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Formulation and Characterization of Herbal Antifungal and Anti-Inflammatory Foot Repair Serum

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KEYWORDS

Structure of Skin, Classification of different dosage form, antifungal activity, anti-inflammatory activity, highly concentrated serum formation

ABSTRACT:

Background: Dry and cracked feet are a result of inadequate moisturizing, increased exposure to pollutants, as well as certain medical conditions like psoriasis, thyroid, diabetes, and eczema so to prepared the highly concentrated foot repair serum.Due to the absence of any oil glands, the feet skin has an ability to crack and dry out. Serum has a thinner viscosity; they not only absorb fast but also reach the deepest layers of the skin to target different parts and provide optimum efficacy. Because of their potent effects and very few adverse effects compared to those of synthetic medications, herbal formulations have consistently attracted significant attention.

Result: The extract of Dimocarpus longan duch and Fragaria × Ananassa were used for the formation of herbal antifungal and anti-inflammatory foot repair serum by using solvent evaporation method and maceration method respectively. The optimization extract were characterised by UV/VIS Spectrophotometer, FTIR Spectrum measurement, HPLC measurement. The required quantity of ingredients like Carbopol 940 (used as a gelling agent), Triethanolamine (used as a pH adjustment), Propylene glycol (used as a humectant), methyl paraben and propyl paraben (used as a preservatives), ascorbic acid (used as a antioxidant) were used for preparation of serum. Various evaluation parameters like Organoleptic testing, pH measurement, Stability study, Spreadability study, Viscosity measurement, Homogeneity. Therefore, present study the main aims to formulate and characterization of herbal antifungal and anti-inflammatory serum for the treatment of cracked heels.

Conclusion: The herbal antifungal and anti-inflammatory foot repaired serum was effectively produced and evaluated using a variety of official parameters, including spreadability testing, pH analysis, and viscosity measurement. Based on all of the investigated herbal serum formulations, M1, M2, and M3 each shown a substantially different rise in skin moisture ($p<0.05$).

INTRODUCTION:

1.1 **Anti-fungal activity :** A fungus-related skin condition called mycosis is referred to as a fungal infection.There are ma

1.2 ny kinds of fungal infections The earth, plants, and even your skin can contain them. In certain cases, they might result in rashes or other skin issues.[1]

1.3

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1.1.1 Classification of fungal infection or mycoses:

Fungal infection are based on three factors: (1) infected site (2) the pathogen's mode of acquisition, and (3) the fungus's type of virulence.Fungal infections, known as mycoses, can be classified into various types based on the degree of tissue involvement and the host's immune response. These types include superficial, cutaneous, subcutaneous, and systemic (deep) infections, and the classification is determined by the specific characteristics of the infection and the way the host's body reacts to the pathogen.

1) The pathogen's mode of acquisition:

Infection-causing fungi can be either internal or external. External fungus can enter the body by a subcutaneous pathway, the skin, or the air. Internally generated infection can take the form of colonization by a member of the natural flora or the recurrence of an earlier infection.

2) The fungus's type of virulence:

Primary pathogens may result in infections in healthy hosts. Pathogens that are opportunistic prompt disease in people with weakened host defence mechanisms.[2]

1.1.1.1 According to infected site:

a) Superficial fungal infection: Superficial fungal infections can be caused by a pathogen that has limited access to the stratum corneum and causes little or no tissue reaction. Superficial mycosis including the tinea versicolor, piedra, and tinea nigra; [3]

b) Cutaneous mycoses: Skin, hair, and nails types of fungal infections are known as cutaneous infections. These fungal infections can be brought on by yeast (Candida species), nondermatophyte fungi, or dermatophytes most frequently.A fungus that causes tinea, a fungal infection, is referred to as a dermatophyte. Hence, dermatophytoses are referred to as tinea infections and are further classified depending on the specific area of the body that is affected, such as tinea pedis for the foot, tinea corporis for the body, tinea cruris for the groin, tinea capitis for the scalp, and tinea unguium for the nails. These are the most commonly encountered dermatophytic infections in the United States.[4] Yeast infections respond primarily to the azoles, whereas dermatophyte infections respond to griseofulvin, allylamines, and azoles. Treatment for nondermatophyte moulds can be more challenging and involve a combination of therapies.[5]

c) Subcutaneous mycoses: Fungal infections known as subcutaneous mycoses primarily affect the dermis and subcutaneous tissue, with rare cases of systemic disease progression. These infections are typically found in tropical regions and typically result from the implantation of common organisms into the skin through local trauma. Immuno suppressed patients are at higher risk for these infections, just like other mycoses. Mycetoma, chromoblastomycosis, and sporotrichosis are the three main subcutaneous mycoses.[6]

1.2 Anti-inflammatory activity:

Anti-inflammatory medicines that minimizes inflammation in the body (redness, swelling, and pain). Anti-inflammatory medicines are prohibit the inflammation.[7] When your body encounters a pathogen (such as viruses, bacteria, or toxic chemicals) or undergoes damage, your immune system is triggered. The immune system deploys inflammatory cells and cytokines, which are substances that stimulate the production of more inflammatory cells, as its first line of defence.. These cells initiate an inflammatory reaction to engulf bacteria and other harmful substances or to initiate the healing process of damaged tissue. This can lead to symptoms such as pain, bruising, swelling, or redness. However, inflammation also affects internal bodily systems that may not be immediately visible.[8]

1.2.1 Inflammation types:

➢ Types of Acute inflammation:

