associated with the absorption.

Accurately weighed amount of extract (100mg) was dissolved and then made up to 100ml with distilled water. Each ml of the stock solution contains 1 mg of extract containing active constituents (primary stock solution). From this primary stock solution, 10 ml was withdrawn and made up to 100 ml with water (secondary stock solution) was taken in standard cuvette and scanned in the range of 200-600nm in a UV spectrophotometer. It exhibits maxima at 273nm and 340. Therefore, further all measurements were taken at 273nm and 340. The result is shown in **Fig. 31 and 33**

Preparation of Standard curve of alcoholic extract of *Emblic officinalis* G. and *Citrus limonum* R. in Phosphate buffer

i) Preparation of phosphate buffer pH 7.4

Phosphate buffer pH 7.4 was prepared as per the method described in I.P.1996 using disodium hydrogen phosphate and sodium hydroxide. The pH was adjusted to 7.4 prior to quantitavie estimation.

ii) Preparation of standard curve of *Emblic officinalis* G. and *Citrus limonum* R. in Phosphate buffer.

Accurately weighed amount of extract (100mg) was dissolved in small quantity of phosphate buffer (pH 7.4) and then made up to 100ml with same buffer. Each ml of the stock solution contains 1 mg/ml of active constituents (primary stock solution).

From this primary stock solution, 10ml was withdrawn and made up to 100ml with buffer (secondary stock solution) individually. From secondary stock solution different working standard solutions i.e., 5,10,15,20,25 and 30 µg/ml were prepared by pipetting 0.5,1,1.5,2,2.5 and 3ml and made up to 10ml with buffer and the absorbance were measured at 273 and 340nm using buffer by UV spectroscopic method. A graph is plotted by using concentration (µg/ml) as X-axis and absorbance at 273 and 340 nm as Y-axis. The results are shown in the **Table 9 and 10**and **Fig. 32 and 35**

Drug- Excipient compatibility studies by FTIR Analysis[57]

Infrared spectrum of any compound or extract gives information about the groups present in that particular compound. The IR absorption spectra of the pure drug and physical admixtures of active constituentswith various excipients in 1:1 ratio were

taken in the range of 4000-400cm^{-1} using Shimadzu and observed for characteristic peaks of drug.

Active constituents-excipient compatability was carried out by FTIR analysis. The obtained spectra of extract and physical admixtures were compared and observed for major peaks are shown in **Table 11 to 20** and **Fig. No.35 to 44**

Formulation of Herbal Antidandruff Gel[58]

Table 4. Formulation of Herbal Antidandruff Gel

S.No.	Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
1	*Emblica officinalis*	0.5ml	-	-	0.5ml	0.5ml	-	-	0.5ml
2	*Citrus limonum*	-	0.5ml	-	0.5ml	-	0.5ml	-	0.5ml
3	*Allium sativum*	-	-	0.5ml	0.5ml	-	-	0.5ml	0.5ml
4	*Zingiber officinalis*	-	-	0.5ml	0.5ml	-	-	0.5ml	0.5ml
5	*Aloe barbadensis*	-	-	0.5g	0.5g	-	-	0.5g	0.5g
6	Carbopol 940	0.30g	0.30g	0.30g	0.30g	-	-	-	-
7	Carbopol 934	-	-	-	-	0.30g	0.30g	0.30g	0.30g
8	Polyethylene Glycol	7g	7g	7g	7g	7g	7g	7g	7g
9	Triethanolamine	0.6g	0.6g	0.6g	0.6g	0.6g	0.6	0.6g	0.6g
10	Propyl Paraben	0.075g	0.075g	0.075g	0.075g	0.075g	0.075g	0.075g	0.075g
11	Glycerine	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
12	Water q.s	50ml	50ml	50ml	50ml	50ml	50ml	50ml	50ml

Procedure

- ✓ Measured quantity of propyl paraben, glycerine and weighed quantity of Polyethylene Glycol were dissolved in about 35 ml of water in beaker
- ✓ Then it was stirred at 100rpm using mechanical stirrer
- ✓ Carbopol 940 and 934 were added slowly to the respective beaker containing above liquid while stirring
- ✓ Triethanolamine (Neutralizing agent) was added slowly with stirring till to attain gel structure
- ✓ Required proportions of aqueousextracts*Emblica officinalis, Citrus limonum, Allium sativum,Zingiber officinalis* and *Aloe barbadensis* were added to the prepared gel and stirred continuously to form proper gel

Physicochemical Evaluation of Herbal Antidandruff Gels[59,60]

Gels were evaluated for their clarity, pH, homogeneity, spreadability, viscosity,drug content, extrudability, *in-vitro* diffusion studies, release kinetics, antimicrobial screening, skin irritation test and*ex-vivo* studies by using standard procedure. All studies were carried out in triplicate and average values were reported.

Clarity[59]

The clarity of various formulations was determined by visual inspection under black and white background and it was graded as follows; turbid, clear, very clear (glassy).

pH[60]

2.5 gms of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of dispersion was measured by using digital pH meter (Elico).

Homogeneity[60]

All formulated gels were tested for homogeneity by visual inspection after the gels have been set in the container for their appearance and presence of any aggregate.

Spreadability[60]

It was determined by wooden block and glass slide apparatus. For the determination of spreadability excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 minutes. Weight (50 gm) was added to pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plates was taken as measure of Spreadability (S). Spreadability was calculated by using the formula:

$$S = ML/T$$

where,

S = Spreadability

M = Weight tide to upper slide

L = Length moved on the glass slide

T = Time taken to separate the slide completely from each other

Viscosity measurement[59]

Viscosity of the gels was determined using a Brookfield viscometer, (Brookfield DV-II + Pro viscometer) by using small sample adapter having spindle number SC4-18/13R. The gel was subjected to a torque ranging from 10 to 100 %.

Drug content[61]

The herbal antidandruff gel of 100mg was dissolved in 50 ml of phosphate buffer 7.4. The volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometerically.

Extrudability[59]

The extrudability test was carried out by using Pfizer hardness tester. A 15gm of gel was filled in aluminium tube. The plunger was adjusted to hold the tube properly. The pressure of 1kg/cm^2 was applied for 30 sec. The quantity of gel extruded was weighed. The procedure was repeated at three equidistance places of the tube. Test was carried out intriplicates.

***In-vitro* diffusion study**[59]

Cellophane Membrane Treatment for Permeation study

Cellophane membrane was boiled in the distilled water for 1 hour and washed with fresh distilled water for three times and kept in ethanol for 24 hours. It was treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at 60^{0}C followed by acidified with 0.2% sulphuric. Finally the membrane was dipped in boric acid buffer pH (9) till it is used for permeation study.

***In-vitro* diffusion study**

The *in- vitro* permeation rate of selected formulations of gel were evaluated by open ended tube through using pH 7.4 as diffusion medium upto 5 hours studies.The cellophane membrane was tied in one end of the tube and then immersed in he receptor compartment containing 200ml of 7.4 buffer solution which was stirred at 100±10 rpm and maintained at 37^{0}C ±2^{0}C. A quantity of 5ml samples were withdrawn from the receptor fluid at the time intervals of 0, 10, 15, 20, 25,30,60,90,120,180,240,300 min. The

release of drug was estimated by using spectrophotometer at 273 and 340 nm and 5ml of phosphate buffer of pH 7.4 was replaced immediately eachtime.

Release Kinetics[62]

Data obtained from *in-vitro* diffusion studies were fitted to various kinetic equations. The kinetic models used are zero order equations (Q=k0t), First order equation

{ln (100 – Q) =ln Q – klt}, Higuchi equation (Q=kt1/2), Hixson and crowell model Qt1/3 Vs t and Qt2/3 Vs – Modified root cube equation. Further, to find out the mechanism of drug diffusion, first 60% drug diffusion was fitted in Korsmeyer and Peppas equation (Q=kptn). Where, Q is the percent of the drug diffusion at time t and k0 and kt are the coefficients of the equations and 'n' are the diffusion exponent. The 'n' value is used to characterize different diffusion mechanism.

The order of drug diffusion can be assessed by graphical treatment of drug diffusiondata.

A plot of cumulative % drug diffusion versus time would be linear if the drug diffusion follows zero order (i.e. Concentration independent diffusion).

A plot of log of % remaining drug versus time would be linear, if the drug diffusion follows first order (i.e. Concentration dependentdiffusion)

The linear equation for zero order drug diffusion plot is: Ct = C0 – Kt

Where,

Ct = concentration remaining at time t, Co = original concentration,

t = time,

K = diffusion rate

The linear equation for first order diffusion plot is

$$\text{LogC} = \frac{\text{logC0Kt}}{2.303}$$

A matrix device as the name implies, consists of drug dispersed homogeneously throughout a polymer matrix. In this model, drug in the outside layer exposed to the

bathing solution is dissolved first and then diffuses out of the matrix. This process continues with the interface between the bathing solution and the solid drug moving towards the interior.

Obviously, for this system to be diffusion controlled, the rate of dissolution of drug particles within the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix. Hydrophilic matrix tablets contain a water swellable polymer.On
[1 – Mt / M] 1/3 =1-kt
Where,

Mt = mass of drug diffusion at time t, M = mass diffusion at the infinite time, K = rate of erosion,

t = time

Thus a plot of [1 – Mt / M] 1/3 versus the time will be linear. If the diffusion of

drug from the matrix is erosion controlled

In order to ascertain whether the drug diffusion occurs by diffusion or erosion, the drug diffusion data was subjected to following modes of data treatments

Amount of drug diffusion versus square root of time (HiguchiPlot)
[1 – Mt / M] 1/3 versustime

Screening of Antimicrobial activity of Herbal Antidandruff gel formulations[63]

16.11.1. Anti Bacterial Activity

Principle

Discs impregnated with known concentration of antibiotics discs are placed on agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18-24 hrs at 37^{o}C. During this period, the antibacterial agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the discs. Organisms which grow up to the edge of the disc are resistant.

Experimental condition

Organisms used **:** *Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes*

Media used **:** Nutrient Agar Media (Muller Hington Agar Media)

Test used **:** Herbal antidandruff formulation F_5, F_6, F_7, F_8

Standard **:** Ciprofloxacin

Procedure

- ✓ The Muller Hington Agar Media was prepared, sterilized and used as the growth medium for bacteria culture, 20ml of the sterilized medium was poured into each sterilized petridish, coverd and allowed to solidify
- ✓ The sterile disc (Whatmann No 2, 6mm diameter) was placed uniformly at equal interval on the inoculated plate. Then it was loaded in the above solidified media
- ✓ About 200 µ/l of sample was loaded in each disc and incubated at 37^0c for 18-24 hours and the Zone of inhibition was measured by using the ruler

6.11.2. Anti-Fungal Activity Principle

Discs impregnated with known concentration of antibiotics discs are placed on Modified Sabouraud's Glucose agar plate that has been inoculated (or) seeded uniformly over the entire plate with a culture of the fungi to be tested. The plate is incubated for 3 days 18-24 hrs at 37^oC. During this period, the antifungal agent diffuses through the agar and may prevent the growth of organism. Effectiveness of susceptibility is proportional to the diameter of inhibition of zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Experimental condition

Organisms used : *Malassezia furfur, Candida albicans*

Media used **:** Saboraud Dextrose Agar (SDA Media)

Test used **:** Herbal antidandruff formulation F_5, F_6, F_7, F_8

Standard **:** Ciprofloxacin

Procedure

- ✓ The Saboraud Dextrose Agar Media was prepared, sterilized and used as the growth medium for fungi culture, 20ml of the sterilized medium was poured into each sterilized petridish, coverd and allowed to solidify
- ✓ The sterile disc (Whatmann No 2, 6mm diameter) was placed uniformly at equal

interval on the inoculated plate. Then it was loaded in the above solidified media

- ✓ About 200 µ/l of sample was loaded in each disc and incubated at 37^0C for 3-4 days and the Zone of inhibition was measured by using the ruler

Skin irritation test[64]

From the above studies, one best formulation (F_8) was selected for skin irritation testing. The skin irritation test was carried out in the department of pharmacology, Periyar College of Pharmaceutical Science, Tiruchirappalli, Tamil Nadu, India. Adult male rabbits weighing between 2 – 3.5 kg were used for skin irritation test. These animals were maintained under identical animal house condition and provided with standard diet and water adlibitum. The animals were acclimatized to laboratory conditions before the test. The test was conducted on unabraied skin of rabbit with 3.18 cm^2 area. They were divided into 2 groups, each containing 3 animals. In the first group (control) only carbopol gel 934 was applied. In the second group (Group 2) Herbal Aantidandruff gel (F_8) containing 1% extract was applied.A thin coat of sample was applied with the help of cotton swab on identical side of the right dorsal surface of rabbits three times per week (but not on 2 consecutive days). The test was carried out for 21 days and observed for erythema and edema after 7^{th}, 14^{th} and 21^{st} days.The results are given in **Table.37** and **Fig.71 to 74** .

***Ex-vivo* studies[62,65]**:

- ✓ *Ex-vivo* skin permeation studies carried out using Goat skull skin
- ✓ The receptor compartment consisted of 400ml of Phosphate buffer (pH 7.4) in 500 ml beaker
- ✓ Temperature was maintained at 37 ± 0.5°C and stirred at 900rpm
- ✓ The Polyherbal Antidandruff gel was placed in Goat abdomen skin and tied to the one end of open-ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magneticstirrer
- ✓ Samples were taken from receptor medium at 0, 5,10,15,20,25,30, 60, 90, 120, 180, 240, 300, 360, 420min
- ✓ Periodically 5ml ofsample was withdrawn and same volume of medium was replaced with freshbuffer
- ✓ All the Samples were assayed spectrophotometrically at 382 nm using PB7.4 pH

as blank

The result is shown in **Table.38** and **Fig.75**

6.14.Stability studies[66,67]:

Stability

Stability is officially defined as the time lapse during which the drug product retains the same property and characteristics that it possessed at the time of manufacture. This process beings at early development phases.

Purpose of stability testing

- To study decomposition kinetics
- To develop stable dosage form
- To estimate shelf-life period or expiry date for drug product
- To ensure safety, efficacy and quality of the active drug substance and dosage form

ICH Guidelines- Specifications

- 5% potency loss from assay of initial batch
- Any specified degradation that exceed specification
- Product failing out of Pharmacopieal limits
- Failure to meet specification for appearance and physical properties

Any one condition is observed then stability of the batch is failed.

Table.5. Stability Storage Conditions

S.No.	Study period	Storage condition	Minimum duration
1	Longer	25 ± 2˚C, 60 ± 5% RH	6months
2	Intermediate	30 ± 2˚C ,60 ± 5% RH	3 months
3	Accelerated	40 ± 2˚C ,75 ± 5% RH	3 months

All the selected formulations were subjected to a stability testing for three months as per ICH norms at a temperature (40˚C $\pm$ 2˚C). All selected formulations were analyzed

for the change in pH, spreadability, homogeneity or drug content by procedure stated earlier.

7. RESULTS AND DISCUSSIONS

Phytochemical studies

The phytochemical studies of *Emblica officinalis, Citrus limonum, allium sativum, Zingiberofficinale, Aloe barbadensis*was done. The presence and absence of Phytoconstituents in the aqueous extract of the above sample was shown in **Table.6** .

