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FORMULATION & EVALUATION OFCISSUS QUADRANGULARIS FOR ANTI-INFLAMMATORY ACTION

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ABSTRACT

Cissus quadrangularis is a common perennial climber in the Vitaceae family that is distributed all over India and is especially common in tropical places. Anti-osteoporotic, antioxidant, analgesic, anti-microbial, anti-obesity, anti-ulcer, adrogenic, anti-hemorrhoidal, anti-diabetic, antifungal, anti-tumor, and bone-healing properties are among the biological actions of the plant. Alkaloids, steroids, flavonoids, tannins, and saponin are all present in the plant. Cissus quadrangularis was used to produce powdered material for the soxhlet apparatus extract preparation. Numerous phytochemical tests were carried out, including those for glycosides, steroids, alkaloids, tannin, flavonoids, and saponins. Herbal therapy is currently widely used due to its therapeutic effects. Numerous research has recently examined the possible medicinal effects and quality compliances of herbal mixtures. Given these facts, the current work involves developing and evaluating herbal medicine, which has received international recognition because of its therapeutic benefits. Recently, a large number of studies looked into herbal formulations to examine their potential therapeutic benefits as well as quality compliances. In light of these facts, the current study focuses on developing and assessing a topical formulation of Cissus quadrangularis. The formulation was prepared using the dispersion process, which involved changing the ratio of constituents. Drug analysis was conducted using a variety of instrumental techniques, including TLC, Fourier-transform infrared spectroscopy, ultraviolet spectroscopy, and more. A number of quality characteristics, including pH, viscosity, spread ability, and in vitro drug release, were also assessed for the formulation. In vitro drug release, spread ability, viscosity, and other quality parameters were determined to be well met by the formulation C2.

KEYWORDS: Cissus quadrangularis, herbal medicine, topical formulation, quality control.

INTRODUCTION

Clinical evidence demonstrates that skin gel is the safest and most effective treatment for various skin-related illnesses. It is also used for community outreach to lessen side effects associated with other conventional dosage forms. An large variety of drug measurement structures, such as semisolids, fluid planning, splashes, and powerful powders, are included in skin drug delivery systems. Gels, creams, and balms are the semisolid foundation materials that are most frequently used for topical medication delivery. A network of cross-connected polymers swelled in a fluid stage medium is referred to as a gel type of measuring structure. Their characteristics are indisputable dependent on the collaboration between the fluid segment and the two strong state polymers. Gels determines there is no steady state stream. An interwoven three-dimensional structure of scattered stage particles is formed by the interaction between the polymer and the fluid scattering medium. For the semisolid state, the growing thickness caused by the interweaving and significant interior grinding is required. Since skin gel prescriptions are less greasy and can be used to administer drugs, they provide an ideal delivery framework.

For skin care and the topical treatment of dermatological diseases, a wide choice of vehicles including solid, semisolids and liquid preparation is available to physician and patients. Within the major groups of semisolid preparations, the use of transparent gel has expanded, both in cosmetics and Pharmaceuticals. Gel is stable one and better vehicle for hydrophobic or water insoluble drugs as Luliconazole. Also, Gel has a high patient acceptability as they possess the advantages. Therefore, they have been recently used as vehicles to deliver various drugs to the skin.

Anatomy of Skin

Skin is the largest organ in the body and covers the body's entire external surface. It is made up of three layers, the epidermis, dermis, and the hypodermis, all three of which vary significantly in their anatomy and function. The skin's structure is made up of an intricate network which serves as the body’s initial barrier against pathogens, UV light, and chemicals, and mechanical injury. It also regulates temperature and the amount of water released into the environment. This article discusses the relevant anatomical structures of the skin’s epidermal layer, its structure, function, embryology, vascular supply, innervation, surgical considerations, and clinical relevance.

1. Epidermis

Layers of Epidermis

The layers of the epidermis include the stratum basale (the deepest portion of the epidermis), stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (the most superficial portion of the epidermis).

Stratum basale, also known as stratum germinativum, is the deepest layer, separated from the dermis by the basement membrane (basal lamina) and attached to the basement membrane by hemidesmosomes. The cells found in this layer are cuboidal to columnar mitotically active stem cells that are constantly producing keratinocytes. This layer also contains melanocytes.

Stratum spinosum, 8-10 cell layers, also known as the prickle cell layer contains irregular, polyhedral cells with cytoplasmic processes, sometimes called "spines", that extend outward and contact neighboring cells by desmosomes. Dendritic cells can be found in this layer.

Stratum granulosum, 3-5 cell layers, contains diamond shaped cells with keratohyalin granules and lamellar granules. Keratohyalin granules contain keratin precursors that eventually aggregate, crosslink, and form bundles. The lamellar granules contain the glycolipids that get secreted to the surface of the cells and function as a glue, keeping the cells stuck together.

Stratum lucidum, 2-3 cell layers, present in thicker skin found in the palms and soles, is a thin clear layer consisting of eleidin which is a transformation product of keratohyalin.

Stratum corneum, 20-30 cell layers, is the uppermost layer, made up of keratin and horny scales made up of dead keratinocytes, known as anucleate squamous cells. This is the layer which varies most in thickness, especially in callused skin. Within this layer, the dead keratinocytes secrete defensins which are part of our first immune defense.

❖ Cells of the Epidermis

i. Keratinocytes

ii. Melanocytes

iii. Langerhans cells

iv. Merkel's cell

i. Keratinocytes

Keratinocytes are the predominant cell type of epidermis and originate in the basal layer,

produce keratin, and are responsible for the formation of the epidermal water barrier by making and secreting lipids. Keratinocytes also regulate calcium absorption by the activation of cholesterol precursors by UVB light to form vitamin D.

ii. Melanocytes

Melanocytes are derived from neural crest cells and primarily produce melanin, which is responsible for the pigment of the skin. They are found between cells of stratum basale and produce melanin. UVB light stimulates melanin secretion which is protective against UV radiation, acting as a built-in sunscreen. Melanin is produced during the conversion of tyrosine to DOPA by the enzyme tyrosinase. Melanin then travels from cell to cell by a process that relies on the long processes extending from the melanocytes to the neighboring epidermal cells. Melanin granules from melanocytes are transferred via the long processes to the cytoplasm of basal keratinocyte. Melanin transferred to neighboring keratinocytes by "pigment donation"; involves phagocytosis of tips of melanocyte processes by keratinocytes.

iii. Langerhans Cells

Langerhans cells, dendritic cells, are the skins first line defenders and play a significant role in antigen presentation. These cells need special stains to visualize, primarily found in the stratum spinosum. These cells are the mesenchymal origin, derived from CD34 positive stem cells of bone marrow and are part of the mononuclear phagocytic system. They contain Birbeck granules, tennis racket shaped cytoplasmic organelles. These cells express both MHC I and MHC II molecules, uptake antigens in skin and transport to the lymph node.

iv. Merkel Cells

Merkel cells are oval-shaped modified epidermal cells found in stratum basale, directly above the basement membrane. These cells serve a sensory function as mechanoreceptors for light touch, and are most populous in fingertips, though also found in the palms, soles, oral, and genital mucosa. They are bound to adjoining keratinocytes by desmosomes and contain intermediate keratin filaments and their membranes interact with free nerve endings in the skin.