The acute inflammation happens in response to a number of circumstances where tissue damage may occur. Infection, reactions to hypersensitivity, chemical or physical agents, and necrosis of tissues are examples of common causes.[9] Resident immune cells, primarily consisting of resident macrophages, dendritic cells, histiocytes, Kupffer cells, and mast cells, initiate the process of acute inflammation in the affected tissue. These cells have the ability to recognize and bind specific molecules, known as pathogen- associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), through surface receptors called pattern recognition receptors (PRRs).PAMPs are substances that are linked to different pathogens but can be separated from host molecules. DAMPs are substances linked to cell damage and host-related injury.[10]

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Features of acute inflammation:

The loss of function, heat, discomfort, itching, and swelling are some of the symptoms of inflammation. Inflammation is a component of the body's intricate biological reaction to harmful substances like irritants, pathogens, and harmed cells.[11]

- **Types of acute inflammation**:

Five distinct symptoms, known as the "cardinal signs," identify acute inflammation: [12]

1. Tumour (swelling)
2. Donor (pain)
3. calor (increased heat),
4. loss of function
5. Rubor(redness)(47)

- **Stages of acute inflammation**

a. Injury response mechanism:

When tissues are damaged due to infection, trauma, toxins, heat, or other causes, an inflammatory reaction, known as inflammation, takes place. Chemicals like prostaglandins, bradykinin, and histamine are released by the damaged cells. These substances cause blood vessels to become permeable, leading to the leakage of fluid into the tissues and resulting in swelling.[13]

b. Inflammation phase

- Beginning right away after the injury, localized swelling is brought on by transudate, a fluid made up of water, salt, and protein that leaks from the injured blood vessels. [14]
- Inflammation regulates bleeding while preventing infection.
- The migration of repair and healing cells to the wound site is made possible by the fluid engorgement.
- During the inflammatory phase, bacteria, pathogens, and damaged cells are removed from the wound region.
- During this stage of wound healing, the presence of white blood cells, growth factors, nutrients, and enzymes leads to the characteristic swelling, heat, pain, and redness.
- Inflammation is a typical phase of wound healing; it only becomes

problematic if it persists for an extended period of time or is severe.

- The inflammatory phase, which typically spans a few days, is characterized by homoeostasis, chemotaxis, and increased vascular permeability. This phase plays a crucial role in preventing further injury, closing the wound, eliminating cellular debris and bacteria, and facilitating cellular migration.[15]

c. Proliferative phase

The Proliferative Phase, the third stage of the healing process, initiates following the removal of debris from the wound. Its main objective is to fill and provide coverage for the wound.

The proliferative phase consists of three separate stages:

[1)] Wound filling, wound border contraction, and wound coverage (epithelialization) are the first three stages. During the first stage, the wound bed is filled with connective tissue, resulting in the formation of glossy and deep red granulation tissue. Simultaneously, new blood vessels are generated. As the wound heals, the edges of the wound contract, causing them to shrink and be pulled towards the center. In the third stage, the proliferation of epithelial cells initiates either from the wound bed or its edges, allowing them to traverse and ultimately cover the wound with epithelium. Typically, this proliferative phase lasts between four to 24 days.[16]

Remodelling, the last step of healing, is when the granulation tissue transforms into a scar and gains increased tensile strength. The quantity of glycosaminoglycans, the water associated with the glycosaminoglycans (GAGs), and proteoglycans decrease as the granulation tissue matures, and the number of capillaries decreases due to capillary aggregation into bigger vessels. The granulation tissue loses metabolic activity and cell density throughout development. Collagen also undergoes type, amount, and organizational changes that improve tensile strength.[17]

The formation of the granulation tissue is finished before the Remodelling phase starts. As a result of mechanical strain and cytokines like TGF-, myofibroblasts may develop into smooth muscle actin (SMA) expressing myofibroblasts. After the healing process is finished, myofibroblasts undergo apoptosis. Collagen III, which is rapidly produced in the extracellular matrix (ECM), is gradually replaced by collagen I. Although collagen I has greater tensile strength, it takes a longer time to be deposited. Both the rate of blood flow and the development of new blood vessels decline. It develops an environment that is totally a vascular and

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acellular[18]

❑ **Causes of acute inflammation:**

1) Allergens,
2) toxic substances,
3) irritants, and
4) foreign bodies that are too large to be digested or harm macrophage phagosomes are some of the causes. Silica and asbestos are two examples of foreign bodies. [19]

➢ **Chronic inflammation:**

Chronic inflammation, characterized as a prolonged and enduring inflammatory state, can last for months or even years. The severity and impact of chronic inflammation are influenced by the original source of injury or inflammation, as well as the body's ability to heal and reverse the damage.[20] Chronic inflammation can start even when there is no damage, and it often lasts longer than it should. Sometimes the cause of the inflammation's recurrence is unclear. Frequent recurrent infections, improper immune responses to healthy tissues, and health conditions like obesity can all play a role in contributing to chronic inflammation.[20]