Table 6. Phytochemical studies

S.No.	Phytoconstituents	Aqueous extracts				
		Emblica officinalis	***Citrus limonum***	***Allium sativum***	***Zingiberof ficinale***	***Aloe barbade nsis***
1.	Alkaloids	+	+	+	+	+
2.	Glycosides	+	+	+	+	-
3.	Saponins	-	-	+	+	+
4.	Tannins	+	+	-	+	+
5.	Phenols	+	+	+	+	-
6.	Reducing sugars	+	+	+	+	+
7.	Amino acids	+	+	+	+	+
8.	Flavonoids	-	+	+	+	+
9.	Terpenoids	-	+	_	+	+
10.	Steroids	+	+	+	-	-

(+) Presence of phytoconstituents (-) Absence of phytoconstituents

The phytochemical studies revealed that the presence and absence of phytoconstituents in the aqueous extracts of *Emblica officinalis, Citrus limonum, Allium sativum, Zingiberofficinale, Aloe barbadensis.*

Identification of Selected Herbs

High Performance Thin Layer Chromatography (HPTLC)

The HPTLC fingerprinting of Aqueous extract of selected parts of *Emblica officinalis* (AEEO), *Citrus limonum* (AECL) were studied individually. The HPTLC fingerprinting was done. The Peaks viewed at system suitability were shown (**Fig.13 and 22**). The four different chromatograms were obtained and photo documentation was done

for individual extracts (**Fig.14 to 17 and 23 to 26**) The 3D display of the chromatogram were obtained and photo documentation were done (**Fig.21 and 30**)

HPTLC Finger Printing of Aqueous extract of *Emblica officinalis* (AEEO)

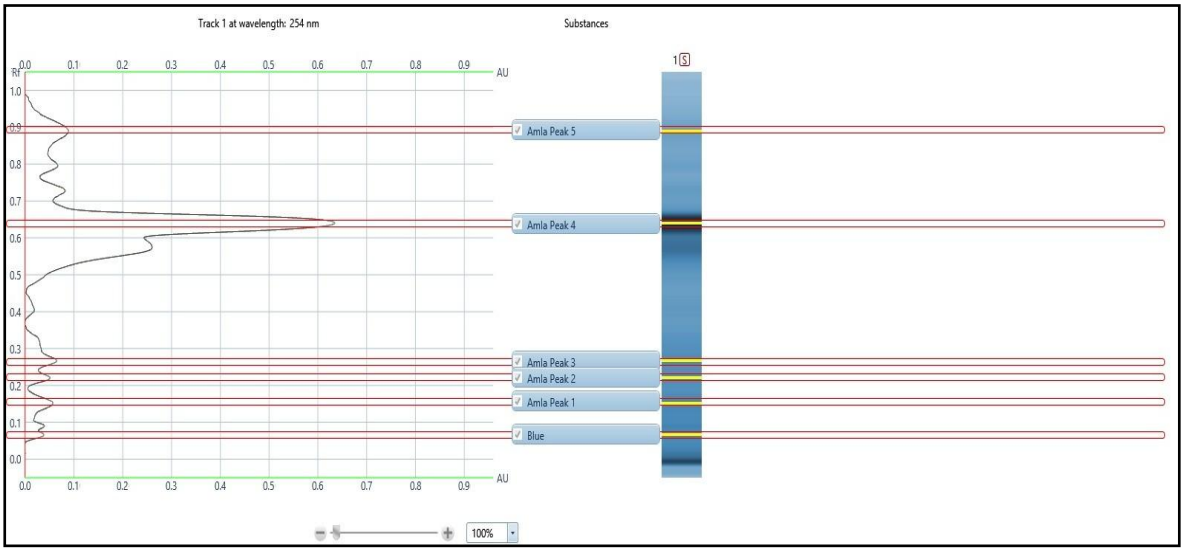


Fig. 13. HPTLC peak at System suitability test for AEEO

Table 7. Rf values from HPTLC Chromatogram of AEEO

S. No.	Track No.	Rf value
1.	Tr.1	0.067
2.	Tr.2	0.157
3.	Tr.3	0.222
4.	Tr.4	0.265
5.	Tr.5	0.638
6.	Tr.6	0.893

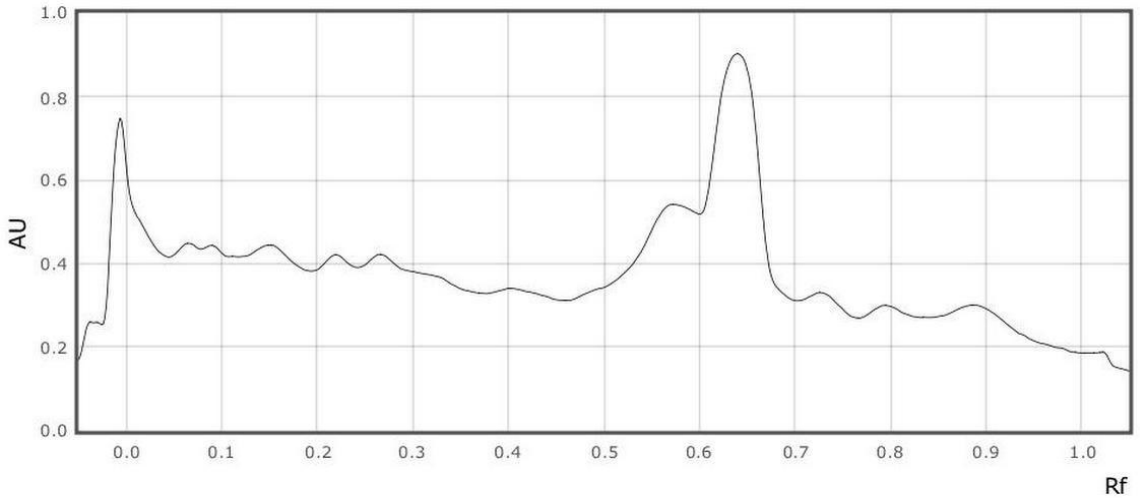


Fig.14 . HPTLC Chromatogram of AEEO at 5 µl Concentration

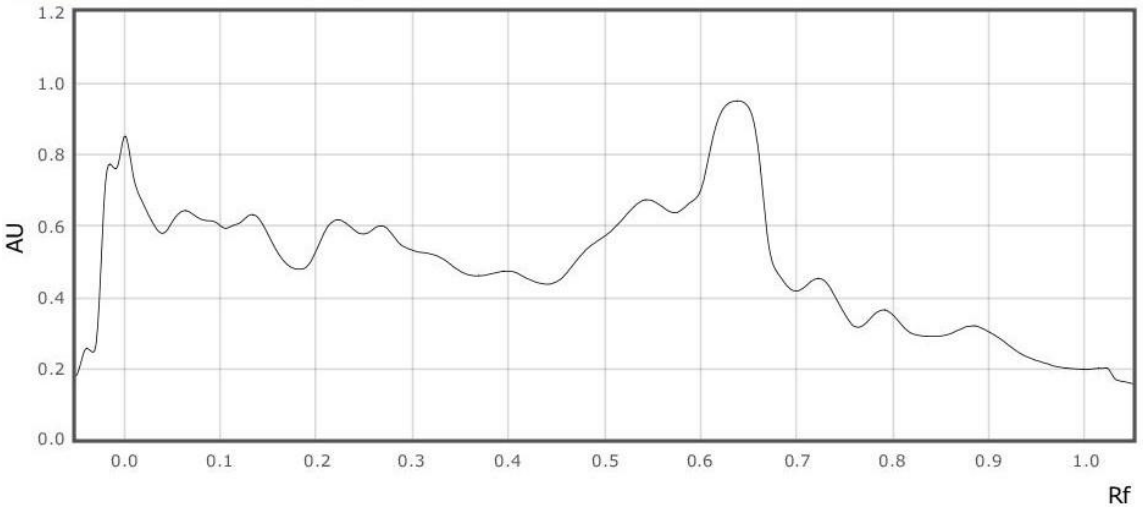


Fig.15 . HPTLC Chromatogram of AEEO at 10 µl Concentration

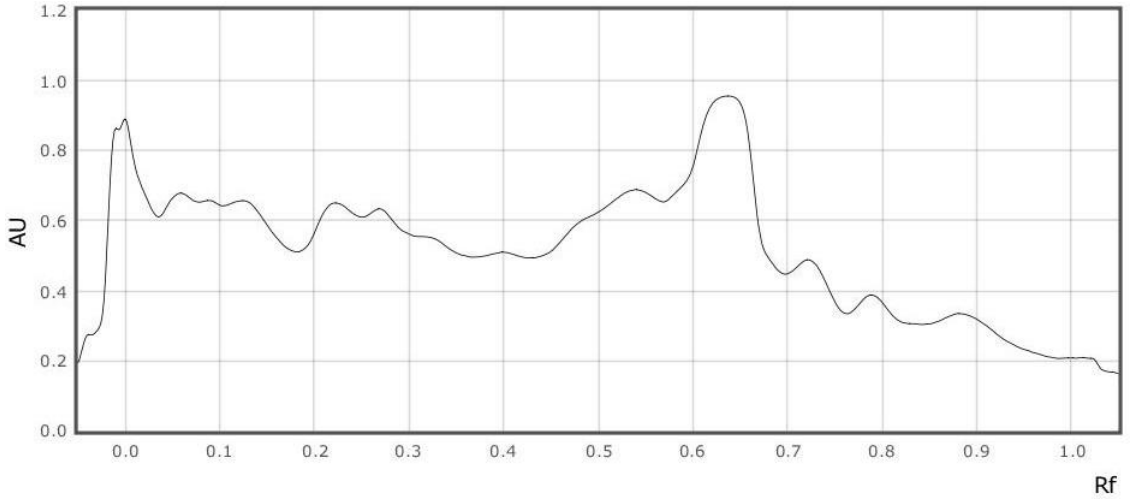


Fig.16 . HPTLC Chromatogram of AEEO at 15 µl Concentration

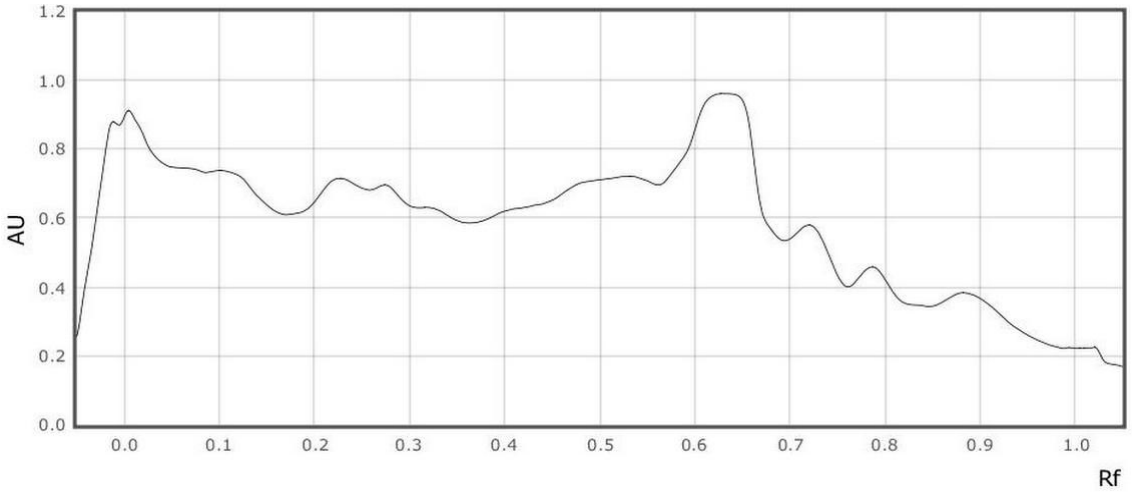


Fig.17 . HPTLC Chromatogram of AEEO at 20 µl Concentration

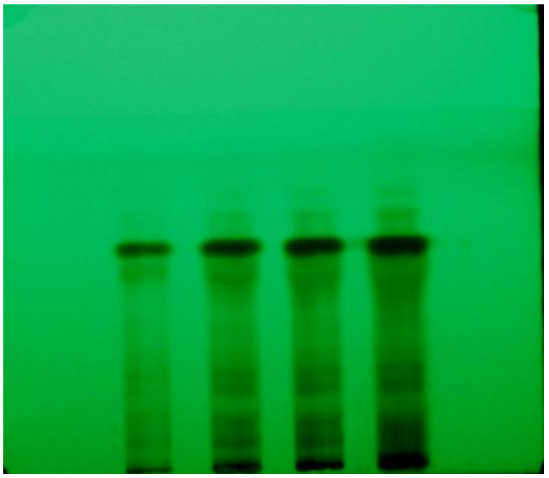


Fig.18. HPTLC of Amla Extract viewed at 254nm

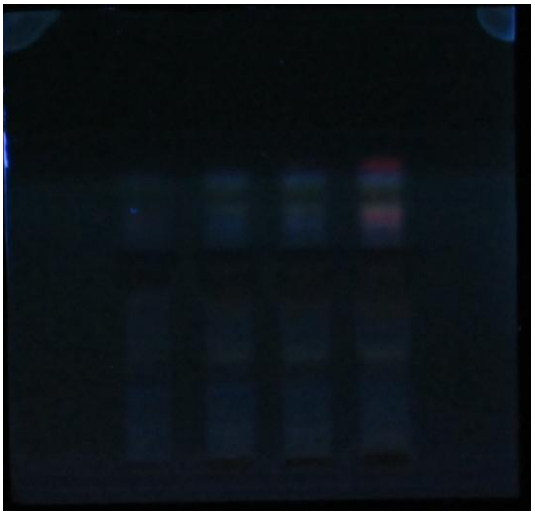


Fig.19. HPTLC of Amla Extract viewed at 366nm

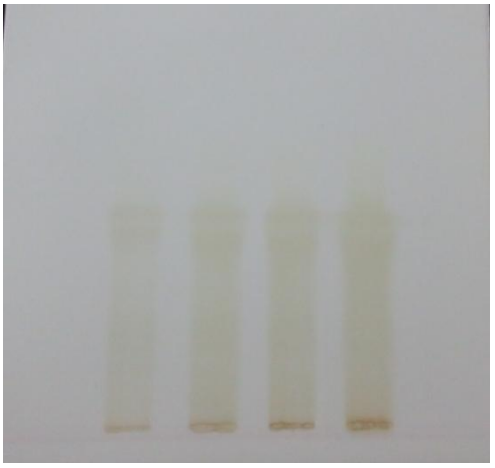


Fig. 20. HPTLC of Amla Extract viewed at Visible Light

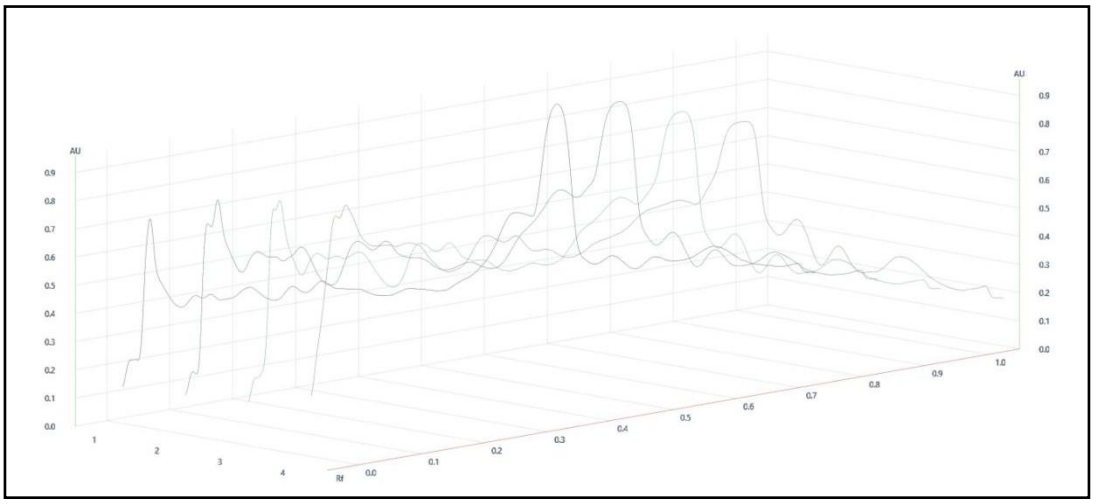


Fig.21 . HPTLC Chromatogram of AEEO (3D)

HPTLC Finger Printing of Aqueous extract of *Citruslimonum*(AECL)