2. Dermis

The dermis is connected to the epidermis at the level of the basement membrane and consists of two layers, of connective tissue, the papillary and reticular layers which merge together without clear demarcation. The papillary layer is the upper layer, thinner, composed of loose connective tissue and contacts epidermis. The reticular layer is the deeper layer, thicker,

lesscellular, and consists of dense connective tissue/ bundles of collagen fibers. The dermis houses the sweat glands, hair, hair follicles, muscles, sensory neurons, and blood vessels.

3. Hypodermis

The hypodermis is deep to the dermis and is also called subcutaneous fascia. It is the deepest layer of skin and contains adipose lobules along with some skin appendages like the hair follicles, sensory neurons, and blood vessels.

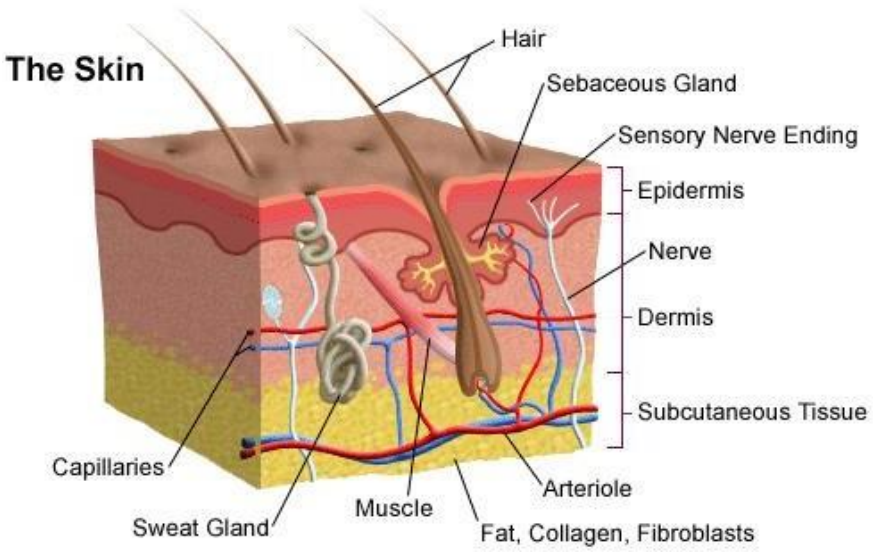

Fig: Skin Anatomy & Physiology.

Herbal drugs are becoming popular day by day for their therapeutic values with less toxic effects. Plants and their derived material have been widely employed as medicine for the prevention and treatment of diseases anciently. The acceptance of herbal medicine increases day by day due to their patient compliance and safety profile.

The development cost of herbal medicine is also low which increases economic benefits. Recently medicinal plants are extensively explored scientifically for their therapeutic and safety benefits. The medicinal values of plant materials may be attributed to the chemical constituent present in them. Drug development through medicinal plants involves the isolation and identification of active constituents.

INFLAMMATION: localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection.

Inflammation is a major problem since ancient times affecting the older population especially.

The joints are most susceptible to inflammation, Inflammation may occur due to the consequences of various diseases; There are many diseases through which inflammation is associated as a major symptom.

Anti-Inflammatory: Anti-inflammatory is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs, also called anti- inflammatories, make up about half of analgesics.

Analgesic: an agent producing diminished sensation to pain without loss of consciousness a drug that is used to relieve pain and produce analgesia.

Chronic inflammation in the joints can damage cartilage, bones, tendons (which attach muscle to bones), or ligaments (which hold joints together) irritate nerves, and produce a long list of symptoms, including pain, swelling, and stiffness. The joint damage may be progressive and irreversible.

Joint inflammation occurs when the immune system or damaged tissue releases chemicals that cause swelling and other symptoms in a joint. It can affect just one joint, such as when a person sustains an injury. However, certain medical Movements of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system. and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are inflammation (inflammation, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cell, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not synonymous: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen. Conditions can lead to multiple instances of joint inflammation throughout the body.

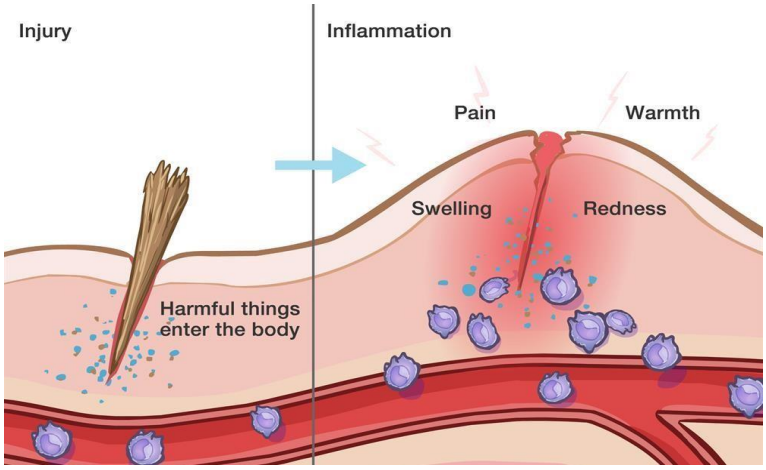

Fig: Inflammation.

The most common symptoms of inflammatory arthritis are **Joint pain** and **stiffness** after periods of rest or inactivity, particularly in the morning. Swelling, redness, and/or a feeling of warmth in the affected joints. Inflammation of other areas in the body, such as the skin or internal organs like the lungs and heart.

