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Formulation and Evaluation of Transdermal Patch in the Aqueous Extract of Anthurium Andraeanum Lind for Treatment of Inflammation

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I. INTRODUCTION

HERBAL MEDICINE:

According to WHO, The field of traditional medicine encompasses the entirety of knowledge, expertise, and methods derived from the theories, beliefs, and experiences inherent to various cultures. These methods can be used to prevent, diagnose, treat, orimprove physical and mental health conditions, as well as to maintain overall health.

Traditional herbal medicines are defined by the World Health Organization as naturally occurring, plant-derived substances that have been utilized to treat sickness within local or regional healing practices. These substances have undergone little to no industrial processing.

Duetoitsnaturaloriginand**lowerriskofadverseeffects**,traditionalherbalmedicine and its preparations have been used extensively for thousands of years in both developed and developing nations.(2)

Theseconsists
1. Herbs
2. HerbalIngredients
3. Herbalpreparations
4. Finishedherbal products

1) **Herbs-** Herbs are any unprocessed plant material, which can be whole, broken up, or powdered, such as leaves, flowers, fruit, seeds, stalks, wood, bark, roots, rhizomes, or other plant parts.
2) **Herbal ingredients-** In their raw form, herbal materials are either whole plants or specific sections of medicinal plants. These consist of herbs, fresh juices, gums, essential oils, fixed oils, resins, and dried herb powders.

These materials may be processed using a variety of regional techniques in some nations, including stir-baking, roasting, or steaming with honey, alcoholic beverages, or other ingredients.

3) **Herbal preparation-** The building blocks of completed herbal products are herbal preparations, which can consist of ground or powdered herbal materials as well as extracts, tinctures, fatty oils, expressed juices, and processed exudates of herbal materials.

Extraction, distillation, expression, fractionation, purification, concentration, fermentation, and other physical or biological processes are used in their production.

They also include concoctions created by boiling or steeping botanical ingredients in alcohol, honey, or other liquids.

4) **Finished herbal products-** Medicinal goods that only include herbal medications or herbal medication mixtures as active ingredients. Herbal concoctions created from one or more herbs may make up these. The phrase "mixed herbal product" can also be used when more than one herb is employed. In addition to the active compounds, they could also contain excipients. Herbal medications may traditionally include naturally occurring organic or inorganic active substances (such as minerals and animal components) that are not derived from plants.

However, finished goods or mixed goods that have been combined with chemically defined active ingredients—such as synthetic chemicals or separated components from herbal materials—are typically not regarded as herbal.

Classification ofHerbal Medicinesas perWHOGuidelines :(3)

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Category 1-Indigenous Herbal Medicines:

This category of herbal medicines is historically used in a local community or region and is very well known through long usage by the local population in terms of its composition, treatment and dosage.

Detailedinformation on this category ofTM, which also includes folkmedicines, may or may not be available. It can be used freely by the local community or in the local region.

Category 2-Herbal Medicinesin Systems:

These medications have a lengthy history of usage, are supported by unique ideas and concepts, and are recognized by national governments. Category of

AYUSH

1. Ayurveda
2. Yoga
3. Unani
4. Siddha
5. Homeopathy

Category 3-Modified Herbal Medicines

These herbal medications are the same as those listed in categories 1 and 2 above, but they have undergone some modification in terms of dosage, form, mode of administration, preparation techniques, and medical indications.

They must satisfy the safety and effectiveness standards set forth by national regulations for herbal medicines.

Category4 -Imported Products with an Herbal Medicine Base:

Allimportedherbalmedications,includingrawmaterialsandfinishedgoods,fall under this category. Herbal medications that are imported need to be registered and sold in their home countries.

The safety and efficacy data must fulfill the standards for the safety and efficacy ofthe recipient country's herbal medicine regulations and be reported to the national authorities of the importing country.

Novel drug delivery systems are the result of advancements in our knowledge of the pharmacokinetic and pharmacodynamics behaviour of drugs, which provide a more logical method for creating the best possible drug delivery system. The carriers used in innovative drugdeliverysystems (NDDS)help keep medication concentrations in therapeutic ranges for extended periods of time.[9]

1.1. There are several advantages of novel drug delivery systems over conventional drug delivery :(10)

1. Thebloodsystem'soratissue'soptimaltherapeutic medicationconcentrationcanbe sustained for an extended amount of time.
2. Apredeterminedrugratethatprolongstheduratio nofthedrug's activity.
3. Thedrug's short half-lifecould beextended.
4. Byfocusingon theplaceofaction, adverseeffects couldbereduced.
5. Lessmedicationwasteandfrequentdosingmaybe possible.
6. Increasedadherencefrompatients.

1.2. HerbalsinNovelDrugDeliverySystem:

The creation of a revolutionary drug delivery method for herbal medications has received a lot of interest during the last few decades. In the modern world, herbal medicines are gaining popularity due to their ability to treat a wide range of illnesses with less side effects and greater therapeutic results. Innovative herbal medicine carriers work by precisely locating the disease.

The utilization of novel drug delivery systems is beneficial as it allows for thedistribution of herbal medications at a predetermined pace and at the site of action, hence minimizing adverse effects and increasing drug bioavailability.

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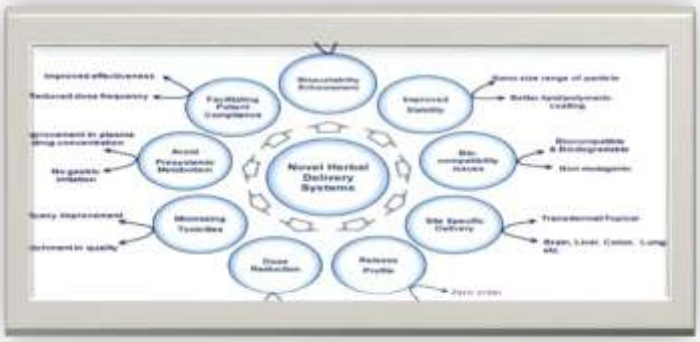

Fig.1.1 - Noveldrugdeliverysystemin Herbal

Many researchers are working to develop novel drug delivery systems using herbs, such as mouth-dispersing tablets, sustained and extended release formulations, mucoadhesive systems, transdermal dosage forms, microparticles, microcapsules, nanoparticles, implants, etc., because they have a lot of potential.[10]

A novel herbal medication delivery system provides new opportunities for thedelivery ofherbal drugs at the appropriate location, concentration, and time. It also provides a scientific means of confirming the standardization of herbal drugs. Herbal remedies werenot thought to be effective for development as innovative formulations due to processing challenges and a lack of scientific support. Contemporary phytopharmaceutical research can address the scientific requirements for herbal medicines to be integrated into innovative drug delivery systems, including but not limited to nanoparticles, microemulsions, matrix systems, solid dispersions, liposomes, and solid lipid nanoparticles. These needs include pharmacokinetics, mechanism of action, site of action, and precise dosage required. The herbal drugs can be utilized in a better form with enhanced efficacy by incorporating them in modern dosage forms. This can be achieved by designing novel drug delivery systems for herbal constituents.

1.2.1. Recentdevelopmentsinnoveldrugdeliversystem ofherbals:[11]

1. Phytosome
2. Liposome
3. Nanoparticles
4. Emulsions
5. Microsphere
6. Ethosome
7. Solid lipid nanoparticles
8. Niosomes
9. Proniosomes
10. TransdermalDrugDeliverySystem
11. Dendrimers
12. Liquid Crystals
13. Hydrogels

1.2.2. ImportanceofNovelDrugDeliverySystemsinHerbal medicines:

A revolutionary approach to drug distribution, the novel drug delivery system overcomes the drawbacks of the conventional drug delivery methods. The potential of the extensive Ayurvedic knowledge base in our nation has only just come to light. Nevertheless, the old and antiquated drug administration method utilized to give the patient the herbal remedy has decreased the medication's effectiveness. The application of innovative drug delivery technology in herbal medicine has the potential to enhance the effectiveness and mitigate the adverse effects of diverse herbal components and herbs. This is the main concept behindaddingacutting-edgedrugdeliverysystemtoherbal remedies. Inordertotacklemore serious ailments, it is crucial to combine Indian Ayurvedic remedies with innovative drug delivery systems. Herbal remedies were long disregarded for the creation of innovative formulations due to a lack of scientific support and challenges with processing, including the extraction, identification, and standardization of individual medicinal components in intricate polyherbal systems. But contemporary phytopharmaceutical research can address the scientific requirements for herbal medicines to be included in novel drug delivery systemslike nanoparticles, micro emulsions, matrix systems, solid dispersions, liposomes, solid lipid nanoparticles, and so forth. These requirements include pharmacokinetics, mechanism of action,

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site of action, precise dose required, and so forth.[12]

To reduce drug loss and degradation, avoid negative side effects, and boost drug bioavailability and the percentage of the drug accumulated in the needed zone, a number of drug delivery and targeting systems are presently being developed.[14]

1.3. SKIN:[14]

Theskincompletelycoversthebodyandcontinueswith themembraneslining the body orifice.

- Itprotectstheunderlyingstructures frominjuryandfrominvasionby microbes.
- Itcontains sensory(somatic)nerveendings ofpain,temperatureand touch.
- Itisinvolvedintheregulationofbody temperature

1.3.1 Structureof theskin

The skin is the largest organ of human body, accounting for about 15% of the whole adult body weight. Skin is one of the most readily accessible parts of the human body for topical administration. Penetration of molecules in the skin mainly occurs through three routes through intact stratum corneum, through the sebaceous follicle and through sweat ducts.

Topicaldrugdeliveryapproachisusedforlocalizedacti ononthebodythroughskin, ophthalmic,rectalandvaginalastopicalroutes. Skin performs various important functions:

- Protectionagainst thephysical,biological andchemicalagents.
- Preventionofexcesslossofwaterfromthebod y
- Vital rolein thethermoregulation
- Enzymeinepidermisand natureofthedrugs

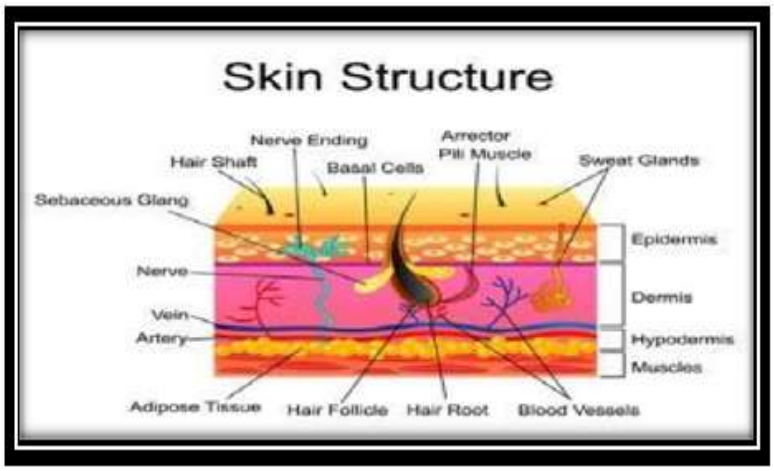

Fig.1.2-SkinStructure

The skin consists of three layers that are the epidermis, the dermis and the subcutaneous tissue. An average human skin surface contain, on an average 40-70 hair folliclesand200-300sweatductsoneverycm²oftheskin.ThepHoftheski nvaryfrom4to 5.6 the skin of an average adult body covers a surface area of about 2m² and receives about one third part of the blood circulating through the body.

1.3.1 A.Epidermis

It is a stratified squamous epithelium layer which is composed primarily of two types of cells dendritic and keratinocytes cells. The epidermis layers harbour a number of other cells such as melanocytes, Merkel cells and Langerhans cells. But the keratinocytes cells type comprises the majority of the cells by far.

- **Stratum germinativum**(basal layer or rowing layer): It contains column-shaped keratinocytes that attach to the zone of basement membrane with their long-axis perpendicular to the dermis.
- **Stratum spinosum**(prickly cell layer or squamous cell layer): It's thicknessvary from5-10cells.Intercellularspaces betweenspinouscellsarebridgedbyabundant desmosomes (adhering spot) to promote coupling between cells of the epidermis and provide resistance to the physical stresses.
- **Stratumgranulosum**(granularlayer):Itconsists oflivingcells;theseare responsible for further synthesis and modification of the proteins involved in keratinization. It is 1-3 cells layer

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in thickness

- **Stratum corneum**(horny layer): The corneocytes are rich in protein and low in lipid content (hydrophilic nature) are surrounded by a continuous extra cellular lipid matrix
- **Malpighian layer** (pigment layer): the layer whose protoplasm has not yet change into horny material

1.3.1. B.Dermis

It lies beneath the epidermis 1.5 -4 mm thick(thickest of the three layers of the skin). It is like home for most of the skin's structures including sweat glands and oil glands, hair follicles, nerve endings, and blood and the lymph vessels. The main components of thedermis are collagen and elastin. It stores much of the body's water supply.

The dermis also contains the scavenger cells from the immune system. In an event that a foreign organism tries to pass through epidermis , these cells will engulf and destroy.

It is an integrated system of fibrous, amorphous and filamentous connective tissue that accommodates stimulus induced entrance by nerve, vascular-networks, fibroblasts, appendages, mast cells. Its thickness ranges from 2000-3000µm. The principal component of the dermis is collagen and it represents **tissue** (Connective 70% of the skin's dry weight.

1.3.1.C.Subcutaneous Tissue

The subcutaneous tissue or hypodermis is not actually considered as a true part of the structured connective tissue, which comprises of loose textured, fibrous, white, connective tissue containing blood and lymph vessels, secretary pores of the sweat gland and the cutaneous nerves. Most investigators consider that drug permeating through the skin enters the circulatory system before reaching the hypodermis, although the fatty tissue could serveas a depot of the drug.

1.3.1. D.Bloodandlymphvessels

Arterioles form a fine network with capillary branches supplying sweat glands, sebaceous glands, hair follicles, and the dermis. Lymph vessels form a network through the dermis.

1.3.1. E.Sensorynerve endings

Sensory receptors (specialized nerve endings) sensitive to touch, temperature, pressure, and pain are widely distributed in the dermis. Incoming stimuli activate different typesofsensoryreceptors.ThePaciniancorpuscleissensitivetodeeppressure.Theskinis an important sensory organ through which individuals receive information about their environment. Nerve impulses, generated inthesensory receptors in the dermis, then to the sensory area of the cerebrum where the sensations are perceived.

1.3.1. F.Sweat glands

These are widely distributed throughout the skin and are most numerous in the palms of the hands, soles of the feet, axillae, and groins. They are formed from epithelial cells.There are two types of sweat gland. The common way opens onto the skin surface through tiny pores, and the sweat produced here is clear, watery fluid important in regulating body temperature.

1.3.2. Functionsoftheskin:[15]

1.3.2. A.Protection:

The skin forms relatively water proof layer, provided mainly by its epithelium, which protects the deeper and more delicate structures. As an important non-specific defence mechanism its act as a barrier against:

- Invasionbymicroorganism
- Chemicals
- Physicalagents
- dehydration

The epidermis contains specialized immune cells calledLangerhans cells, which are a type of microphage. Due to the presence of the sensory nerve endings in the skin the body reacts by reflex action to unpleasant or painful stimuli, protecting it from further injury.

1.3.2. B.Regulationofbodytemperature:

Body temperature remains fairly constant at about 36.8℃ across a wide range of environmental temperature ensuring that the optimal range for enzyme activity required for metabolismismaintained.Inhealth,variationsareusuallylimitedtobetween0.5and0.75℃, although it rises slightly in the evening, during exercise and in women just after ovulation.

1.3.2. C.Heatproduction:

When metabolic rate increases, body temperature rises, and when it decrease body temperature falls. Some of the energy diffusion during metabolic activity is in the form of heat and the most active organs produced in the body and heat lost to the environment .The principal organs

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involved are

- Skeletal muscles - contraction of skeletal muscles produces a large amount of heatand the more strenuous the muscular exercise, the greater the heat produced
- Theliver isverymetabolicallyactive andheatisproducedasa by-product.Metabolic rateandheatproductionareincreasedafter eating
- Thedigestiveorgansproduceheatduringperistal sisandduringthechemical reactions involved in digestion.

1.3.2. D.Heatloss

Most heat loss from the body occurs through the skin. Small amounts are lost in expired air, urine, and faeces. Only heat loss through the skin can be regulated. Heat loss by the outer routes cannot be controlled.

Heat loss through the skin is affected by the difference between body and environmental temperatures, the amount of the body surface exposed and the type of clothes worn. Air insulates against heat loss when trapped in layers of clothing and between the skin and clothing. For this reason several layers of light weight clothes provide more effective insulation against low environmental temperatures than one heavy garment.

1.3.3. E.Drugtransport acrossskin

` There are mainly two important layers in the skin epidermis and dermis. Bloodvessels are profusely distributed beneath the skin in the subcutaneous layer.
Therearetwoprimarymechanisms intended fordrug absorption throughthe skin

1)Intercellular 2)Transcellular

The most common route of delivery is through the sebaceous route. Permeation tends to occurthroughintercellularmatrix,butviatrans cellularpathwayithasbeenshowntoprovide a quicker alternative route for highly polar molecules.

1.3.3 Routesofpenetration:[17]

The diffusion may enter the blood vessels either directly through the epidermis or indirectly through diffusion. Primarily through the shunt pathway, this includes the sweat ducts, sebaceous glands, and hair follicles. Due to this, there are two primary entry points

1.3.4. Trans-cornealpenetration:

❖ **Intracellularpenetration:**

Drug molecules move through the stratum corneum cellular structure. It typically occurs with hydrophilic drugs. Water builds up close to the protein filaments outer surface as the stratum corneum hydrates. This immobilized water appears to be permeable to polar molecules.

❖ **Intercellularpenetration:**

Non-polar substances travel along the intercellular pathway. These molecules disperse throughoutthenon-aqueouslipidmatrixthatisabsorbedinthespacebetweentheproteinfilaments.

❖ **Transappendegeal penetration:**

The shunt pathway is another name for this (Bodde et al.). In this pathway, the drug molecule may pass through hair follicles, the pilosebaceous apparatus sebaceous pathway, or the salty sweat glands' aqueous pathway (Barry, 1987). The transappendegeal pathway has a relatively small surface area (less than 0.1% of the total surface), which is why it is regarded as being of minor importance. However, for large polar compounds, this route might be significant. The relative ability of the penetrant to partition into each skin phase, which is particularly important determines the route through which permeation occurs .The transdermal permeation can be.

1. Represented as a composite of a series of events occurring in this order. Adsorptionofapenetrant molecule ontothesurfacelayersofstratumcorneum.
2. Diffusionthroughstratumcorneum andthroughviableepidermis.
3. Finallythroughthepapillarydermisintothemicrocirculation.

The peripheral circulation is sufficiently quick, and the viable tissue layer and capillaries are relatively permeable. The rate-limiting step is therefore diffusion through the stratum corneum (Scheuplein, 1965). It functions as a passive diffusion medium, the stratum corneum. Therefore, the various skin tissue layers can be represented by a simple multilayer model for transdermal drug diffusion.

1.4 Transdermaldrugdelivery system:

Transdermal drug delivery systems (TDDS) are designed to deliver the drug substances from the surface of the skin through its various layers into the systemiccirculation. TDDS is the delivery of drugs through the skin to achieve

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systemic effects. Transdermal patches control the delivery of drugs at controlled rates by employing an appropriate combination ofhydrophilicandlipophilic polymers.