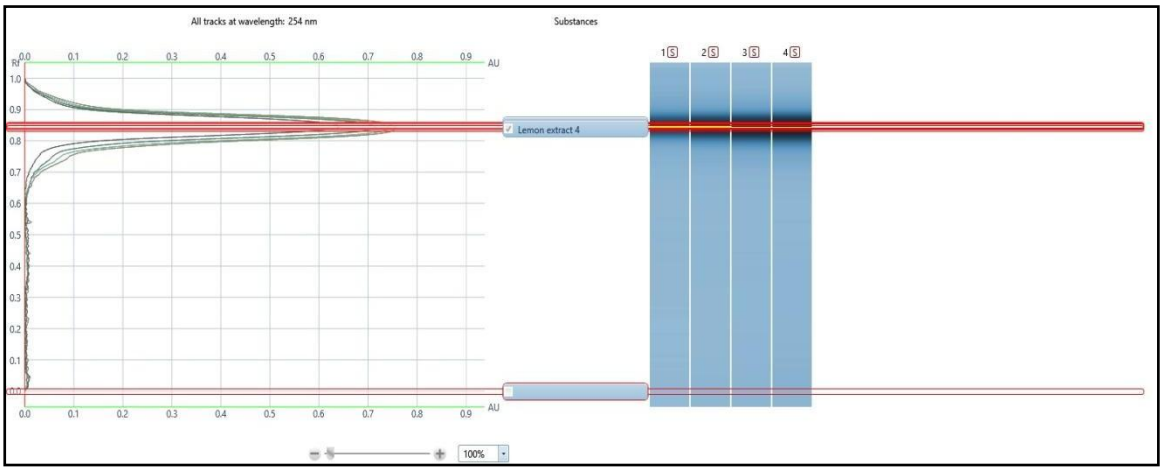


Fig.22. HPTLC peak at System suitability test for AECL

Table 8. Rf values from HPTLC Chromatogram of AECL

S. No.	Track No.	Rf value
1.	Tr.1	0.844
2.	Tr.2	0.846
3.	Tr.3	0.849
4.	Tr.4	0.839

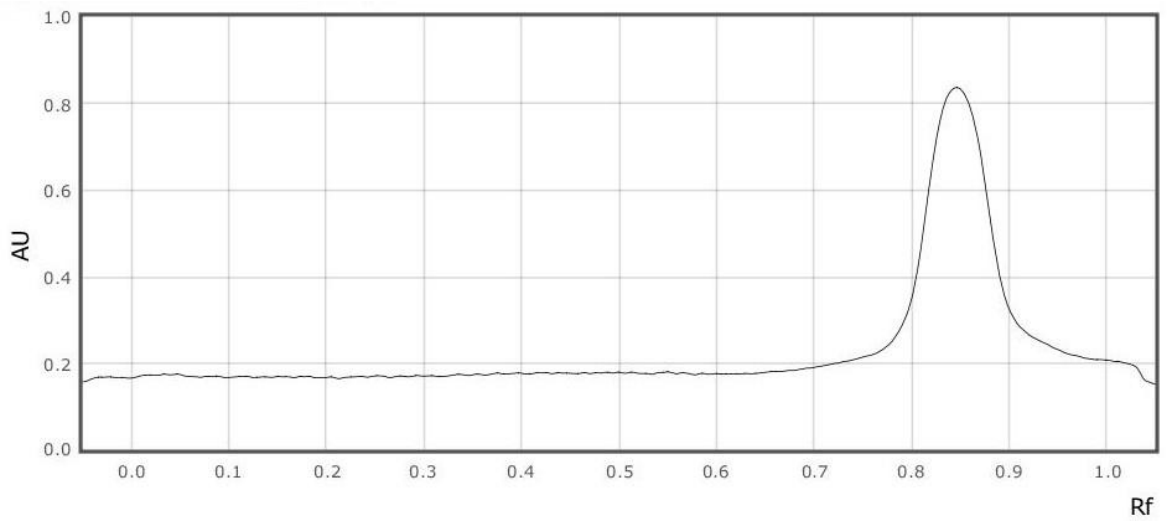


Fig.23. HPTLC Chromatogram of AECL at 5 µl Concentration

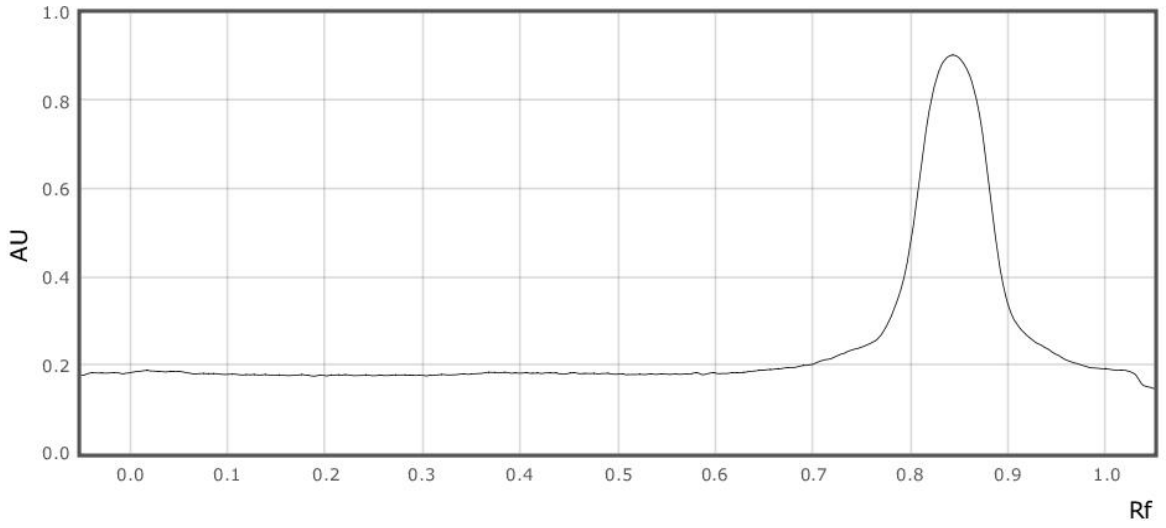


Fig.24 . HPTLC Chromatogram of AECL at 10 µl Concentration

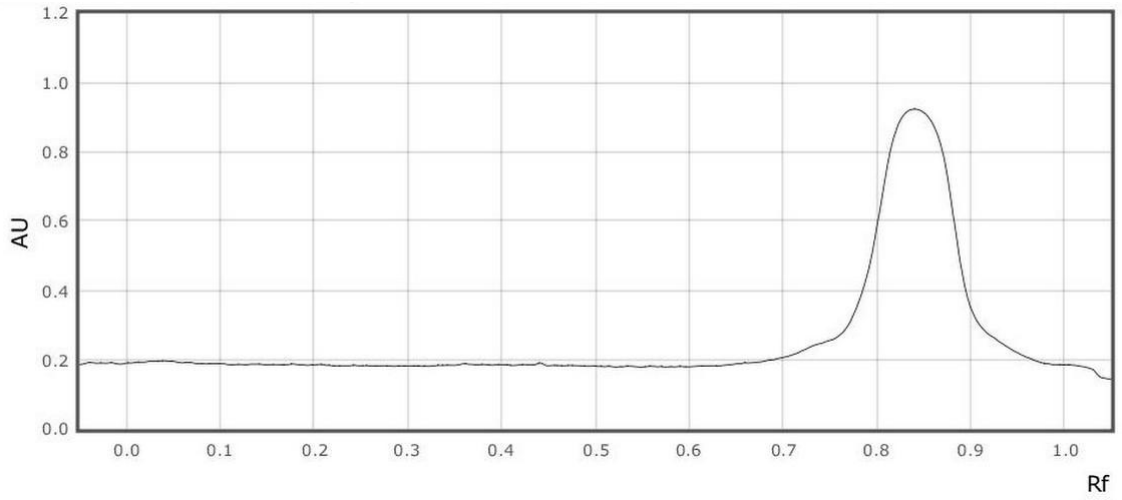


Fig.25 . HPTLC Chromatogram of AECL at 15 µl Concentration

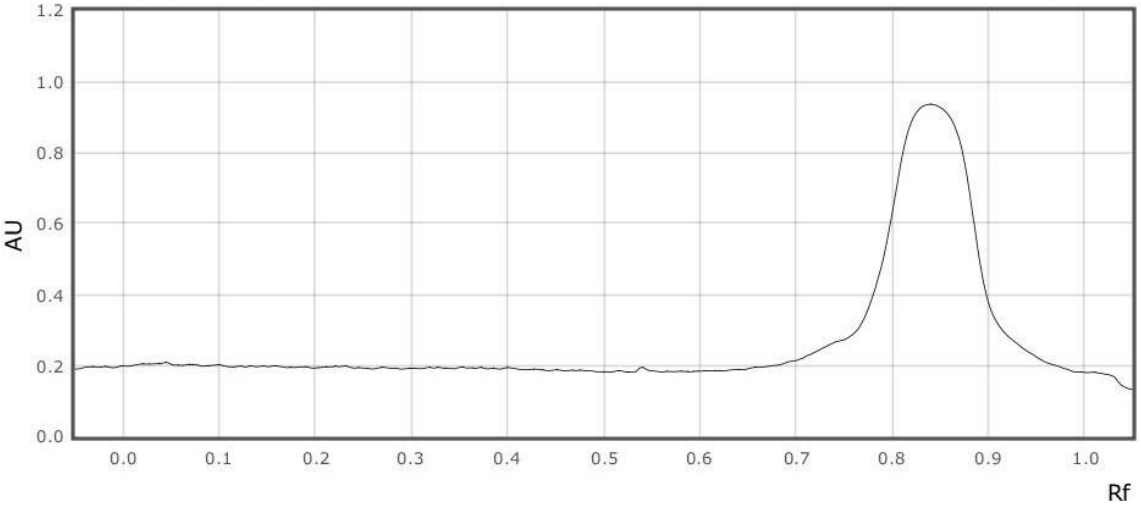


Fig.26. HPTLC Chromatogram of AECL at 20 µl Concentration

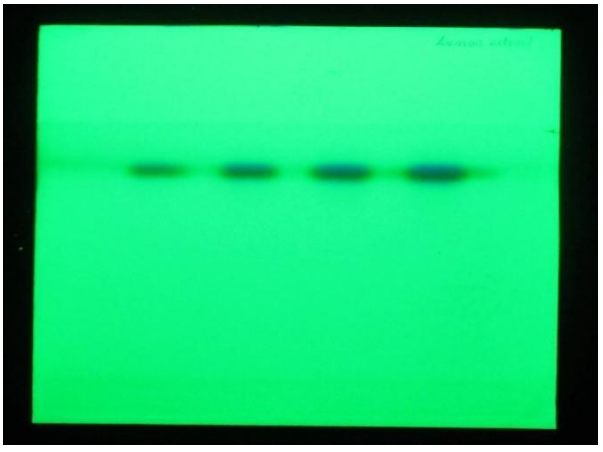


Fig.27. HPTLC plate viewed at 254nm

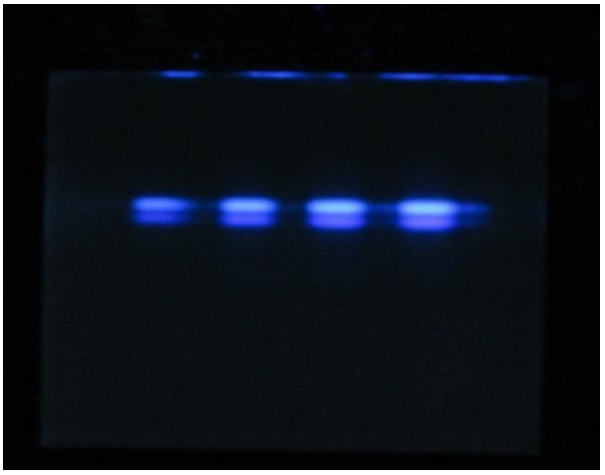


Fig.28. HPTLC plate viewed at 366nm

Fig.29.HPTLC plate viewed at visible light

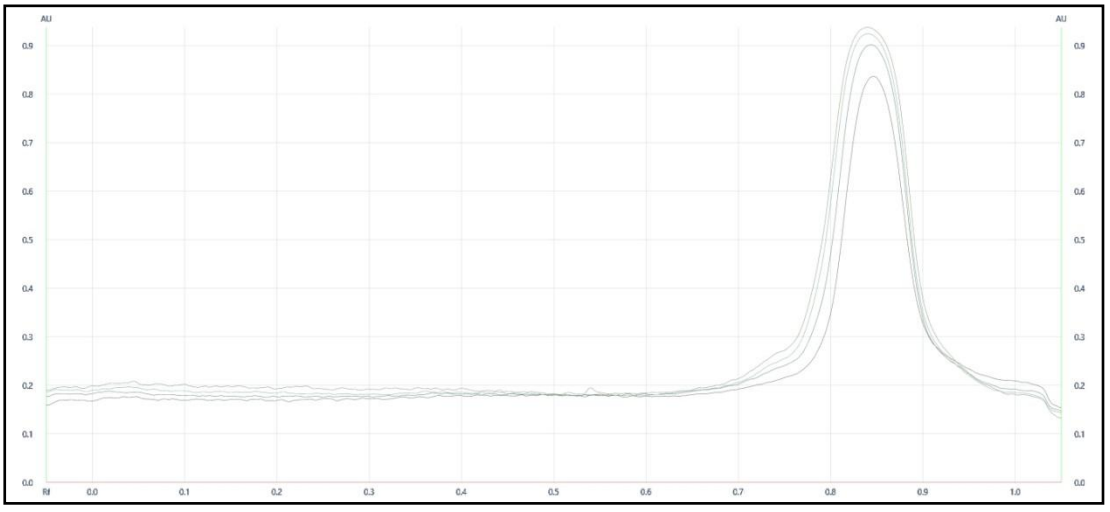


Fig.30. HPTLC Chromatogram of AECL (3D)

UV Analysis

Absorption maxima (λ max) of Aqueous extract of*Emblica officinalis*. Gaertn

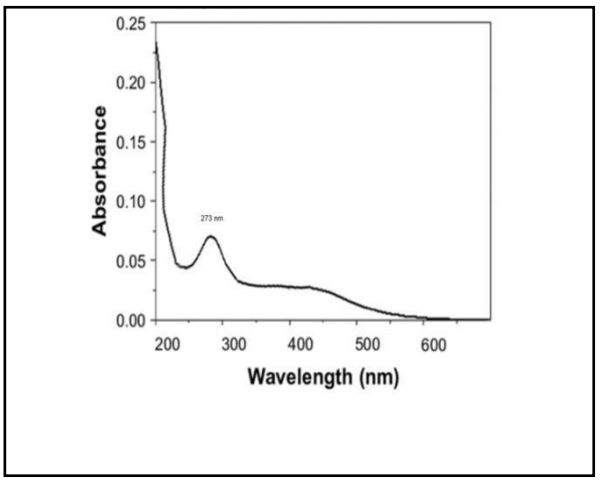


Fig.31.Absorption maxima (λ max) of Aqueous extract of *Emblica officinalis.*Gaertn

The sharp peak observed at 273nm, further measurements were taken at 273nm.

Standard curve of Aqueous extract of *Emblica officinalis*. Gaertn Table 9.