Swollen joints happen when there's an increase of fluid in the tissues that surround the joints. Joint swelling is common with different types of arthritis, infections, and injuries. A swollen joint is a symptom of the following health conditions: **Osteoarthritis (OA)**

Pathogenesis

1. Tissue injury or insult: This triggers the release of various signaling molecules like histamine, prostaglandins, and cytokines, which act as chemical messengers.
2. Vascular changes: Signaling molecules cause vasodilation (widening) of blood vessels, increasing blood flow to the affected area. This leads to redness and warmth.
3. Increased vascular permeability: Blood vessels become leaky, allowing plasma proteins andimmune cells to migrate into the tissue, causing swelling and edema.
4. Immune cell recruitment: Chemokines, another type of signaling molecule, attract various immune cells, including neutrophils, macrophages, and lymphocytes, to the site of injury.
5. Phagocytosis and antigen presentation: Immune cells engulf and destroy pathogens or damaged cells in a process called phagocytosis. They also process and present antigens from the invaders to activate the adaptive immune system.
6. Resolution and repair: If the insult is successfully cleared, the inflammatory response subsides, and the tissue undergoes repair and regeneration. However, if the insult persists or the immune response becomes dysregulated, chronic inflammation can develop.

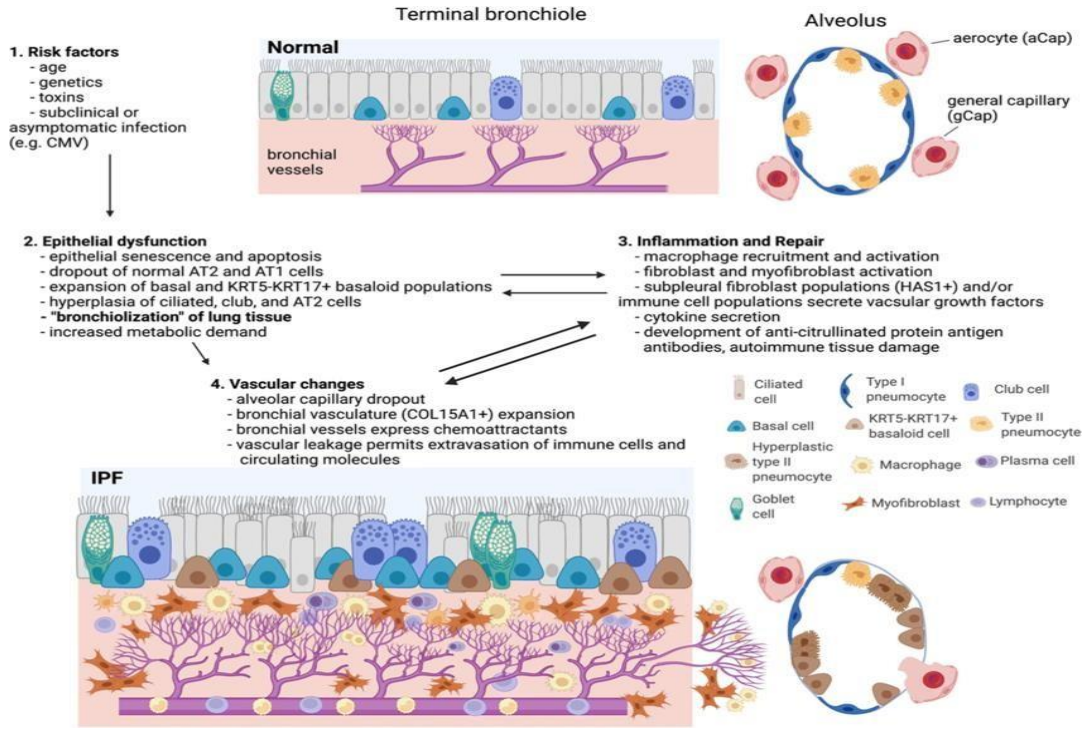

Fig. Pathogenesis.

Stages of Inflammation

1. **Vasodilation and Increased Permeability:** Blood vessels near the affected area widen (vasodilation). Increased permeability allows proteins, nutrients, and immune cells to move from the bloodstream to the tissue.
2. **Emigration of White Blood Cells (Leukocytes):** White blood cells (leukocytes), especially neutrophils and macrophages, move from the bloodstream to the site of injury or infection. Chemotaxis guides the movement of these cells toward chemical signals released by damaged tissues.
3. **Phagocytosis:** White blood cells, particularly macrophages and neutrophils, engulf and digest foreign particles, damaged cells, and debris through a process called phagocytosis.
4. **Resolution and Repair:** Anti-inflammatory signals help resolve the inflammation. Macrophages play a key role in releasing anti-inflammatory signals and promoting tissue repair. Tissue repair may involve regeneration of damaged cells or the formation of scar tissue.

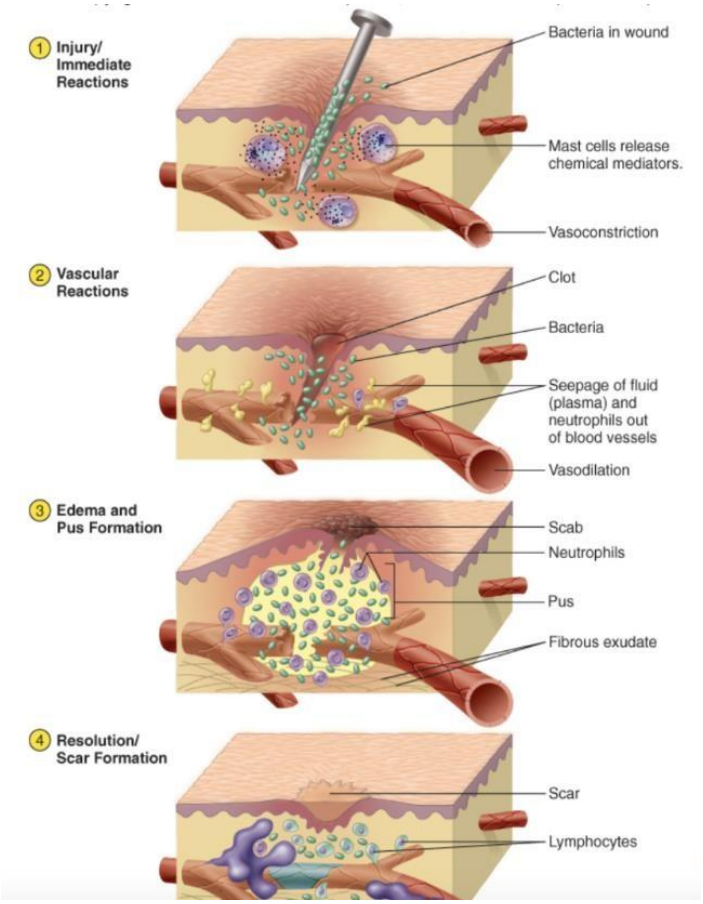

Fig. Stages of Inflammation.

