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Original Article

Formulation And Evaluation Of An Anti-Inflammatory Transdermal Patch For Honey Bee Stings

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ABSTRACT

Honey bee stings cause local pain, swelling, and inflammation due to venom containing melittin and phospholipase A2. The present study aims to formulate and evaluate an anti-inflammatory transdermal patch for effective management of honey bee stings. The patch was formulated using natural polymers HPMC, PVP K30, and PVA with Boswellia serrata extract as an anti-inflammatory agent. The formulated patches were evaluated for folding endurance, pH, adhesion test, and photosensitivity. The results indicated good physicochemical properties and potential for sustained drug delivery. The developed transdermal patch offers a patient-friendly, non-invasive approach for treatment of honey bee sting inflammation.

Keywords: anti-inflammatory agent, transdermal patch, honey bee stings, photosensitivity, non-invasive approach.

INTRODUCTION

Introduction to Honey Bee

Honey Bee is a social insect belonging to the family Apidae. Honey bees are beneficial insects because they help in pollination and production of honey, beeswax, and royal jelly. However, they sting humans and animals as a defense mechanism.(1)

Types of Honey Bees

1. Queen bee
2. Worker bee
3. Drone bee

Only the female worker bee possesses a stinger and can sting.

Introduction to Honey Bee sting

Fig no 1: Honey Bee sting

Honey bee sting is a common insect injury that injects venom into human skin. Bee venom contains melittin, histamine, phospholipase enzymes, and peptides that produce inflammatory reactions.(2)

A honey bee sting is a common defensive injury caused by the female worker honey bee when it feels threatened or disturbed. During the sting, the bee injects venom into the skin through a barbed stinger. This venom contains various biologically active

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substances that produce pain, redness, swelling, itching, and inflammation. In some individuals, bee stings may also cause severe allergic reactions known as anaphylaxis.(3)

Honey bee stings are common in rural and agricultural areas and may occur accidentally during outdoor activities, farming, gardening, or handling bee hives. Although a single sting usually causes mild local symptoms, multiple stings can lead to serious toxic effects and medical emergencies.(4)

Clinical Symptoms

- Pain
- Burning sensation
- Redness
- Swelling
- Itching
- Local inflammation

Severe Reaction

- Allergic reaction
- Tissue damage
- Anaphylaxis

Skin :

Skin is the largest and one of the most important organs of the human body. It forms the outer protective covering of the body and acts as a barrier between the internal organs and the external environment. The skin covers approximately 1.5–2 square meters of body surface area in an adult human and accounts for nearly 15% of total body weight.(5)

The skin performs several important physiological and protective functions such as protection against microorganisms, prevention of water loss, regulation of body temperature, sensation, excretion, immune defense, and absorption of drugs through the transdermal route.

In pharmaceutical and transdermal drug delivery systems, the skin plays a very important role because drugs can be administered through the skin to produce local or systemic therapeutic effects. (6)

Anatomy of Skin

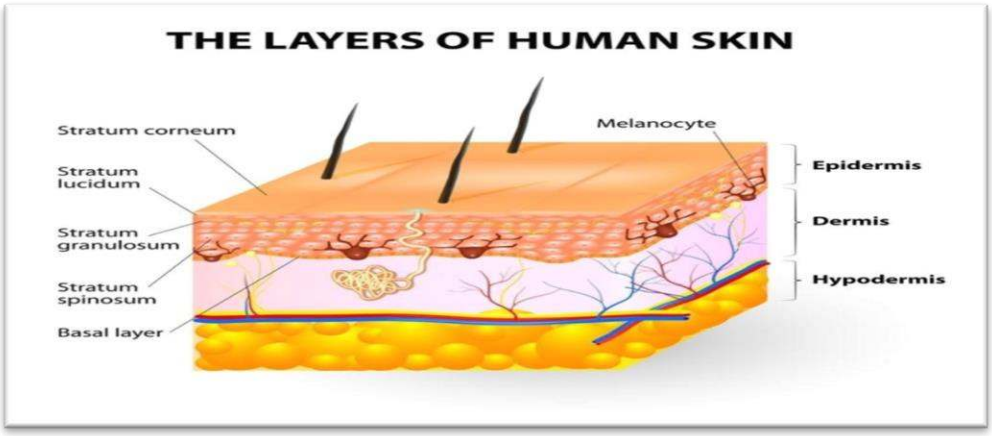


Fig no : 2 layers of human skin

➢ **The skin is composed of three main layers:**

1. Epidermis

2. Dermis

3. Hypodermis (Subcutaneous tissue)

Each layer has a distinct structure and specific functions.

1. Epidermis

The epidermis is the outermost layer of the skin. It acts as the first line of defense against physical injury, chemicals, microorganisms, and dehydration. It is made up of stratified squamous keratinized epithelium.(7)

The epidermis does not contain blood vessels and receives nutrients by diffusion from the dermis.