Standard curve of Aqueous extract of *Emblica officinalis.* Gaertn

Concentration (µg/ml)	**Absorbance at 273nm Average ± SD**
0	0.000 ±0.001
5	0.257±0.001
10	0.430 ±0.001
15	0.593 ±0.001
20	0.748 ±0.001
25	0.908±0.001
30	1.031 ±0.004

Mean± SD : n= 3

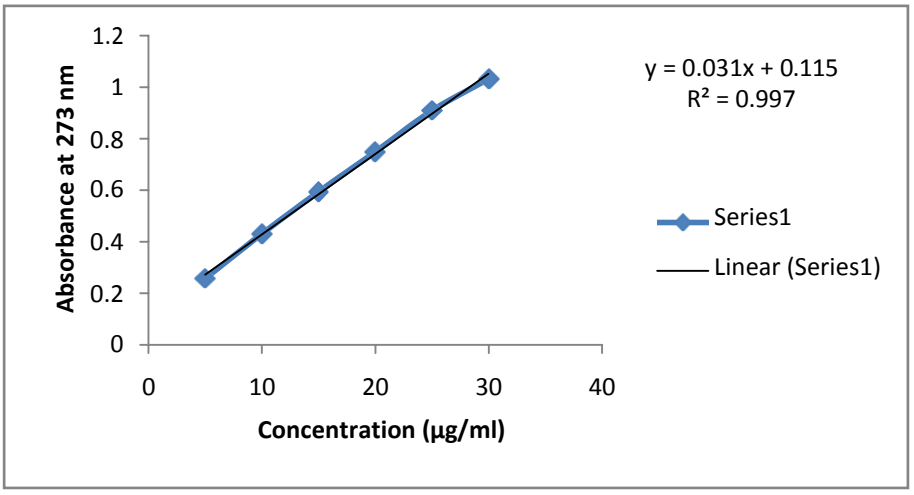


Fig.32 . Standard curve of Aqueous extract of *Emblica officinalis*. Gaertn

The standard curve has good regression coefficient r^2= 0.997 and it shows the linearity

Absorption maxima (λ max) of Aqueous extract of *Citrus limonum*. Risso

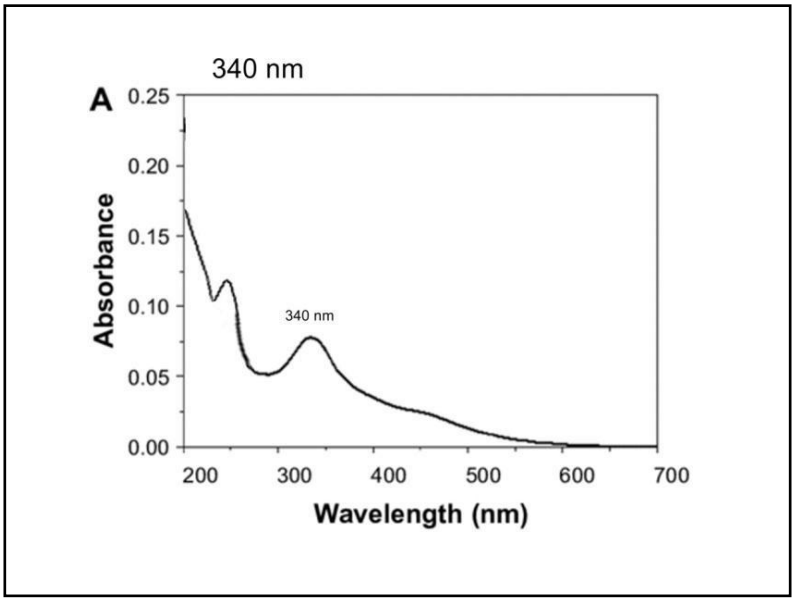


Fig.33. Absorption maxima (λ max) of Aqueous extract of *Citrus limonum*. Risso

The sharp peak observed at 340nm, further measurements were taken at 340nm.

Standard curve of Aqueous extract of *Citruslimonum*. Risso

Table 10. Standard curve of Aqueous extract of *Citruslimonum*. Risso

Concentration (µg/ml)	Absorbance at 340nm Average ± SD
0	0.000 ±0.001
	0.310 ±0.001
10	0.420 ±0.001
15	0.509 ±0.001
20	0.793 ±0.001
25	0.943 ±0.001
30	1.257 ±0.005

Mean± SD : n= 3

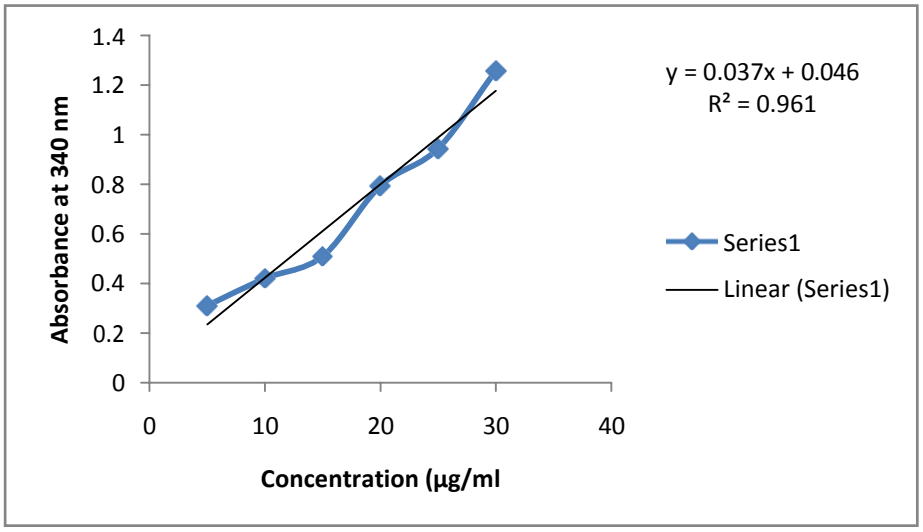


Fig.34 . Standard curve of Aqueous extract of *Citruslimonum*. Risso

The standard curve has good regression coefficient r^2= 0.961 and it shows the linearity

Compatibility study FTIR studies

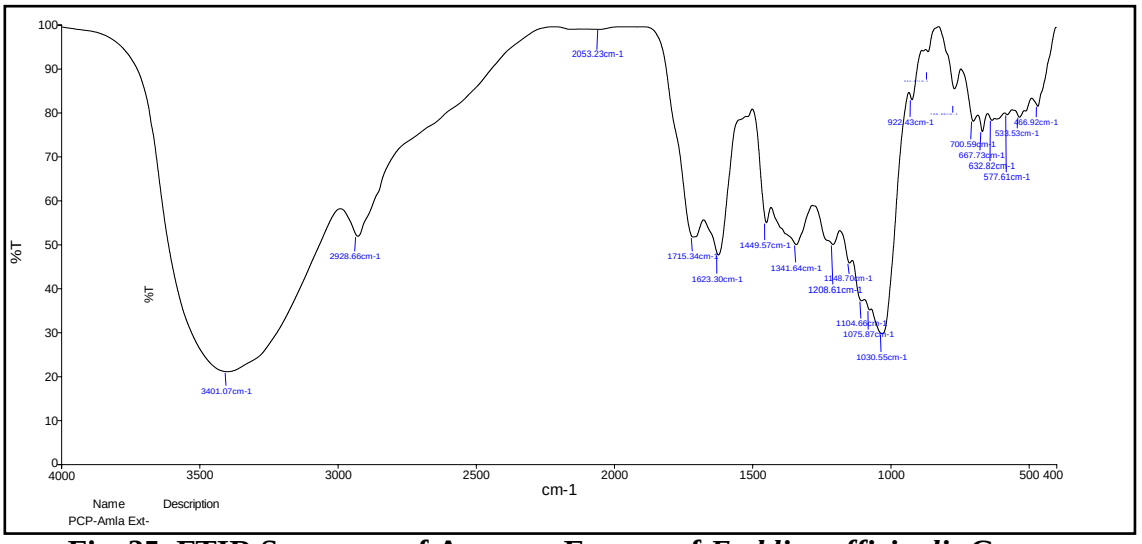


Fig .35. FTIR Spectrum of Aqueous Extract of *Emblica officinalis.*Gaertn

Table 11. FTIR Interpretation of Aqueous Extract of *Emblica officinalis.*Gaertn

Wave number (cm^{-1})	Functional Group
3401.07	O-H stretching
2928.66	C-H Stretching
2053.23	N=C=S Stretching
1715.34	C=O Stretching
1623.30	C=C Stretching
1449.57	C-H Bending
1208.61	C-O Stretching
1075.87	C-O Stretching

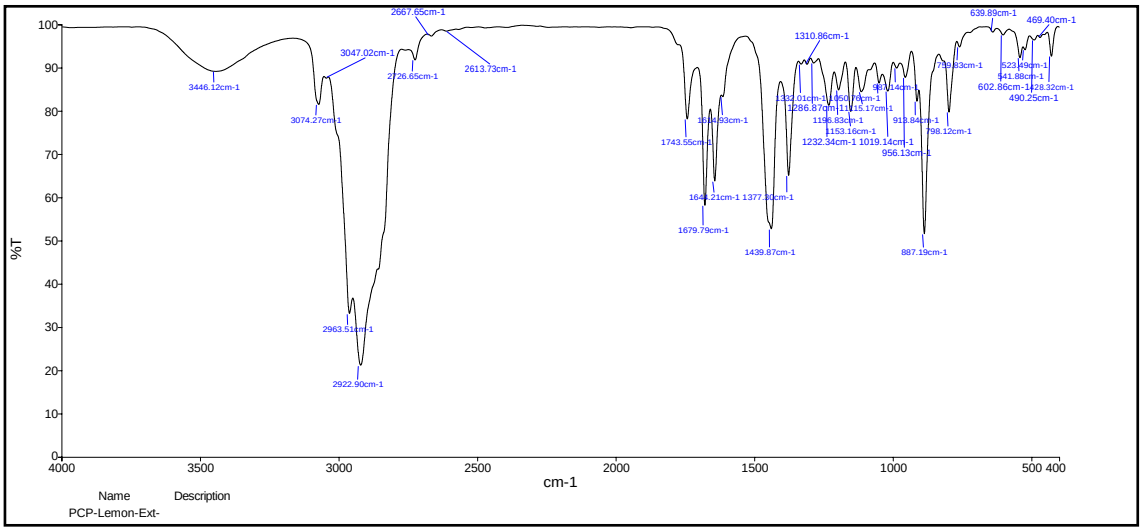


Fig.36. FTIR Spectrum of Aqueous Extract of *Citrus limonum.* Risso

Table 12 . FTIR Interpretation of Aqueous Extract of *Citrus limonum.* Risso

Wave number (cm^{-1})	Functional Group
3446.12	O-H stretching
3047	C-H Stretching
2963.51	C-H Stretching
1743.55	C-O Stretching
1614.93	C=C Stretching
1286.87	C-O Stretching
1153.16	C-O Stretching
1050	C-O Stretching

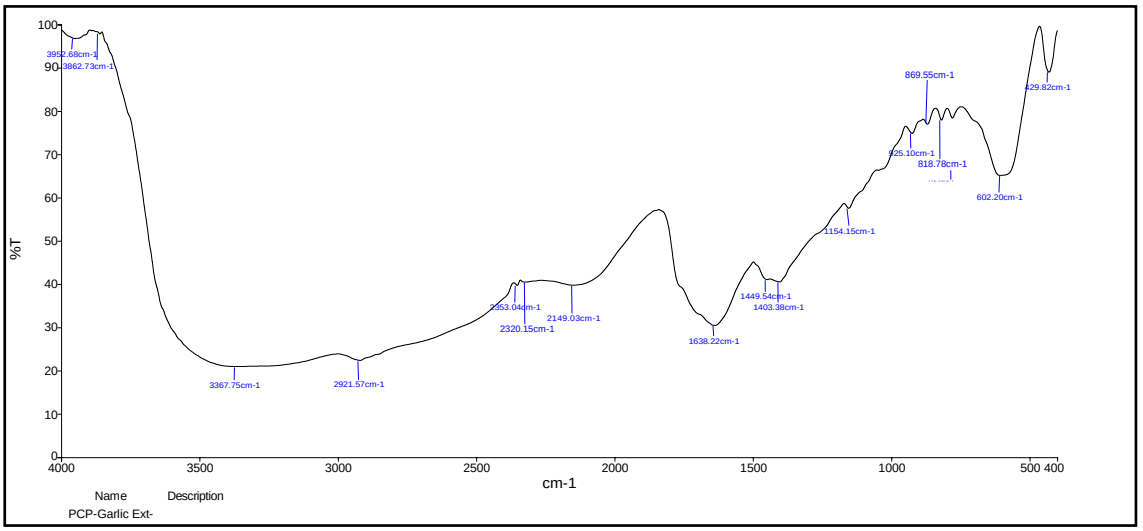


Fig No.37. FTIR Spectrum of Aqueous Extract of *Allium sativum*. Linn

Table 13. FTIR Interpretation of Aqueous Extract of *Allium sativum*. Linn

Wave number (cm^{-1})	**Functional Group**
3367.75	O-H stretching
2921.57	C-H Stretching
2320.15	N-H Stretching
2149.03	S-C≡N Stretching
1638.22	C=C Stretching
1449.54	C-H Bending
1403.38	S=0 Stretching

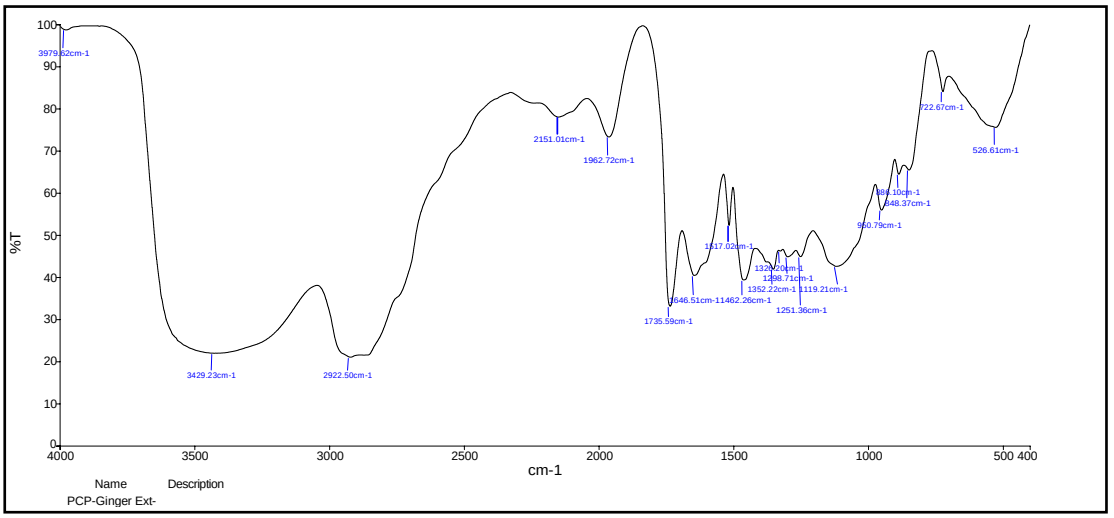


Fig . 38.FTIR Spectrum of Aqueous Extract of *Zingiberofficinale*. Roscoe

Table 14. FTIR Interpretation of Aqueous Extract of *Zingiberofficinale*. Roscoe

Wave number (cm^{-1})	**Functional Group**
3429.23	O-H stretching
2922.50	C-H Stretching
2151.01	S-C≡N Stretching
1962.72	C-H Bending
1735.59	C=O Stretching
1646.51	C=N Stretching
1326.20	O-H Bending

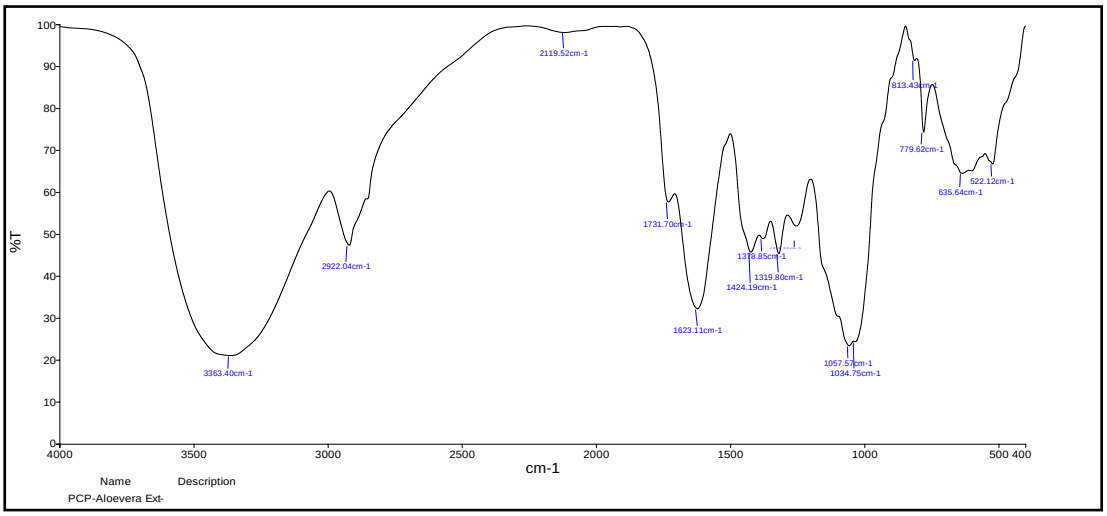


Fig . 39. FTIR Spectrum of *Aloe barbadensis*

Table 15. FTIR Interpretation of *Aloe barbadensis*

Wave number (cm^{-1})	**Functional Group**
3363.40	O-H stretching
2922.04	C-H Stretching
2119.52	C≡C Stretching
1731.70	C=O Bending
1623.11	C=C Stretching
1378.85	O-H Bending