Cissus Quadrangularis L. is a fleshy plant found in major parts of the world, especially in Asia, Africa, and a few other warm tropical regions. It is one of the common food items in India. Cissus quadrangularis is also used for various treatments like fracture healing, antiulcer, anti-helminthic, antifungal, anti-hemorrhoidal, analgesic, antibacterial properties, etc. It also serves in the best way to treat various infirmities such as hemorrhoids, leprosy, epilepsy, dyspepsia, skin burns, dysentery, bowel complaints, to increase appetite, etc.

Fig: Cissus Quandrangularis.

It is commonly known as a "bone setter"; helps in bone empowerment and also reduces pain, the plant is also referred to as "**Hadjod**" because of its ability to join bones.

Traditionally this plant has been reported to possess various medicinal uses in gout, piles, tumours, peptic ulcers, and leukorrhea.

The identification and isolation of plant material utilize various analytical techniques including spectroscopy like, Ultraviolet & Infrared Spectroscopy and chromatographic techniques like; TLC. The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated.

The complexity of plant extracts can be simplified by isolating and separating them using different techniques based on their solubility pattern.

Looking towards the importance of the plant; the aim of the present study was to prepare and evaluated topical herbal formulation of Cissus quadrangularis as an anti-inflammatory agent especially in gout to support the traditional claim of the plant.

This Review Article throws light on various recent knowledge of scientific research in various aspects of this plant, which mainly incorporate remarkable pharmacological activities such as anti-ulcer, anti-bacterial, anxiolytic, antipyretic, antidiabetic, bone healing, antioxidant and anti-inflammatory properties, and phytochemicals studies.

Cissus quadrangularis commonly known as **Hadjod** is a perennial plant of the family Vitaceae.

SYNONYM - Adamant creeper, Square stalked vine, veldt grape, devil's backbone, adamant creeper, asthisamharaka, hadjod and pirandai, Sannalam, Nalleru, Vajravelli, Magara Valli.

Biological Source -It consists of the dried inner bark of the Cissus Quadrangularis.

Kingdom	**Plantae (plants)**
Subkingdom	Trophobionts (vascular plants)
Super division	Spermatophyta (seed plants)
Division	Magnoliophyte (flowering plants)
Class	Magnoliopsida (dicotyledons)
Subclass	Rosedale
Order	Rhamnus's
Family	Vitaceae (grape family)
Genus	Cissus L. (tree line)
Species	Cissus quadrangularis Ljj

Morphological Characters

Colour: Green

Odour: Pungent smell

Taste: Astringent

Texture: Rough

Size: Height of 1.5 m. Branches withinternodes 8-10 cm (3-4 in) long and 1.2-1.5 cm (0.5-0.6 in) wide. Toothed trilobeleaves 2-5 cm (0.8-2.0 in) wide appear at the nodes.

Fig. Cissus Quandrangularis.

Shape: Succulent climber with green stems and branches. The stems are quadrangular in shape.

Microscopic Characters

Epidermis single layer, 6-10 layers of parenchymatous cells, 3-5 Layers of chlorenchyma cells, Cambium: Present, Vascular Bundle: Exarch type, Pith: Large central evident pith Large central evident pith. Microscopic studies were done by preparing a thin hand section of Cissus quadrangularis stem. The section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid, and mounted with glycerin. A separate section was prepared and stained with iodine solution for the identification of starch grains. Microscopic studies of stem showed epidermis single layered, covered externally with thick cuticle; epidermal cells thin-walled, cork and single layered cork cambium; four patches of Collenchymatous cells present in cortical region, Collenchymatous vascular bundles capped by sc1erenchymatous sheath cambium, idioblasts containing raphides and isolated acicular crystals of calcium oxalate present in the outer region of cortex. Powder of the dried leaves was used for the observation of powder microscopical characters. The powered drug was separately treated with phloroglucinol and HCL solution, glycerin and iodine to determine the presence of Beaded epidermal cell, Sclariform vessels, Collenchymas Phloem fiber, Raphides and

Acicular crystals of calcium oxalate, Fibres , trichomes and starch grains.

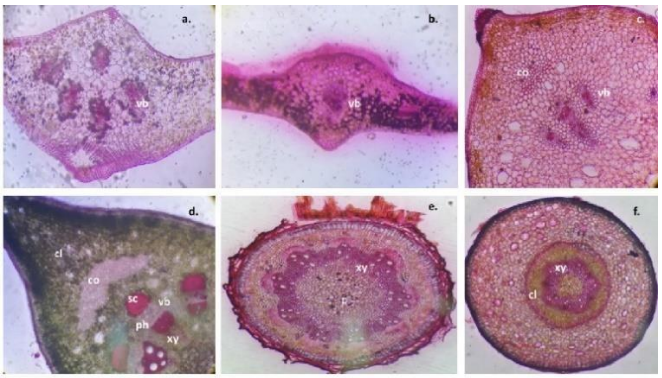

Fig. Microscopic Study.

T.S. of stem is four angled with four thick and long wings. The central part of the stem is 1.8 mm thick; the wings are 1.4 mm thick and 6 mm long. The stem consists of an epidermal layer of circular, thick walled, darkly stained and with thick cuticle. The sub epidermal cells at certain places have undergone tangential divisions forming periderm like layer of cells. The ground tissue is parenchymatous, circular thin walled and compact Scattered in the ground tissue are wide, circular secretory cavities. The extreme margins of the wings have sclerenchyma cells. The vascular system consists of multi stranded bundles located in groups within the outer part of the wings. In each wing there is an arc of 3 radially stretched collateral vascular bundles. Each bundle has a having a thick cap of sclerenchyma cells. The xylem strand includes wide, circular, angular thin-walled vessels surrounded by thick wall xylem fibres.