"Transdermal therapeutic systems are defined as self-contained, discrete dosageforms which, when applied to the intact skin, deliver the drug(s), through the skin, at a controlled rate to the systemic circulation."[18]

Now a days herbal drugs are more used as they are safe because of having less side effectsorlowcost.Andpeopletrustherbaldrugsmorethan Allopathy.HerbalTransdermal patches can develop valuable assessment and drug safety by additional site specific the way and temporal position in the body's imperative to reduce the number and size of doses required to achieve the objective of systemic medication during topical application to the intact skin surface. [20]

1.4.1. HerbalTransdermal patch:

Table1.1-ListofMarketedAvailableherbalTransdermal Patches

S.No	Brand	ActiveMaterial(Plant Source)	Indications
01.	Restopatch	Jatamansi/Musk(Nardostachys rhizome extract and Sarpagandha/Serpentine root jatamansi) wood (Rauwolfiaserpentina) flower extract	Helpsfallasleep fasterandeasier
02.	Boswelliaturmeric patch	ShallakiGuggul(Boswelliaserrata) Haridra (Curcuma longa)	Rheumatoidarthritis,osteo arthritis,joint pain
03.	Relyonherbalpain relievingpluspatch	Menthol, Methyl salicylate and Camphor,Boswellia,Eucalyptus, Capsaicin	Rheumatoidarthritis, osteoarthritis, joint pain
04.	RelyonMosquito RepellentPatch	Neemoil,NirgundiOil, Citronella Oil & Vitamin B1	Protectionfrombug bitesandmosquitoes
05.	RelyonEve	Boswelliaserrata	Menstrualpainrelief patch

1.4.1. Typesoftransdermalpatches:[22]

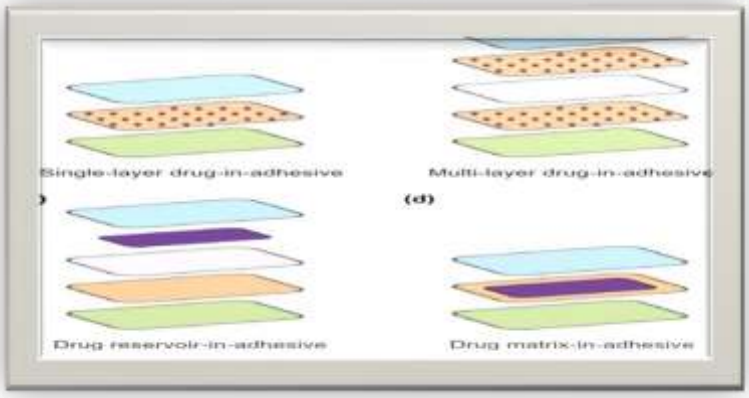

Fig.1.3 – Typesoftransdermalpatches

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a) **Single layer drug-in-Adhesive Patches:** In this technique, the medication stays in contact with the skin-attached sticky layer. In the adhesive layer, the medicine is released and the layers of the skin and other materials are attached together.

b) **Multi-layer drug-in-Adhesive Patches:** The single-layer drug in adhesive, which includes introducing the medicinedirectly into theadhesivelayer,is comparable to themulti-layer drug in adhesive. In this technique, the medication is immediately released from the reservoir by one of the layers. This patch features both a long-lasting backing and a short- term liner layer.

c) **Reservoir type patches:** The reservoir transdermal system, as opposed to the single-layer and multilayer drug-in-adhesive systems, has a separate drug layer. This system has a compartment for fluids containing a medicine solution or suspension that is kept apart from the liner by an adhesive and membrane. The backing layer also supports this patch scheme. The rate of release in the reservoir system is zero.

d) **Matrix type patches:** The drug layer of the semisolid matrix that makes up the matrix system is in direct contact with the liner layer and contains the drug as a solution or suspension. The drug layer is partially covered by the adhesive layer in this device.

e) **Vapor patches:** The adhesive layer in this kind of patch system not only holds the various layers together but also lets out vapor. These brand-new patches are widely used to release essentialoilsforupto6 hours.Thesepatches aremostlyutilizedincasesofdecongestionand release essential oils. There are many different kinds of vapor patches on the market that are designed to increase sleep quality and lessen cigarette smoking.

1.4.2. Idealproperties:

- Two-yearmaximumshelflife.
- Smallpatch size(lessthan40 cm^2).
- Convenientdosingfrequency(oncedailytooncep er week). Acceptableintermsofappearance(i.e.,clear,whit ecolour).Easyreleaseliner removal (e.g., for youngsters and elderly patients).
- Simplepacking(i.e.,the fewestpouchesandstepsneededtoinstallthesyste m).
- Sufficientskinadherence,i.e.,nofalloffthroughou tthedosageintervalandsimple removal without causing skin trauma.
- No cold flow, or residue, around the patch's edge during storage, after application to the skin, or underneath the patch after removal.

1.4.3. Advantage:(29)

- Thefirst passof hepatic metabolism is avoided.
- Controlleddeliverythatisprolongedandsustained .
- Easyaccessto thedesiredareaorunhealthy area.
- Deliver a generally constant and sustained medication concentration in the plasma as compared to traditional systems, which frequently exhibit fluctuations in concentration.
- Makesitpossible tousemedications withbriefbiological half-lives.
- The simplicity of stopping the dose in the event of any adverse effects, either systemic or local.
- Itissimpletostopreceivingtherapyatanytime.
- Thereisnovariationinthebioavailabilityofmedic ationsadministeredorallydueto factors like pH, intestinal motility, meal intake, etc.
- Appropriateadministrationthatispainless.
- In a wide range of unfavorable patient populations, an absorption rate can remain stable.
- Increasingpharmacologicalandphysiological return.
- Preserveasteadylevelof powerfulmedicinesintheplasma.
- Increasedpatientcompliancebecausedifferentdo seprofilesarenotincluded.
- Theabilitytomorecarefullytargetthespot wheremedicineis delivered.
- Offerself-administrationthatisappropriate.
- Easeofdoseinterruptionintheeventofanyadverse effects,eithersystemicor local.
- Transdermaladministrationisanexcellentoptionf ormedicationsthatupsetthe stomach and intestines because it minimizes these organs direct contact.
- Whilethevolumeandareaofgelapplicationarespe cifiedinapatch,thisisnot always the case with other applications.
- Due to the accumulation of plasma levels at the conclusion of the dosing interval, adverse effects were condensed, and therapy was improved.
- The convenience with which drug delivery can

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be stopped by simply removing the skin patch.

1.4.4. Disadvantage:(36)

- TheTDDSisonlyappropriateformedicationsthat aremoderatelypowerful.
- Thereisachanceoflocalirritabilityandarrhythmia .Themedicationsmaybe denatured by epidermis generated enzymesor enzymes derived from microorganisms found on the skin.
- Theonlymedicationsthatactbyrequiringverylow plasmaconcentrationsare suitable for usage.
- The drug's physicochemical characteristics must be favorable for stratum corneumpenetration.
- On a single person, between individuals, and withaging, the skin's barrier function varies from one place to another.
- Variationsinskin-surfaceabsorptioneffectiveness.
- Adhesionproblemswithsomeskintypes,suchase xcessivelyoilyskin.
- Difficulty in determining the maximum amount of time a patch can be left in anylocation owing to permeability variations (often not more than 7 to 10 days).
- Ifamedicationdosegreaterthan10mg/dayisrequir edforitstherapeutic application, transdermal delivery will be very difficult.

1.4.5. Moderntechniquesoftransdermaldrugdeliverysystem:(41-51)

A)Structure-basedenhancementtechniques:

- **Transdermal patch:**

The device that is loaded with drug candidate and typically applied to the skin to deliveraparticulardose ofmedication acrossthe skin and into theblood circulation is known as a transdermal patch or skin adhesive patch.

- **Microfabricatedmicro needles**:

These are the tools with characteristics of both a transdermal patch and a hypodermic needle that can deliver medication and effectively cross the membrane .The system consistsofadrug reservoirand a fewmicroneedlesthat extendfrom thereservoirand help deliverthe drug by penetrating the stratum cornea and epidermis.

Macroflux:

These are objects with a surface area of roughly 8 cm^2 and 300 microprojections per cm^2, with a maximum individual microprojection length of 200 m.

There are three different varieties of Macroflux. One of them is the Dry-Coated Macroflux system, which consists of a microprojection array coated with medication and attached to an elastic polymer adhesive backing

- **Metered-DoseTransdermalSpray(MDTS):**

It is a liquid preparation in the form of a solution that is applied topically and is composedofavolatileornon-volatilevehiclethatcontainsafullydissolvedmedicationin the solution.

When using MDTS, the drug is more effectively absorbed through the skin and at a sustained level. The MDTS may have the following benefits: **A)** It enhances deliverypotential without causing skin irritation because it is non-occlusive**. B)** A higher level of acceptability. **C)** Dose adaptability and Simple production

B.Electrically-basedenhancement techniques:

- **Iontophoresis:**

It involves applying a small amount of current (a few mill amperes) to the skin only in a specific area while the electrode stays in contact with the formulation that needs to beapplied. Iontophoretic delivery of lidocaine is regarded as a nice approach for rapid onset of anesthesia, and pilocarpine delivery can be used as an example to induce sweat in the diagnosis of cystic fibrosis.

- **Ultrasounds:**

Intheseprocedures,the drugsubstanceiscombinedwithacouplingagent (typicallya gel, cream, or ointment) to facilitate the transfer of ultrasonic energy from the system to the skin. The lipids in the stratum cornea must be ruptured in order for the medication to pass through the biological barrier.

- **Photomechanical waves:**

Due to the development of transient channels, photomechanical waves significantly contributed to the stratum cornea becoming highly permeable to drug substance.

- **Electroporation:**

Applying high-voltage pulses to the skin causes disruption through electroporation. Most frequently, high voltages (>100 V) and brief treatment times (milliseconds) are used. The creation of transient pores during electroporation is

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thought to be the cause of the increased skin permeability.

The technology has been successfully applied to increase the skin permeability of molecules with varying lipophilicity and size, such as proteins, oligo nucleotides, small molecules, and peptides.

❖ **Electro-osmosis:**

When a voltage difference is applied to a porous membrane that already has some charge, a bulk fluid or volume flow occurs without the presence of concentration gradients. Electro osmosis is the name of this procedure.

C) Velocitybasedenhancementtechniques:

❖ **Needle-FreeInjections:**

This transdermal delivery system uses a dependable energy source to propel liquid or solid particlesthroughtheskin's outerlayersat supersonicspeeds in order to deliverthedrug. The mechanism entails pushing compressed gas (helium) through a nozzle in such a way that the resulting drug particles are entrained within the jet flow and move at a fast enough speed to penetrate.

❖ **PowderjectDevice:**

By using a high-speed gas flow, the solid drug particles are propelled across the skin. This is made up of a gas canister that lets helium gas at a high pressure enter a chamber at the end of which is a drug cassette with powdered drug between two polycarbonate membranes. Following release, the gas expands quickly and forms a powerful motion that moves like a wave as it travels down the nozzle due to the instantaneous rupture of both membranes that is frequently observed. A 600-900 m/s speed range is used for this.

1.4.6. VariousmethodsforpreparationTDDS:(57-61s)

1.4.6.A. AsymmetricTPXmembranemethod:

A sample patch may be made for this using a heat-sealed polyester film (type 1009, 3m) with a backing membrane that has a concave of 1 cm in diameter. A TPX poly (4- methyl-1-pentene) asymmetric membrane is used to cover the concave membrane, which is then sealed with an adhesive.

1.4.7.B.CircularTeflonmould method:

Solutions with different ratios of polymers are utilized in an organic solvent. Half as much of the same organic solvent is used to dissolve the calculated amount of medication. The second half of the organic solvent is used to dissolve enhancers at various concentrations before they are applied. TheplasticizerdiN-dutylphthalateisincludedinthedrugpolymersolution. Theentire mixture must be agitated for 12 hours before being placed into a Teflon mold. In order to managesolventvaporizationinalaminarflowhoodmodelwithanairspeedof0.5m/s,the molds must be put on a flat surface and covered with an inverted funnel. For 24 hours, the solvent is allowed to evaporate. Before evaluation, the dried films must be kept for a further 24 hours at 250.5°C in a dessicator containing silica gel to prevent aging effects. Within a week of preparation, the type films must be reviewed.

1.4.7.C.Mercurysubstratemethod:

This method involves dissolving the medication and plasticizer in a polymer solution. The solution mentioned above should be stirred for 10 to 15 minutes to provide a uniform disperse on before being placed over a mercury surface that has been leveled and covered with an inverted funnel to prevent solvent evaporation.

1.4.7.D. By using IPMmembranes method:

In this procedure, the medication is dissolved in asolution of water and carbomer 940 polymers that contains propylene glycol, and it is then swirled for 12 hours in a magnetic stirrer. Triethanolamine is to be added in order to neutralize the dispersion and make it viscous. If the drug's solubility in an aqueous solution is particularly poor, buffer pH 7.4 can be employed to create a solution gel. The IPM membrane will incorporate the gel produced.

1.4.7.E.ByusingEVACmembranes"method:

Polyethylene (PE) and ethylene vinyl acetate copolymer (EVAC) membranes can be used as rate control membranes to prepare the target transdermal therapeutic system. Propylene glycol is used to make gel when the drug is not soluble in water. Propylene glycol is used to dissolve the drug; carbopol resin will then be added to the solution and neutralized with a 5% w/w sodium hydroxide solution. The drug is applied to a backing sheet that covers thedesignated areaand is in theform ofagel. Tocreatealeak-proofdevice, arate-regulating membrane will be positioned over the gel, and the edges will be heated to seal.

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1.4.7.F.Aluminiumbackedadhesivefilm method:

If the loading dose is greater than 10 mg, transdermal drug delivery systems may result in unstable matrices. The method of using adhesive film with aluminum backing is appropriate.

Chloroform is the preferred solvent for its preparation because it is soluble in the majority of drugs and adhesives. Chloroform is used to dissolve the drug, and then adhesive material is added and dissolved in the drug solution. Aluminum foil lines an aluminum frame made of mold, and tightly fitting cork blocks are used to blank off the ends.

1.4.7. Factorsaffectingtransdermalpermeation .(61)

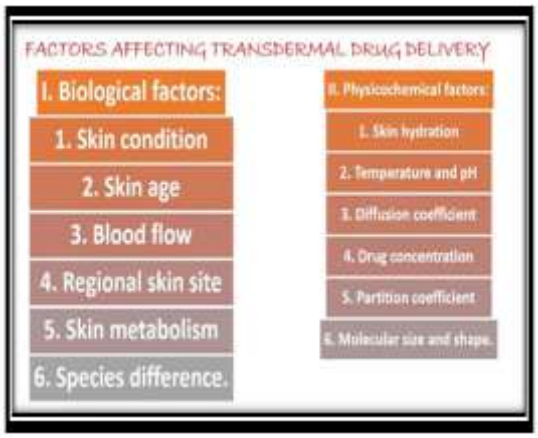

Fig.1.4-Factorsaffectingtransdermal permeation

1.4.8. A.Biological factors:

❖ **Skinconditions:**

Theintact skin serves as abarrier,but manyagents such as acids and alkalis can penetratethe skin by piercing the barrier cells. In addition, many solvents can access the complex, dense structure of the horny layer. Lipid fraction is removed by solvents like methanol and chloroform, creating artificial shunts that make it easy for drug molecules to pass through.

❖ **Skinage:**

There is no discernible difference between the skin of adults and children and that of older people in terms of permeability. Children exhibit toxic effects due to their greater surfacearea per unit of body weight, which led to severe side effects from powerful steroids, boric acid, and hexachlorophene.

❖ **BloodSupply:**

Transdermalabsorptionmaybeimpactedby changesinperipheralcirculation.

❖ **Regionalskinsite:**

Site-specificdifferences exist in skin thickness, stratum corneum type,andappendage density. These components have a big impact on penetration.

❖ **Skin metabolism:**

Steroids, hormones, chemical carcinogens, and some drugs are all processed by the skin. So the effectiveness of a drug that has penetrated the skin has been determined by skin metabolism.

❖ **Species differences:**

Species differences in skin thickness, appendage density, and keratinization of the skin all affect penetration.

1.4.9.B.Physicochemicalfactors:

❖ **Skin hydration:**

The permeability of skin increases significantly when it comes into contact with water. The most essential element in increasing skin permeation is hydration. Therefore, humectant use occurs during transdermal delivery.

❖ **TemperatureandpH:**

With temperature changes, drug permeation multiplies ten times. With a drop in temperature, the diffusion coefficient falls. Based on the pH and pKa or pKb values, weak acids and bases separate. The drug concentration in skin is based on the percentage of unionized drugs. So, temperature and pH play a major part in drug penetration.

❖ **Diffusioncoefficient:**

The drug diffusion coefficient affects drug penetration. The properties of the drug, the diffusion medium, and their interactions determine the drug's coefficient of diffusion at a constant temperature.

❖ **Drug concentration:**

The flux has an opposite relationship with the gradient of concentration across the barrier,and the gradient will be higher if the drug concentration is higher across the barrier.

❖ **Partition coefficient:**

For effective action, the ideal partition

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coefficient (K) is needed. High-K drugs are not yet ready to leave the Lipid layer of skin. Additionally, drugs with low K levels won't permeate.

❖ **Molecularsizeandshape:**

Small molecules absorb drugs more quickly than large ones; the relationship between molecular weight and drug absorption is inverse.

1.4.9. C.Environmentalfactors:

❖ **Sunlight:**

Sunlight causes blood vessel walls to thin, which results in bruising in areas that have been exposed to the sun with only minor trauma. A freckle, or solar lentigo, is the most obvious pigment change brought on by the sun.

❖ **Cold Season:**

Result frequently in dry, itchy skin. As a reaction to the drying effects of the weather, skin produces more oil. A good moisturizer will aid in reducing the effects of dry skin. Finally, consuming a lot of water can keep your skin hydrated and radiant.

❖ **AirPollution:**

Acne or spots can result from dust blocking pores and increasing bacteria on the skin's surface and on the face. This has an impact on drug delivery via the skin. Invisible chemical pollutantsintheaircaninterferewiththeskin'snaturald efensemechanism bydestroyingthe natural oils that keep the skin hydrated and flexible.

❖ **EffectofHeatonTransdermalpatch:**

Transdermal drugs absorb more readily when heated. The patient should be warned to keep heated objects, such as heated water bags or hot water bottles, away from the patch application site. Even a high body temperature may increase the effectiveness of drugs delivered transdermally. In this situation, the patch needs to be taken away immediately.Until they are ready to be used, transdermal drug patches are kept in their original containers and kept in a cool, dry place.

1.4.10. Various approaches Used in the Development of Transdermal Drug Delivery Systems :

Four different approaches have been utilized to obtain Transdermal drug delivery systems:

1.4.10.A.MembranePermeationControlledSystems:

In this type of system, the drug reservoir is totally encapsulated in a shallow compartment moulded from a drug -impermeable metallic plastic laminate and a rate controlling membrane, which may be micro porous or non-porous. The drug molecules are permitted to release only through the rate-controlling membrane.

In the drug reservoir compartment, the drug solids are either dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium such as silicone fluid to form a paste like suspension. A thin layer of drug compatible, adhesive polymer like siliconeorpolyacrylateadhesivemaybeappliedtothee xternalsurfaceoftheratecontrolling membranetoachieveanintimatecontactofthetransder malsystemandskinsurface.Therate of drug release from this type of system can be tailored by varying the polymer composition, permeability coefficient and thickness of the rate limiting membrane, and adhesive. Themajor advantage of membrane permeation controlled transdermal system is the constant release of drug. However, a rare risk also exists when an accidental breakage of the rate controllingmembranecan resultin dosedumpingorarapidreleaseof theentiredrugcontent.