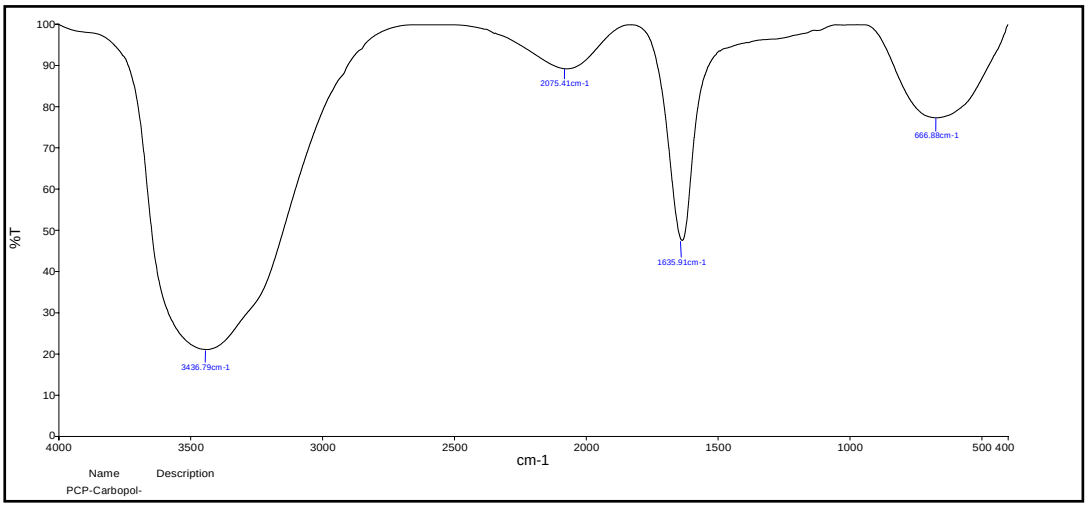


Fig . 40. FTIR Spectrum of Carbopol 934

Table 16. FTIR Interpretation of Carbopol

Wave number (cm^{-1})	**Functional Group**
3436.79	O-H stretching
2075.41	N=C=S Stretching
1635.91	C=C Stretching
666.88	C=C Bending

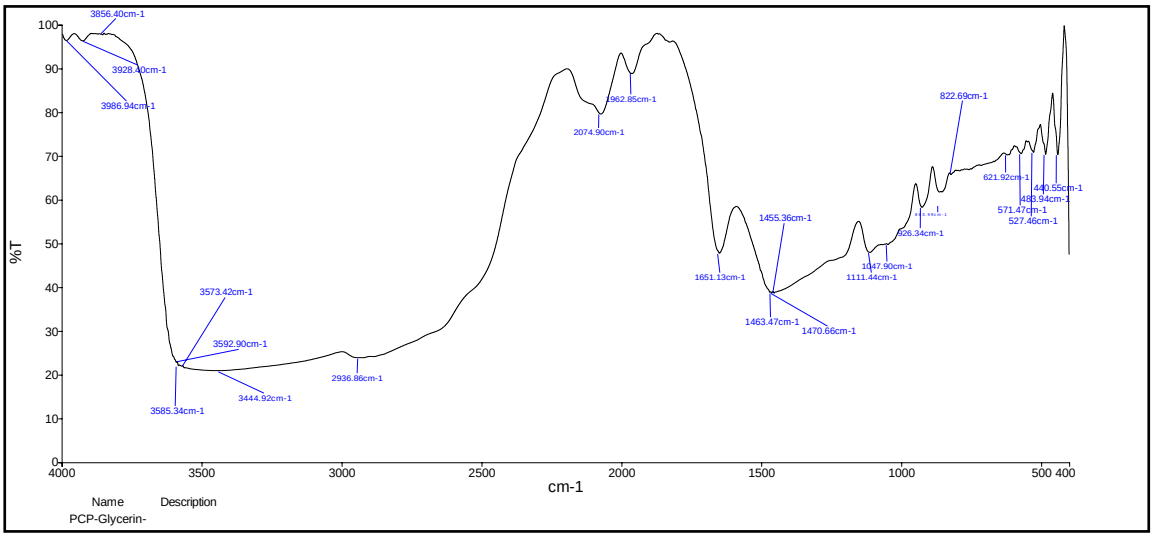


Fig .41.FTIR Spectrum of Glycerin

Table 17. FTIR Interpretation of Glycerin

Wave number (cm^{-1})	**Functional Group**
3444.92	O-H stretching
2933.86	C-H Stretching
2074.90	N=C=S Stretching
1962.85	C-H Bending
1651.13	C=N Stretching
1111.44	C-O Stretching

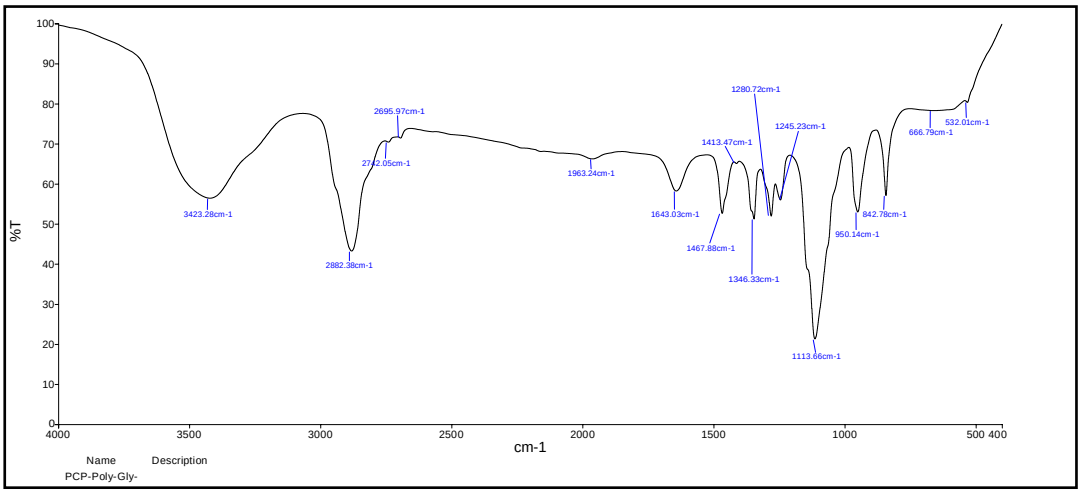


Fig. 42. FTIR Spectrum of Polyethylene glycol

Table 18. FTIR Interpretation of Polyethylene glycol

Wave number (cm^{-1})	Functional Group
3423.28	O-H stretching
2882.38	C-H Stretching
1643.24	C=N Stretching
1414.22	OH Bending
1346.33	O-H Stretching
1280.72	C-O Stretching
1245.23	C-N Stretching
1113.66	C-O Stretching

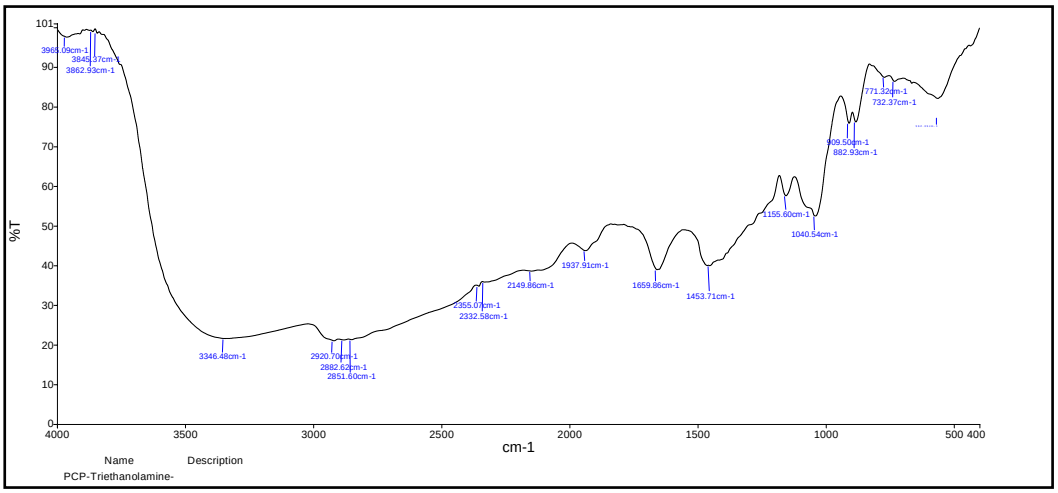


Fig . 43. FTIR Spectrum of Triethanolamine

Table 19. FTIR Interpretation of Triethanolamine

Wave number (cm^{-1})	Functional Group
3346.48	N-H stretching
2920.70	N-H Stretching
2882.62	C-H Stretching
2851.60	C-H Bending
2149.86	N=N=N Stretching
1659.86	C-H Bending
1453.71	C-H Bending

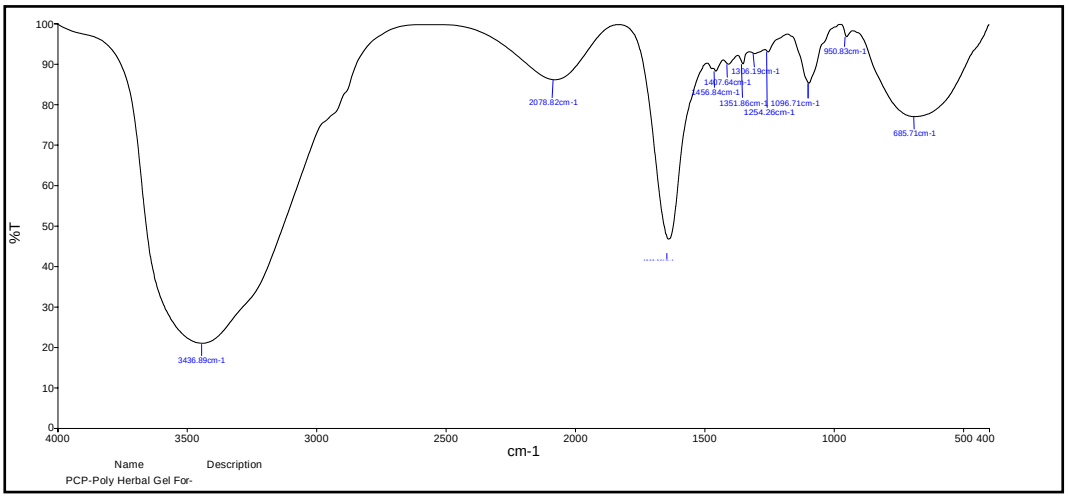


Fig. 44. FTIR Spectrum of Herbal Antidandruff Gel

Table 20. FTIR Interpretation of Herbal Antidandruff Gel

Wave number (cm^{-1})	Functional Group
3436.89	O-H stretching
2078.82	N=C=S Stretching
1639.52	C=C Stretching
1351.86	O-H Bending
1254.26	C-O Stretching
1306.19	C-N Stretching
1096.71	C-O Stretching

There are no extra peaks seen other than the normal peak in the spectra of the mixture of the extracts containing active constituents and excipients so there is no evidence of interaction with the drug and polymers and they are compatible with each other.

Physico chemical evaluation of Herbal Antidandruff Gel

Table 21. Physico chemical evaluation of Herbal Antidandruff Gel

Formulations	Clarity	pH	Homogeneity	Spreadability (g.cm/sec)	Extrudability	Viscosity (cps)	% Drug Content
F1	Turbid	6.9	Not Good	10.08	+	8823	70.92
F2	Turbid	6.8	Not Good	12.89	+	8818	75.30
F3	Turbid	6.7	Not Good	12.27	+	8951	68.53
F4	Turbid	6.9	Not Good	13.86	+	8890	72.95
F5	Clear	7.1	Good	18.75	+ +	9632	79.82
F6	Clear	6.9	Good	20.55	+ +	9826	83.02
F7	Clear	7.0	Good	22.39	+ +	9142	78.92
F8	Clear	7.2	Good	18.07	++	9122	85.46

+ Satisfactory , ++ Excellent

Eight batches of Herbal Antidandruff Gel formulations were prepared by using Carbopol 940 and Carbopol 934 were subjected to various physicochemical evaluations

Based on the clarity,pH, homogeneity,spreadability,viscosity,percentage drug content and extrudability formulations $F_{5,}$ $F_{6,}F_{7,}F_{8}$ were selected for further studies.

Optimized formula of Herbal Antidandruff Gel

Table 22. Optimized formula of Herbal Antidandruff Gel

S. No.	Ingredients	F_5	F_6	F_7	F_8
1.	*Emblica officinalis*	0.5ml	-	-	0.5ml
2.	*Citrus limonum*	-	0.5ml	-	0.5ml
3.	*Allium sativum*	-	-	0.5ml	0.5ml
4.	*Zingiber officinalis*	-	-	0.5ml	0.5ml
5.	*Aloe barbadensis*	-	-	0.5g	0.5g
6.	Carbopol 934	0.30g	0.30g	0.30g	0.30g
7.	Polyethylene Glycol	7g	7g	7g	7g
8.	Triethanolamine	0.6g	0.6	0.6g	0.6g
9.	Propyl Paraben	0.075g	0.075g	0.075g	0.075g
10.	Glycerine	3ml	3ml	3ml	3ml
11.	Water q.s	50ml	50ml	50ml	50ml

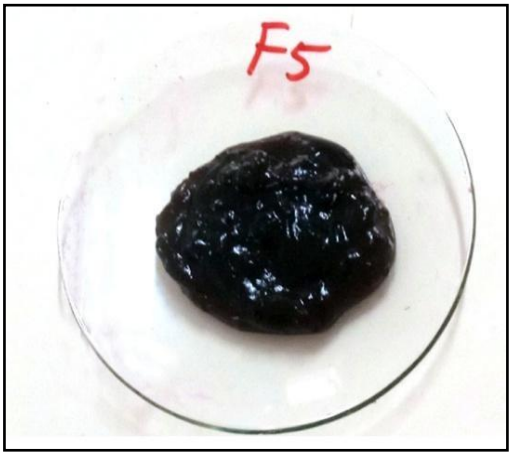
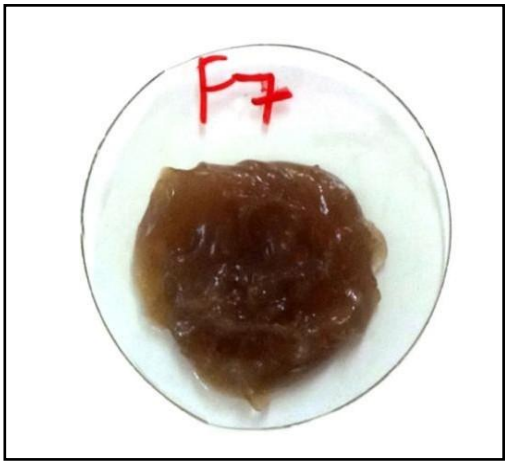