1. Anti-Inflammatory Effects

USES

Inhibition of Pro-Inflammatory Mediators: Cissus quadrangularis has been investigated for its ability to inhibit pro-inflammatory mediators, such as cytokines (e.g., TNF-α, IL-1β), which play a crucial role in the inflammatory response.

Downregulation of Inflammatory Pathways: Research suggests that Cissus quadrangularis may downregulate signaling pathways associated with inflammation, including NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells).

2. Joint and Bone Health

Osteoarthritis and Rheumatoid Arthritis: Due to its anti-inflammatory properties, Cissus quadrangularis has been studied for its potential role in managing symptoms associated with

osteoarthritis and rheumatoid arthritis, conditions characterized by inflammation in the joints. Bone Fracture Healing: Some studies suggest that Cissus quadrangularis may contribute to bone health and fracture healing, potentially by reducing inflammation and promoting bone regeneration.

3. Connective Tissue Support

Tendon and Ligament Health: Cissus quadrangularis has been explored for its effects on tendons and ligaments. It may contribute to the maintenance and repair of connective tissues, potentially alleviating inflammation in these areas.

4. Antioxidant Properties

Free Radical Scavenging: The plant contains antioxidant compounds that may help neutralize free radicals, reducing oxidative stress, which is often associated with inflammation.

5. Wound Healing

Topical Application: Some studies suggest that applying Cissus quadrangularis extracts topically may promote wound healing. This could be attributed to its anti-inflammatory and tissue-regenerating properties.

6. Pain Management

Analgesic Effects: Cissus quadrangularis has been investigated for its potential analgesic effects, which may contribute to pain relief associated with inflammatory conditions.

7. Safety and Tolerability

Low Toxicity: Studies have generally reported that Cissus quadrangularis is well-tolerated with low toxicity. However, individual responses may vary, and it is important to consider potential interactions with medications.

Chemical Constituents

Root Powder Often Provides a Steady Source of Mineral Resources Including Potassium 67.5 Mg, Calcium 39.5 Mg, Zinc 3,0 Mg, Sodium 22.5 Mg. Iron 75 Mg. Lead 3.5 Ing, Cadmium 0:25 Mg, Copper 0.5 Mg and Magnesium 1.15 Mg

1. Phytosterols: Plant sterols with potential anti-inflammatory and antioxidant properties.
2. Flavonoids: These are polyphenolic compounds with antioxidant effects. They contribute to the plant's ability to combat oxidative stress.
3. Triterpenoids: These compounds are known for their anti-inflammatory and

immunomodulatory properties.

4. Carotenoids: These are pigments with antioxidant properties that contribute to the plant's overall free-radical scavenging abilities.
5. Vitamins and Minerals: Cissus quadrangularis may contain various vitamins andminerals, such as ascorbic acid (vitamin C), carotene (precursor to vitamin A), and calcium.
6. Calcium fluoroborate: A compound found in Cissus quadrangularis, which may contribute to its potential bone health benefits.
7. Phenolic Compounds: Various phenolic compounds, including resveratrol, which is also found in grapes and has antioxidant properties.
8. Quercetin: A flavonoid with antioxidant and anti-inflammatory properties.

AIM

Aim and Objective

To Prepare and Evaluate Herbal Anti-Inflammatory Gel Using Cissus Quadragularis Powder Extract.

Objective

- To carry out an extraction process to obtain extraction Hadjod powder
- To prepare formulation and evaluation of Anti-Inflammatory herbal gel.
- To carry out the study of anti-inflammatory activity of joint pain and muscle pain.
- To Develop an optimal gel formulation by adjusting concentrations of Cissus quadrangularis extract, gelling agents, and additives for enhanced stability and consistency.
- To Evaluate the anti-inflammatory activity of the herbal gel through in vitro assays, focusing on the inhibition of key inflammatory markers.
- To Conduct skin irritation studies and assess systemic safety to ensure the gel's compatibility with skin and overall tolerability.
- To Investigate the transdermal absorption of active compounds to understand their bioavailability and systemic exposure.
- To Evaluate the ease of application, spread ability, and duration of action to enhance user compliance and optimize the gel's practicality.

PROCEDURE

Material and Methodology

- The plant material Cissus quadrangularis was collected then shade dried, homogenized to fine powder, and stored in an airtight container.
- All chemicals and reagents used were of analytical grade.

Extraction

1. Sample Preparation
- Obtain dried and finely ground Cissus quadrangularis plant material. Ensure that it is representative of the sample want to extract.
2. Weighing the Sample
- Weigh an appropriate amount of the plant material. The amount will depend on the expected yield and the concentration of the compound.
3. Soxhlet Apparatus Setup
- Assemble the Soxhlet apparatus, including the Soxhlet extractor, condenser, and round-bottom flask.
- Place the weighed Cissus quadrangularis sample into a thimble made of filter paper or other suitable material.
4. Solvent Selection
- Choose a solvent suitable for extracting the desired compounds from Cissus quadrangularis. Common solvents include ethanol
5. Loading the Apparatus
- Place the sample thimble into the Soxhlet extractor.
- Add a sufficient amount of the selected solvent i.e ethanol to the round-bottom flask.
6. Extraction Cycle
- Begin the extraction by heating the flask. The solvent vaporizes, rises through the Soxhlet arm, and condenses in the condenser.
- The condensed solvent drips onto the Cissus quadrangularis sample in the Soxhlet extractor, extracting compounds from the plant material.
7. Continuous Extraction
- Allow the Soxhlet apparatus to operate in a continuous loop until a significant amount of the desired compounds has been extracted. This process may take severalhours.
8. Completion
- Once the extraction is complete, the concentrated extract containing the extracted

compounds is collected in the flask.

9. Recovery

- Recover the solvent from the extract using evaporation techniques.

10. Analysis

- Analyze the extracted compounds using suitable analytical methods, such as chromatography, to identify and quantify the components of interest.

Fig. 6: Cissus Quandragularis Powder Extraction Process.