1.4.9. B.Adhesive Dispersion TypeSystems:

Thissystemisasimplifiedformofthe membrane permeation controlledsystem.Here the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer e.g., poly(isobutylene)orpoly(acrylate)adhesiveandthens preadingthemedicatedadhesive,bysolvent casting or hot melt, on to a flat sheet of drug impermeable metallic plastic backing to form a thin drug reservoir layer.

On the top of the drug reservoir layer, thin layers of non -medicated, rate controlling adhesive polymer of a specific permeability and con stant thickness areapplied to produce an adhesive diffusion - controlled delivery system.

1.4.10.C.MatrixDiffusionControlledSystems:

In this approach, the drug reservoir is prepared by ho mogenously dispersing drug particles in a hydrophilic or lipophilic polymer matrix. The resultant medicated polymer is then moulded into a medicated disc with a defined surface area and controlled thickness. The drug reservoir can be formed by dissolving drug and polymer in a common solvent followed by solvent evaporation in a mould at an elevated temperature

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and/or vacuum.

The drug reservoir containing polymer disc is then pasted on to an occlusive baseplate in a compartment fabricated from a drug impermeable plastic backing. The adhesive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc. The advantage of this system is the absence of dose dumping since polymer cannot rupture.

1.4.10.D.MicroReservoir Type:

This system is a combination of the reservoir and matrix diffusion type drug delivery systems. The drug reservoir is formed by first suspending the drug solids in an aqueous solution of water soluble liquid polymer and then dispersing the drug suspension homogenously in lipophilic polymer viz. silicone elastomers by highenergy dispersion technique to form several discrete, unleachable microscopic spheres of drug reservoirs.

Thequickstabilizationofthisthermodynami callyunstabledispersionisaccomplished by immediately cross -linking the polymer chains in-situ, which produces a medicated polymer disc with a constant surface area and fixed thickness. Positioning the medicated disc at the center and surrounding it with an adhesive produce a transdermal therapeutic system.

1.5. BasicComponentsofTransdermalDrugDeliverySystems:[62]

1.5.1.A.Polymermatrix:

This primarily aids in the medication's release from transdermal patches that are dependent on or managed by the polymer. The drug releases at a sluggish rate due to the formation of a very dense matrix when the polymer concentration increases. The foundation of a transdermal drug delivery system is polymer. The concentration and other physiochemical characteristics of the drug as well as the polymer determine the drug's diffusion throughout the polymer matrix and its rate of release.

Idealpropertiesofpolymermatrixare:

1. Itmustbeinert andnotreactwithanymedication.
2. Itcannotbreak downin thepresenceof the medicationand excipients.
3. Itshouldn'taffectthemedication's stability.
4. Itoughtto beaccessiblewith ease.
5. Itoughttobereasonablypriced.
6. Itcan'thaveanyantagonisticeffectsof anykind.
7. Itshouldn'tcausehypersensitivityreactionsofany kind.

Examplesofpolymermatrix:-
Hydroxypropylmethylcellulose,PVA(polyvinylAlcohol), PVC (polyvinyl chloride), Starch, PVP, Polyethylene etc.

1.5.1. B.Active ingredient:

The most crucial element of transdermal patches is the drug reservoir. It needs to be chosenwithextreme caution.Becauseionizeddrugmoleculeshavepoor skinpenetration and permeation, drugs that ionize quickly are not good ingredients to use in transdermal patches.

Idealpropertiesforactiveingredient are:

1. Itshouldnotirritatehumanskin.
2. Itsbiologicalhalf-life oughttobebrief.
3. It must possess sufficient potency to deliver the necessary pharmacological effect.
4. When taken as prescribed, it shouldn't cause any kind of hypersensitivityreaction.
5. Itoughttobenaturallynon-toxic.
6. Thedrugmust haveaffinities forboth hydrophilic and lipophilic phases.

1.5.1.C. Penetrationenhancers:

These are the compounds that increase the skin's permeability to drugs by improving the skin's properties. Drugs can enter the skin through three different pathways: polar, non- polar, and polar/nonpolar. Changing one of these routes improves penetration. Protein conformational alterations can modify the polar pathway. Lipid rigidity can be changed to modify the non-polar pathway.

Idealpropertiesofpenetrationenhancersare:

1. Theskin layer shouldn'tbepermanently harmed.
2. Itoughttopossesspharmacologicalinertness.
3. Itoughtnottobeharmful.
4. Itoughttobeallergy-free.
5. Itoughttobeaction-oriented.
6. Itmustnotcauseirritation.

1.5.1.D. Surfactants:

These are included when a drug exhibits hydrophilic qualities. They improve the drug's polar pathway transport. There's no use of cationic surfactants. They are thought to irritate skin the most. Pluronic F127 is a non-ionic surfactant

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example. One instance of an anionic surfactant is sodium lauryl sulfate, or SLS.

1.5.1.E. Solvents:
Itisused as asolublethesolute.
Example-Ethanol,Methanol,Glycerol,Propylene glycol.

1.5.1.F. Plasticizers:
Theyareemployedtolessenorminimizethepolymerfilm'sbrittleness.Theysupplythe polymeric film with flexibility and elasticity.
Highconcentrationsofplasticizercausethe filmtobecomemoistandsticky.

Idealpropertiesofplasticizerare:
1. Itmustbeacceptable.
2. Itmustnotirritateorreactnegatively.
3. Itmustpossesspharmacological inertness.
4. Itshouldn'thavean impactonthedrug'sstability.
5. Itshouldbereasonablypriced.
6. Itmustbesimpleandaccessible.

Examples of plasticizers are- Glycerol, Propylene glycol, Dibutyl Phthalate, Polyethylene glycol.

1.5.1.G. Drugreservoir component:
This is a constituent that comprises a single polymer or a blend of polymers in diverse ratios and concentrations.

1.5.1.H. Backing laminates:
This facilitates giving and receiving support. They ought to stop the release of medication from surfaces that are not in contact with the skin. It ought to work well with the medication and excipients. Flexibility, strength, and elasticity should all be taken into account when choosing.

Thetransdermal drugdelivery systemgainsappearance, flexibility,andocclusionsas a result. While choosing the excipients for backing laminates, Compatibility is something to think about. High flexibility backing laminates are the most appropriate.
Example of backing laminates is:- Metallic Plastic Laminates, Polyurethane, 'Aluminiumfoil.

1.5.1 I. Adhesive layer:
Thislayeradheresthetransdermaldeviceonsurfaceofskinatpropersiteandposition.

Idealpropertiesofadhesivelayersare:
1. Itshouldbeabletoadherewithlittleforce.
2. Itshouldn'taffecthowquicklythemedication releases.
3. Thedrug'ssolubilityshouldn't beimpacted.
4. Itmustnotcauseskinirritation.

1.4.16.J.Release liners:
Before transdermal patches are applied to the skin, these protective layers are removed. They aid in preventing medication loss while being transported and stored.
Examplesofreleaselinersare: - Teflon,Silicon,Polyesteretc.

1.6. Inflammation:(63)
The localized response (reaction) of vascularized living tissues to both endogenous and external triggers is called inflammation. The Latin word "inflammare," which means to burn, is where the word originates. The basic purpose of inflammation is to restrict tissue damageand to identifyand eradicatethe causing culprit.

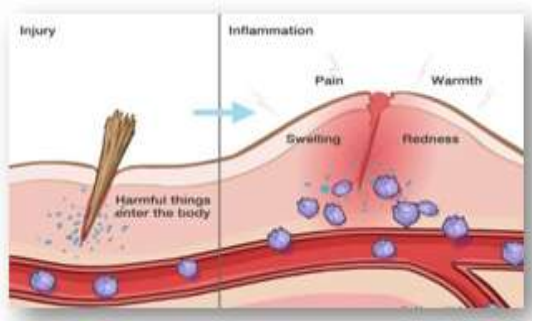

Fig.1.5 - Inflammation

Therefore,
aninjury'sphysiological(protective)responseisinflammation. Asaresult of violence or some illnesses, inflammation should be viewed as a therapeutic intervention rather than a sickness in and of itself.

1.6.1. Causes:
Causesofinflammationareapparentlycausesof diseasessuchas:
1. **Physicalagents**- mechanicalinjuries,alterationintemperaturesand pressure, radiation injuries.
2. **Chemicalagents**- includingtheincreasinglistsofdrugsand toxins.
3. **Biologicagents(infectious)** - bacteria,viruses,fungi,parasites.
4. **Immunologic disorders**- hypersensitivity reactions, autoimmunity, Immunodeficiency

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states etc.

5. **Genetic/metabolicdisorders**-examplesgout,diabetesmellitusetc…

1.6.2. Classification:

Inflammationisclassifiedcrudelybasedondurationoft helesionandhistologic appearances intoacute and chronic inflammation.

❖ **Accordingtothe course:**

1. Acute,
2. Sub-acute
3. Chronic

❖ **Accordingtothepredominantphase:**

1. Alterative
2. Exudative
3. Proliferative(productive)

❖ **Accordingtothecausativefactors:**

1. Trivial,
2. Specific

1.6.3. Acuteinflammation:

Acute inflammation can occur for afew minutes, hours, ordays and is a temporary, transientresponsetoaninflammatoryagent.Neutrophi l-dominatedleukocytesmovetothe site of damage, and fluids and plasma proteins are released.

1.6.3.1. Thefollowingfiveindicators ofacuteinflammation are:

1. **Redness**(rubor)which,asincellulitis,iscausedbyt hedilatationoftinyblood vessels within injured tissue.
2. **Heat**(calor)whichresultsfromincreasedbloodflo w(hyperemia)duetoregional vascular dilation
3. **Swelling**(tumor)whichisduetoaccumulationoffl uidintheextra vascularspace which, in turn, is due to increased vascular permeability.
4. **Pain** (dolor), which partly results from the stretching & destruction of tissues due to inflammatory edema and in part from pus under pressure in, as abscess cavity. Some chemicalsofacuteinflammation,includingbrady kinins,prostaglandinsandserotonin are also known to induce pain.
5. **Lossoffunction**:Theinflamedareaisinhibitedby painwhilesevereswellingmay also physically immobilize the tissue.

1.6.3.2. Eventsofacute inflammation:

Acuteinflammationiscategorizedintoanearly vascularandalatecellular responses.

1) The**Vascular response**hasthefollowingsteps:

a. Immediate(momentary)vasoconstrictioninseco ndsduetoneurogenicorchemical stimuli.
b. Vasodilatationofarteriolesandvenulesresultingi nincreasedbloodflow.
c. Due to increased vascular permeability, there is a slowdown and stasis in blood flow following the phase of enhanced blood flow, which is most noticeably observed in the post-capillary venules. Extravascular tissues are flooded with protein-rich fluid due to the increased vascular permeability. As a result, red blood cells have now filled the previously dilatedbloodarteries,causingstasis.Exudateisthe termfortheprotein-richfluidthatisnow present in the extravascular area. Clinically, the exudates presence manifests as edema. The vascular events of acute inflammation are mediated by chemical mediators.

2) **Cellular response**

Thecellularresponsehasthefollowingstages:

a. Migration,rolling,pavementing&adhesiono f leukocytes
b. Transmigrationof leukocytes
c. Chemotaxis
d. Phagocytosis

Normally, plasma occupies the peripheral zone and blood cells, especially erythrocytes in venules,arerestrictedtothecenter(axial)zone.Anincre asingnumberofneutrophilsgather at the endothelium surfaces (peripheral zone) due to enhanced vascular permeability.

A) **Migration,rolling,pavementing,andadhesion ofleukocytes**

❖ Marginationisaperipheralpositioningof whitecellsalong theendothelial cells.

❖ Subsequently, rows of leukocytes tumble slowly along the endothelium in a processknown as rolling.

❖ Intime,theendotheliumcanbevirtuallylinedbyw hitecells.Thisappearanceis called pavementing.

❖ Thereafter, the binding of leukocytes with endothelial cells is facilitated by cell adhesion molecules such as selectins, immunoglobulins, and integrins etc. which result in adhesion of leukocytes with the endothelium.

B). Transmigration ofleukocytes

❖ Leukocytes escape from venules and small veins but only occasionally from capillaries. The movement of leukocytes by extending pseudopodia through the vascular wall occurs by a process called diapedesis.

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- The most important mechanism of leukocyte emigration is via widening of inter- endothelial junctions after endothelial cells contractions. The basement membrane is disrupted and resealed thereafter immediately.

C). Chemotaxis:

Chemotaxis is a unidirectional attraction of leukocytes from vascular channels towards the siteofinflammationwithinthetissuespaceguidedbych emicalgradients(includingbacteria and cellular debris).

- The most important chemotactic factors for neutrophils are components of the complement system (C5a), bacterial and mitochondrial products of arachidonic acid metabolism such as leukotriene B4 and cytokine, Interleukin-L(IL-8). All granulocytes, monocytes and to lesser extent lymphocytes respond to chemotactic stimuli.
- How do leukocytes "see" or "smell" the chemotactic agent? This is because receptors on cell membrane of the leukocytes react with the chemo-attractants,resulting in the activation of phospholipase C that ultimately leads to release of cytosolic calcium ionsand these ions trigger cell movement towards the stimulus.

D)**Phagocytosis**

The process of phagocytosis involves specialized cells engulfing and internalizing particulate material, such as injured cells, invading pathogens, and tissue debris. Tissue macrophages, monocytes, and polymorphonuclear leukocytes—especially neutrophils—are examples of these phagocytic cells.

Phagocytosisinvolvesthreedistinctsteps.

1) **Theleukocytes'recognitionandattachmentoftheparticle tobeingested:**

If the material to be phagocytosed is coated with specific plasma proteins known as opsonins, phagocytosis will be facilitated. These opsonins facilitate the attachment of the particulate matter to the cell membrane of the phagocyte.

2) **Engulfment :**

Engulfmentoccurs whenpseudopods,or extensionsof the cytoplasm,flow aroundthe target to be engulfed. This process finally leads to the particle being completely enclosed within the phagosome that the phagocytic cell's cytoplasmic membrane creates. The ingested particle is exposed to the degradative lysosomal enzymes as a result of the phagosome and lysosome fusing to produce a phagolysosome.

3) Killing or degradation

Thefinalstageofbacterialphagocytosisistheirdestruction anddeath.Therearetwo methods for eliminating germs.

a) Oxygen-independentmechanism:

Some of the components of polymorphonuclear leukocytes' primary and secondary granules mediate this. These consist of defenses, lysozymes, lactoferrin, and the protein known as "bactericidal permeability increasing protein" (BPI).

Compared to oxygen-dependent methods, lysosomal enzyme-mediated bacterial death is likely less effective. Nonetheless, the lysosomal enzymes are necessary for the phagosome to break down dead organisms.

b)Oxygen-dependentmechanism:

Therearetwo types of oxygen-dependent killing mechanisms

i) **Non-myeloperoxidase dependent**

Microorganisms are killed by oxygen when reactive oxygen species such hydrogen peroxide (H_2O_2), super oxide (O_2), hydroxyl ions (HO^-), and perhaps single oxygen (O_2) are formed. These organisms outer orbits contain a single, unpaired electron that reacts with chemicals in the cell membrane or nucleus to produce harm.

ii) **Myeloperoxidase–dependent**

The lysosomal enzyme myeloperoxidase, which changes , into hypochlorous acidin the presence of halide ions, is responsible for the bactericidal action ofThe neutrophil's , most effective bactericidal killing system is the(H_2O_2). Moreover, a comparable method works well against helminthes, viruses, fungus, and protozoa. Similar to vascular events, chemical mediators initiate or trigger cellular activities, such as adhesion, transmigration, chemotaxis, and phagocytosis.

IV.Chemicalmediatorsofinflammation Sources of mediators:

Thechemicalmediatorsofinflammation canbederivedfrom plasmaor cells.

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a) Plasma-derived mediators:

i) Complementactivation

- Increasesvascularpermeability(C3a,C5a)
- Activateschemo-taxis(C5a)
- Opsoninization(C3b,C3bi)

ii) FactorXII(Hagemanfactor)activation

Itsactivationresultsinrecruitment offoursystems:thekinin,the clotting,the fibrinolysis and the compliment systems.

b) Cell-derivedchemical mediators:

Cell-derivedchemicalmediatorsinclude:

Most mediators perform their biologic activities by initially binding to specific receptors on target cells. Once activated and released from the cells, most of these mediators are short lived. Most mediators have the potential to cause harmful effect.

1.6.3.3. Morphologyofacuteinflammation:

Characteristically, the acute inflammatory response involves production of exudates. Anexudateisanedemafluidwithhighproteinconcentration,whichfrequentlycontains inflammatory cells. Atransudateissimplyanon-inflammatoryedemacausedbycardiac,renal, Under-nutritional & other disorders.

Therearedifferentmorphologictypesofacute inflammation:

1) Serous inflammation

This is characterized by an outpouring of a thin fluid that is derived from either the blood serum or secretion of mesothelial cells lining the peritoneal, pleural, and pericardial cavities.

❖ **Abscess**

Anabscessisan accumulationofpuswithtissue destructionandacavity format.

2) Fibrinousinflammation

Moresevereinjuriesresultingreatervascular permeabilitythatultimately leadsto exudation of larger molecules such as fibrinogens through the vascular barrier.

❖ Fibrinousexudate ischaracteristic of inflammation inserous body cavities suchas the pericardium (butter and bread appearance) and pleura.

❖

Courseoffibrinousinflammationinclude:

❖ Resolutionby fibrinolysis

❖ Scarformationbetween parietalandvisceralsurfacesi.e.theexudatesgetorganized

❖ Fibrousstrandformationthat bridgesthepericardialspace.

❖

Typesof FibrinousInflammation:

 Fibrinous ParenchymalInflammation

 FibrinousSerosalInflammation

 Fibrinous MucosalInflammation(Croupous andDiphtheria).

❖ **FibrinousParenchymal inflammation:**

InflammationDefinition:Exudativeinflammationwithexudationoffibrinonthe inner surfaces of the Pulmonary parenchyma (pulmonary alveoli).

❖ **FibrinousSerosalInflammation:**

Exudativefibrinousinflammationsofthesero usmembranesmayoccurasareaction of the serosa to other underlying disorders (serositis) or in the presence of tissue injury occurring in the serosa (such as infarction).

❖ **FibrinousSerosalInflammationMorphology**

 Serosawillappear dullwhereslightamounts offibrin are present;

 Massiveexudationofserumwillproducevillousdeposits offibrin(asinfibrinous pericarditis or "hairy heart").

 Later the fibrin deposits are absorbed by histiocytes and transformed into scar tissue, creating adhesions between the layers of the serosa.

❖ **FibrinousMucosalInflammation:**

Generalpathogenesis:Infibrinousinflammationsinthemucosa,thefibrinous exudation process is usually preceded by superficial necrosis.

Types:

 CroupousType

 DiphtheriaType

❖ **Croupous Type:**

Exudative inflammation in which a wide area of fibrinous exudate forms an easily removablepseudomembranecoveringthenecrosis,whichislimitedtothemucosal epithelium, Example: Diphtheria laryngotracheitis.

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❖ **Diphtheria Type:**

Exudative inflammation in which necrosis extending into the sub mucosa is covered by a wide area of fibrinous exudate in the form of an adhesive pseudo membrane that canonly be forcibly removed.

Example:Diphtherictonsillitisandpharyngitis,Dysentery.

•Antibioticassociatedcolitis(pseudo membranouscolitis).

3) Suppurative(Purulent)inflammation

Thistypeofinflammation is characterizedby theproductionofalargeamount of pus.

Pusisathickcreamyliquid,yellowish orbloodstainedincolorandcomposed of:

- Alargenumberofliving ordead leukocytes (pus cells)
- Necrotictissuedebris
- Living and deadbacteria
- Edemafluid

Thereare twotypesofSuppurativeinflammation:

A) Abscessformation:

An abscess is a circumscribed accumulation of pus in a living tissue. It isencapsulated by a so-called pyogenic membrane, which consists of layers of fibrin, inflammatory cells and granulation tissue.