Fig.45 . Formulation F_5

Fig.46 . Formulation F_6



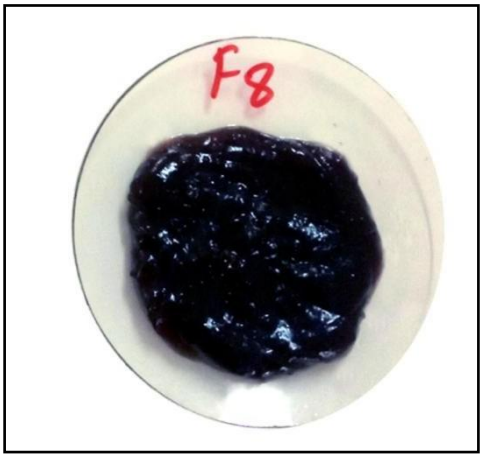


Fig.47 . Formulation F_7

Fig.48 . Formulation F_8

Physiochemical Evaluation of Best Four formulations

Table.23 . Clarity Test

Formulations	Clarity
F_5	Clear
F_6	Clear
F_7	Clear
F_8	Clear

Table 24 . pH

Formulations	pH
F_5	7.1
F_6	6.9
F_7	7.0
F_8	7.2

Table 25. Homogeneity Test

Formulations	Homogeneity
F_5	Good
F_6	Good
F_7	Good
F_8	Good

Table 26.Spreadability Test

Formulations	Spreadability
F_5	18.75
F_6	20.55
F_7	22.39
F_8	18.07

Table 27 .Extrudability Test

Formulations	Extrudability
F_5	79.82
F_6	83.02
F_7	78.92
F_8	85.46

Table 28 .Viscosity

Formulations	Viscosity
F_5	9632
F_6	9826
F_7	9142
F_8	9122

Table 29. Drug content

Formulations	Drug content
F_5	79.82
F_6	83.02
F_7	78.92
F_8	85.46

***In vitro* release studies**

1. Comparative *In vitro* release profile of F_5, F_6, F_{8A}, F_{8B} formulation

Table 30. Comparative *In vitro* release profile of F_5, F_6, F_{8A}, F_{8B} formulation

S. No.	Time (in min)	% of release of F_5 formulation	% of release of F_6 formulation	% of release of F_{8A} formulation	% of release of F_{8B} formulation
1	0	0.000	0.000	0.000	0.000
2	5	4.577± 0.128	15.437±0.945	4.990±1.350	14.100±1.108
3	10	9.250 ±0.824	22.553±3.085	7.307±1.666	22.000±1.155
4	15	18.840±0.277	28.967±0.447	15.710±1.467	27.643±0.904
5	20	26.247±0.967	36.333± 1.480	22.937±0.998	34.763±1.644
6	25	32.093±0.340	40.780±0.435	29.873±2.830	43.093±1.322
7	30	38.820±1.688	48.220±1.446	38.500±1.267	50.817±0.981
8	60	47.573±0.637	58.173±1.615	45.843±1.554	57.570±1.445
9	90	58.000±0.386	68.533±0.996	56.667±1.576	68.977±1.520
10	120	73.100±1.097	79.627±1.873	71.773±1.356	77.413±1.987
11	180	81.000±1.528	84.333±1.564	83.000±1.528	84.977±0.989
12	240	66.333±1.333	74.667±1.987	63.333± 1.667	74.000±0.577
13	300	45.500±0.987	42.257±0.765	48.000± 1.528	43.000±1.528

Mean ± S.D: n=3

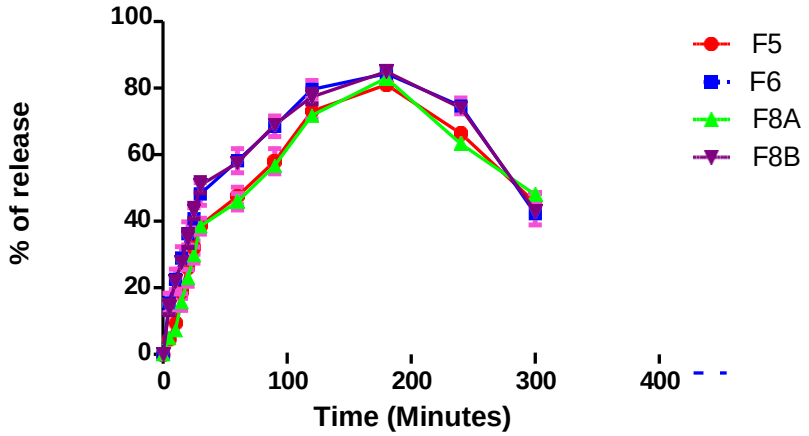


Fig.49. Comparative *in-vitro* release study of F_5, F_6, F_{8A}, F_{8B}

7.8.2. Diffusion Kinetics

The optimized gel formulations of (F_5, F_6, F_{8A}, F_{8B}) were fitted to various kinetic equations to determine the mechanism of drug diffusion rate as indicated by maximum r^2 value.

Table 31. Diffusion kinetics of F_5 formulation

S.No	Cummulative % release (Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	4.577	5	2.236	0.661	0.699	1.98
3	9.25	10	3.162	0.966	1	1.958
4	18.84	15	3.873	1.275	1.176	1.909
5	26.247	20	4.472	1.419	1.301	1.868
6	32.093	25	5	1.506	1.398	1.832
7	38.82	30	5.477	1.589	1.477	1.787
8	47.573	60	7.746	1.677	1.778	1.72
9	58	90	9.487	1.763	1.954	1.623
10	73.1	120	10.954	1.864	2.079	1.43
11	81	180	13.416	1.908	2.255	1.279
12	66.333	240	15.492	1.822	2.380	1.527
13	45.5	300	17.321	1.658	2.477	1.736

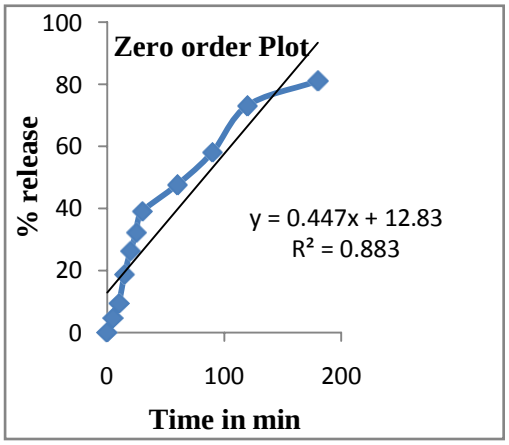


Fig.50. Zero order kinetic plot F_5

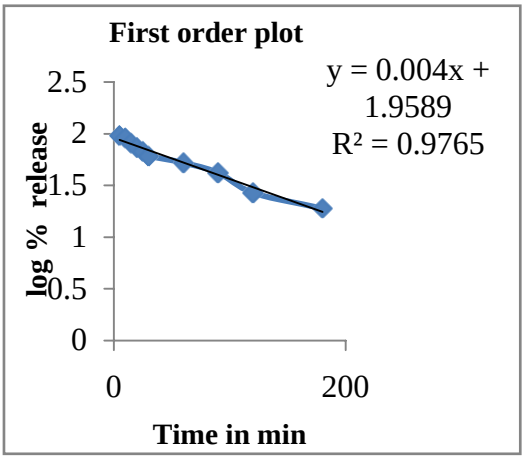


Fig.51. First Order Kinetic Plot of F_5

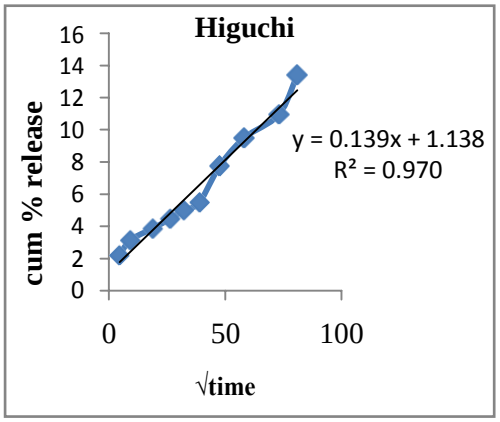


Fig.52. Higuchi plot of F_5

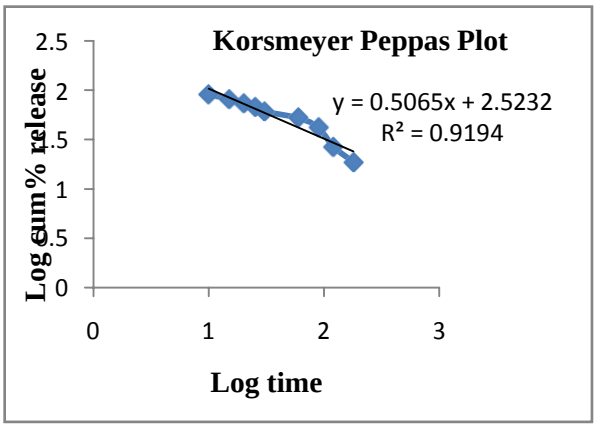


Fig. 53.KorsmeyerPeppas Plot of F_5

Table 32. Diffusion kinetics of F_6 formulation

S.No	Cummulative % release (Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	15.43667	5	2.236	1.189	0.699	1.927
3	22.55333	10	3.162	1.353	1	1.889
4	28.96667	15	3.873	1.462	1.176	1.851
5	36.3333	20	4.472	1.560	1.301	1.804
6	40.78	25	5	1.610	1.398	1.772
7	48.22	30	5.477	1.683	1.477	1.714
8	58.173333	60	7.746	1.765	1.778	1.621
9	68.533333	90	9.487	1.836	1.954	1.498
10	79.626666	120	10.954	1.901	2.079	1.309
11	84.33334	180	13.416	1.926	2.255	1.195
12	74.66666	240	15.492	1.873	2.380	1.404
13	42.25667	300	17.321	1.626	2.477	1.762

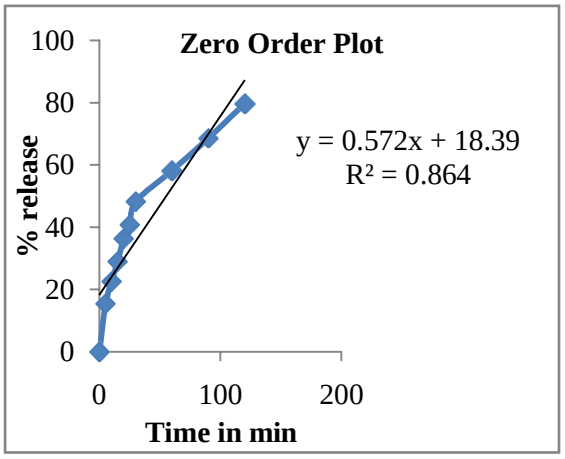


Fig.54. Zero order plot of F_6

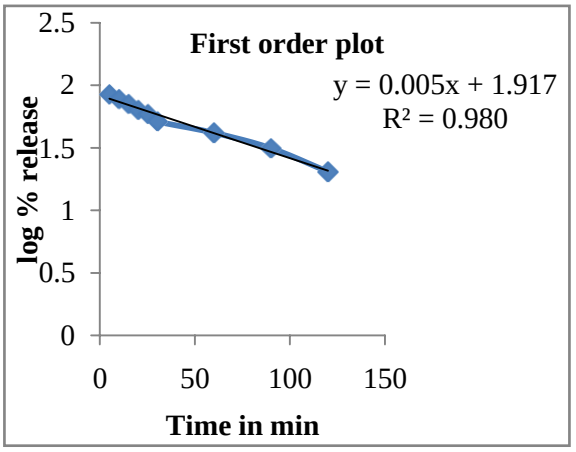


Fig.55.First order plot of F_6

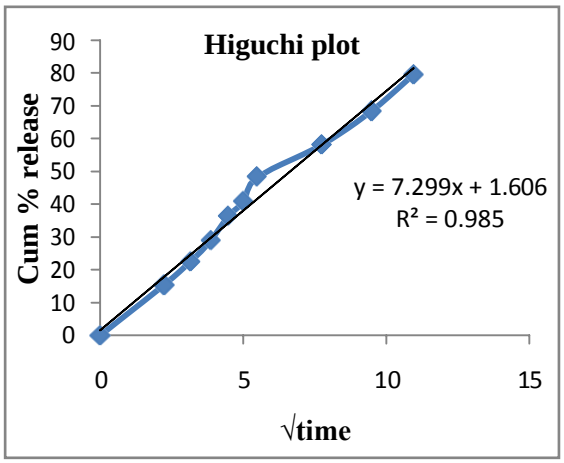


Fig.56. Higuchi plot of F_6

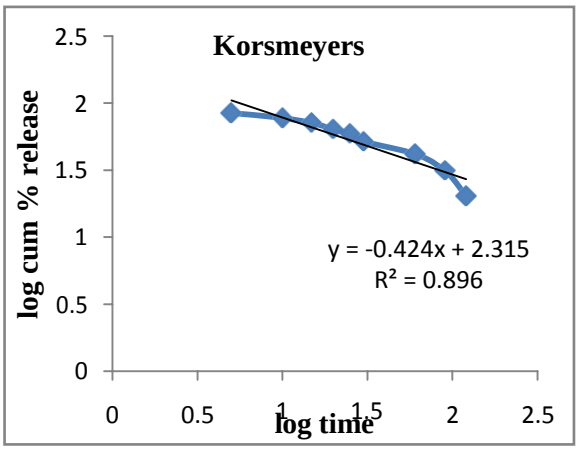


Fig.57.Korsmeyer plot of F_6

Table 33. Diffusion kinetics of F_{8A} formulation

S.No	Cummulative % release (Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	4.99	5	2.236	0.698	0.699	1.978
3	7.306667	10	3.162	0.864	1	1.967
4	15.71	15	3.873	1.196	1.176	1.926
5	22.93667	20	4.472	1.361	1.301	1.887
6	29.87333	25	5	1.475	1.398	1.846
7	38.5	30	5.477	1.585	1.477	1.789
8	45.84333	60	7.746	1.661	1.778	1.734
9	56.66667	90	9.487	1.753	1.954	1.637
10	71.77333	120	10.954	1.856	2.079	1.451
11	83	180	13.416	1.919	2.255	1.230
12	63.33333	240	15.492	1.802	2.380	1.564
13	48	300	17.321	1.681	2.477	1.716

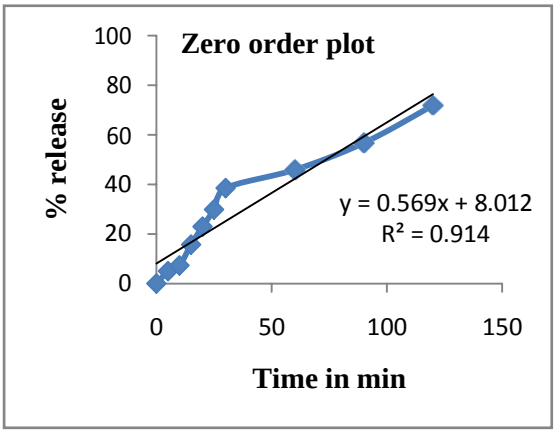


Fig.58. Zero order plot of F_{8A}

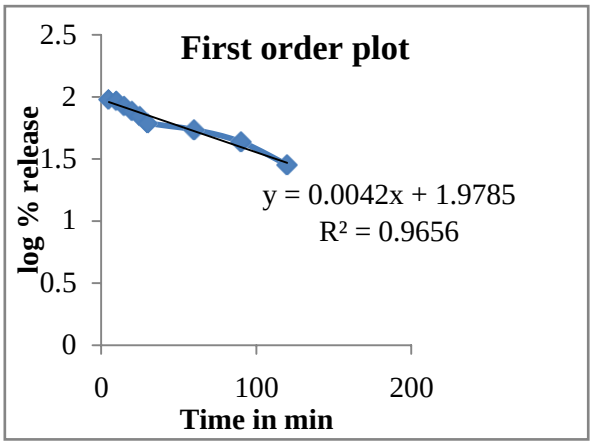


Fig.59. First order plot of F_{8A}

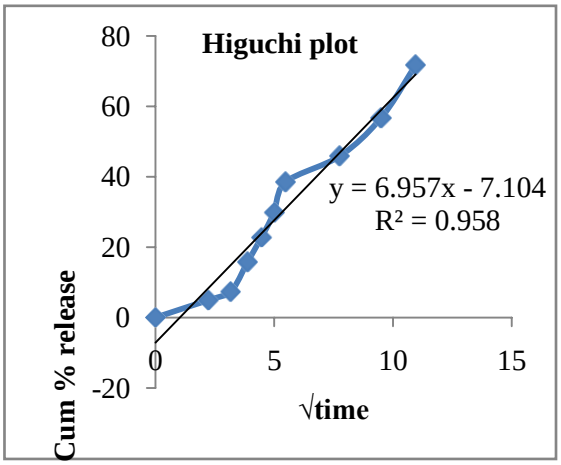


Fig.60. Higuchi plot of F_{8A}

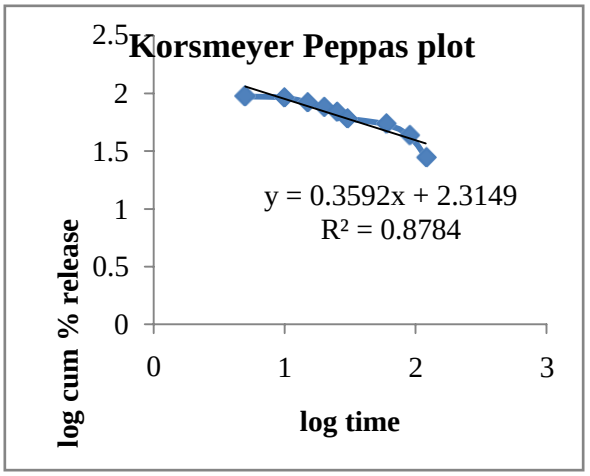


Fig.61. Korsmeyer Peppas plot of F_{8A}

Table.34. Diffusion kinetics of F_{8B} formulation

S.No	Cummulative % release (Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	14.1	5	2.236	1.149	0.699	1.934
3	22	10	3.162	1.342	1	1.892
4	27.64333	15	3.873	1.442	1.176	1.859
5	34.76333	20	4.472	1.541	1.301	1.814
6	43.09333	25	5	1.634	1.398	1.755
7	50.81667	30	5.477	1.706	1.477	1.692
8	57.57	60	7.746	1.760	1.778	1.628
9	68.97667	90	9.487	1.839	1.954	1.492
10	77.41333	120	10.954	1.889	2.079	1.354
11	84.97667	180	13.416	1.929	2.255	1.177
12	74	240	15.492	1.869	2.380	1.415
13	43	300	17.321	1.633	2.477	1.756