1.3 Excipients

A medicine is made up of two main components: active pharmaceutical ingredient and excipient. Most, if not all, medicines would be impossible to manufacture without the use of excipients. Excipients are ingredients that are added to medications to improve production, patient acceptability, stability, and release control, among other things.The majority of a drug product is typically made up of excipients, with very small amounts of the active molecule. . Excipients were previously referred to as inactive components. Excipients are essential in the formulation of a dosage form. These are the ingredients that comprise the dosage forms, along with the Active Pharmaceutical Ingredients. explains all the different excipient types and sources, and also including their uses and how they can be applied to a variety of tasks. Excipients are best suited to a specific dosage form.The luminal fluids of the digestive system are an example of an aqueous environment where many active pharmaceutical ingredients (APIs) have poor solubility and sluggish dissolution rates. Specialized dosage forms can be developed to increase dissolution rate through a variety of processes, or excipients can be added to the formulation to aid in drug dissolution. Excipients are substances present in dosage forms that do not serve as the active ingredient. As per the World Health Organization's definition, an active pharmaceutical ingredient (API) is a substance incorporated in a final pharmaceutical product that is intended to produce pharmacological effects or have an immediate impact on the diagnosis, cure, protection, treatment, or prevention of diseases, as well as to directly contribute to the recovery, correction, or improvement of health." Pharmaceutical excipients are essential for absorption of drugs in the body. Excipients usually don't have any medicinal qualities. Its typical goal is to make the drug product manufacturing easier and ultimately make it easier for the drug to be absorbed physiologically. The function of excipients in taste, disintegration, lubricity, flowability, and antimicrobial defence may be aided. A crucial step in the drug manufacturing process is choosing the right excipient to support the design of your pharmaceutical formulation.

1.3.1 Role of excipients in the formulation of medicine:

Tablets, capsules, oral liquids, topical creams and gels, transdermal patches, injectable products, implants, eye products, nasal products, inhalers, and suppositories are among the numerous options available for administering medications. Pharmaceutical excipients, which are compounds added to pharmaceutical dosage forms, serve purposes beyond their direct therapeutic effects. These purposes may include aiding in manufacturing, providing protection, offering support, enhancing stability, improving patient acceptability, or increasing bio-availability. They might also help identify the product and improve its general usability or safety while being stored.[21]

1.3.2 Active pharmaceutical ingredient excipients :

Excipients are substances created to interact with active pharmaceutical ingredients (APIs) and improve their properties. Excipients are a useful tool for drug formulators because they can be made to promote a variety of ingredient qualities. They can be used to bulk up solid formulations that contain small amounts of APIs for long-term stabilization or to improve the activity of the active ingredient in the final dosage form, such as by facilitating drug absorption, lowering viscosity, or increasing solubility.[22]

1.3.2.1.1 Serum:

A composition with a lot of active components is called a serum. One or more specific concerns may be the focus of a

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serum. A serum does not include as many components as a moisturizer or cleanser. Instead, it offers stronger actives that penetrate the skin more effectively and profoundly.[23] A highly concentrated preparation, serum can be made from water or oil like any other cream. A serum has a small number of components that are intended to maximize the availability of the active substance, which might be a vitamin, growth factor, botanical extract, etc. Because serum has a thinner viscosity, they not only absorb fast but also reach the deepest layers of the skin to target different parts and provide optimum efficacy.[24] Because of their potent effects and very few adverse effects compared to those of synthetic medications, herbal formulations have consistently attracted significant attention.[25]

Types of serum

- **Hydrating serum:** HA serums absorb into the skin and function to moisturize from within the layers of the skin by drawing in water almost like a sponge, in contrast to other moisturizers that tend to sit on top of the skin. This means that moisturizing serums can maintain healthy skin and soften fine wrinkles on the skin's surface.[26]
- **Anti-aging serum:** With the aim of enhancing the appearance of your skin, serums that assist elegant aging are skin care products that contain active ingredients that target the outward indications of aging, such as fine lines, wrinkles, and age spots.[27]
- **Exfoliating serum:** Severe lack of moisture, fine lines, wrinkles as well as hyperpigmentation, exfoliating serums are the most effective choice. An exfoliating serum's acids will aid in removing dead skin cells and battling skin issues like discoloration, dark spots, and early indications of aging.[28]
- **Firming serum** An anti-aging innovation in skin structure and contour is called as Firming Serum. A more defined face contour can be achieved with the help of Firming Serum, which is specifically made to noticeably tighten and firm the skin.[29]
- **Brightening serum** A skin-brightening serum is a cosmetic item containing potent ingredients intended to significantly lessen dullness and discoloration. They are occasionally referred to as skin lightening or skin brightening (not skin whitening) products.[30]

1.4 Transdermal drug delivery system:

The transdermal drug delivery system (TDDS) is a controlled drug delivery method that seeks to administer medication through the skin at a predetermined and controlled rate. This approach offers advantages such as prolonged therapeutic impact, reduced side effects, increased bioavailability, improved patient compliance, and ease of discontinuing drug therapy.The stratum corneum is believed to be the rate-limiting barrier in transdermal penetration for the majority of molecules. For most molecules, the stratum corneum is thought to be the rate-limiting barrier in transdermal permeation. Appendageal, transcellular, and intercellular routes are the three primary routes by which drugs can enter cells.[31] approximately 74% of medications taken today are taken orally and are not as effective as desired. Transdermal drug delivery systems were developed to enhance such characters. Transdermal drug delivery is the process of administering drugs through the skin to produce a systemic effect, in contrast to traditional topical drug delivery methods. Transdermal drug delivery systems (TDDS) are specific formulations where a significant portion of the drug is transported into the systemic blood circulation, while the remaining portion is targeted to viable epidermal and/or dermal tissues of the skin for localized therapeutic benefits. The safety, effectiveness, and quality of the transdermal drug delivery system depend greatly on the adhesive. Topical therapeutic agent administration has many benefits over traditional oral medicaments.[32]

1.5 Topical drug delivery system

A drug's clinical effectiveness when applied topically is influenced by both the drug's accessibility to the target site and its pharmacologic characteristics. The majority of drugs intended sites of action used to treat dermatological conditions is located within the living tissue of the skin. The skin topmost layer are thin and that is known as stratum corneum, it is important impediment for drug delivery by dermal. Clinical drug usefulness is frequently constrained by its inability to cross the stratum corneum, which it needs to reach its target site.[33] The benefits of topical drug delivery include ease of administration, a compliant patient, increased compliance, and eliminating first-pass metabolism. lower rates of absorption and cosmetic considerations are disadvantages.[34]