B) Acutediffuse(phlegmonous) inflammation:

This is characterized by diffuse spread of the exudate through tissue spaces. It is caused by virulent bacteria (eg. Streptococci) without either localization or marked pus formation.

❖ **Empyema:**

Suppurativeinflammationinabodycavity.

Pathogenesis:

AnempyemausuallyoccurswhenaSuppurativeinflammationofanorganbreaks through into an adjacent cavity.

Phlegmon:

DiffuseSuppurativeinflammationthatspreadsprimarilyinloosefibrous connective tissue.

4) Catarrhalinflammation:

This is a mild and superficial inflammation of the mucous membrane. It is commonly seen in the upper respiratory tract following viral infections where mucous secreting glands are present in large numbers.

5) Pseudomembranousinflammation:

The basic elements of pseudo membranous inflammation are extensive confluent necrosis of the surface epithelium of an inflamed mucosa and severe acute inflammation of the underlying tissues. The fibrinogens in the inflamed tissue coagulate within the necrotic epithelium.Thefibrinogen,whichcontaintheneutrophilicpolymorphs,redbloodcells, bacteriaandtissuedebrisformafalse(pseudo)membranewhichformsawhiteorcolored layer over the surface of inflamed mucosa.

PseudomembranousinflammationisexemplifiedbyDiphtheriticinfectionofthe pharynx or larynx.

❖ **Hemorrhagic Inflammation:**

Definition: Exudativeinflammationinvolvingmicrovascularinjurywithmassive microvascular bleeding, producing an exudate with high erythrocyte content.

Biologicpurpose: Exudativeinflammationduetosevere vascularinjury.

Morphology: Theinflamedareaisusually necroticand filledwith blood.

1.6.3.4. Effectsofacuteinflammation:

A. Beneficialeffects

1. **Dilution of toxins**: The concentration of chemical and bacterial toxins at the site of inflammation is reduced by dilution in the exudate and its removal from the site bythe flow of exudates from the venules through the tissue to the lymphatics.
2. **Protective antibodies**: Exudation results in the presence of plasma proteins including antibodies at the site of inflammation. Thus, antibodies directed against the causative organisms will react and promote microbial destruction by phagocytosis or complement-mediated cell lysis.
3. **Fibrinformation**:Thispreventsbacterialspreadandenhancesphagocytosisby leukocytes.
4. **Plasmamediatorsystemsprovisions**:Thecomplement,coagulation,fibrinolytic,&kinin systems are provided to the area of injury by the process of inflammation.
5. **Cell nutrition**: The flow of inflammatory exudates brings with it glucose, oxygenand other nutrients to meet the metabolic requirements of the greatly increasednumber of cells. It also removes their solute waste

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products via lymphatic channels.

6. **Promotion of immunity**: Micro-organisms and their toxins are carried by the exudates, either free or in phagocytes, along the lymphatic's to local lymph nodes where they stimulate an immune response with the generation of antibodies and cellular immune mechanisms of defense.

B. Harmful effects

Tissuedestruction: Inflammationmayresultintissue necrosiswhichmay,inturn,incite inflammation.

1. **Swelling:** The swelling caused by inflammation may have serious mechanical effects atcertainlocations.Examplesinclude acute epiglottitiswithinterferenceinbreathing; acute meningitis and encephalitis with effects of increased intracranial pressure.
2. **Inappropriateresponse:**Theinflammatoryreactionseeninhypersensitivityis inappropriate (i.e. exaggerated).

1.6.3.5. Causeofacute inflammation

Acuteinflammationmay end upin:

1. Resolution i.e. complete restitution of normal structure and function of the tissue, eg. lobar pneumonia.
2. Healingbyfibrosis(scarformation).
3. Abscessformation.However, ifitis leftuntouched,it mayresult in:-

a) **Sinus formation** - when an abscess cavity makes contact with only one epithelial lining.

b) **Fistula formation**: when an abscess tract connects two epithelial surfaces. Orvery rarely to septicemia with subsequent metastatic abscess in heart, kidney,brain etc.

1.6.4. Chronicinflammation

A protracted inflammatory process (weeks or months) characterized by simultaneous tissuedamage, active inflammation, and healing attempts is called chronic inflammation.

1.6.4.1. Causesofchronicinflammation:

1. Persistentinfections

Chronic inflammation is a common side effect of several intracellular infection- related bacteria, including fungus, leprosy, and tuberculosis. These organisms cause delayed hypersensitivity reactions and are not very harmful.

2. **Prolongedexposure to non-degradable but partiallytoxic substances:**either foreign materials like asbestos and silica, or endogenous lipid components that cause atherosclerosis.
3. **Progression from acute inflammation:** Acute inflammation almost always progresses to chronic inflammation following.
4. **Autoimmunity.**Autoimmunediseasessuchasrheumatoidarthritisandsystemiclupus erythematosis are chronic inflammations from the outset.

1.6.4.2. Cellsofchronic inflammation:

1. Monocytesand Macrophages
2. T-Lymphocytes
3. B-lymphocytesandPlasmacells
4. Mastcellsandeosinophils
5. Neutrophils.

1.6.4.3. Classificationofchronicinflammation:

Chronicinflammationcanbeclassifiedintothefollowing**twotypes**basedonhistologic features:

1) Nonspecificchronicinflammation:

This involves a diffuse accumulation of macrophages and lymphocytes at site ofinjury that is usually productive with new fibrous tissue formations. E.g. Chronic cholecystitis.

2) Specificinflammation(granulomatous inflammation):

Granulomatous inflammation is characterized by the presence of granuloma. A granuloma is a microscopic aggregate of epithelioid cells. Epithelioid cell is an activated macrophage, with a modified epithelial cell-like appearance (hence the name epithelioid).The epitheloid cells can fuse with each other & form multinucleated giant cells. So, even though, a granuloma is basically a collection of epithelioid cells, it also usually contains multinucleated giant cell & is usually surrounded by a cuff of lymphocytes and occasional plasma cells.

Twotypesofgiant cells:

A. Foreignbody-typegiantcellswhichhaveirregularlyscatterednucleiinpresence of indigestible materials.

B. Langhansgiantcellsinwhichthenucleiarearrangedperipherallyinahorse–shoe pattern which is seen typically in tuberculosis, sarcoidosis etc…

Giantcellsareformedbyfusionofmacrophagesperhapsbyaconcertedattemptof two or more cells to engulf a single particle.

Pathogenesis:

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Therearetwotypes of granulomas,which differin theirpathogenesis.

A. Foreign bodygranuloma

B. Immunegranulomas

Majorcausesofgranulomatiousinflammation include:

a) Bacterial: Tuberculosis,Leprosy,Syphilis.

b) Fungal:Histoplasmosis,Cryptococcosis,Coccidioidomycosis,andBlastomycosis.

c) Helminthic:Schistosomiasis.

d) Protozoal: Leishmaniasis, Toxoplasmosis.

e) Chlamydia: Lymphogranulomavenerum.

f) Idiopathic: Acidosis,Primarybiliarycirrhosis.

Thesystemiceffects ofinflammation include:

a. Fever
b. Endocrine& metabolic responses
c. Autonomicresponses
d. Behavioralresponses
e. Leukocytosis
f. Leukopenia
g. Weightloss

II. REVIEW OF LITERATURE

1. **AbimashazhniJ.R et al; (2016)** screened the phytochemical constituents and anti-microbial activity of different parts of Anthuriumandraeanum. Powdered palnt materials were extracted with different solvents and studied showed the presence of alkaloids,flavonoids,phenols,Phlobatannins,steroids and tannins.
2. **Jaime Teixeira da Silvaa A et al; (2014)** A successful tissue culture protocol for anthurium would allow for the mass clonal propagation of this plant to serve the floriculture pot-plant and cut-flower markets. Success has been achieved in anthurium tissue culture using several explant types, and the morphological and cytogenetic stability of such regenerants has also been tested. This review provides a detailed analysis of the conditions required for the successful culture of anthuriums in vitro. Besides micropropagation, this review also highlights selected current applications of in vitro anthurium biotechnology such as anther culture, polyploidy production, genetic transformation, and their importance in breeding work, synthetic seed technology and cryopreservation.
3. **Manjunath S et al., (2018)** he Design, Development and Evaluation of Herbal Transdermal Patches for Anti-inflammatory Activity. In this study, various transdermal matrix patches containing Commiphoramukul of variable combination of ethyl cellulose/polyethylene glycol with enhancer (menthol: limonene) were prepared. The prepared patches were studied with respect to physicochemical characters, drug-excipient interaction, dissolution, skin permeation, stability and in vivo anti-inflammatory studies. The combination of ethyl cellulose and polyethylene glycol produces smooth flexible films. Dissolution and in vitro skin permeation studies revealed that the cumulative amount of drug permeated was decreased as the polyethylene content of the film increased. (79)
4. **ChaowalitM et al., (2022)** formulated herbal transdermal patches for anti-inflammatory of Lysiphyllumstrychnifolium stem extract into optimized polyvinyl alcohol blended flim as a matrix layer for controlled drug release. Its ideal characteristics are ease of flim formation, biocompatibility, and biodegradability in particular it must be compatible with the drug and safe for human use.(80)
5. **Sarunya T et al., (2022)** in her work development and clinical trials on anti-inflammatory effect of transdermal patch containing a combination of Kaempferiaparviflora and curcuma longa extracts. It's widely reported to have a potent anti-inflammatory effect. The study develops transdermal delivery of a combination of both extracts in matrix- patch formulation with five different volatile oil which possess analgesic and anti-inflammatory properties.(81)
6. **Prachi D.B et al., (2023)** Development and evaluation of anti-inflammatory transdermal patches of colocasiaesculenta plant extract. Hence this study aims to formulate an anti-inflammatory transdermal patch using different polymers such as ethyl cellulose, hydroxy propyl methylcellulose with plasticizers poly-ethyl glycol 400. Patches were prepared through solvent casting method. It was concluded from the research that, a herbal extract of the plant with HPMC showed moderate anti-inflammatory action and controlled drug release, thus can be selected for the development of transdermal patches for effective uses.(82)
7. **Arora P et al., (2002)** prepared matrix type

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transdermal patches containing diclofenac diethylamine using different ratios of PVP and ethyl cellulose by solvent evaporation technique. The drug matrix films were casted on a polyvinyl alcohol backing membrane and physical studies (moisture content, moisture uptake, and flatness), in vitro release studies were studied. They concluded that diclofenac diethylamine can be formulated into the transdermal matrix type patches for sustaining its release characteristics and the polymeric composition (PVP/EC, 1:2) was found to be the best among the formulations studied.[83]

8. **Rajagopal K et al., (2005)** formulated and evaluated matrix type transdermal patches of nimesulideby using different polymers alone or in combination, dibutyl phthalate as the plasticizer and aluminium foil as the backing membrane. In-vitro release studies of the prepared formulations were performed by keshary-chien diffusion cell through cellophane membrane and excised mice skin in phosphate buffer solution (pH7.4). Both the studies showed that (2:2) hydroxy propyl methyl cellulose (HPMC) and ethyl cellulose (EC) combination may be the suitable polymer combination for development of transdermal drug delivery system (TDDS) of Nimesulide. The patches were further evaluated for physicochemical characteristics and skin-irritancy test.[84]
9. **Alam MI et al., (2009)** developed low-dose, matrix-type transdermal patches containing celecoxib for the treatment of osteoarthritis. The patches were designed to be used over a period of 24 h. Different ratios of ethyl cellulose/polyvinyl pyrrollidone (EC/PVP) were used for the development of the system. All of the prepared patches were subjected to physicochemical evaluation, in vitro drug release, permeation, and anti-inflammatory studies (in vivo). The release rates and flux increased linearly when an increase in the fraction of PVP was mixed with the formulations.[85]
10. **Patel PR et al., (2009)** in their work they developed a matrix type transdermal therapeutics system containing drug aceclofenac with different ratios of hydrophilic (hpmc) and hydrophobic (ethyl cellulose) polymeric systems by solvent evaporation techniques. The results indicated that the formulation containing 15% oleic acid and 10% isopropyl myristate gives better penetration of aceclofenac through rat skin.[86]
11. **ShashikantBarhate D et al.,(2009)** developed a transdermal delivery system of ketoprofen. The transdermal patches of ketoprofen were prepared by mercury substrate method using polymer Eudragit RS100, Eudragit RL100, HPMC K100M, HPMC E5 and HPMC K4M. Propylene glycol and oleic acid used as a skin permeation enhancer and dibutyl phthalate and polyethylene glycol-400 used as a plasticizer. The prepared patches will evaluated for thickness, folding endurance, tensile strength, drug contain uniformity, in-vitro permeation study. Drug polymer interactions determine by FTIR and standard calibration curve of ketoprofen were determine by using UV estimation. In vitro release study was performed by using Franz-diffusion cell. Patches prepared with HPMC with enhancer propylene glycol showed higher % moisture transmission. The formulated transdermal patch by using EudragitRS100, EudragitRL100, HPMC K100M, HPMC E5 and HPMC K4M showed good physical properties. It was observed that the formulation containing HPMC E5 showed ideal zero-order release kinetics.[87]
12. **Parthasarathy G et al., (2011)** developed transdermal drug delivery system of Naproxen with Ethylcellulose and Hydroxy propyl methyl cellulose polymer in various concentrations. Transdermal films were fabricated by matrix technique with various polymer proportions using dibutylphthalate as plasticizer. These transdermal drug patches were characterized for their thickness, tensile strength, content uniformity, in-vitro release. The release profiles were found to be varied with various concentrations of Ethylcellulose Polymer. The sample of patches prepared with 2:8 and 8:2 ratios of Ethyl cellulose and Hydroxy propyl methyl cellulose shows highest and lowest in-vitro release of Naproxen respectively.[88]
13. **ThusharaBindu D et al., (2011)** prepared transdermal patch of ibuprofen by using gelatin, sodium benzoate and glycerine as main ingredients. In order to increase its permeability, permeation enhancers like olive oil, coconut oil and sun flower oil were used and the patches were characterized by evaluating physicochemical parameters like thickness, weight variation, folding endurance, drug content, breaking strength and ex vivo

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release study. From this study, it was concluded that olive oil is the better permeation enhancer for Ibuprofen transdermal patch.[89]

14. **SatyanarayanPattnaik et al., (2011)** developed a suitable transdermal matrix patch of ketorolac tromethamine with different proportions of polyvinyl pyrrolidone (PVP) and ethyl cellulose (EC) using a D-optimal mixture design. The prepared transdermal patches were subjected to different physicochemical evaluation. The surface topography of the patches was examined by scanning electron microscopy (SEM). The drugpolymer interaction studies were performed using Fourier transform infrared spectroscopic (FTIR) technique. A correlation between in-vitro drugrelease and in-vitro skin permeation was established and the criterion of desirability was employed to optimize the formulation. The results of the physicochemical characterization and in-vitro permeation of the prepared patches were promising to formulate transdermal patches with PVP/EC combinations.[90]
15. **Das A et al., (2017)** in her work she formulated a transdermal patch of indomethacin using patchouli oil as a penetration enhancer to increase transdermal permeation of drug from matrix system across rat epidermis; many researches has done to investigate the permeation enhancing effects of the essential oils. [91]
16. **Nilesh M et al., (2018)** Formulation development and evaluated of transdermal patch of piroxicam for treating dysmenorrhea and pain. The transdermal patches of piroxicam were prepared using the combination of polymers, hydroxypropyl methylcellulose E15, polyvinylpyrrolidone (PVP) K30, and ethyl cellulose in different concentration with sodium lauryl sulfate as the permeation enhancer and polyethylene glycol 400 as the plasticizer was used for the formulation of transdermal drug delivery system which demonstrated sustained release of the drug through the patches. Piroxicam is an NSAID utilized for treating dysmenorrhea in the form of tablet formulation. The transdermal patch of piroxicam was fabricated by using sustained release hydrophilic and lipophilic polymers containing permeation enhancer and thoroughly evaluated.[92]
17. **AvniPravinbhai D et al.,(2022)**The study has been used to provide information regarding the use of anti-inflammatory transdermal patches. The degree of postoperative analgesic frequency of adverse events and patient compliance has been compared with transdermal diclofenac patches and tablets. The transdermal system has been considered as the innovative delivery method used for providing relief in various pain. In this study, diclofenac has been used in both oral and transdermal forms.[93]
18. **Michael HQ et al.** studied the release of permeation enhancers from transdermal drug delivery system of drug -in-adhesive type using known enhancers from eight types of adhesive polymers. They showed that, enhancers released completely from the adhesive and the release rate depended on the types of adhesives used especially among the acrylic polymers. They also showed that acrylic adhesive and polyisobutylene adhesive showed slower drug release rate than silicon adhesive.[94]
19. **Manish Kumar et al.,** fabricated matrix diffusion controlled transdermal drug delivery system of meloxicam by solvent evaporation technique using various concentration ratios of polymer (pectin). These transdermal drug delivery systems were characterized for their thickness, weight variation, folding endurance, swelling index, content uniformity, compatibility, in-vitro release and skin irritation studies of the drug from the polymeric matrix. Meloxicam was found to be compatible with pectin as revealed by Fourier Transform Infrared Spectroscopy (FTIR) studies and showed satisfactory physiochemical characteristics. In-vitro release studies were carried out with modified Franz diffusion cell using pH 7.4 phosphate buffer as receptor medium and it showed controlled release of drug..[95]
20. **Nathiya et al., (2018)** disclosed the molecular formula, weight, and biological activity of the bioactive substances. This study was beneficial for screening new medications that would not typically be available through the pharmacy services system.[96]
21. **Stephanie Flore DjuichouNguemnang et al, (2019)** evaluated anti-inflammatory properties by in vitro screening method by inhibition tests for cyclooxygenase, 5- lipoxygenase, protein denaturation, extracellular ROS production, and cell proliferation; while antiarthritic

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properties were evaluated in vivo in rats using the zymosan A-induced monoarthritis test and the CFA-induced polyarthritis mode. [97]

III. AIM & OBJECTIVES

3.1. Aim:

The aim of the study is to formulate and evaluate the herbal transdermal patch for the treatment of Inflammation by using whole plant parts in the aqueous extract of Anthuriumandraeanum.

3.2 Objective:

- To explore the herbs used in the treatment of Inflammation.
- To develop herbal Transdermal patches and perform its evaluation parameters using whole plant parts extracts of Anthuriumandraeanumfor the effective treatment of Inflammation.
- To scientifically validate developed formula
- To perform in vitro screening of the developed formulation for its efficiency towards
- Anti-inflammatory
- Anti- microbial action

- Loss on drying
- Total ash value
- Water soluble ash value
- Acid insoluble ash value
- Sulphated ash value
- Water soluble extractive value
- Alcohol soluble extractive value

IV. CHROMATOGRAPHIC EVALUATION

- Thin Layer Chromatography

4.1 Preformulation Studies

- Absorption maxima
- Physical Appearance
- Solubility
- FT-IR spectroscopy

4.2 Formulation of Transdermal Patch

4.3 GC-MS studies

4.4 Evaluation Parameters

- Organoleptic characteristics
- Uniformity of Weight
- Thickness of the Patch
- Drug content determination
- Folding endurance
- Percentage moisture uptake
- Percentage moisture content
- Determination of surface pH
- Flatness test.