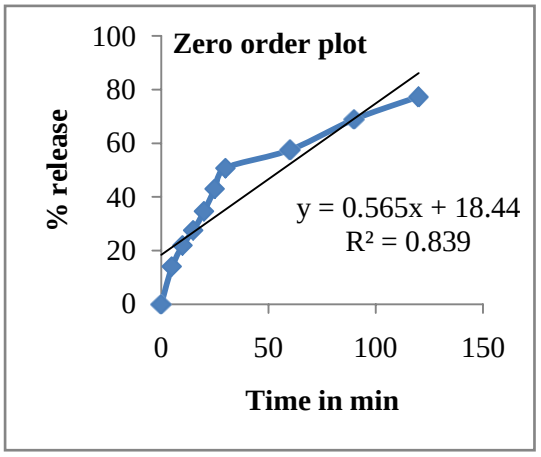


Fig.62. Zero order plot of F_{8B}

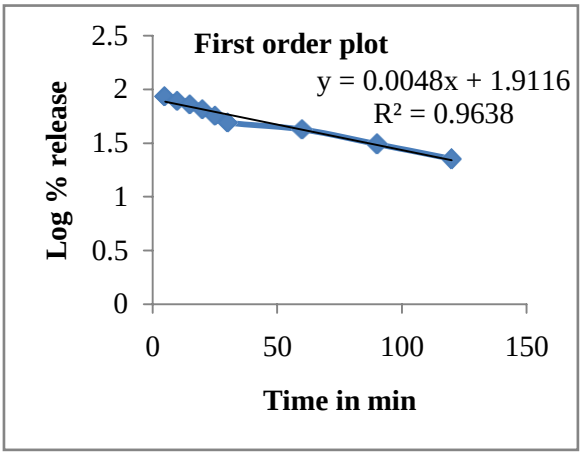


Fig.63. First order plot of F_{8B}

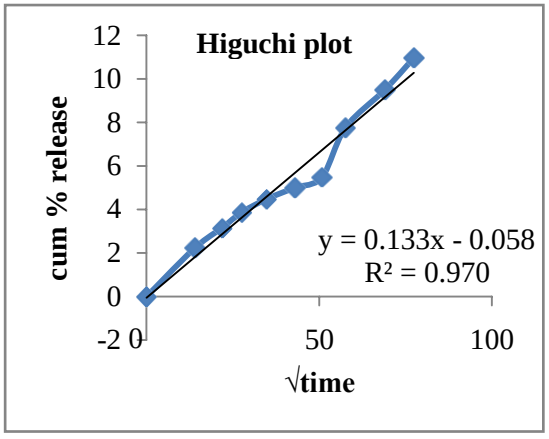


Fig.64. Higuchi plot of F_{8B}

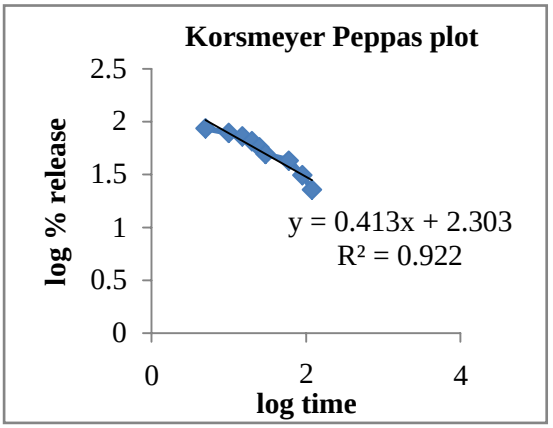


Fig 65. Korsmeyers Peppas plot of F_{8B}

Table.35. Diffusion kinetics

Formulation code	Correlation coefficient(r^2)				'n'-Diffusion Exponent
	Zero order	First order	Higuchi	Korsmeyerpeppas	
F_5	0.883	0.976	0.970	0.919	0.954
F_6	0.864	0.980	0.985	0.896	0.875
F_{8A}	0.914	0.965	0.958	0.878	0.963
F_{8B}	0.839	0.963	0.970	0.922	0.960

Release kinetic study revealed that the F_5,F_6, F_{8A} and F_{8B} follows Zero order and non fickian diffusion model. So they were subjected to antimicrobial screening.

Screening of Antimicrobial activity of Optimized Gel formulation

The anti-microbial activity for the given sample was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test microorganism of *Malassezia furfur* was obtained from Institute of Microbial technology, Chandigarand other test organisms *Candida albicans Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and Fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 Units/disc for *Candida albicans and* Ketoconazole 100 units/disc *Malassezia furfur*).

For Fungi

After72h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no fungal growth around the patch. The figures are shown in Fig 66,67 and the results are shown in Table 36

For Bacteria

After 24h the plates were observed. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the patch. The Figures are shown in fig. 68, 69 and 70 and the results are shown in Table 3.6

Fungi

Fig.66.*Malassezia furfur*

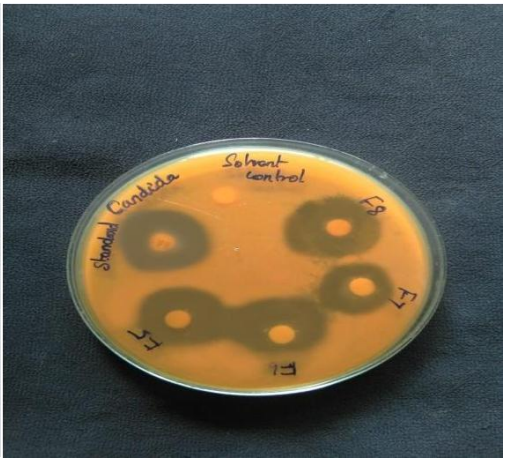


Fig. 67. *Candida albicans*

Bacteria

Fig.68. *Escherichia coli*

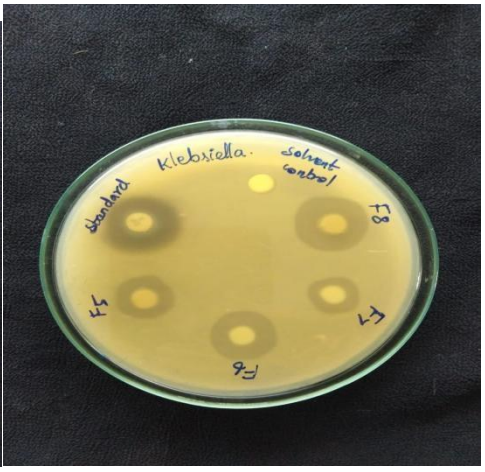


Fig.69. *Klebsiella aerogenes*

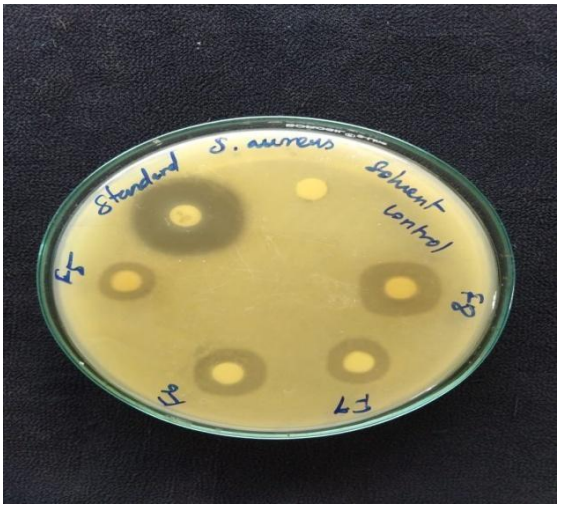


Fig.70. *Staphylococcus aureus*

Table 36.Screening of Antimicrobial activity

S.No	Name of the Organism	Zone of Inhibition in mm					
		Sample				Solvent Control	Standard
		F_5	F_6	F_7	F_8		
1.	*Malassezia furfur* (MTCC 1765)	27	28	23	33	Nil	35
2.	*Candida albicans* (NCIM 3102)	25	27	20	29	Nil	32
3.	*Staphylococcus aureus* (NCIM 2079)	12	16	15	20	Nil	35
4.	*Escherichia coli* (NCIM 2065)	18	22	20	24	Nil	38
5.	*Klebsiella aerogenes* (NCIM 2098)	17	20	15	22	Nil	30

Sample

F_5- Herbal Antidandruff Gel containing Aqueous extract of *Emblica officinalis*

F_6- Herbal Antidandruff Gel containing Aqueous extract of*Citrus limonum*

F_7- Herbal Antidandruff Gel containing Aqueous extract of Adjuvants such as *Allivum sativum, Zingiberofficinale, Aloe barbadensis*

F_8- Herbal Antidandruff Gel containing Aqueous extract of *Emblicaofficinalis,Citrus limonum* and adjuvants

Standard Ciprofloxacin- 5µg /disc for bacteria

Nystatin- 100 units / disc for *Candida albicans*

Ketoconazole- 100 units/disc for *Malassezia furfur*

Solvent DMSO

When compared to F_5, F_6, F_7 the formulation F_8 showed greater inhibition against *Malassezia furfur, Candida albicans, Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes.* So formulation F_8 has been selected for skin irritation , *ex-vivo* and stability studies.

Skin Irritation studies

Skin irritation test was carried out on 2 groups of healthy male rabbits each containing 3 rabbits as per the procedure explained already. The animals were numbered from one to six. After each application the skin surface was observed for erythema and edema after 7^{th}, 14^{th} and 21^{st} days. Thus it concluded that the Herbal Antidandruff Gel (F_8) have acceptable skin complications. The control group did not show any skin irritation.

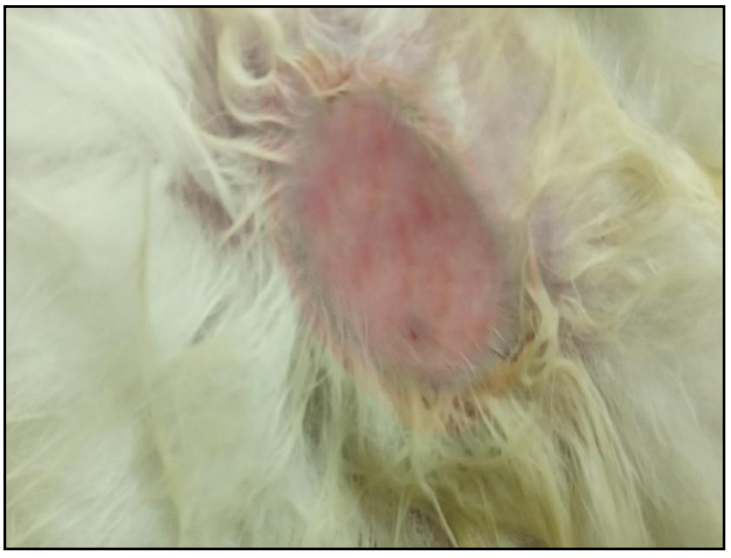


Fig. 71. Control- Carbopol 934

Fig. 72. After 7 days skin irritation study

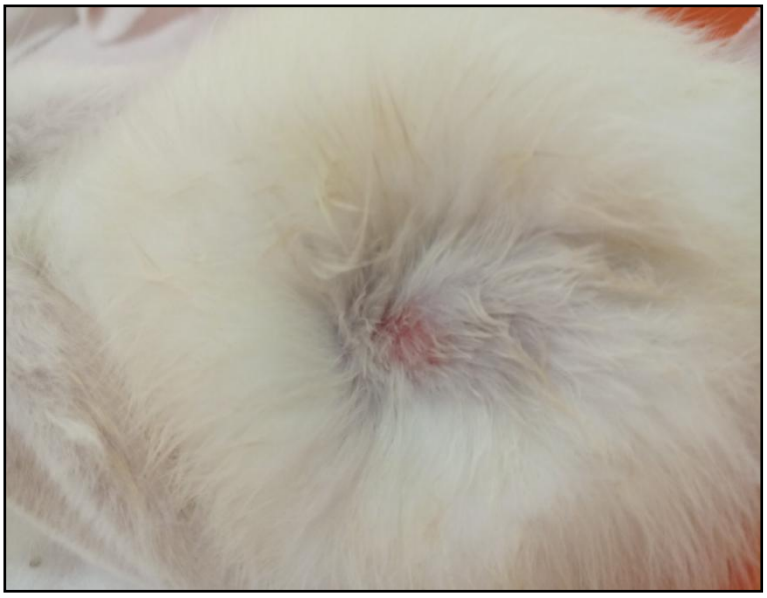


Fig.73. After 14 days of skin irritation study

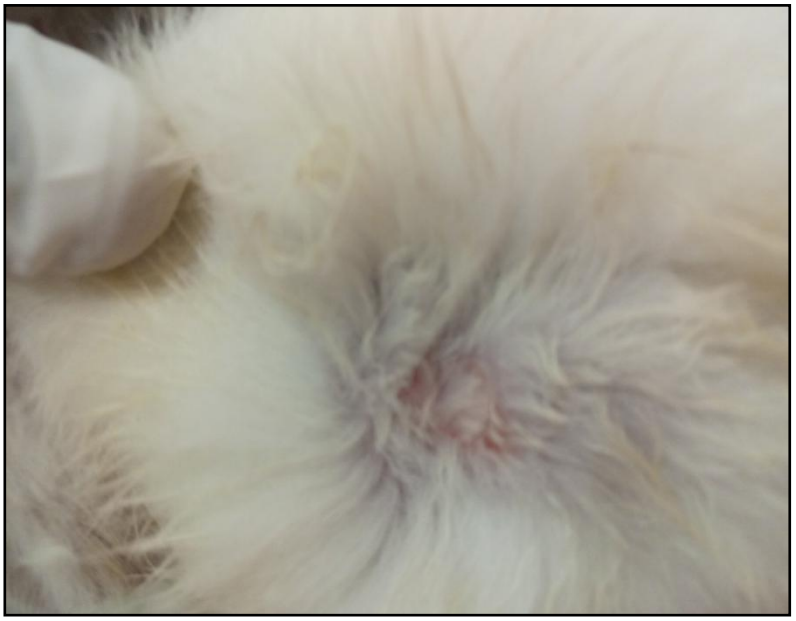


Fig.74.After 21days of skin irritation study

Table 37. Grading of skin irritation study

Name of the Animal	**Animal number**	**7th day**	**14th day**	**21st day**
Group 1	1	0	0	0
	2	0	0	0
	3	0	0	0
Group 2	1	0	0	0
	2	1	0	0
	3	0	0	0

0 – No erythema, 1 – Minimally perceptible erythema (faint pink), 2 – Marked erythema (red),

3 – Fiery red erythema with edema.