Procedure for Preparation Of Gel

1. Weighing of Ingredients: Weigh the required amount of Carbopol 934, PEG 4000, and Sodium benzoate using a precision weighing balance.
2. Dispersing Carbopol: Sprinkle the weighed Carbopol 934 into the distilled water with constant stirring. Allow it to hydrate and disperse for about 30 minutes. This process is known as the "wetting" phase.
3. Heating and Mixing: Heat the dispersion to aid in the dissolution of Carbopol. Continue stirring until a clear gel base is formed.
4. Cooling: Allow the gel base to cool to room temperature. Ensure that it remains a homogeneous solution.
5. Weighing and Addition of PEG 4000: Weigh the required amount of PEG 4000. Add the PEG 4000 to the gel base while stirring continuously until it is fully dissolved.
6. Adding Sodium Benzoate: Weigh the required amount of Sodium benzoate and add it to

the gel base. Stir until fully dissolved.

7. Adding Ethanolic Extract: Incorporate the ethanolic extract of Cissus quadrangularis into the gel base. Adjust the quantity based on the desired concentration of the plant extract.
8. Adjusting pH: Measure the pH of the gel using a pH meter. If necessary, adjust the pHto the desired range using a neutralizing agent such as Triethanolamine (TEA). Add the neutralizing agent slowly while monitoring the pH.
9. Final Mixing: Stir the gel thoroughly to ensure uniform distribution of all components. Continue mixing until you achieve a smooth and homogeneous gel.
10. Testing and Adjustments: Check the consistency and appearance of the gel. If needed, make adjustments to the formulation by adding small amounts of water or neutralizing agent to achieve the desired properties.
11. Packaging: Transfer the final gel into suitable containers for storage and use.

Preparation of Gel

Table: % Composition.

Ingredient	C1(W/W)	C2(W/W)	C3(W/W)
Ethanolic extract of Cissus quadrangularis	5	5	5
Carbopol 934	0.375	0.5	0.625
PEG 4000	1.25	1.25	1.25
Sodium benzoate	0.25	0.25	0.25

Table: Prepartion of Gel.

SR.NO	Name of Ingredient	Quantity Taken
1	Ethanolic extract of Cissus quadrangularis	5 gm
2	Carbopol 934	0.50 gm
3	Polyethylene glycol 4000 (w/w)	1.25gm
4	Sodium benzoate	0.25 gm

Chromatographic Method of Analysis

Stepwise Procedure for TLC

- Step 1 - TLC Plate (rectangular) was washed with Acetone and kept in hot air oven for30 minutes at 100, to activate the plate.
- Step 2 - After activation of plate a very fine line was drawn on the paper at one end, simultaneously 8 ml Toluene and 2 ml Ethyl acetate are taken in a 500 ml beaker and shaken well and a petri plate was placed over it.
- Step 3 - Spots of Standard Cissus quadrangularis ethanolic extract and Formulation 2 was given on TLC plate with the help of capillary on the line.
- Step 4 - Then the TLC plate was places in the beaker containing solvent system in such a

way that, solvent system level remains below the spots. And solvent system is allowed to develop on the plate and then the lid was closed.

- Step 5 - Once the solvent reached particular height then the plate was taken out and dried.
- Step 6 - The plate was observed under U.V. lamp in short wavelengths. Following are the images of TLC plate after completion of above steps:

Phytochemical Test

A. Test for Proteins

Biuret Test: Test solution was treated with equal volume of 10% sodium hydroxide solution and two drops of 1% copper sulphate solution, mixed well and observed for the formulation of violet/pink colour. If it is so, presence of proteins was detected.

B. Test for Saponins

Foam Test: In this test 0.5gm of extract was added in 10-20 ml of water, shaken for few minutes formation of frothing which persisted for 60-120 seconds, shows presence of saponins.

C. Test for Carbohydrates

Benedict's Test: Test solution was mixed with one or two drops of Benedict's reagent and it is boiled in water bath, wait for few minutes and observe the formation of reddish-brown precipitate which indicates the positive result for the presence of carbohydrates.

Molisch's Test: Filtrate was treated with one or two drops of alcoholic α-naphthol solution in a test tube. It resulting the formation of the violet ring which is present at the junction which is indicates the presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dil. HCL, neutralized with alkali and heated Fehling's A and B solutions. Formation of red precipitate which shows the presence of reducing sugar.

D. Test for Alkaloids

Wagner's Test: A fraction of extract was treated with three to 3-5 drops of Wagner's reagent it resulting the formation of reddish-brown precipitate. This shows the presence of alkaloid.

Mayer's Test: Filtrates were treated with Mayer's reagent. Formation of a yellow-coloured precipitate which indicates the presence of alkaloid.

E. Test for Phenols

Ferric Chloride Test: Extracts was treated with 3-4 drops of ferric chloride solution. It resulting the formation of bluish black colour which indicates the presence of phenols.

F. Test for Flavonoids

Shinoda Test: Crude extract was mixed with few fragments of magnesium ribbon and add drop wise con. HCL in that mixture. Wait for few minutes, then it shows the pink scarlet colour which is indicates the flavonoids.

Alkaline Reagent Test: Crude extract was mixed with 2 ml of 2 % solution of NaOH. An intense yellow colour was formed which turn colourless on addition of few drop of dilute acid which indicates the presence of flavonoids.

G. Test for Steroids

Liebermann Burchard Test: Extract was mixed with 1-2 drops of acetic anhydride, boil it and cool it. After the cooling add concentrated sulphuric acid from the side of test tube the observed the formation of a brown ring at the junction of two layers. Green coloration of the upper layer which indicates positive test for steroids.

H. Test for Glycosides

Liebermann's Test: Crude extract was mixed with each of 2 ml chloroform and 2 ml acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added. A colour change from violet to blue to green indicated the presence of glycoside.

Salkowski's Test: Crude extract was mixed with 2 ml chloroform, then 2ml of concentrated H2SO4 was added carefully and shaken gently. A reddish-brown colour indicates the presence of steroidal ring. i.e., glycone portion of the glycoside.

Keller-Killiani Test: Test solution was treated with 1-2 drops of glacial acetic acid and ferric chloride solution mixed well. Then add few concentrated sulphuric acid, it resulting the formation of two layers. Lower layer is reddish-brown layer and upper layer is acetic acid layer which turns in bluish green which indicates the positive test for glycosides.

Evaluation Parameter

All prepared gel formulations were subjected to evaluation using parameters like physical appearance, pH, viscosity, spreadability, stability study, Chromatographic analysis using TLC

method.

- Physical appearance of formulation

The gel formulations were visually inspected for colour, odour, consistency, grittiness, uniformity stickiness and homogeneity.