4.5 Stability studies

- Cold and heat test
- Estimation of heavy metals by AAS
- HPTLC fingerprinting analysis of Herbal Transdermal Patch

4.6 In-vitro analysis of HTP

- Anti-inflammatory activity by albumin denaturation method
- Anti-microbial activity by agar well diffusion method

V. PLANT & POLYMER PROFILE

5.1. Plant profile:[106]

Fig.5.1 - Anthuriumandraeanum

Anthurium are gorgeous tropical plants with lustrous, dark green, rectangular, heart-shaped leaves that grow best in low light. The long-lasting, beautiful flower bracts have prutruding pale yellow, tail-like flower spadix and are colored red, rose, pink, and white. The flower bracts glisten like they've been polished and have a puckered look. Anthuriums bloom for four to six months a year on average, with each plant bearing blooms almost continually. Ever flower can be left on the plant for around six weeks or, if cut and put in a vase with water, for several weeks.

1. General information:

- **Scientific name:** Anthuriumandraeanum
- **Common name(s):** Tailflower, Flamingo-Flower
- **Family:** Araceae
- **Plant type:** perennial; herbaceous

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- **USDA hardiness zones:** 10B through 11
- **Planting month for zone 10 and 11:** year round
- **Origin:** not native to North America
- **Availability:** somewhat available, may have to go out of the region to find the plant.

2. Scientific classification:

- **Kingdom**–Plantae
- **Clade**– Angiosperms,Monocots.
- **Order**–Alismatales
- **Family**–Araceae
- **Sub-family**-Pothoideae
- **Genus**–Anthurium Schott
- **Species**-A.andraeanumLind
- **Class**– Liliopsida
- **Phylum**– Tracheophyta
- **Variety**-A.andraeanumvar.andraeanum

A.andraeanum var. atropurpureumpynaert

3. Description:

- **Height:** 2 to 3 feet
- **Spread:** 2 to 3 feet
- **Plant habit:** upright
- **Plant density:** open
- **Texture:** coarse
- **Foliage:**
- **Leaf arrangement:** alternate
- **Leaf type:** simple
- **Leaf margin:** revolute
- **Leaf shape:** sagittate (arrow)
- **Leaf venation:** brachidodrome; pinnate
- **Leaf type and persistence:** evergreen
- **Leaf blade length:** 8 to 12 inches
- **Leaf color:** green
- **Fall color:** no fall color change
- **Fall characteristic:** not showy
- Flower
- **Flower color:** pink; white; salmon
- **Flower characteristic:** year-round flowering
- Fruit
- **Fruit shape:** elongated
- **Fruit length:** unknown
- **Fruit cover:** fleshy
- **Fruit color:** red
- **Fruit characteristic:** inconspicuous and not showy

5. Therapeutic uses:

A Belize healer reports on the use of anthurium in stem **for discomforts of arthritis and rheumatism**: Leaves are cut and boiled in a pot and the user seats over it with a blanket.

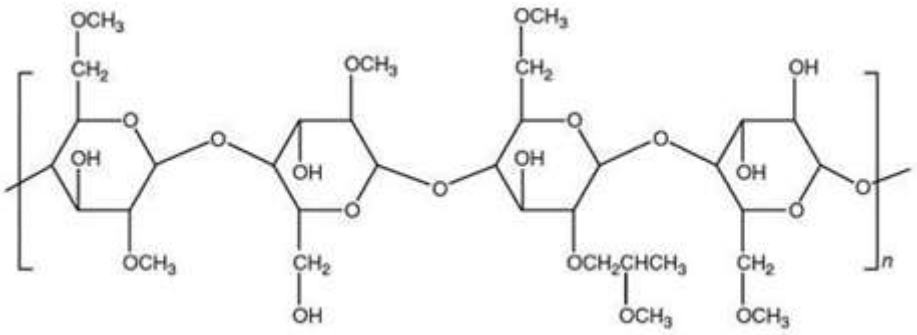
Poultice also used for **muscle aches and cramps**: Leaves are wrapped around the neck and back.

- Anti-inflammatory
- Anticancer
- Anti-microbial

5.2. <u>Polymer profile</u>:

5.2. A. Hydroxy propyl methyl cellulose:

- **British pharmacopoeia:**Hypromellose
- **United State Pharmacopoeia:**Hydroxy Propyl Methyl cellulose
- **Synonyms:**Methocel, HPMC
- **Chemical Name:** Cellulose, 2-Hydroxypropyl methyl ether
- **Empirical Formula:** HPMC is a partially o-methylated and o- (2-Hydroxy propylated)
- **Molecular Weight:** Approximate 10000 to 1500000.
- **Functional Category:** Tablet binder, coating agent, and film former.
- **Structural Formula:**
- **Pharmacopoeia:** BP and USP.



- **Description:**Odourless, tasteless, white or creamy white Fibrous or granular powder.
- **pH:** 5.5 to 8.0.
- **Aqueous Viscosity:** (1% w/v) HPMC K4M =

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3000 to 5600 cp. HPMC K15M = 11250 to 21000 cp.

- **Solubility:** Soluble in cold water, insoluble in alcohol, Ether, and chloroform, but soluble in a Mixture of methylene chloride and Methanol.
- **Stability:** Stable in dry condition from pH 3.0 to 11.0.
- **Storage Condition:** It is hygroscopic in nature. Should be stored In well-closed container, in a cool and dry Place.
- **Incompatibilities:** Incompatible with some oxidizing agents. Since it's non-ionic, hydroxypropyl methyl Cellulose will not complex with metallic Effect.
- **Safety:** It's generally regarded as a nontoxic and Non-irritant material although excessive oral Consumption may have a laxative effect.
- **Application:** HPMC is widely used in oral and topical Pharmaceutical formulations. In oral Products, it primarily used tablet binder and Extended release matrix.

5.2. B. Poly Vinyl alcohol

- **Synonyms:** PVOH; Poly (Ethenol), Ethenol, homopolymer; PVA; Polyviol; Vinol; Alvyl; Alcotex; Covol; Gelvatol; Lemol; Mowiol; Mowiflex, Nelfilcon A, Polyviol and Rhodoviol
- **Chemical formula:** (C2H4O) x
- **Molecular weight:** 86.09g/mol
- **Functional category:** film former.
- **Description:**Colourless, Non- toxic thermoplastic, adhesive.
- **Aqueous viscosity:** (10% w/v) PVA K4M = 5000 to 6000cp.
- **pH:** 5-7
- **Solubility:** cold water soluble.
- **Density:** 1.19–1.31 g/cm3
- **Melting point:** 200 °C (392 °F; 473 K)
- **Incompatibility:** virtually unaffected by organic solvents like hydrocarbon.
- **Storage condition:** 00 to 300 and store under away from direct sunlight.
- **Stability:** Thermal stability 3200C
- **Safety:** Acute oral toxicity is low.
- **Log P:** 0.26 Refractive index: 1.477 @ 632 nm
- **Uses:** It is used as backing membrane.

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VI. MATERIALS AND METHODS

6.1. MATERIALS USED:

Table 6.1 - Chemical used in formulation & Manufacturer

Sl.no	Chemicals	Manufacturer	Use in formulation
01	HPMC	BRM Chemicals	Binder
02	PVA	Himedialaboratories.pvt.Ltd	Flim former
03	PEG-400	BRM Chemicals	Plasticizer
04	Tween 80	Himedialaboratories.pvt.Ltd	Surfactant
05	Ethanol	Jiangyintenghuaco.Ltd	Solvent
06	Glycerine	Himedialaboratories.pvt.Ltd	Lubricating agent

6.2. INSTRUMENTS USED: The instruments used are listed in table 6.2.

Table 6.2 - Instruments used in Formulation, Evaluation and their manufacturer

S.No	Instruments	Manufacturers
01.	Weighing balance	Scale Tec
02.	PH meter	Alpha vision plus
03.	Hot plate	Hasthas scientific instrument/Chennai.
04.	Water bath	Hasthas scientific Instrument/Chennai.
05.	Magnetic stirrer	Conxport
06.	Autoclave	Labsol enterprises
07.	Laminar air flow	Aeromech
08.	UV spectrophotometer	LABMAN
09.	Dessicator	Cabinets manufacturers india
10.	Vacuum pump	Value
11.	Refrigerator	LG
12.	Humidity controlled oven	REMI
13.	Soxhlet apparatus	Raja enterprises india
14.	Hot air oven	Genuine
15.	BOD incubator	Genuine
16.	Dissolution apparatus	Scientific Engg corp. Delhi
17.	Tensiometer	Mex tech
18.	Atomic absorption spectroscopy	Shimazdu
19.	Flatness measurement apparatus	India MART
20.	Folding endurance Tester	India MART
21.	Vernier caliper	India Tools $ Instruments
22.	GC-MS	India MART
23.	IR-Spectroscopy	Deeksha Analytical Pvt Ltd
24.	HPTLC	Anamatrix
25.	Muffle Furnace	Meditech

6.3. Collection and authentication of Plant material:

6.3.1. Collection of plant:

The plant Anthuriumandraeanum were collected and grown on the well irrigated and well manure (organic compound) land in the Medicinal garden at Kalpetta, Wayanad (dist), Kerala.

6.3.2 Authentication of Plant material:

The collected specimens were botanically identified and authenticated by Dr. A.Balasubramanian, Executive director Department of Botany, in ABS HERBAL GARDENS,SALEM-636003. The whole plant was identified as Anthuriumandraeanumbelongs to the family Araceae. (Ref.no :AUT/JKKMMRF/280)

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Fig.6.1 & 6.2- Dried Plant parts and coarse powder dried

6.3.3. Macroscopy:

Whole plants parts of Anthuriumandraeanumwere collected and different organoleptic features vizshape, size, colour, type, odour, taste were observed. These parameters are considered useful in the qualitative control of the crude drug and evaluated as per WHO guidelines.

6.3.4. Physio-chemical evaluation :[107,108]

Shade dried powdered whole plant of Anthuriumandraeanumwere used for the determination of the physio-chemical constants in accordance with the WHO guidelines.

6.3.4. A. Determination of Loss on Drying:

Ten grams of the sample materials were placed in an evaporating dish covered with tar (without first drying). Avoid using a high-speed mill to prepare the samples. After five hours at 105°C in the drying room, the sample in the tarred evaporating dish was weighed. Every hour, the process of drying and weighing is repeated until the difference in weight between the two subsequent measurements is less than 0.25%. When there is no more than 0.001g difference between two successive weigh-ins following 30 minutes of drying and 30 minutes of cooling in a desiccator, the weight is considered constant. The percentage moisture content is contrasted with the sample that was air dried.

6.3.4. B. Determination of Ash values:

The inorganic residue that remains after burning and is made up of inorganic salts that are either naturally present in the drug or have been purposefully added to it as a means of adulteration, is known as the ash value of a crude drug.

It is employed to assess the grade and purity of the raw medication in powdered form.

❖ **Total Ash:**

The whole ash method is intended to quantify the overall amount of material left over after burning.

They consist of two types of ash: non-physiological ash, which is the remains of foreign material stuck to the surface of the plant, and physiological ash, which comes from the plant tissue itself. Ten grams of sample material is placed in the silica crucible to red hot for thirty minutes, and then let it cool in a dessicator. In a tarred silica dish, precisely weigh the crushed drug and incinerate it at a temperature of no more than 450°C until the sample is carbon-free. Then, chill it in a dessicator and weigh it. The entire ash percentage was computed.

❖ **Water Soluble Ash:**

Fig.6.3 -Water soluble ash

The entire amount of ash was retrieved, and after five minutes of boiling in 25 milliliters of water, the insoluble material was collected in ashless filter paper, cleaned in hot water, and ignited for fifteen minutes at a temperature not to exceed 450. The weight of this residue in milligrams should be deducted from the weight of all the ash. It was computed to find the amount of water-soluble ash in milligrams per gram of air-dried material.

❖ **Acid Insoluble Ash:**

After boiling the entire ash with diluted hydrochloric acid, the residue is recovered, and the insoluble materials that remain are burned and quantified. This quantifies the amount of silica that is there, particularly in the form of siliceous earth and sand.25 milliliters of diluted hydrochloric acid were added to the crucible that held the sample's

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complete ash. After gathering the insoluble material on ashless filter paper (Whatmann 41), hot water was used to wash the mixture until the filtrate was neutral.

After being dried on a hot plate and burned to constant weight, the filter paper containing the insoluble materials was returned to the original crucible. Let the residue cool in an appropriate dessicator for half an hour, then weigh it right away. Calculations were made about the acid-insoluble ash content in relation to the air-dried medication.

Sulphated Ash:

- To determine how much of a sample's residual material hasn't volatilized, utilize the Sulphated ash test. These tests are typically performed to find out how much inorganic material is present. A silica crucible was heated to a red temperature for ten minutes, then cooled in a dessicator before being weighed. A 2g sample was precisely weighed, softly ignited, and then completely burned. After cooling, the residue was wet with 1 milliliter of sulfuric acid, heated gradually until no more white vapors were produced, and then burned at 800 ± 25 degrees Celsius until all black particles were gone. After allowing the crucible to cool, heat and a few drops of sulfuric acid were applied. Then lit again, let it cool, then measured. Until two subsequent weigh ins differ by more than 0.5 mg, this procedure was repeated.

6.3.4. C. Determination of Extractive values:

When the components of a medication cannot be easily assessed by any other method, extractive values are helpful for the assessment of phytoconstituents. These values also reveal the makeup of the active ingredients in a crude medication.

- **Determination of Water Soluble Extractive:**

A 5g sample that had been air dried and coarsely powdered was weighed, and it was macerated for 24 hours in a closed flask with 100ml of chloroform water (95ml distilled water and 5ml chloroform). After being repeatedly shaken for six hours, it was left to stand for eighteen. After that, it was quickly filtered while being careful not to lose any solvent. Twenty-five milliliters of the filtrate were evaporated until they were completely dry in a shallow dish with a tarred bottom. The dish was then dried in a hot air oven at 105°C for an hour, cooled in desiccators for thirty minutes, and then weighed. After repeating the procedure until a constant weight was reached, the percentage of water soluble extractive value was computed using the medicine that had been air dried as a reference.

- **Determination of Alcohol Soluble Extractive:**

After 5g of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours, it was shaken frequently for six hours and left to stand for eighteen hours. After that, it was rapidly filtered, being careful not to lose any solvent, and 25ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish. The dish was then cooled in desiccators and weighed again, repeating the process until a constant weight was reached. The percentage of alcohol soluble extractive value was calculated with reference to the air dried drug.

6.4. Extraction of plant material:[107]

200g of dried powdered of Anthuriumandraeanum were carefully packed in a Soxhlet apparatus and extracted continuously by aqueous extraction over the course of eighteen hours . The extract was concentrated under a rotary vacuum evaporator while still hot, filtered, dried, and stored in a dessicator until needed again.

Fig.6.4 -Soxhlet apparatus & Rotary vacuum evaporator

6.4.1. Preliminary Phytochemical screening:[88]

The extract obtained from whole plant of Anthuriumandraeanum Lind were then subjected to qualitative chemical tests to determine the presence

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of phytoconstituents.

PHYTOCHEMICAL TESTS:

6.4.1. A.DETECTION OF CARBOHYDRATES AND GLYCOSIDES

Small quantity of extract was dissolved separately in distilled water and filtered. The filtrate was subjected to various tests to detect the presence of different carbohydrates.

a. Molisch's test:

Filtrate was treated with alcoholic solution of alpha-Naphthol and a few drops of conc. Sulphuric acid were added through the sides of the test tube. The formation of violet ring at the junction of the liquids was indicates the presence of carbohydrates.

b.Fehling's test:

Filtrate was treated with few ml of dilute hydrochloric acid and heated on a water bath for 30 minutes. After hydrolysis the solutions were neutralized with sodium hydroxide solution. To the neutralized solutions, equal quantities of Fehling's A & Fehling's B solutions were added and heated on a water bath for a few minutes. Formation of red-orange precipitate was indicates the presence of reducing sugars.

C.Benedict's test:

Filtrate was treated with 5ml of Benedict's reagent and heated on a water bath for a few minutes. The formation of red-orange precipitate was indicates the presence of reducing sugars.

Another small portion of extract was hydrolyzed with dilute hydrochloric acid for a few hours (2 to 4 h) in a water bath and subjected to various tests to detect the presence of different glycosides.

D .Liebermann- Burchard's test:

Hydrolysate was treated with a few drops of acetic anhydride, boiled and cooled. Few drops of sulphuric acid were added through the sides of the test tube. Formation of a brown ring at the junction of two liquids and green colour in the upper layer indicates the presence of glycosides.

E.Legal's test:

Hydrolysate was treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of blood red colour indicates the presence of cardiac glycosides.

F .Keller Killiani's Test (Cardiac glycoside):

To 2 ml of extract, glacial acetic acid, one drop of 5% ferric chloride and concentrated sulphuric acid were added. There is the absence of cardiac glycosides.

6.4.1. B.DETECTION OF ALKALOIDS

Small portions of solvent-free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

a. Mayer's test:

Filtrate was treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate was indicates the presence of alkaloids.

b. Dragendroff's test:

Filtrate was treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate was indicates the presence of alkaloids.

c. Wagner's test:

Filtrate was treated with solution of iodine in potassium iodide (Wagner's reagent) and formation of brown precipitate was indicates the presence of alkaloids.

d. **Hager's test**:

Filtrate was treated with a saturated solution of picric acid (Hager's reagent) and formation of yellow precipitate was indicates the presence of alkaloids.

6.4.1.C .DETECTION OF PHYTOSTEROLS

Extract was refluxed separately with solution of alcoholic potassium hydroxide till complete saponification took place. Saponified mixtures were diluted with distilled water and extracted with solvent ether. Ethereal extract was evaporated to dryness and the residue subjected to Liebermann- Burchard's test.

Liebermann- Burchard's test

Residues were treated with a few drops of acetic anhyride; boiled, cooled, and 1 ml of sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two liquids and green colour in the upper layer indicates the presence of steroids and tri terpenoids.

6.4.1.D. DETECTION OF FIXED OILS AND

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FATS

a .Spot test:

A small quantity of extract was pressed separately between two filter papers. Formation of oil stains on the filter paper indicates the presence of fixed oil.

b. Saponification test:

A few drops of 0.5 N alcoholic Potassium hydroxide was added to a small quantity of extract along with a drop of phenolphthalein. Mixture was heated on a water bath for 1 to 2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

6.4.1.E. DETECTION OF SAPONINS

About 1ml of extract was diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. Formation of any froth above the surface was indicates the presence of saponins.

6.4.1.F. DETECTION OF PHENOLIC COMPOUNDS AND TANNINS

Small quantities of extract was diluted separately in water and were tested for the presence of phenolic compounds and tannins.

a. Ferric chloride test:

To the test solutions, a few drops of 5% ferric chloride solution were added. Formation of a bluish-black or greenish-black colour was indicates the presence of phenolic compounds and tannins.

b. Gelatin test:

To the test solutions a few drops of 1% gelatin solution in 10% sodium chloride was added. Formation of white precipitate was indicates the presence of tannins.

c. Lead acetate test:

To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate was observed for the presence of tannins. Formation of a yellow precipitate was indicates the presence of flavonoids.

d. Aqueous bromine test:

To the test solution, a few drops of aqueous bromine solution were added. Formation of a yellow precipitate was indicates the presence of tannins.

6.4.1.G. DETECTION OF PROTEINS AND FREE AMINO ACIDS

Small quantities of extracts were diluted separately in water and tested for the presence of proteins and free amino acids by subjecting the extracts to various tests.

a. **Millon's test**: To 2ml of the test solutions, 2ml of Millon's reagent were added and heated. Formation of white precipitate that gradually turns red was indicates the presence of proteins and amino acids.

b. **Biuret test**: To the test solutions, a few drops of 0.7% Copper Sulphate solution was added. Formation of a purplish violet colour was indicates the presence of amino acids.

c.**Ninhydrin test**: To the test solutions, a few drops of Ninhydrin solution was added in a water bath. Formation of a bluish colour was indicates the presence of amino acids.