- **Advantages of topical drug delivery system:**

Because topical medications are intended for localized pain relief with negligible systemic side effects, they have a much better profile for adverse effects. This specifically refers to drug classes with minimal systemic absorption.[35]

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1.6 Cracked heels:

Due to the absence of any oil glands, the feet skin has an ability to crack and dry out . The crack on skin that is a result of this dryness. Dry and cracked feet are a result of inadequate moisturizing, increased exposure to pollutants, as well as certain medical conditions like psoriasis, thyroid, diabetes, and eczema.[36] The beginning of cracked heels may occur when the skin around your heels becomes thick and dry. Dry, thick skin can crack or fissure under additional pressure, leading to heel fissures. Heel fissures can occur in anyone, but certain factors increase their likelihood, such as: Wearing open-toed shoes, such as sandals, Bathing or showering in hot water, Using abrasive soaps, Having skin that is cold and dry, Cold and dry weather, Standing for extended periods of time.[37] Due to its structure and function, heel skin is particularly vulnerable to dryness. A thick corneum layer makes up the heel skin, which helps it withstand the pressure of body weight. It undergoes delayed healing and excessive pigmentation while responding to the physical stimulus.[38] Heel fissures are splits or cracks in the epidermis that can result from anhidrosis (dry skin, which is also known as sclerosis), and they may or may not be accompanied by hyperkeratosis. Deep dermal fissures may enlarge and become painful. The rim of the heel is typically where callus formation occurs. The skin is typically dry and may have a thick callus, which is visible as a dis coloured area of skin that is yellow or dark brown, particularly along the inside border of the heel. In addition to excessive exposure or a lack of moisturizing, it is a sign of poor foot care.[39]

2. DRUG PROFILE:

2.1. Longan lychee peel:

Figure 1 : Figure of longan lychee fruit [40,41]

Table 1 : Drug profile of Longan Lychee Peel

Class	Details
Common name	Longan lychee
Kingdom	Plantae
Family	Sapindaceae
Genus	*Dimocarpus*
Species	*D.longan*
Binomial name	*Dimocarpus longan*
Chemical constitutes of longan lychee peel	Gallic acid,catechin,ellagic acid
Pharmacological activity	Antioxidant, anti-inflammatory effect, anti-fungal

2.2. Strawberry fruits:

Figure 2 : Figure of strawberry fruits[42,43]

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Table 2 : Drug profile of *Fragaria* × *ananassa*

Class	Details
Common name	Strawberry fruit
Kingdom	Plantae
Family	Rosa ceae
Genus	*Fragaria*
Species	*F.* × *ananassa*
Binomial name	*Fragaria* × *ananassa*
Chemical constitutes of strawberry fruit	Cyanidin-3-glucoside , Vitamin- c Quercetin-3-glucoronide, Ellagic acid
Pharmacological activity	Anti-inflammatory ,antioxidant

3. MATERIAL AND METHOD

3.1. Materiales: Longan lychee peel, Strawberry fruit,Carbopol 940, HPMC (hydroxy propyl methyl cellulose),propylene glycol,ropylene glycol,Propyl Paraben,Methyl Paraben,Ascorbic acid, Hyaluronic acid, disodium EDTA

3.2. Methology:

3.2.1. Extraction approaches of medicinal plants:

The preparation of crude plant extracts serves as the foundation for the isolation and study of medicinal plants, hence extraction is the first fundamental step in this research. cleaning up the chemical components found in plants.[44,45] The investigation of medicinal plants begins by focusing on the pre-extraction and extraction procedures, which are vital steps in the processing of bioactive components from plant materials**.** low-volume manufacturing Enterprises (SME) levels commonly employ traditional methods like Soxhlet extraction and maceration.

3.2.2. Plant sample pre-extraction preparation:

It covers how to pre-treat plant material to extract secondary metabolites with high biological activity. Both choosing the extraction process and properly preparing the material for extraction are crucial. This procedure should stop the growth of bacteria and fungus as well as the deterioration of medicinal chemicals. Plant material can be prepared using a variety of techniques, including as drying, fermentation, freeze-drying, convection drying, microwave vacuum drying, and enzymatic procedures. Weighting, volume measurement, mixing, dilution, heating, cooling, fractionation, purification, and preservation are some of the procedures that are involved.[46] The first step in the investigation of medicinal plants is the preparation of plant samples, which aims to protect the bio molecules present in the plants prior to the extraction process. The plant samples, such as leaves, barks, roots, fruits, and flowers, can be in fresh or dried form. Other treatments, such as grinding and drying, applied to the plant materials also play a role in preserving the phyto chemicals within the final extracts.

3.2.3 Dried vs fresh samples:

Both fresh and dried samples of medicinal plants are employed in research. Dried samples are recommended in most cases because they are easier to prepare for research.

3.2.4.Grinded vs. powdered samples:

When particle size is reduced, there is more surface contact between the samples and the extraction solvents. While grinding created smaller, coarser samples, powdered samples contain more uniform, smaller-sized particles that are better able to contact the extraction solvents that are present on their surfaces. The target analytes must come into contact with the solvent in order for the extraction to be efficient, and the optimum particle size is less than 0.5 mm.[47]

3.2.5. Extraction process:

To prevent dust and other unwanted materials, the fruits' peels were carefully washed in tap water. Fruits and peels that were free of dust were allowed to dry in the experimental lab's shade. The dried peels were then ground into a fine powder using a mechanical grinder. Finally, by sieving the powdered peels, a moderately fine powder was extracted and used for extraction

a) Extraction of Gallic acid from Longan Lychee Peels:

- **Selection and collection of materials:**Fresh fruits of dimocarpus longan duch (longan lychee peel) collected from ICAR- National Research Center Litchi Bihar.