- pH measurement

The pH of prepared gels was determined using a digital pH meter. The pH meter was calibrated before each use with standard pH 5 and pH 7 buffer solutions.

- Viscosity

Viscosity of the prepared gels was measured by a Brookfield viscometer at 100 rpm, using spindle number 6. Viscosities were recorded at room temperature.

- Spreadability

Spreadability is an important property of topical formulation for patient compliance. About 0.5 gm of gel was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 100 gm was allowed to rest on the upper glass plate. The increase in the diameter due to the spreading the gels was noted.

- Syringeability

Treatment of severe cases requires administration of the drug directly periodontal pocket by using an injectable system fast relief. In this view, syringeability of gel formulations was noted.

- Stability Study using Cooling and heating test

The stability of the formulation was studies against temperature changes, after 48 hours of preparation. Gel formulation was placed at 45 for 48 hours and then 48 hours at 4 for three cycles. At the end of stability study, the samples were analyzed for their physical appearance as colour, pH and viscosity.

- Chromatographic method of analysis

The Cissus quadrangularis ethanolic extract obtained is subjected to Thin Layer Chromatography (TLC), for separating compounds and to observe presence of different compounds in the extract. Selection of solvent system is a important part of TLC. Ethanol is used as a solvent in this test.

As per spot separation in TLC plate the RF value of Cissus quadrangularis was calculated by formula,

$$\text{RF Value} = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by Solvent}}$$

RESULTS

Identification of Morphological Features of Cissus Quadrangularis

- Leaf - leaves simple or lobbed, sometimes 3-folialate, dentate
- Stem: stem of varying lengths; stern quadrangular, 4-winged, internodes constricted at nodes; internodes 4-15 cm long and 1-2 cm thick; buff colored with greenish tinge, angular portion reddish-brown.
- Flower: Flowers bisexual, tetramerous, in umbellate cymes, opposite to the leaves, Calyx cup-shaped, obscurely 4-lobed.
- Fruit: Fruit globose or obovoid fleshy berries, one seeded, dark purple to black; seeds ellipsoid or pyriform. Flowering and fruiting time May-June.

Powder Microscopy

SR. No.	Features	Observation
1.	Nature	Coarse powder
2.	Colour	Cream colour light yellow
3.	Odour	Slightly characteristic
4.	Taste	Slightly characteristic

Fig: powder microscopy

Microscopic

Sr. No.	Features	Observation
1.	Beaded epidermal cell	Present
2.	Sclariform vessels	Present
3.	Collenchymas cell	Present
4.	Phloem fiber	Present
5	Raphides and Acicular Crystals ofcalcium oxalate	Present
6.	Fibres	Present
7.	Pitted Parenchymatous cells	Present

Physical apperance

Table Physical Apperance.

Sr.no.	Property	Inference
1.	Colour	Dark brown
2.	Greasiness	Non greasy
3.	Spreadability	Good
4.	Homogeneity	Homogenous
5.	Syringeability	Good

Phytochemical Tests

Sr.no	Test	Petroleum ether	Chloroform	Methanol
1.	Test for Proteins 1)Biuret test 2)Xanthoproteic test 3)Millon's test	 - - +	 + - -	 - - +
2.	Test for Saponins 1)Foam test	 +	 -	 +
3.	Test for Carbohydrates 1) Benedict's test 2) Molisch test 3) Fehling's test	 + - -	 - + -	 + - +
4.	Test for Alkaloid 1)Wagner's test 2)Mayer's test	 + -	 + -	 + +
5.	Test for Phenol 1)Ferric chloride test	 -	 -	 +
6.	Test for Tannins 1)Gelatin test 2)Braymers test	 - +	 + -	 + -
7.	Test for Flavonoids 1) Shinoda test 2)Alkaline Reagent test	 + +	 - -	 + +
8.	Test for Steroids 1)Liebermann Burchard test	 +	 +	 +
9.	Test for Glycosides 1)Liebermann's test 2)Salkowski's test 3)Keller-Killiani test	 - + +	 - + +	 + + +

Table: Phytochemical Tests

Note: + indicates presence and – indicates absence of phytoconstituent

Physico-Chemical Parameters

Sr. No.	Parameter	Values (%) w/w
1.	Loss on drying	2.5
2.	Total ash	12.5
	Acid insoluble ash	2.5

	Water soluble ash	8.6
	Sulphated ash	5.0
3.	Extractive values	
	Petroleum ether extractive	18.0
	Water soluble extractive	3.0
	Alcohol soluble extractive	2.0

Quantitative Chemical Evaluation

Powder analysis with Chemical agents

Reagent	Colour observed
Powder as such	Whitish yellow
Powder + conc. HCl	Brown
Powder + conc. HNO3	Yellow
Powder + conc. H2SO4	Brown
Powder + Glacial acetic acid	Light yellowish white
Powder + 5% NaOH	Brown
Powder + 5% KOH	Yellowish brown
Powder + 5% ferric chloride	Light yellow
Powder + picric acid (Saturated solution)	Yellow
Powder + ammonia	Light brown

Fluorescence Analysis of Powder Drugs

Chemicals/Reagents	**Fluorescence Observed**	
	Short Wavelength	**Long Wavelength**
Powder as such		
Powder + 1N NaOH 1N Methanol	Cream	Brown
Powder + 1N NaOH in water	Brown	Reddish brown
Powder + 50% HCl	Green	Reddish brown
Powder + 50% HNO3	Green	Brown
Powder + 50% H2SO4	Dark green	Brown
Powder + Petroleum ether	Light brown	Dark brown
Powder + Chloroform	colorless	Brown
Powder + picric acid	Yellow	Yellowish brown
Powder + 5% ferric chloride solution	Green	Reddish brown
Powder + 5% iodine	Greenish brown	Black
Powder + methanol	colorless	Light brown
Powder+ HNO3+NH3	Yellowish green	Reddish brown

Physical Test of Formulation

- **Spreadability**

Sr.no	**Formulation**	**Spreadability (gm.cm/s)**
1.	C1	26.04
2.	C2	22.73
3.	C3	24.84

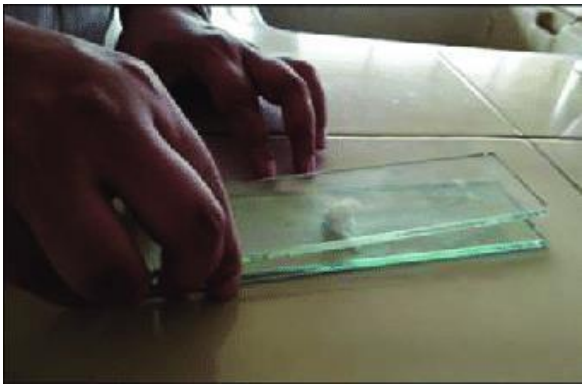

Fig. Spreadabilty.