DETECTION OF GUMS & MUCILAGE

About 10ml of extract was added to 25ml of absolute ethanol with constant stirring. Precipitate was examined for its swelling properties and for the presence of carbohydrates.

6.4.1.H.DETECTION OF FLAVONOIDS

a**. Shinoda test**: To the test solutions, a few fragments of magnesium metal were added along with concentrated hydrochloric acid, and heated. Formation of magenta colour was indicates the presence of flavonoids.

b. **Alkaline reagent test**: To the test solutions a few drops of sodium hydroxide solution was added. Formation of an intense yellow colour that turns less intense on addition of acid was indicates the presence of flavonoids.

6.5.Chromatographic evaluation:[108]

6.5.1Thin Layer Chromatography (TLC)

TLC is a very useful method for identifying the chemical constituents of an extract by separating them.

• TLC profiles created for an extract from a specified solvent system together with additional parameters may be applied to a comparative qualitative assessment of herbal medications as a finger print.

• TLC is a crucial analytical tool for classifying, identifying, and estimating various natural product classes. This method uses the differential migration of a solute between two phases—a stationary phase and a mobile phase—to separate the various components. In this case, the stationary phase

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functions as an adsorbent and adsorption are the separation principle.

❖ **Separation of Components**

A calibrated capillary tube was used to spot the extracts, which were dissolved in their respective solvents and placed one centimeter above the bottom of a prepared TLC plate. The specks ranged in diameter from 2 to 3 mm and were all of equal size.

❖ **Selection of Mobile phase**

The selection of solvent or mobile phase depends upon various factors as mentioned below:

1) Nature of substance to be separated.
2) Nature of stationary phase(Polar/Non polar)
3) Mode of chromatography(normal/reverse phase)
4) Extent of separation to be achieved (analytical/preparative).

Increasing order of polarity was used to select the solvent systems. The solvent systems were chosen based on the results of the chemical tests and the types of phyto constituents that were present. The various spots that appeared in each system were identified using iodine staining and certain reagents. Some phytoconstituents in the extracts were identified using the following solvent systems.

❖ **Stationary Phase**

Precoated TLC plates of E –Merck were used for the study.

6.6. Preformulation Studies:[103]

The first step in the systematic process for developing pharmacological dosage forms is called the Preformulation testing. It is the study of the physical and chemical characteristics of a pharmacological ingredient both by itself and in combination with excipients. Preformulation testing's overarching goal is to produce data that formulators can utilize to create manufacturably stable, bioavailable dosage forms. The subsequent Preformulation research is conducted.

✠ Finding the absorption maxima
✠ Physical appearance
✠ Solubility
✠ Infrared spectroscopy studies(compatibility studies)

6.6.1. Finding the absorption maxima (λmax):

To identify the medication, the absorption maximum was discovered. Certain details regarding the chromophoric portion of the molecules have been obtained through the use of ultraviolet visible spectrophotometry. When exposed to light in the visible and ultraviolet portions of the spectrum, organic molecules in solutions absorb specific wavelengths of light based on the kind of electronic transition involved in the absorption. A standard cuvette was filled with the extract solution (5, 10, 15, 20, 25 µg/ml)
in distilled water, and it was scanned in the 200–800 nm range using a UV spectrophotometer.

6.6.2. Physical appearance &Solubility

Colour, taste, texture and solubility were determined for the active ingredient and excipients.

6.6.3. Infra-Red Spectroscopy

A popular method for examining materials in their gaseous, liquid, or solid phases is Fourier Transform Infrared spectroscopy, or FTIR. It is predicated on the interplay of inherent vibrations of the chemical bonds separating the atoms that make up matter with electromagnetic radiation. Not every molecule's potential oscillations will produce an absorption band in the infrared (IR) spectrum. IR spectroscopy is used to determine a substance's functional group and clarify its structure. It can also be used to determine whether or not a particular sample of an organic material is the same as another. It is also employed in quantitative analysis, impurity detection, and reaction progress study.

After placing the sample (with the aid of a sample holder) directly in the direction of the infrared beam, 128 scans were obtained for every measurement spanning the 500–4000 cm−1 spectral range, with a resolution of 4 cm−1.

6.7. Preparation of Transdermal Patch:

Table 6.3- Ingredients and Ratio of Transdermal patch Preparation

S.NO	INGREDIENTS	FORMULATION
01.	HPMC	2gm
02.	PVA	4ml
03.	Polyethylene glycol-400	5ml
04	Aqueous extract ofAnthuriumandraeanum	0.5gm

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05.	Glycerine	0.1ml
06.	Ethanol	10ml
07.	Distilled Water	20ml
08.	Tween 80	0.02ml

- Initially weigh the above required ingredients for the formulation.
- In a clean 100 ml beaker add 20ml of water and 10ml ethanol as a solvent mix well using magnetic stirrer.
- Add 2gms of HPMC and 4ml PVA used as a polymer mix well until it dissolved in solvent.
- Add 5ml PEG- 400 as a plasticizer and 0.02ml Tween 80 as a solubilizing agent.
- Finally add 0.5gm extracted crude drugs with constant stirring 10-15 mins.
- After 10 mins then the formulation becomes viscous then it was added to the mould in glass petri plates which were lubricated with glycerine
- Then petri plates are kept in hot air oven for 24hrs at 35^0 C.

6.8. GC-MS Study:

Gas Chromatography:

The components of a mixture are separated using this physical separation technique by differences in their distribution between two phases, one of which is mobile and moves through the mixture in a specific direction, and the other of which is fixed.

Mass Spectrometry:

Mass spectrometry is an effective analytical method that may be used to determine the composition and structure of various molecules, measure known materials, and identify novel substances within a sample.

Combination of Gas Chromatography with Mass Spectrometry

The hybrid analytical method known as gas chromatography-mass spectrometry (GC-MS) combines the detection qualities of MS with the separation powers of GC to increase the effectiveness of sample examinations. Volatile components in a sample can be separated by GC, however MS aids in fragmenting the components and identifying them based on mass. A wide number of novel applications for GC-MS in various fields are made possible by its improved sample identification ,higher sensitivity ,expanded range of analyzable samples, and faster findings.

GC-MS is utilized in production, quality control, and research and development in the pharmaceutical business. It is employed in the process of identifying contaminants in active medicinal components. Compound synthesis and characterization also employ GC-MS.

6.9. Pharmacological screening:

6.9.1. Anti-inflammatory activity-Inhibition of albumin denaturation:[94]

Principle

Protein denaturation is the process by which proteins are subjected to external stressors or compounds, such as heat, an organic solvent, a strong acid or base, or concentrated inorganic salt. As a result, proteins lose their secondary and tertiary structures. When denatured , the majority of biological proteins cease to function biologically. Inflammation is known to be caused by protein denaturation. The ability of the sample to inhibit protein denaturation was investigated as part of the inquiry into the mechanism of the anti-inflammatory effect. It worked well to prevent albumin denaturation caused by heat.

Materials used

Diclofenac, Bovine Serum Albumin (BSA) was procured from Sigma Aldrich, USA. 10x Phosphate Buffered Saline (PBS) was purchased from Himedia, India.

Procedure

Inflammation is primarily brought on by denaturation of proteins. With a little modification, the method of Mizushima, Kobayashi, and Sakat et al was used to assess the inhibition of protein denaturation. To evaluate sample formulations (500, 250, 100, 50, and 10 µg/mL), 500 µL of 1% bovine serum albumin was added. After being left at room temperature for ten minutes, this combination was heated to 51°C for twenty minutes. After bringing the resultant mixture to room temperature, the absorbance at 660 nm was measured. Diclofenac was used as a control that worked well. The following formula was used to determine the % inhibition of protein denaturation during the triplicate experiment:

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% Inhibition= (A1-A2) /A1x 100)

Where A1 is the absorbance of the control, A2 is the absorbance of the test sample and A1is the absorbance of the control.

- **Statistical analysis**

By measuring the % inhibition of protein denaturation at various concentrations, the anti-inflammatory activity was measured. The drug Diclofenac served as a positive control. Software called Graph Pad Prism 6.0 (USA) was used to calculate the numbers.Values are measured as SD ± Mean

6.9.2. Anti-microbial screening by agar –well diffusion method:

Principle

- The antimicrobial agents found in the sample were let to permeate the medium and engage in interactions with newly seeded test organisms on a plate. Because there will be a confluent lawn of growth, the resulting zones of inhibition will be evenly round. One can measure the zone of inhibition diameter in millimeters.

6.9.3.Anti-bacterial Screening:[96,97]

Materials used

(Staphylococcus aureus- 902) were purchased from MTCC, Chandigarh, India. Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution was purchased from Hi Media, India. Test samples, petri-plates, test tubes, beakers conical flasks were from Borosil, India. Spirit lamp, double distilled water.

6.9.4.Agar-well diffusion Method

Nutrient Agar Medium:

To produce the medium, 2.8g of Nutrient Agar Medium (Hi Media), which is sold commercially, was dissolved in 100ml of distilled water. The dissolved medium was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. While still molten, the autoclaved mixture was thoroughly mixed and then transferred to 100mm petriplates, with 25–30 ml each plate.

Procedure

- In a 24-hour period, petri dishes with 20 milliliters of nutritional agar media were seeded. The McFarland standard was used to adapt the culture of the bacterial strains to a 0.5 OD value (Staphylococcus aureus - 902).
- After cutting wells, various sample concentrations (5, 2.5, 1, and 0.5 mg/ml) were applied. After that, the plates were incubated for 24 hours at 37°C.

- **Statistical analysis**

By measuring the diameter of the inhibitory zone that formed around the wells, the antibacterial activity was measured. The antibiotic gentamicin served as a positive control. Software called Graph Pad Prism 6.0 (USA) was used to calculate the numbers.Values are measured as SD ± Mean

6.9.5.Antifungal Screening[96,97]

Principle

- In a plate that had just been seeded with the test organisms, the anti-fungal compound found in the sample was let to diffuse out into the medium and interact. Because there will be a confluent lawn of growth, the resulting zones of inhibition will be evenly round. Millimeters can be used to measure the diameter of the zone of inhibition.

Materials used

- Potato dextrose agar medium, Griseofulvin solution , test samples, test tubes, beakers conical flask, spirit lamp, double distilled water and petri-plates.

6.9.6.Agar-well diffusion Method:

Potato Dextrose Agar Medium

- In 100 milliliters of distilled water, 20 grams of potato infusion, 2 grams of dextrose, and1.5grams of agar were dissolved to create the potato dextrose agar medium.
- The dissolved medium was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. While still molten, the autoclaved mixture was thoroughly mixed and then transferred onto 100mm petri plates (25–30 ml/plate).

Procedure

A 72-hour culture of the fungal strain Aspergillusniger was seeded into petri plates with 20 milliliters of potato dextrose agar medium. The wells were then sliced, and various concentrations of sample PHTP 2(60:40) (5, 2.5, 1 and 0.5 mg/ml) were applied. After that, the plates were incubated for 72 hours at 28°C.

The diameter of the inhibition zone that developed around the wells served as an indicator of the anti-fungal action. A positive control was employed, namely griseofulvin. Software called Graph Pad Prism 6.0 (USA) was used to calculate

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the numbers.

6.9.7 Evaluation of Transdermal patches:[98,99]

1. Organoleptic characteristics:

Visual checks for colour, clarity, flexibility, texture, appearance and odour were made on each created patch.

2. Uniformity of weight:

To do this, five distinct patches of every group were weighed, the uniform size was chosen at random, and the average weight of three was determined. The patch used for the experiments has been dried at 60˚C for four hours before the test.

3. Thickness of the Patch

A digital vernier caliper was used at several spots along the patch to measure its thickness. Three randomly chosen patches were utilized from each formulation. The thickness of a single patch was averaged out.

4. **Drug content determination**

A 2cm by 2cm patch was immersed in 100ml of ethanol and shaken constantly for a full day. The entire mixture was then ultrasonically treated for fifteen minutes. The drug content is determined by spectrophotometric estimation of the drug at a wavelength of 281nm after filtration.

5. Folding Endurance

One patch was folded repeatedly in the same spot until it broke in order to ascertain this. The value of folding endurance was determined by counting how many times the patch could be folded in the same way without breaking.

6. Percentage Moisture uptake

The patches were precisely weighed and then put in desiccators with aluminum chloride. The patches were removed and weighed after a full day. The difference between the final and original weight was used to compute the percentage of moisture uptake in relation to the starting weight. It is computed using the formula that follows.

$$\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{X100}$$

7. Percentage Moisture content

The patches were weighed and stored in calcium chloride-filled desiccators. The patches were removed and weighed after a day. This formula was used to determine the percentage moisture content.

$$\textbf{ercentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{X100}$$

8. Determination of surface pH

The patches were allowed to swell by being in contact with 1 milliliter of distilled water for two hours at room temperature. The electrode was then brought in touch with the patch's surface and allowed to equilibrate for one minute in order to record the pH.

9. Tensile strength

Tensile strength is measured with a tensiometer. A patch is attached to the tensiometer assembly, the weight required to break the patch is computed, and the elongation of the patch as a result is measured (using the instrument's pointer). By averaging three patch readings, one may find the tensile strength of a patch. The tensile strength of the patch is:

Tensile strength = break force / a ´ b (1 + ΔL/L)

Where, **a** = patch width, **b** =patch thickness, **L** = patch length, **ΔL** = patch elongation at breakage point, and break force=weight (kg) required for patch breakage.

10. Swellability:

Every patch (labeled W1) is weighed separately, put in a different 2% agar gel plate, and then incubated at 37±1°C before being checked for any physical alterations. The patches are taken off the gel plates and any extra surface water is wiped away with filter paper at regular intervals of one hour to three hours. The swollen patches (labeled W2) are weighed again, and the following formula is used to determine the Swelling Index (SI):

$SI = (W_2 – W_1)/W1*100$

11. Flatness test

A transdermal patch is divided into three longitudinal strips: one from the center, one from the left, and one from the right. The length of each strip is measured.

The following is the formula for calculating flatness:

$\text{Constriction (\%)} = (I_1 – I_2) \times 100$

Where,I_1=strip initial length,I_2=strip final length.

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6. 10. Stability Studies :[98,99]

Stability

The period of time that a medicinal product maintains the same qualities and attributes that it had when it was first manufactured is known as its stability. This process is in its early stages of development. In many cases, instability in contemporary formulations is not noticeable until after a long period of time has passed under normal circumstances. It is customary to subject formulated products to severe stress conditions in order to evaluate their stability. This accelerates deterioration and decreases the amount of time needed for testing. Humidity and temperature are common high stressors. As a result, inadequate formulation will be eliminated. The stability condition chart is mentioned in table 6.4

Strategy of stability testing

1. The investigation of the kinetics of drug breakdown.
2. The creation of a consistent dose form.
3. One requirement of stability testing is the establishment of the expiration date for pharmaceutical products that are sold commercially.
4. At least three primary batches of the therapeutic product should have data from the trial given.
5. Pilot scale manufacturing is the minimal requirement for the batches.
6. From a safety perspective, it is crucial that the patient receives a consistent dosage of medication for the duration of the product's shelf life.

Table 6.4- Stability condition chart

Intended Storage Condition	Stability Test Method	ICH Test Temperature and Humidity (period in months)	WHO Test Temperature And Humidity (period In Months)
Room Temperature	Long term	25±20°C/60 ±5%RH	25±20°C/60± 5%RH or 30±20°C/65± 5%RH 30±20°C/75± 5%RH
	Intermedia te	30±20°C/65 ±5%RH	30±20°C/65± 5%RH
	Accelerated	40±20°C/75 ±5%RH	40±20°C/75± 5%RH
Refrigerated	Long term Accelerated	50°C/ambient 25±20°C/60 ±5%RH	5±30°C 25±20°C/60± 5%RH or 30±20°C/65± 5%RH
Freezer	Long term	-200°C/ambient	-200°C±50°C

6.11. Acclerating test:

In accordance with the ICH recommendations [7], the best following parameters: 40 ± 2°C and 75 ± 5% relative humidity (RH) are used to verify the medications' capability for stability in the most optimally adapted conditions.

6.12. Cold and heat test: In order to observe any layering, demulsification, mildew, or alterations in color and consistency, the transdermal patches were placed in a test tube with a plug and kept at 550°C for six hours, followed by twenty-four hours of storage at -150°C in a refrigerator.

6.13. Cellophane Membrane Treatment:

The cellophane membrane was boiled for one hour in distilled water (DW), then it was cleaned three times with new D.W. and stored for twenty-four hours in ethanol. It was cleaned with D.W., treated with 0.3% sodium sulfite, and then acidified with 0.2% sulfuric acid after soaking in distilled water for two minutes at 60°C. The membrane was lastly immersed in boric buffer (pH 9) before to being used in the permeation investigation.

6.14.Drug Permeation Studies

Distilled water was used as the diffusion medium in an open-ended tube to measure the in vitro diffusion rate of developed transdermal patches for up to eight hours of research. Once the receptor compartment holding 200ml of 7.4 buffer solution was filled, the cellophane membrane was tipped in one end of the tube. The temperature was

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kept at 37°C±2°C and swirled at a medium pace. The same volume of diffusion medium was replaced with fresh samples at regular intervals after the samples were removed. Shimadzu UV1700 UV-visible spectrophotometer, calibrated to 260–280 nm, was used to evaluate the materials.

6. 15.Analysis of Heavy Metals by Atomic Absorption Spectrophotometry :[85,32]

The identification of heavy metal elements and certain non-metal elements in their atomic state is accomplished through the use of an atomic absorption spectrophotometer.

When characteristic wave length light from a cathodic discharge lamp travels through atomic vapour produced by an element being studied that has been atomized to the ground state, it is absorbed. Determining the degree of decreased radiation light intensity is how the assay of the element under investigation is tested. The general guideline for absorption spectrophotometry is followed by atomic absorption. Comparing the test preparation's absorbance to that of the reference preparation completes the assay.

❖ **Estimation of Mercury by AAS :**

In distilled water from a 1000 ppm solution, mercury standards of 0.001, 0.002, 0.003, 0.004, 0.005, and 0.01 mg/l were created. Test sample weighed precisely and placed in 300 ml BOD bottles. Concentrated sulfuric acid in 50 milliliters Each bottle was filled with 25 milliliters of nitric acid and 15 milliliters of potassium permanganate solution (5%), which was let to stand for 15 minutes. Each tube received 8 milliliters of a 5% potassium persulphate solution, which was heated for two hours at 95°C in a water bath. To lessen the excess permanganate, cool and add 6 ml of sodium chloride hydroxylamine Sulphate solution. 5 ml of stannous chloride solution were added following the decolorization process. Biological Oxygen Demand (BOD) bottle was quickly fastened to the aeration device, creating a closed system. Remove the BOD bottle from the aeration equipment to create a closed system once the reaction has finished. The aforesaid method was used to prepare the blank and standard. As directed by the particular task requirements, set the AAS. The blank, standards, and sample solutions were aspirated and measured absorbance at 253.7 nm.

❖ **Estimation of Lead by AAS**

Nitric acid (1:499) was used to prepare lead standards of 0.01–0.02, 0.04–0.08, 0.06–0.08, and 0.1 mg/l in a 1000 ppm solution. Weighed the test sample precisely in a beaker, added 50 ml of hydrochloric acid, and digested the mixture until it was only three-quarters of its original volume. Solutions were chilled and made up to 100 ml with distilled water. The blank and standard solutions were prepared in the above manner. As directed by the particular task requirements, set the AAS. The blank, standards, and sample solutions were aspirated. measured absorbance at 283.3 nm.