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➢ **Drying and Grinding** :

- To extract plant or fruit components, drying is a crucial step.
- Active components, intermediates, and metabolic reactions can all be found in fresh plant or fruit materials.
- The processing of plant materials prior to extraction therefore requires drying

➢ **Extraction process:**

- Extraction of *Dimocarpus longan duch* by using solvent extraction method (dried powder of longan lychee peel was prepared)
- It was extracted in 70 ml ethanol by Soxhlet extraction at 75°C
- Then collected mixture will be filtered through whatman filter paper and kept for the evaporation of ethanol.
- The final extraction of longan lychee peel was obtained and which mentioned in result section(144)[48]

B) **Extraction of cyanidin-3-glucoside (anthocyanin glucoside) from strawberry fruit:**

➢ **Maceration Process :**

- 5 gm of strawberry fruit sample is crushed in a homogenizer, it is diluted with methanol at a ratio of 1:1 and left for 7 days .
- solution centrifuged at 15000 rpm for 10 min.
- Then collected mixture has filtered by using whatman filter paper and kept for the evaporation
- Final extract of *Fragaria* × *Ananassa* was obtained.[49]

3.2.6. Preparation of sample:

3.2.6.1. UV spectroscopic method development

A. Preparation of standard stock solution

- 10 mg of longan lychee peel and strawberry fruits were accurately weighted and transferred in 10 ml volumetric flask.Added 10 ml of diluent then sonicated. 1000 µg/ml of solution-A longan lychee peel and solution-B strawberry fruits .1 ml of this solution-A and B were diluted to 10 ml with distilled water 100 µg/ml.Further as respective concentration want 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10ppm of longan lychee peel extract.
- Preparation of diluents: Distilled water used as diluent.

B. Preparation of the calibration curve

- From the standard stock solution range from 0.2, 0.4, 0.6, 0.8, 1 µg/ml were transferred in series of 10 ml volumetric flask followed by making volume up to 10 ml with distilled water which gives concentration of longan lychee peel extract 2, 4, 6, 8, 10ppm respectively.
- Choice of wavelength: It showed absorbance at a wavelength of 272 nm for longan lychee peel, so it was selected max.

❑ **Determination of wavelength by UV-VISIBLE spectrophotometer**

A 10 mg of longan lychee peel and strawberry fruit standard was weighed and transferred to a 10 ml separate volumetric flask along with 10 ml distilled water. After that take 1 ml solution from it and again add 10 ml methanol in separate. As respectively make the both flask was shaken a bit to give the stock solution and the wavelength was measured with a scan of 200 to 800 nm.

3.2.6.2 HPLC Spectrum measurements:

❑ **Preparation of mobile phase:**

Water and Aceto nitrile HPLC grade were mixed in the proportion of 80:20 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication**.**

❑ **Preparation of diluents**

Mobile phase was used as diluents.

❑ **Selection of Mobile Phase**

The final mobile phase selected was a mixture of water and ACN in the ratio 80:20

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Table 3 : Optimization of Mobile Phase

SR.NO.	COMPOSITION	RATIO	REASON
1	ACN-Methanol	50-50	High baseline noise
2	Water –ACN	50-50	Peak was showing different retention time
3	Water –ACN	40-60	Peak showing tailing
4	Water –ACN	60-40	Peak was showing different retention time
5	Water-ACN	80-20	The peak obtain was sharp and was having good resolution.

❑ **Selection of Detector and Detector Wavelength**:UV-visible detector was selected, as it is reliable and easily determines the correct wavelength and was set at 272 nm as the detection wavelength.

❑ **Selection of the Stationary Phase:** On the basis of reverse phase HPLC mode the stationary phase with C8 bonded phase i.e.inertsil C18 – silica based (250 mm × 5.6 mm), 3 µm was selected.

❑ **Optimization chromatographic conditions:** Final chromatographic condition mentioned in below table no. 4

Table 4: Final chromatographic condition

CHROMATOGRAPHIC NAME	CHROMATOGRAPHIC CONDITION
Stationary Phase	Inertsil C18 (250 nm × 4.6 mm), 3 µm
Mobile Phase	Water – ACN (80-20)
Detection Wavelength	272nm
Flow rate	1 ml/min
Injection volume	10 µL

7) Preparation of blank formulation of serum:

- For the preparation of blank formulation of serum first of all to take the Carbopol 940 which is dissolved in water and stirred by magnetic stirrer at 1000 rpm until it has homogenized.
- Triethanolamine added into the formulation and adjust the pH.
- Required quantity of ascorbic acid dissolved in distilled water
- Take the Methyl paraben and propyl paraben as a preservatives in propylene glycol
- Then this solution added to the previous mixture.

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- Phytosome complex suspension added to the gel formulation.
- Final mixture of formulation was stirred by using magnetic stirrer at 1000 rpm for 20 min.(129)

A) Role of ingredients used in serum formulation:

- Carbopol 940:In this formulation Carbopol 940 are white powder. It is a highly effective rheology modifier that can produce high viscosity and produces clear gels or hydro-alcoholic gels and creams that sparkle. Clear gels, hydro alcohol gels, and creams are perfect applications for its short flow, non-drip characteristics.[50]
- Ascorbic acid:In this formulation ascorbic acid used in wound healing, improve iron absorption from plant-based foods, and support the immune system. It functions as an antioxidant.[51]
- Propylene glycol: In this formulation propylene glycol is act as a humectants. It is also used as solvent to properly mix the ingredients in product formulations.[52]
- Methyl paraben: In this formulation methyl paraben used as preservatives.
- Propyl paraben: In this formulation propyl paraben used as preservatives and also act as antifungal and antimicrobial agent.
- Triethanolamine: In this formulation Triethanolamine are viscous liquid which is used as Ph adjustment, emulsifying agent.[53]

1.1 Evaluation parameters:

Evaluation of herbal foot repair serum by various physicochemical parameter like (colour,odour), Ph testing, viscosity, spreadability, extrudability mentioned below.