- Viscosity

The Viscosity was recorded at 37 using Brookfield Viscometer

Sr.no	Formulation	Viscosity (cps)
1.	C1	10814
2.	C2	6620
3.	C3	5300

Fig Viscosity

- pH Value

Sr.no	Formulation	pH Value
1.	C1	5.85
2.	C2	6.8
3.	C3	5.5

Fig. pH.

Stability Study using Cooling and heating test

- **Chromatographic studies**

For TLC, the solvent system used was Toluene: Ethyl acetate in the ratio of 8:2Following are the it Stages of TLC plate after complection of above steps:

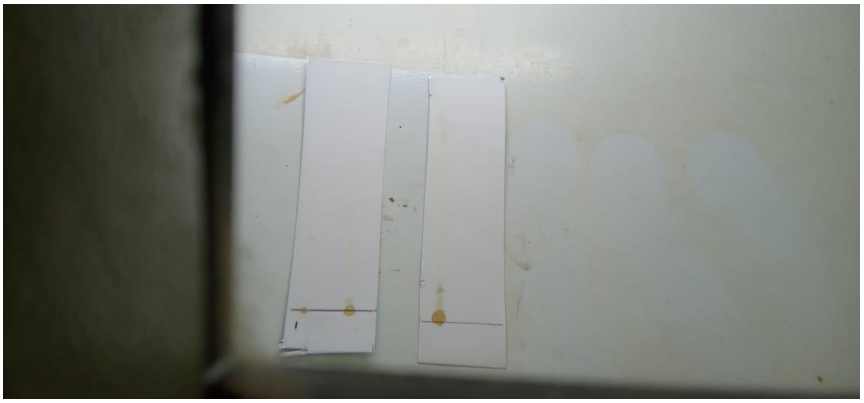

Fig. 10: TLC Plate in Day Light.

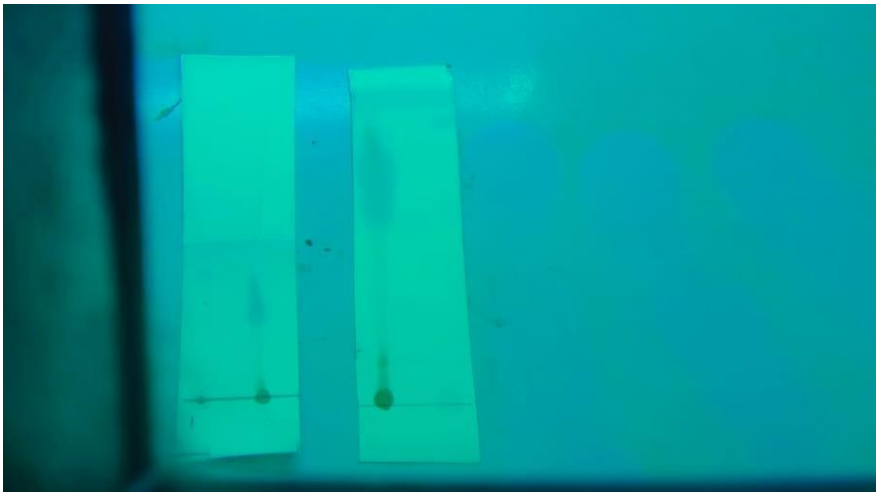

Fig. 9: TLC Plate in Short UV Wavelength.

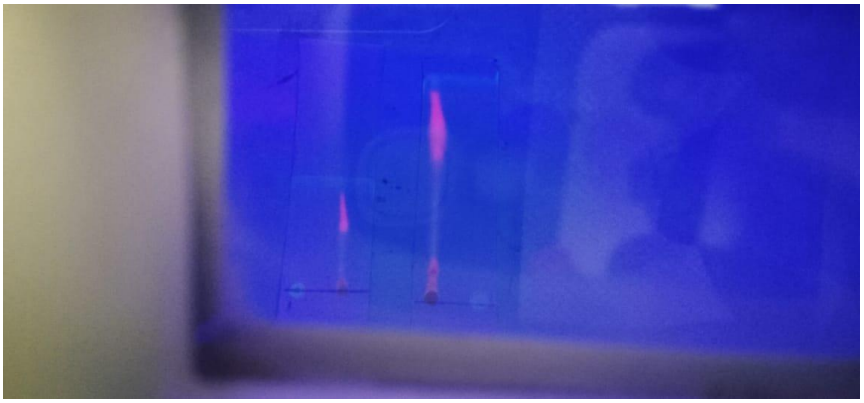

Fig. 8: TLC Plate in Long UV Wavelength.

CONCLUSION

The entire plant of Cissus quadrangularis, a member of the Vitaceae family, was subjected to pharmacological evaluation for its traditional medicinal uses in treating various diseases and disorders. Procured from the local market in Pimpri-Chinchwad, Pune, the plant underwent assessment according to WHO guidelines. Pharmacognostical and physicochemical parameters were employed to ensure the authenticity, quality, and purity of the crude drug, providing valuable insights for identification and authentication. Phytochemical studies revealed the presence of alkaloids, steroids, flavonoids, tannins, proteins, glycosides, and saponin compounds in various extracts of Cissus quadrangularis. Considering these findings, the isolation, purification, and characterization of these phytochemicals could pave the way for future medicines with transformative potential. Additional studies are recommended to explore the antioxidant, antiobesity, antiulcer, and antifungal activities of the plant. Morphological studies indicated the distinctive green color, characteristic odor, and slightly characteristic taste of the stems. Microscopic analyses unveiled the stem's unique features, including single-

layered epidermis, collenchymatous cells, and vascular bundles. Physicochemical evaluations, such as total ash value and extractives, provided insights into the quality of the herbal drug. Additionally, chemical reagent tests, quantitative analyses, and behavioral observations highlighted the diverse chemical composition of Cissus quadrangularis stems. Formulation analyses, including spreadability, viscosity, and pH values, offered further insights into potential applications of the plant in pharmaceutical formulations.

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