❖ **Estimation of Arsenic by AAS**

50ml of the digest sample was taken in a 200ml beaker. 5ml of concentrated hydrochloric acid and 5ml of sodium iodide solution were added and mixed well. After 30 minutes, 0.5ml of sodium borohydride solution was added. The test sample, 5g, was taken in a 200ml beaker. 10ml of 2.5N sulphuric acid and 50 ml of 5% potassium persulphate were added. Boiled until the final volume was reduced to 10 ml and then make up to 50ml in standard flask. Arsenic standard solution 0.005, 0.0075, 0.01, 0.02, 0.04 & 0.05mg/l in distilled water from 1000ppm solution were prepared. aspirated the blank, standards, and sample solution while adhering to the work instructions for the AAS. The absorbance at 193.7 nm was measured.

❖ **Estimation of Cadmium by AAS**

Nitric acid (1:499) was used to create cadmium standards of 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/l from a 1000 ppm solution. Weigh the test sample precisely in a beaker, then distil it with 50 ml of hydrochloric acid until the solution is reduced to one-fourth of its original volume. Cool solutions were prepared with 100 milliliters of distilled water. The aforesaid method was used to prepare the standard and blank solutions. As directed by the particular task requirements, set the AAS. The blank, standards, and sample solutions were aspirated measured absorbance at 228.8 nm.

6. 16. HPTLC finger printing of Herbal Transdermal Patch:[143, 144]

❖ **Sample Preparation:**

A volumetric flask containing 0.1g of precisely weighed herbal transdermal patch was filled with 10ml of ethanol to dissolve it, and then Whatmann filter paper No. 1 was used to filter the mixture for HPTLC profiling.

❖ **Application of Sample:**

Precoated HPTLC plates (Merck) that

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were readily accessible in the market were utilized for the investigation. The Linomat V applicator was used to apply solutions at different concentrations to the corresponding HPTLC plates, which were then dried.

❖ **Development of Plates**

After being developed to the desired distance in an appropriate solvent system, the plates were taken out of the chamber, dried, and scanned.

❖ **Detection**

The generated plates were examined in both daylight and ultraviolet light to identify the constituents.

❖ **DensitometricScanning:**

For both the quantitative and qualitative analyses, the produced plates were scanned at an appropriate wavelength. Peak locations and heights were measured to determine the unknown sample concentration and compare the results with the standards.

VII. RESULTS AND DISCUSSION

The final results of several experiments are arranged and examined to offer a strong case for the research that was done.

7.1.Macroscopy:

Organolepticcharactersofpowdereddrug:

Anthuriumandraeanumpowder.

❖ **Nature**–Coarse powder
❖ **Taste**–Burning taste
❖ **Odour**–Characteristic
❖ **Colour**–Brown

7.2 Physio-chemical constants:

Physio chemical parameters were measured using the procedures outlined in the materials and methods, and the results were tabulated . A drug's ash levels indicate the earthy matter or inorganic makeup as well as any other contaminants that may be present. The main application of the extractive values is to identify medicine that has been contaminated or exhausted.The percentage yield was shown in table 7.1.

7.3 ExtractionofPlant Material:

Table. 7.1- Percentageyieldof aqueous plantextractsofAnthuriumandraeanum

S.NO	Extracts	ColourandConsistency	Percentageyield(w/w)
1	Aqueous	Brown&sticky	22.07%

7.4 PHYSICO-CHEMICALSTANDARDS

During the development of new formulations, we must rely on physicochemical standards to optimize stability, solubility and effectiveness which is indicated in table 7.2.

Table 7.2 -DatashowingthePhysico-chemical standards

S.No	PhysicochemicalStandards	Observation(% w/w)
		Anthuriumandraeanum
01.	TotalAsh	17 ± 0.09
02.	AcidInsolubleAsh	4.58 ± 0.16
03.	WaterSolubleAsh	6.40 ± 0.48
04.	SulphatedAsh	0.52 ± 0.37
05.	Loss on Drying	0.5 ± 0.08
06.	WaterSoluble Extractive	4.86 ± 0.90
07.	AlcoholSolubleExtractive	3.16 ± 0.39

7.5.Phytochemical screening:

The qualitative phytochemical screening of the extract was performed to identify the main groups of chemical constituents (glycosides, alkaloids, tannins, saponins, terpenoids, carbohydrate, cardiac glycosides, anthraquinone glycosides, flavonoids, and phenols) present intheextractsusingthecolorreactionsandmentionedin thebelowtable7.3.

7.5.1. PhytochemicalanalysisofextractofwholeplantofAnthuriumandraeanum

Table 7.3 -Phytochemical standards

S.No	Test	Aqueous Extract
01.	Alkaloids	+

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02.	Glycosides	-
03.	Tannins	+
04.	Flavonoids	+
05.	Steroids	+

(+) **Presence**,(-)**Absence**

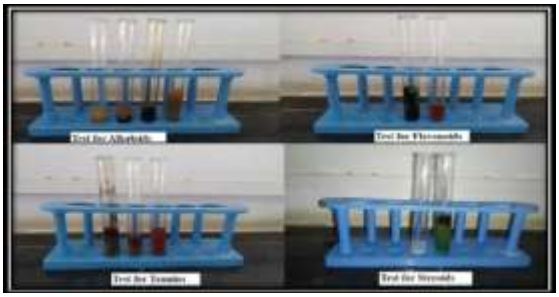

Fig.7.1- Reportof ChemicaltestforAnthuriumandraeanumExtract

❖ **ThinLayerChromatography(TLC)**

ThinLayer Chromatography tests were conducted on the aqueous extracts of Anthuriumandraeanumusing established techniques to verify the presence of different phytoconstituents in the plant extracts. A table displaying potential Rf values for aqueous extract is shown below in table 7.4.

Table 7.4- ThinLayerChromatographyofAnthuriumandraeanum

S.No	Chemical Constituents	Mobile Phase	Detecting Method	Typeof Extract	Number ofSpots	RfValues
01	Alkaloids	Toluene :Ethyl acetate: Diethyl amine:(7:2:1)	UVat 366nm	Aqueous extract	3	0.32 0.7 0.9
02	Tannins	Toluene:Ethyl acetate:Formic acid (24: 16:1.2)			3	0.26 0.68 0.84
03	Flavonoids	Chloroform: Methanol (9:1)			4	0.28 0.64 0.84 0.98
04	Steroids	Ethyl acetate: Methanol:Water (20:2.5:2.5)			2	0.4 0.8

7.6. Preformulationstudies:

Aqueousextract ofAnthuriumandraeanumsolubleinwater.

❖ **Absorptionmaxima (λmax)ofAEAA:**

TheplantextractsconductedUVspectroscopy&thespectrashowtheabsorbance unit &λmax shown in Table 7.5.

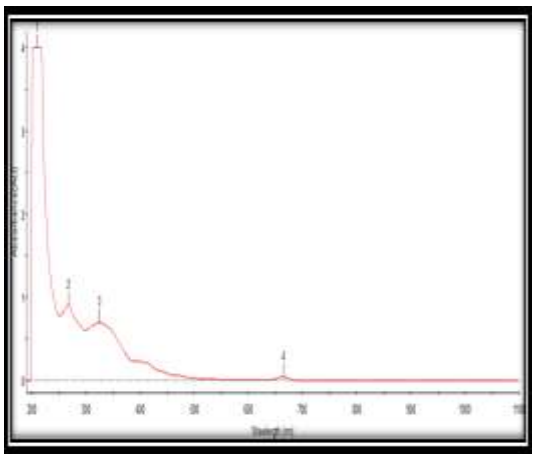

Fig 7.2-UVspectrumofAEAA

Table 7.5-λmaxofAEAA

S.No.	Wavelength (nm)	AbsorbanceUnit(AU)
01.	209.40	4.000
02.	267.50	0.909
03.	324.45	0.700
04.	664.50	0.050

The absorbance for the whole plant extract was observed at different wavelength to confirm its preformulation stabilities. Thesharppeakobserved at267.50nm.

❖ **Physical appearance**

Colour: Light brown
Taste: Bitter taste
Solubility: Freely soluble in Distilled Water

❖ **Compatibilitystudy:**

FT-IRStudies:

FT-IRspectroscopywasperformed forAEAA,HPMCandHTP. The active pharmaceutical ingredients can exist either in

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crystalline or amorphous state. Based on the interpretation,the FTIR spectrum of the herbal transdermal patch showed the characteristic peak of Anthuriumandraeanum herbal transdermal patch involving the active ingredients depicted in table 7.6 , 7.7 ,7.8.

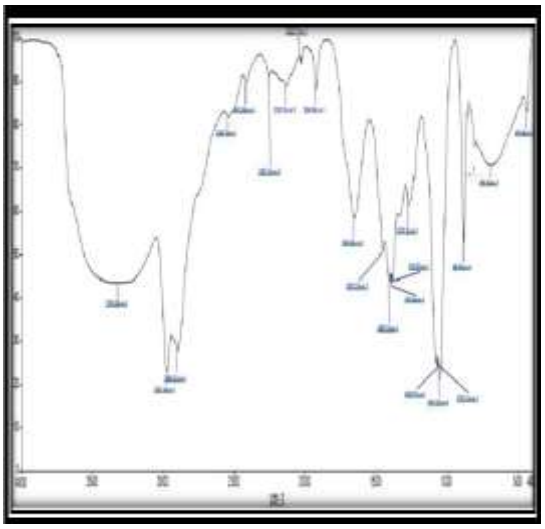

Fig 7.3- FT-IRSpectrumofAEAA

Table7.6- FT-IRspectrum ofAEAA

S.NO	Wavenumber(cm^{-})	Assignment
01.	3316.03	N-Hstretching
02.	2972.16,2900.27	C-Hstretching
03.	1924.36	C-Hbending
04.	1652.08	C=Cstretching
05	1451.03,1406.12	C-Cstretching (in-ring)
06.	1393.44,1382.52	S=Ostretching
07.	1065.70	C-Nstretching
08.	688.00	C-Br stretching

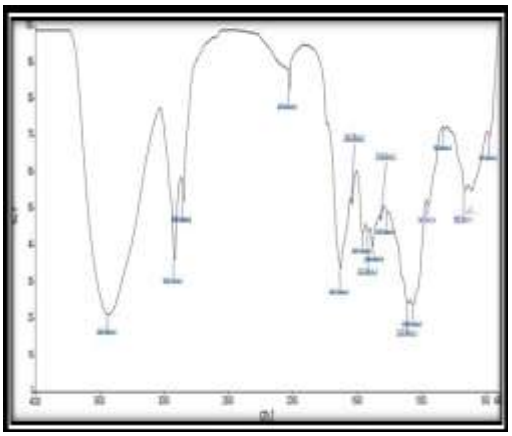

Fig 7.4- FT-IRSpectrumofHPMCE5LV

S.NO	Wavenumber(cm^{-1})	Assignment
01.	3432.94	O-Hstretching
02.	2920.12	C-Hstretching
03.	1631.29,1457.16	C-Cmultiplebondstretching
04.	1316.42	O=Hstretching

Table7.7- Interpretationof FT-IRSpectrumofHPMCE5LV

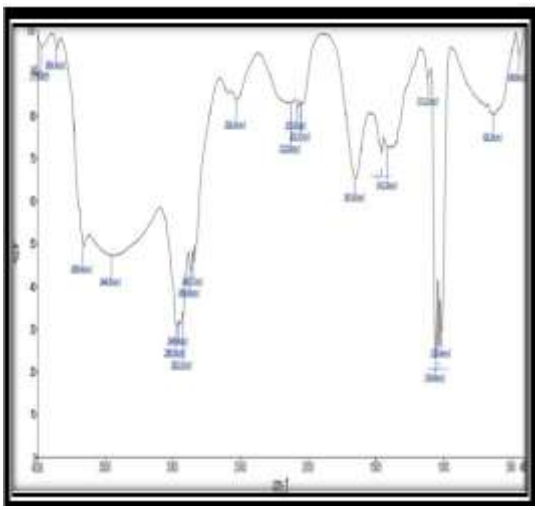

Fig 7.5- FT-IRSpectrumofHTP

The interpretation of infrared spectra involves the correlation of absorption bonds in the spectrum of absorption bands in the spectrum of

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unknown compound with the known absorption frequencies for types of bonds.IR spectroscopy used to establish whether a given sample of an substance is identical with other or not depicted in figure 7.5.

Table 7.8-Interpretation of FT-IRSpectrumofHTP

S.NO	Wavenumber(cm^{-1})	Assignment
1	3446.93	N-H stretching
2	2865.88	C-Hstretching
3	2524.47	S-Hbending
4	2122.88	C=Cstretching
5	1651.6	C=Ostretching(in- ring)
6	1113.21	O-H stretching
7	1013.44, 1054.68	C-Nvibration

ThefindingsoftheFT-IRgraphsforthedrug,excipients,andformulationsrevealedthat noextrapeakorbroadeningofpeakswasseen,whichsuggeststhatthedrug andexcipients are compatible. FT-IR thermogram suggest that the drug is compatible and there were no chemical interactions between polymers.IR spectrum of HTP reveals that only excipients alone are present and none other substances are not involved in formulation in shown in above table 7.8.

7.7 Formulationdevelopment:

The goal of the solvent casting approach was to successfully construct transdermal patches of Anthuriumandraeanumin order to increase the combined bioavailability of herbal drugs. Transparent, smooth, flexible, and homogeneous qualities were observed in the prepared film shown in figure 7.6.

Fig 7.6 - TransdermalpatchofAnthuriumandraeanum

7.6. GC-MSStudy

Bycomparingtheresultswiththeinstrumentlibrary,the GC-MSanalysisofthe produced HTP revealed the existence of the following components .

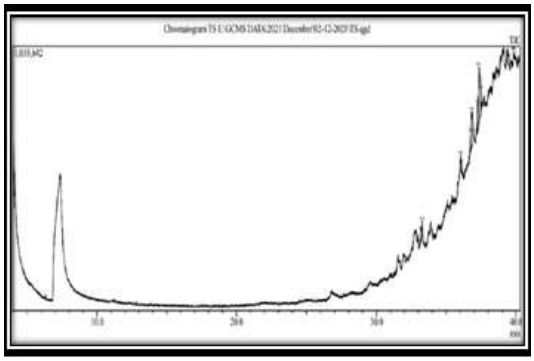

Fig7.7 - GC-MS Chromatogram

.01	33.175	TIC	4.8	5.19	4.63	9,12-Octadecadiynoicacid, tert-butyldimethylsilylester
02.	33.252	TIC	3.59	6.92	2.59	(-)-Isolongifolol,TBDMS Derivative
03.	36.039	TIC	6.47	7.12	4.55	Silane, diphenyl(2,2-dichloroethoxy)ethoxy
04.	36.74	TIC	3.07	8.4	5.4	Betadoradecin
05.	37.25	TIC	20.71	14.72	3.53	Ledenealcohol
06.	36.844	TIC	11.15	11.87	2.45	ethyl 3-methyl-2-oxocyclopentanecarboxylate

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07.	37.359	TIC	34.54	23.98	7.21	Tetracosamethyl Cyclododecasiloxane
08.	37.475	TIC	4.96	10.14	4.7	2-Cyclohexen-1-one,4-(3-hydroxybutyl)-3,5,5-trimethyl
09.	39.335	TIC	3.5	4.96	7.04	(3r,4r,5r)-5-hydroxycaryophyll-8(13)-ene- 3,4-Epoxide

Table 7.9 - InterpretationofGC-MSSpectrum ofHTP

GC-MS systems offer sensitive and reproducible results. It increases the confidence of analytical results of the formulation of HTP. It is used to detect the compounds present in HTP.

7.10. Pharmacologicalscreening:
7.11Anti-inflammatoryactivity-Inhibition ofalbumin denaturation:

The anti-inflammatory properties of HTP was assessed using the egg albumin denaturation method. The formulation of HTP showed the highest rate of inhibition.

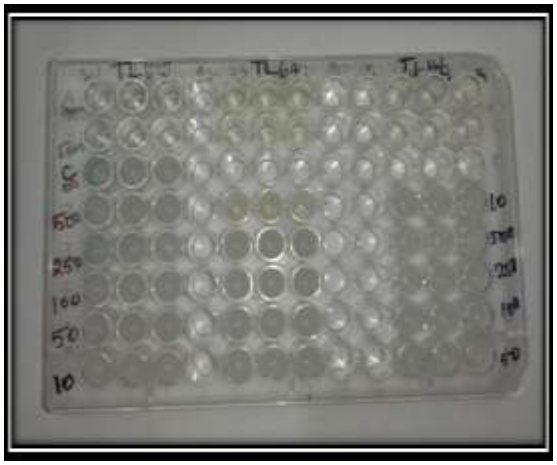

Fig 7.8– Albumin denaturationInhibitioneffectofHTPatdifferentconcentration

Table 7.10 - OpticalDensityforHTP

S.No.	Testedsample concentration (µg/ml)	ODValueat660nm (in triplicates)		
1.	Control	2.120	2.121	2.165
2.	500µg/ml	0.653	0.627	0.233
3.	250µg/ml	1.829	1.791	1.857
4.	100µg/ml	1.864	1.874	1.873
5.	50µg/ml	1.888	1.946	1.934
6.	10µg/ml	1.977	1.953	1.966

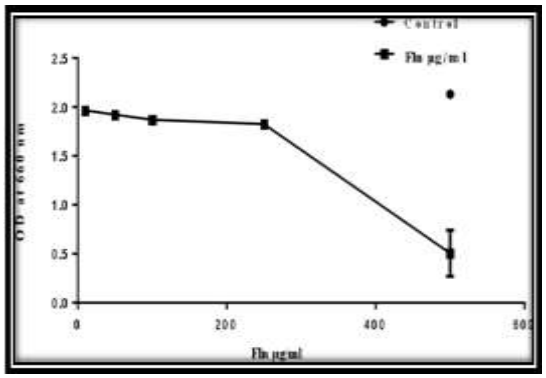

Fig 7.9 -ODat660nm of HTPµg/mlforalbumin denaturation

Table 7.11- PercentageofInhibition ofHTPandDiclofenac foralbumindenaturation

S.No	Concentration (µg/ml)	% Inhibition of albumin denaturation ±SEM	
		Standard	HTP
1	10	17.26 ± 0.347	15.45 ± 0.347
2	50	26.90 ± 0.347	24.48 ± 0.347
3	100	41.16 ± 0.200	40.75 ± 0.347
4	250	51.60 ± 0.347	44.37 ± 0.200
5	500	67.86 ± 0.347	60.24 ± 0.200

Results are shown as mean ±SEM. SEM:Standard error of the mean

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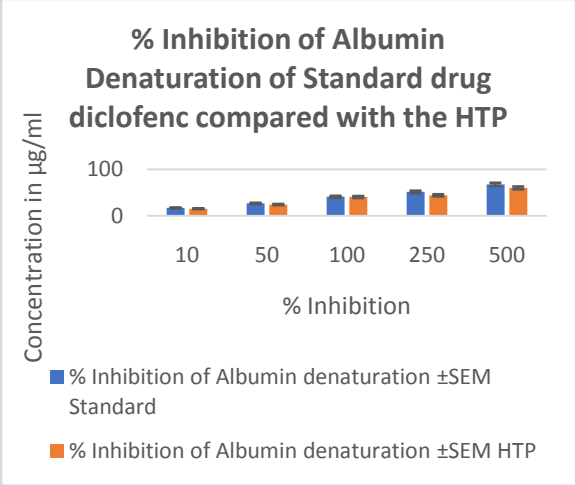

Fig 7.10 - PercentageInhibitionofalbumindenaturationfor HTP and standard

All the data were analysed statistically by one way ANOVA using Graph pad Prism software.The descriptive data were expressed as mean ± standard error of the mean.The percentage rate of inhibition is directly proportional to the sample concentration in (µg/ml).Diclofenac was used as the control drug for estimating anti-inflammatory activity.