- **Physicochemical parameter:**

Check the color of the sample of the serum formulation; it should be a glossy, milky white. apply a small amount of a serum formulation onto your skin and feel it for smooth, uniform texture and a non-greasy finish.

- **pH measurement:**

By the use of digital pH meter the pH test will be measure. in this test dipper of digital pH meter will be dipped into the formulation of serum and recorded the pH value of the serum formulation. pH of skin having acidic pH (4-6) so the pH of serum formulation having acidic pH.

- **Homogeneity test:**

In this test serum formulation will be spread onto the glass slide and observe the serum formulation will be proper distributed onto the glass slide.

- **Spreadability:**

First of all to take the filter paper and calculate area of filter paper(A1) and weigh of filter paper(W1).Then take BD 5 ml syringe and put the 20 drops of sample onto the filter paper. When last drops hits the filter paper then start the timer and count down for 10 min. At that time (during 10 min) liquid will spread over the filter paper. After drying for ten minutes, cut the remaining filter paper with scissors and weigh it. Note down the area of spreadable portion of the filter paper(A2) and weigh the remaining portion of filter paper that is (W2).and measure the spreadability by using specific formula that is mentioned below.

% SPREADABILITY = (A2/A1)100

- **Determination of Viscosity:**

Viscosity measure by using rheological viscomete r(Brookfield viscometer) at 100 rpm, use the S64 model type of spindle. In this test serum will be put into the large mouth container and then spindle dipped into the serum formulation and check the viscosity of serum formulation.

- **Stability testing :**

In this test serum formulation were placed at various temperatures for the 3 month period. After 3 month check the serum formulation and analyzed.

4. RESULT AND DISCUSSION:

4.1 Result of extract process of longan lychee peels:

❑ **Selection and collection of longan lychee peels**: Fresh fruits of dimocarpus longan duch (longan lychee peel) collected from ICAR- National Research Center

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Litchi Bihar. Longan lychee peel is a variety of lychee. Which shown in below figure no. 3.

Figure 3 : Selection and Collection of materials

❑ **Drying and grinding :**

First of all peel of longan lychee was grinding by mixture to form the powder. Powder form of longan lychee peel was dried by hot air oven at 60 C˚ for 2 to 3 hour. Dried powder of longan lychee peel was shown in below figure no. 4

Figure 4 : Drying and Grinding of longan Lychee Peels

❑ **Extraction of Gallic acid from longan lychee peels**

Final extract of longan lychee peel was obtained by using Soxhlet method which shown in below figure no. 5

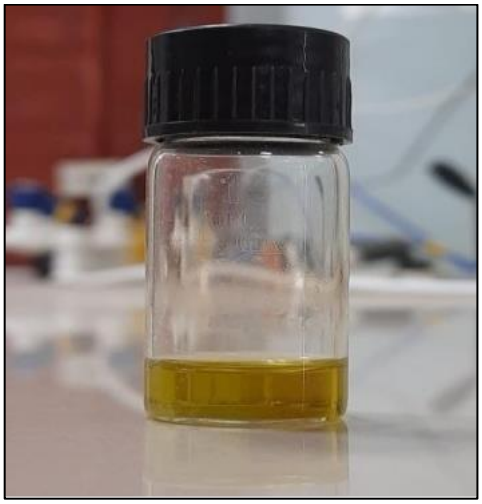

Figure 5 : Final extract of longan lychee peel

4.2. Result of extraction process of strawberry fruit

Final extract of *Fragaria* × *ananassa* was obtained by maceration process which shown in below figure no. 6.

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Figure 6 : Final extract of strawberry fruit

4.3. Evaluation of extract:

4.3.1. UV/VIS spectrophotometer analysis

4.3.1.1. longan lychee peel:

Estimation of Gallic acid have been identified using UV Spectrum obtained in the specific range of 200-400 nm. Maximum wavelength of Gallic acid was obtained to be 272 nm.

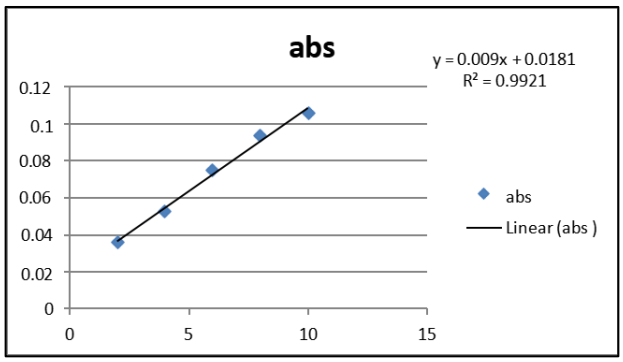

Figure 7: Calibration curve of Gallic acid

Table 5 : Calibration curve of Gallic acid

Concentration(µg/ml)	Absorbance at 272 nm
2	0.035689
4	0.052345
6	0.074565
8	0.093264
10	0.105478

The absorbance obtained for the standard curve was plotted in the figure and linearity was observed by the interception forming straight line and R2 value was 0.994 for Gallic acid. Hence, it was concluded that the calibration curve was linear.