In Group 2 showed Minimally perceptible erythema (faint pink). The Group 1, carbopol gel base showed no erythema and edema. Thus it concluded that the Herbal Antidandruff gel (F_8) have acceptable skin complications. The control group did not show any skin irritatio

***Ex -vivo* study**

Fig. 75. *Ex-vivo* study

Table 38. *Ex-vivo* Skin Permeation of F_8

S. No.	Time (in min)	% of release of F_{8A} formulation	% of release of F_{8B} formulation
1	0	0.000	0.000
2	5	4.437±1.445	5.013±1.150
3	10	9.847±0.987	9.733±1.385
4	15	12.347±1.923	12.373±1.393
5	20	16.573±0.765	15.823±0.876
6	25	20.853±1.623	20.310±1.523
7	30	22.103±1.743	25.063±1.401
8	60	34.067±0.345	38.383±1.765
9	90	42.270±1.234	43.730±0.453
10	120	48.983±2.2	51.667±1.202
11	180	60.397±1.876	63.050±0.580
12	240	47.797±1.443	49.793±1.473
13	300	38.157±1.157	44.213±1.867

Mean ± S.D: n=3

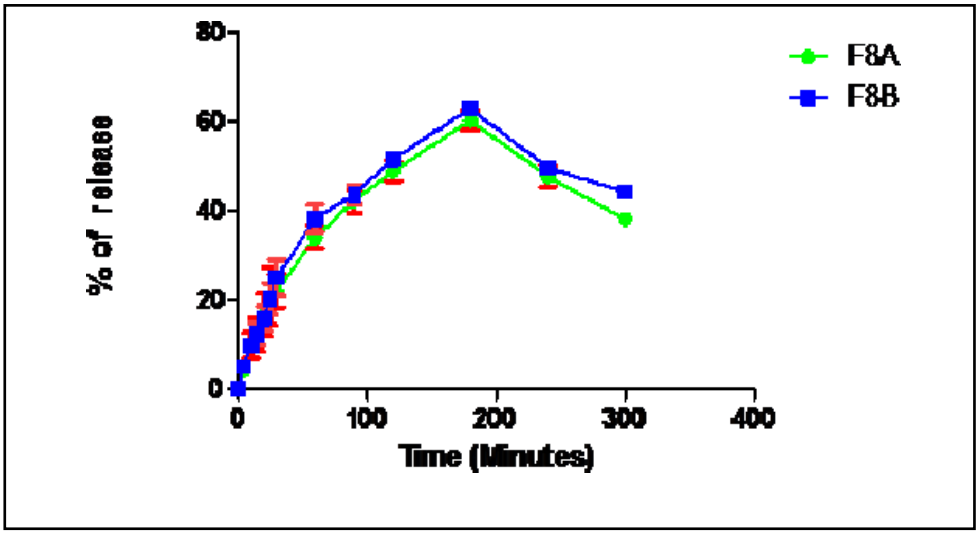


Fig. 76. *Ex-vivo* Skin Permeation of F_{8A} and F_{8B}

***Ex-vivo* Diffusion Kinetics**

Table 39.*Ex-vivo*Diffusion kinetics of F_{8A} formulation

S.No	Cummulative % release (Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	4.436666	5	2.236	0.647	0.699	1.980
3	9.816667	10	3.162	0.992	1	1.955
4	12.34667	15	3.873	1.092	1.176	1.943
5	16.57333	20	4.472	1.219	1.301	1.921
6	20.85333	25	5	1.319	1.398	1.898
7	22.10333	30	5.477	1.344	1.477	1.892
8	34.06667	60	7.746	1.532	1.778	1.819
9	42.27	90	9.487	1.626	1.954	1.761
10	48.98333	120	10.954	1.690	2.079	1.708
11	60.39667	180	13.416	1.781	2.255	1.598

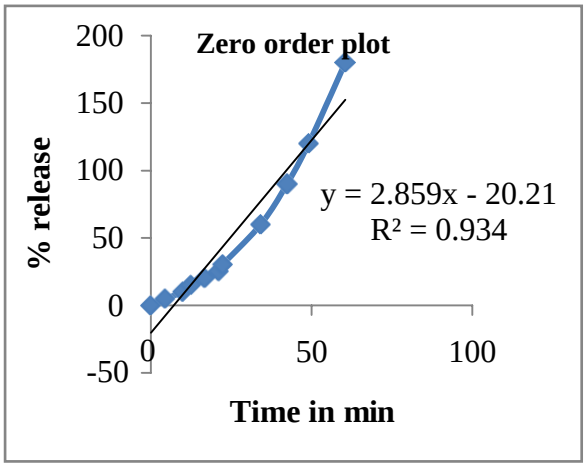


Fig.77.Zero order plot of F_{8A}

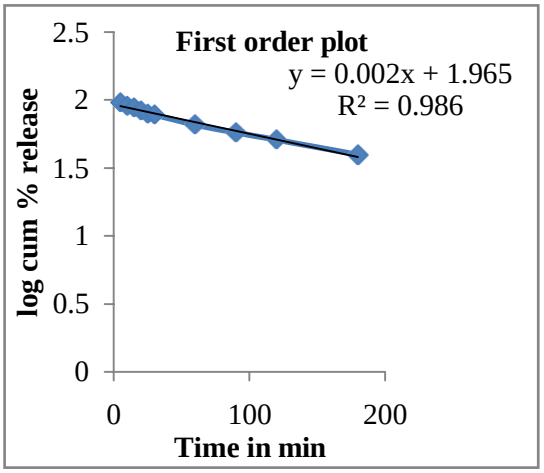


Fig.78. First order plot of F_{8A}

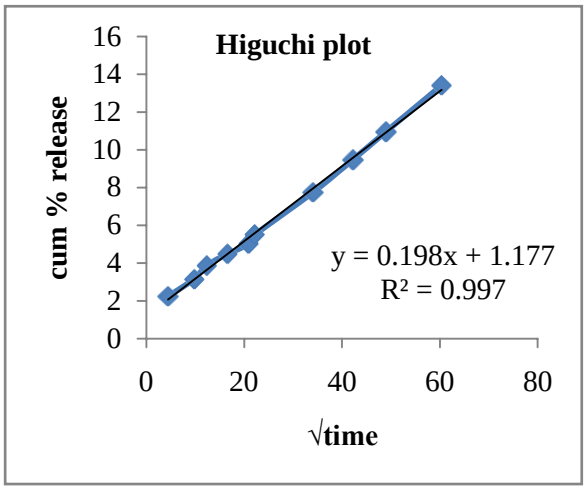


Fig.79. Higuchi plot of F_{8A}

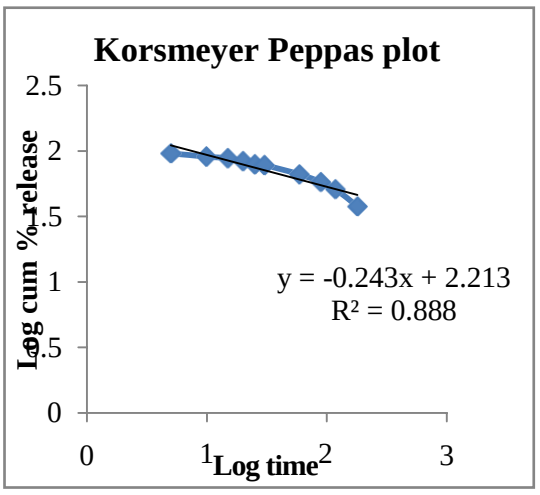


Fig.80. Korsmeyer Peppas plot of F_{8A}

Table 40. *Ex-vivo*Diffusion kinetics of F_{8B} formulation

S.No	Cummulative % release (Q)	Time (T)	Root T	Log (%) release	Log T	Log % remaining
1	0	0	0	0	0	2.000
2	5.013333	5	2.236	0.700	0.699	1.978
3	9.733334	10	3.162	0.988	1	1.956
4	12.37333	15	3.873	1.092	1.176	1.943
5	15.82333	20	4.472	1.199	1.301	1.925
6	20.31	25	5	1.308	1.398	1.901
7	25.06333	30	5.477	1.399	1.477	1.875
8	38.38334	60	7.746	1.584	1.778	1.790
9	43.73	90	9.487	1.641	1.954	1.750
10	51.66667	120	10.954	1.713	2.079	1.684
11	63.05	180	13.416	1.800	2.255	1.568

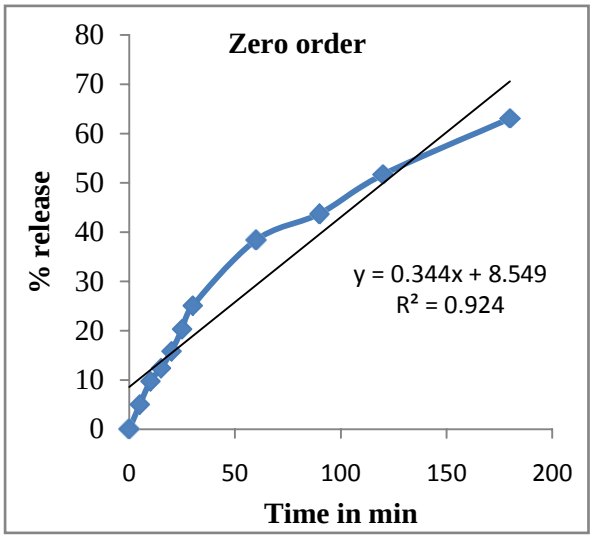


Fig. 81. Zero order plot of F_{8B}

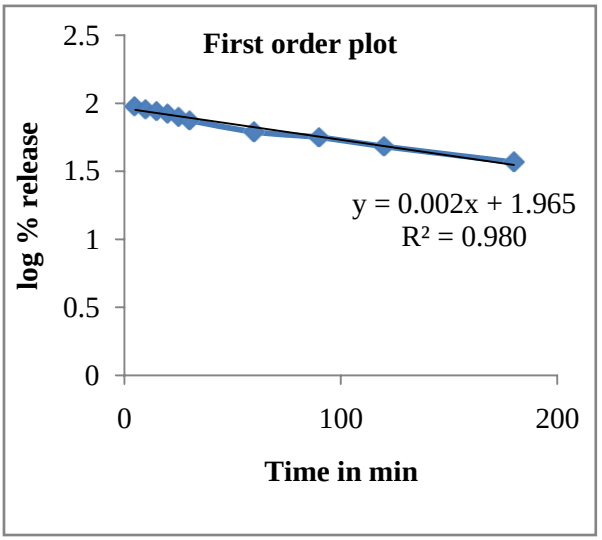


Fig.82. First order plot of F_{8B}

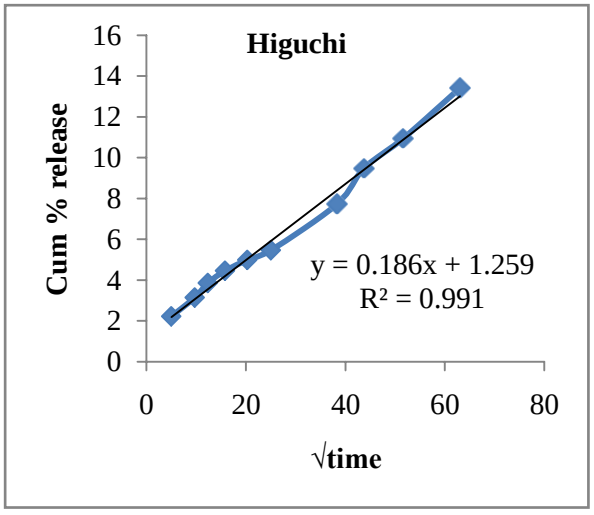


Fig.83. Higuchi plot of F_{8B}

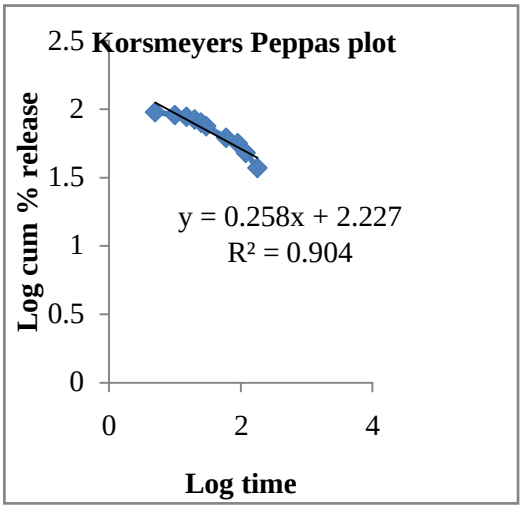


Fig.84.korsmeyers Peppas plot of F_{8B}

Stability study of Herbal Antidandruff Gel F_8

Table 41.Stability study of F_8

S. No	Parameter	Observation						
		Initial	At the end of 1st month		At the end of 2nd month		At the end of 3rd month	
			RT	40±2°C & RH 70±5%	RT	40±2°C & RH 70±5%	RT	40±2°C & RH 70±5%
1.	Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
2.	pH	7.2	7.0	7.2	7.2	7.2	7.2	7.1
3.	Spreadibility	18.07	18.06	18.07	18.07	18.07	18.07	18.07
4.	Extrudability	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
5.	% drug content	85.46	85.46	85.44	85.46	85.46	85.46	85.46

The stability studies of Herbal Antidandruff Gel of formulation F_8 was carried out for three months. During this period, the formulation were stable and showed no significant changes in visual appearance, pH, Spreadability, Extrudability, % drug content.

14. SUMMARY AND CONCLUSION

Eight batches of Herbal Antidandruff gel were formulated. All the formulated gels were subjected to Physiochemical evaluations such as Clearance, pH, Homogeneity, Spreadability, Extrudability, Viscosity, Drug content were evaluated. Based on the Physicochemical evaluations formulation $F_{5,}$ $F_{6,}$ F_7 and F_8 were selected as the optimized gel formulation.

Based on the phytochemical screening as well as HPTLC method on Aqueous extract of *Emblica officinalis* and *Citrus limonum* are rich in bioactive compounds. However, further studies are needed in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds responsible for antidandruff activity.

The FTIR graphs of active constituents, excipients and formulations results showed that there is no extra peak (or) broadening of peaks were observed and thus it indicates that there was no incompatibility between active constituents and excipients.

For the above selected formulations, i*n-vitro* release profiles were performed. The data obtained from *in vitro* release profile after 5 hours was fitted with various kinetic equations to determine the mechanism of active constituents release and release rate as indicated by higher correlation coefficients (r^2). The active constituents release from gel formulation follows zero order and non-fickian diffusion. Base on the i*n-vitro* release profile it was found that release of active constituents from prepared gels followed first order kinetics.

To confirm the release mechanism, the data of F_5, F_6, F_7, F_8 release were applied to Korsmeyer- peppas equation to find out the release exponent 'n', which indicates the mechanism of drug diffusion from the gel formulation. Then they were subjected to Screening of antimicrobial activity.

The Antimicrobial screening result showed that the formulation F_8 was highly inhibit the fungi and bacterial growth around the patch. So F_8 was selected for further evaluations such as Skin irritation , *Ex- vivo* and stability studies.

The Skin irritation study of F_8 gel formulation produced no erythema and edema in the animals during 21 days of study. Hence it is concluded that Herbal antidandruff gel is safe to use. The *Ex-vivo* studies showed the diffusion property of the herbal antidandruff gel formulation (F_8) through the skin at the end of 5 hours.

The stability study were performed for the selected formulation (F_8) by both the technique as per the ICH guidelines. The gel was subjected to stability study at $40^oC \pm 2^oC$ and 75±5% RH, samples were withdrawn on 1 month, 2 month ,3 month and analysed. The result shown that the product is stable for 3 months without change in physical changes .

Since the antimicrobial studies has given encouraging results in enhancing the antidandruff activity of F_8 formulation , it is concluded that the F_8 Herbal antidandruff gel may be subjected to further *in-vivo* and clinical trials.

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