7.11.Anti-microbialactivitybyagarwelldiffusionmethod:

❖ **AntibacterialScreening**

Thetestorganismsthatwereseededinteracted withtheantimicrobialcomponentsthat were present in the samples by allowing them to diffuse into the media. There was aconfluent lawn of growth, resulting in uniformly circular zones of inhibition. Millimeters can be used to measure the diameter of the zone of inhibition. Sample data were collected and compared to standard drugs. Compared to the conventional medication, HTP demonstrated almost equal inhibition against Staphylococcus aureus shown in table 7.12.

Table 7.12 - Zone of inhibition of HTP against Staphylococcus aureus

S. No.	Name of the test organism	Name of the Test sample	Zoneofinhibition(mm)SD ± Mean				
			5mg/ml	2.5mg/ml	1mg/ml	0.5mg/ml	PC
1.	Staphylococcusaureus – 902	HTP (60:40)	15.5±0.7	12.25±0.35	12.2±0.28	4.15±0.21	15.25±0.35

Valuesareexpressedasmean±SD(SD–StandardDeviation)

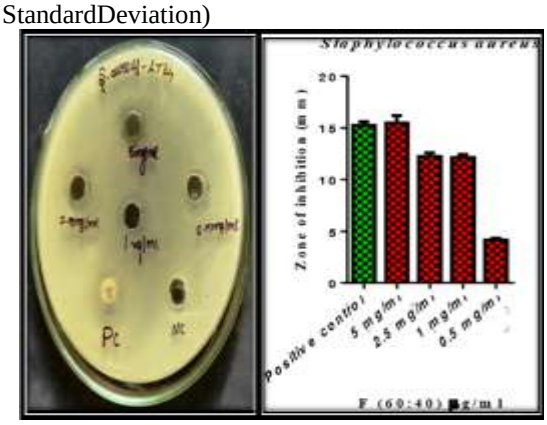

Fig 7.11 - Zone of inhibition of HTP against Staphylococcus aureus

❖ **Anti-fungal Screening**

The test organisms that were seeded interacted with the antimicrobial components that were present in the samples by allowing them to diffuse into the media. There was a confluent lawn of growth, resulting in uniformly circular zones of inhibition. Millimeters can be used to measure the diameter of the zone of inhibition. Sample data were collected and compared to standard drugs. Compared to the conventional medication, HTP demonstrated greater inhibition against Aspergillusniger shown in table 7.13.

Table 7.13 - Zone of inhibition of HTP against Aspergillusniger

S. No	Name of the testorganism	Nameofthe testsample	Zone of inhibition(mm) SD± Mean				
			5 mg/ml	2.5 mg/ml	1 mg/ml	0.5 mg/ml	PC
1.	Aspergillus	HTP	14.5±0.7	12.25±0.35	0	0	12.5±0.7

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	niger	(60:40)					

Valuesareexpressedasmean±SD(SD - StandardDeviation)

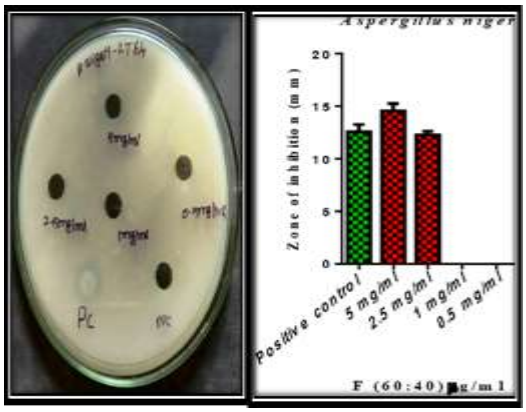

Fig 7.12 -Zone ofinhibitionofHTPagainstAspergillusniger.

Fig 7.11 and 7.12 revealed the mean inhibition zones produced by whole plant extract against test bacteria and fungi.The mean inhibition produced by the test sample at 5 mg/ml, 2.5 mg/ml,1mg/ml, 0.5 mg/ml are 15.5±0.7, 12.25±0.35, 12.2±0.28, 4.15±0.21 respectively and for positive control was 15.25±0.35 for anti-bacterial activity against Staphylococcus aureus.

The mean inhibition produced by the test sample at 5 mg/ml, 2.5mg/ml,1mg/ml, 0.5mg/ml are,14.5±0.7 ,12.25±0.35 respectively and for positive control was for 12.5±0.7. No zone of inhibition was observed for 1mg/ml, 0.5mg/ml for anti-fungal activity against Aspergillusniger.

7.10.Physicochemicalevaluationofpatches:

The physiochemical properties of the optimized herbal transdermal patches, including their physical appearance, weight uniformity, thickness, folding endurance, moisture content, moisture uptake, drug content, tensile strength, drug permeation studies, surface pH, swellability, flatness, and stability, were assessed.

❖ **Organoleptic characterization:**

The transdermal patches are tested for organoleptic properties, which provides information about sensory properties of transdermal patches and helps to ensure its quality and effectiveness shown in table 7.14.

Table7.14 -OrganolepticcharacterizationofHTP

Parameter	HerbalTransdermalPatch
Colour	Brown
Odour	Characteristic
Texture	Smooth
Flexibility	Flexible

Table 7.15- Physicochemical evaluationparameters for HTP

S.No	Parameters	Values
1	Uniformity of weight	0.42 ± 0.74 g
2	Thickness	0.46 ± 0.87 mm
3	Drug content	98 %
4	Folding endurance	253 ± 0.46
5	Percentage moisture uptake	2.95 ± 0.65 %
6	Moisture content	1.246 ± 0.04 %
7	Surface pH	6.25 ± 0.18
8	Tensile strength	6.138 ± 0.74 kg/mm^3
9	Swellability	2.004
10	Flatness	99.99 ± 0.02

Physicochemical evaluationparameters for HTP

- The weight of the prepared patches shows low standard deviation values which indicates the patch was uniform in weight.
- The thickness of the patch measured by Vernier caliper indicate that the patch has uniform thickness.
- The drug content provides information about the amount of drug present in transdermal patches for diffusion.
- Folding endurance of the patch was good which indicates patch can withstand more physical stress without damage.
- Percentage moisture uptake of polymer matrix will increase the formation of pores that enhance porosity.
- Moisture content indicates that patch has specific amount of the moisture content of the patch that can influence both the mechanical properties and drug release, moisture content and formulation was permeable to water vapour.
- The pH of the formulation was found to be satisfactory in the range of 5 to 7, so that it doesn't cause any irritation to the skin.
- Tensile strength of the patch indicates that the

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formulation is strong but not brittle.

- The swellability of the patch was determined for time interval of 1 hr and it indicates that Increase in time will increase the swellability of the patch.
- Flatness of the patch indicates that the patch is reproducible and maintains a satisfactory smooth surface.

❖ **Stability**

In accordance with ICH recommendations, the best optimized transdermal patch underwent a three-month stability assessment under the following conditions. Temperatureof 40 ± 20^{o}C and relative humidity (RH) of 70 ±5% to verify the stability potential of the medications included in the best-optimized formulation depicted in table 7.16.

Table 7.16 -Acceleratedstabilitystudydata

Parameter	**Roomtemperature**	**40±2^{0}C&70±5% RH**
VisualAppearance	Brown	Brown
Initial	Nochange	Nochange
Attheendof1stmonth	Nochange	Nochange
Attheendof2ndmonth	Nochange	Nochange
Attheendof3rdmonth	Nochange	Nochange
Colour	Brown	Brown
Initial	Nochange	Nochange
Attheendof1stmonth	Nochange	Nochange
Attheendof2ndmonth	Nochange	Nochange
Attheendof3rdmonth	Nochange	Nochange
Texture	Smooth	Smooth
Initial	Nochange	Nochange
Attheendof1stmonth	Nochange	Nochange
Attheendof2ndmonth	Nochange	Nochange
Attheendof3rdmonth	Nochange	Nochange

The formulation of an aqueous extract of Anthuriumandraeanum: stability studies transdermal patch was applied for three months in accordance with the protocol stated in the table.No.7.16.The formulations appearance, color, texture, and drug content did not significantly alter during thistime and they remained stable.

❖ **Coldandheattest:**

The Cold and heat test of formulation is tested using Hot air oven at 55^{0}C for 6hrs and Refrigerator at -15^{0}C for 24hrs shown in table 7.17.

Table 7.17- Coldand Heattest forHTP

Condition	**Observation**
Cold	No Change
Heat	No Change

Thereisnoevidenceofchangesinformulation.Therefo retheresultofColdandheat test ensured that the product is remaining stable over time.

7.11.Invitrodrugreleaseprofile:

Every hour after that, the medication release from the patches decreased. The transdermalpatchwasshowntodelivermedicinesinali nearfashion forup toeighthours.At 7hours, there was a noticeable peak in the cumulative drug release, which climbed gradually. Drug release (in absorbance) is plotted against time.

Table 7.18 – In vitrodrugreleaseprofile

S.No.	**Time(min)**	**Absorbance**
1.	0	0
2.	30	0.0681
3.	60	0.0983
4.	120	0.1563
5.	180	0.2830
6.	240	0.4681
7.	300	0.6734
8.	360	0.8964
9.	420	1.1693

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10.	480	1.1747

7.10. Heavy metal analysis

There is less heavy metal in the HTP analysis than are allowed. The following Table displays the mean concentration of heavy metals (ppm) ± standard deviation of three replicates for each heavy metal level found in the HTP.

Table 7.19 -Heavy metal analysis of HTP

S.No.	Nameofthemetal	Observation (mg)
1.	Mercury	1.151 ± 0.031
2.	Lead	2.4 ± 0.031
3.	Arsenic	0.304 ± 0.035
4.	Cadmium	0.012 ± 0.150

7.10. Highperformancethin layer chromatography:

The HTP was exposed to finger printing by HPTLC. It displays unique Rf values depending on the concentration. The sample's chromatogram at various wavelengths was reported, and a table with potential R_f values was created.

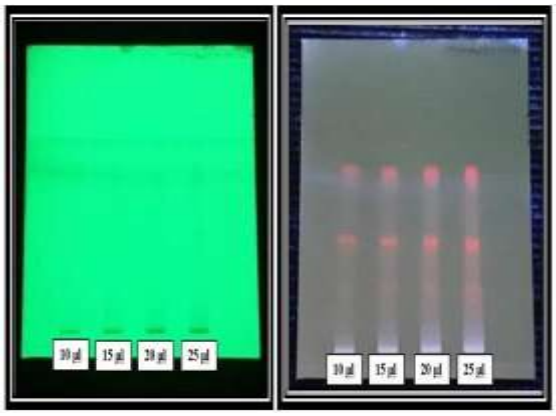

Fig 7.13 -HPTLCChromatogramviewedat366nm

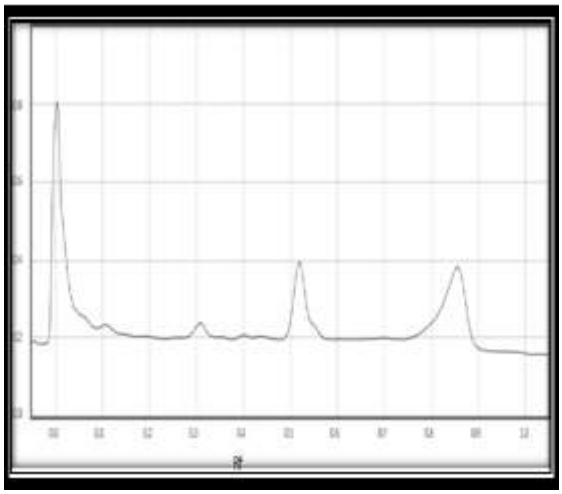

Fig 7.14-HPTLCChromatogramviewedat254nm

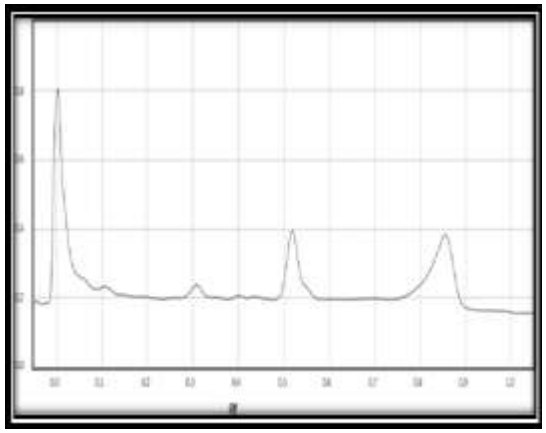

Fig 7.15-ChromatogramofHTP(10µl)at366nm

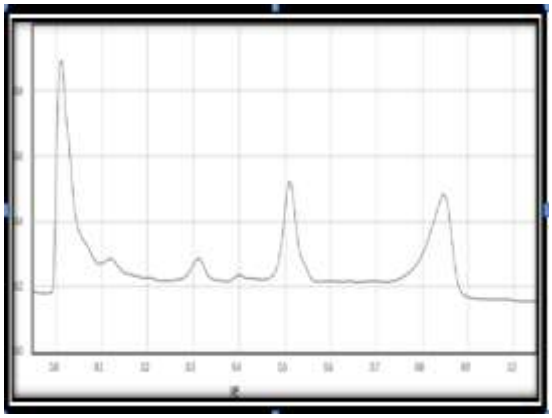

Fig 7.16-ChromatogramofHTP(15µl)at366nm

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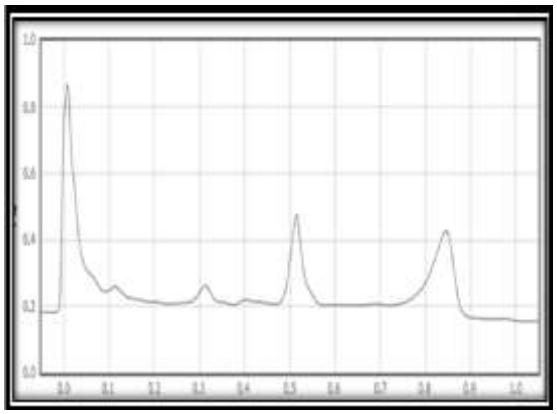

Fig 7.17-ChromatogramofHTP(20µl)at366nm

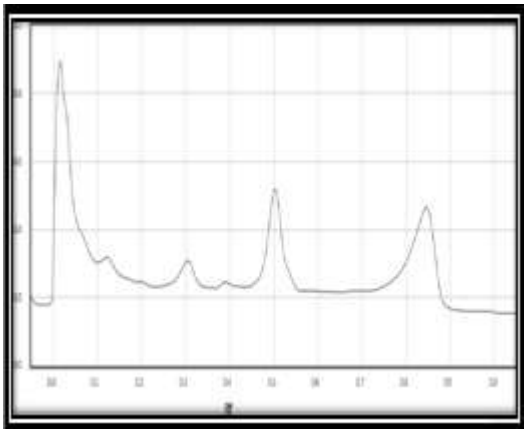

Fig 7.18 -Chromatogramof HTP (25µl) at 366nm

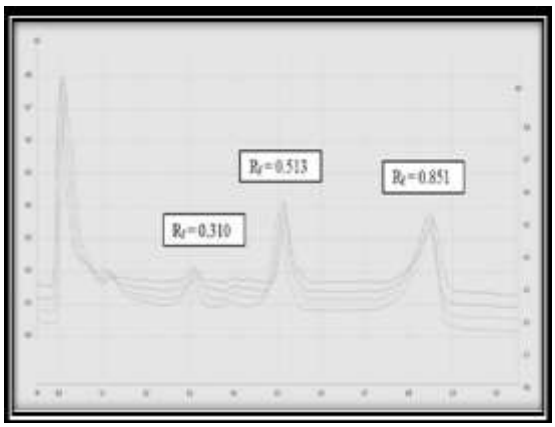

Fig 7.19-HPTLCofTransdermalPatch (Alltracksatwavelength)366nm

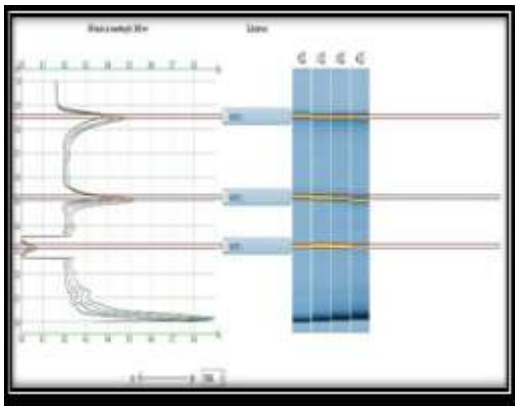

Fig7.20 - HPTLCofTransdermalPatch(Alltracksatwavelength) 366nm

The HPTLC fingerprinting of transdermal patch revealed the presence of phytoconstituents and it indicates their repeatability and reproducibility

Table 7.20 - Data showing the Rf values of Transdermal Patch at 366 nm

S.No.	PeakNo.	λ	Rfvalueof sample
1.	Peak1	366nm	0.310
2.	Peak2		0.513
3.	Peak3		0.851

VIII. SUMMARY AND CONCLUSION

Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as theyhave less side effects as compared with modern medicines. The drugs of Ayurvedic origin can be utilized in a better form with enhanced efficacy by incorporating in modern dosageforms.Phytotherapeuticsdeliverthecomponentsinanovelmannertoincreasepatient compliance and avoid repeated administration. More plants and their combinations should be explored to get reliable, safe and effective formulations that can compete with syntheticdrugs.

In the present study, the medicinal plant **Anthuriumandraeanum**was established for the

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treatment of inflammation, it was selected and formulated as transdermal patches.

Prior to preformulation and formulation development, the selected aqueous plant extract were subjected to phytochemical screening after successive solvent extraction. Qualitative chemical examination of extracts revealed the presence of alkaloids, flavonoids, steroids and tannins. Based on phytochemical studies, aqueous extract was selected and subjected to formulation development.

The various formulation parameters, Drug-Polymer ratio and permeation enhancers were optimized to get thin, transparent, smooth, stable and high permeable transdermal patches.TheFTIRgraphsofdrug, excipientsand formulationsshowedthat there arenoextra peaks (or) broadening of peaks and thus it indicates that there is no incompatibility between the drug and excipients.

HTP of aqueous extracts of Anthuriumandraeanumtransdermal patches were preparedbysolventcastingtechnique.TheGC-MSanalysisshowedthepresenceof differentphytocomponents ofmedicinal importancein theaqueous extractof HTP.

From the optimization, best formulation HTP was selected based on in vitro anti - inflammatory activity in terms of albumin denaturation assay.

Also the selected HTP was found to possess significant antimicrobial property. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes.

Optimized formulation was evaluated for Percentage Moisture uptake, Percentage Moisture content, Thickness, Folding Endurance, Percent Elongation, Tensile strength and in vitro drug release. The formulation showed optimum % Moisture uptake, Moisture content, Thickness, folding endurance, Percent elongation, and Tensile strength.

The stability study results showed that there is no significant change from its initial nature till the period of three months at 40˚C ±2˚C/75 5% RH.

Theheavymetal analysisresultshowedthattheHTPdoesnotcontaintoxiclevel of tested heavy metals such as mercury, lead, arsenic and is safe for administration.

The present pilot study has revealed that the formulated HTP will be a better drug of choice for the treatment of Inflammation as compared to the conventional dosage forms. The potency of the developed formulation shall be subjected to in vivo screening methods in future and may be developed as transdermal patches for effective treatment against Inflammation.

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