4.3.1.2. Estimation of strawberry fruit:

Estimation of Cyanidin-3-glucoside have been identified using UV Spectrum obtained in the specific range of 200-400 nm. Maximum wave length of Cyanidin-3-glucoside was obtained to be 274 nm.

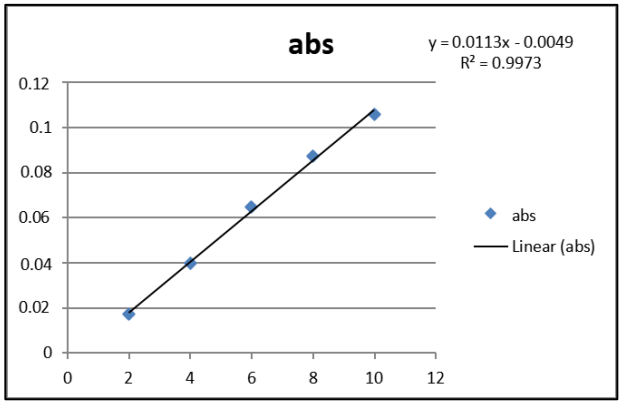

Figure 8 : Calibration curve of Cyanidin-3-glucoside

Table 6: Calibration curve of Cyanidin-3-glucoside

Concentration	Absorbance
2	0.0167521
4	0.0393218
6	0.0645432
8	0.0870783
10	0.1054782

The absorbance obtained for the standard curve was plotted in the figure and linearity was observed by the interception forming straight line and R2 value was 0.997 for Cyanidin-3-glucoside. Hence, it was concluded that the calibration curve was linear.

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FTIR Spectrophotometer analysis:

4.3.2.1. FTIR spectrum of Gallic acid:

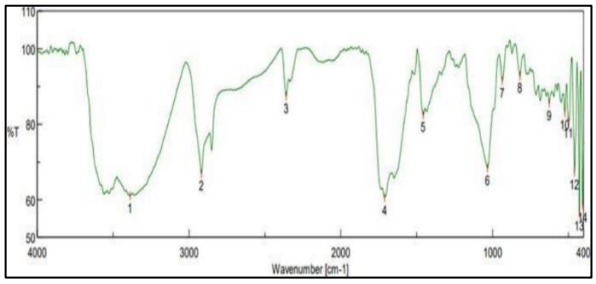

Figure 9 : FTIR spectrum of Gallic acid

Table 7 : FTIR spectrum of observed data comparison with the official pharmacopoeias

Peak no	Standard	Observed	Functional group
1	3402.75	3388.32	-O-H stretching
2	2928.04	2918.73	Aromatic C-H stretching
4	1624	1711.51	C=O
5	1450	1456.96	Benzene
6	1054.73	1033.66	C-O stretching

4.3.2.2. FT-IR Spectrum of Cyanidin-3-glucoside

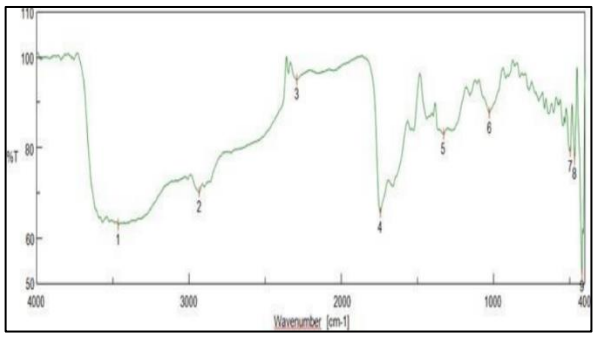

Figure 10: FTIR Spectrum of Cyanidin-3-glucoside

Table 8 : FTIR spectrum of observed data comparison with the official pharmacopoeias

Peak no.	Standard	Observed	Functional group
1	3428.33	3485.46	-O-H Stretching
2	2937.05	2933.2	-C-H Stretching
4	1715.64	1745.26	-C=O
5	1419.83	1328.71	-C-H
6	1054.38	1027.87	-C-O

4.3.3. HPLC Spectrum measurement:

4.3.3.1. HPLC Spectrum measurement for Gallic acid

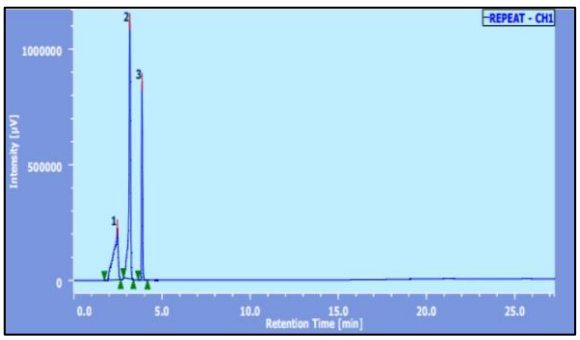

Figure 11 : HPLC Spectrum measurement of Gallic acid

Table 9: HPLC Spectrum measurement of Gallic acid

Peak no.	Peak name	Mobile phase	Flow rate	Area	Retention time
2	Gallic acid	Water-ACN (80-20)	1mL/min	6838042	3.167
3	Catechin	Water-ACN (80-20)	1mL/min	3092474	3.858

4.3.3.2. HPLC Spectrum measurement for cyaniding-3-glucoside

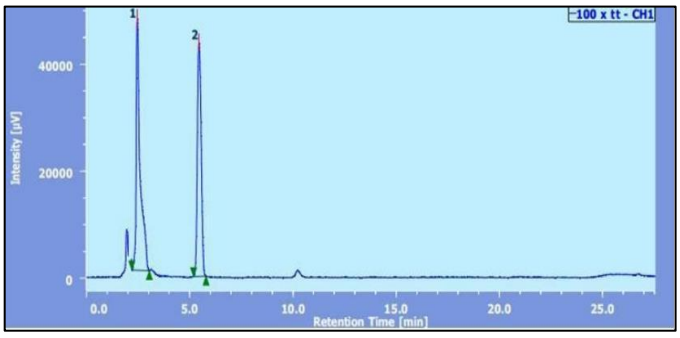

Figure 12 : HPLC Spectrum measurement of Cyanidin-3-glucoside

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Table 10 :HPLC Spectrum measurement of Cyanidin-3-glucoside

Peak no.	Peak name	Mobile phase	Flow rate	Area	Retention time
2	Cyanidine -3-glucoside (anthocyanin)	Water-ACN (50-50)	1mL/min	567592	5.314
1	Delphinidine-3-glucoside	Water-ACN (50-50)	1mL/min	656090	2.458

4.4.Preparation of serum:

Figure 13 : Blank preparation of serum formulation

Quantity required for the formulation of herbal antifungal and anti-inflammatory foot repair serum which mentioned in below table no.11

Table 11 : Composition of foot repair serum:

Composition	M1 for 100%	M2 for 100%	M3 for 100%
Extract of longan lychee peel	1.5	2	3
Extract of strawberry fruit	1	2.5	3
Carbopol 940	3	3	3
Ascorbic acid	0.05	0.06	0.05
Propylene glycol	15	15.5	14
Methyl paraben	0.15	0.15	0.15
Propyl paraben	0.02	0.02	0.02
Triethanolamine	QS	QS	QS
Distilled water	100	100	100

4.5 Evaluation of serum formulation:

1. Physical appearance:

The result of physical appearance testing of serum formulation can be mentioned in below table no.12.

Table 12 : Organoleptic result of the serum formulation

Parameter	Results
Color	Transparent
Smell	Unpleasant
Consistence	Thinner viscous

2. pH measurement: pH measurement of serum formulation mentioned in below table no. 13

Table 13 : Determination of pH of serum formulation

Sr.no.	Time interval	M1	M2	M3
1	Initial	6.54	6.48	6.50
2	8th	6.46	6.35	6.41
3	16th	6.41	6.40	6.38
4	24th	6.39	6.45	6.28

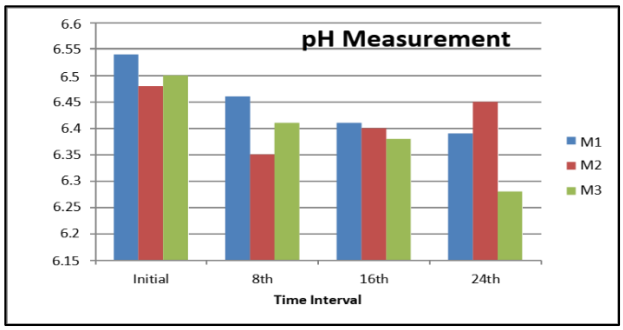

Figure 14: Graph of pH measurement

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1. Spreadability testing: Spreadability testing of the serum formulation mentioned in below table no.14

Table 14 : Measurement of spreadability testing

Sr.no.	Interval	Initial area	Time
1	Initial day	6.2 cm	10 sec
2	5th day	6.5 cm	10 sec
3	10th day	6.7 cm	10 sec

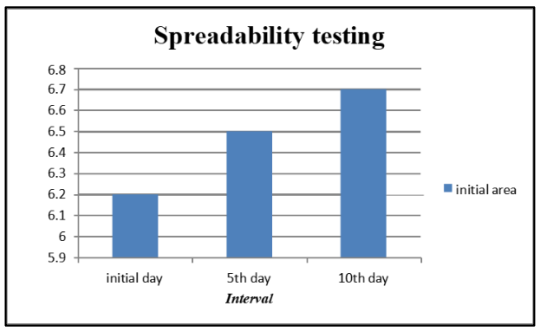

Figure 15 : Graph of Spreadability test

1. Determination of Viscosity: Determination of viscosity for the serum formulation mentioned in table no.15.

Table 15 S: Measurement of viscosity

Sr.no.	No.of days	M1	M2	M3
1	1st day	212596	215916	217856
2	5th day	230416	232153	236856
3	10th day	256834	277835	300836

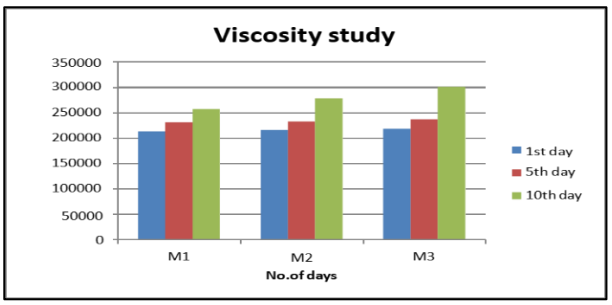

Figure 16: Graph of viscosity study

CONCLUSION

The herbal antifungal and anti-inflammatory foot repaired serum was effectively produced and evaluated using a variety of official parameters, including spreadability testing, pH analysis, and viscosity measurement. Based on all of the investigated herbal serum formulations, M1, M2, and M3 each shown a substantially different rise in skin moisture ($p<0.05$). All formulations produce a milky white in color, non-greasy, non-oily, and uniform contents when the completed serum's texture is evaluated. Without any pH adjustments, every composition provided a pH value that was within the range of normal skin pH. The skin was successfully used for the spreadability investigation for all formulations. Due to its stability and ability to provide the largest percentage of moisture increased, the M2 formulation of herbal antifungal anti-inflammatory serum is the optimum formulation according to the study.

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