Layers of Epidermis

a) Stratum Corneum

- Outermost layer of dead keratinized cells
- Main barrier to drug penetration
- Prevents water loss and microbial entry

b) Stratum Lucidum

- Thin transparent layer present in thick skin
- Found mainly in palms and soles

c) Stratum Granulosum

- Contains keratohyalin granules
- Involved in keratin formation

d) Stratum Spinosum

- Contains living keratinocytes
- Provides strength and flexibility

e) Stratum Basale (Germinativum)

- Deepest epidermal layer
- Produces new skin cells by mitosis
- Contains melanocytes responsible for melanin production

➢ **Functions of Epidermis**

1. Protection against microorganisms and chemicals

2. Prevention of dehydration

3. Formation of keratin

4. Protection from UV radiation

5. Barrier for transdermal drug delivery (8)

2. Dermis

The dermis is the middle and thickest layer of the skin located beneath the epidermis. It is mainly composed of connective tissue containing collagen and elastin fibers.

The dermis provides strength, flexibility, nourishment, and sensory functions to the skin.

Layers of Dermis

a) Papillary Layer

- Upper thin layer of dermis
- Contains capillaries and nerve endings
- Supplies nutrients to epidermis

b) Reticular Layer

- Thick deeper layer
- Contains collagen and elastin fibers
- Provides elasticity and tensile strength

➢ **Functions of Dermis**

1. Provides mechanical strength

2. Maintains elasticity of skin

3. Supports blood circulation

4. Contains sensory receptors for pain, pressure, touch, and temperature

5. Helps in wound healing and thermoregulation

3. Hypodermis (Subcutaneous Tissue)

The hypodermis is the deepest layer beneath the dermis. It mainly consists of adipose tissue and loose connective tissue.

➢ **Functions of Hypodermis**

1. Stores fat as energy reserve

2. Provides insulation against heat loss

3. Protects underlying organs from injury

4. Connects skin with muscles and deeper tissues

Factors Affecting Topical Absorption of Medications:

A-Physiological Factors of Skin

1. Skin ness
2. Lipid content and Part of Skin
3. Density of Sweat gland
4. Skin PH
5. Blood Flow
6. Hydration of Skin
7. Disease state and Inflammation of Skin

B-Pysiochemical Factors of Drug

1. Distribution Coefficient
2. Molecular Weight
3. Degree of Ionization (unionized drugs gets absorbed well)
4. Effect of Excipients

Physiological Functions of Skin

1. Protective Function

1. Protects the body against:
2. Mechanical injury
3. Chemicals
4. Microorganisms
5. UV radiation

2. Thermoregulation

1. Maintains body temperature through:
2. Sweating
3. Vasodilation
4. Vasoconstriction

3. Sensory Function

1. Skin contains receptors for:
2. Touch
3. Pain
4. Pressure
5. Temperature

4. Excretory Function

1. Sweat glands excrete:
2. Water
3. Salts
4. Urea

Skin and Transdermal Drug Delivery

Transdermal Drug Delivery System (TDDS) is a method of delivering drugs through the skin in the form of a medicated patch. The drug penetrates through different layers of the skin and produces local or systemic therapeutic effects. TDDS provides controlled and sustained release of drugs over a prolonged period of time. It improves patient compliance, avoids first-pass metabolism, and reduces gastrointestinal side effects associated with oral drug delivery (9)

Principle of TDDS

Drug diffuses from the patch → Through Stratum Corneum → Via Epidermis & Dermis → Reaches dermal blood capillaries → Systemic circulation → Therapeutic effect. In transdermal drug delivery systems (TDDS), drugs are administered through adhesive patches applied on the skin surface.(10)

Fick's Law of Diffusion is the basic principle: (11)

$$J = \frac{D \times K}{h} (Cd - Cr)$$

Where

J = Flux,

D = Diffusion coefficient,

K = Partition coefficient,

h = Skin thickness,

Cd = Donor concentration,

Cr = Receptor concentration

Applications of TDDS

1. Pain Management: Fentanyl patch, Diclofenac patch
2. Hormone Replacement: Estrogen, Testosterone patch
3. Smoking Cessation: Nicotine patch
4. Motion Sickness: Scopolamine patch
5. Herbal/Anti-inflammatory: Boswellia, Turmeric, Menthol patch - like yours
6. Cardiovascular: Nitroglycerin patch for Angina.(12)

The drug penetrates through:

1. Stratum corneum
2. Epidermis
3. Dermis
4. Blood circulation

This method provides:

- Controlled drug release
- Improved patient compliance
- Reduced dosing frequency
- Avoidance of first-pass metabolism (13)

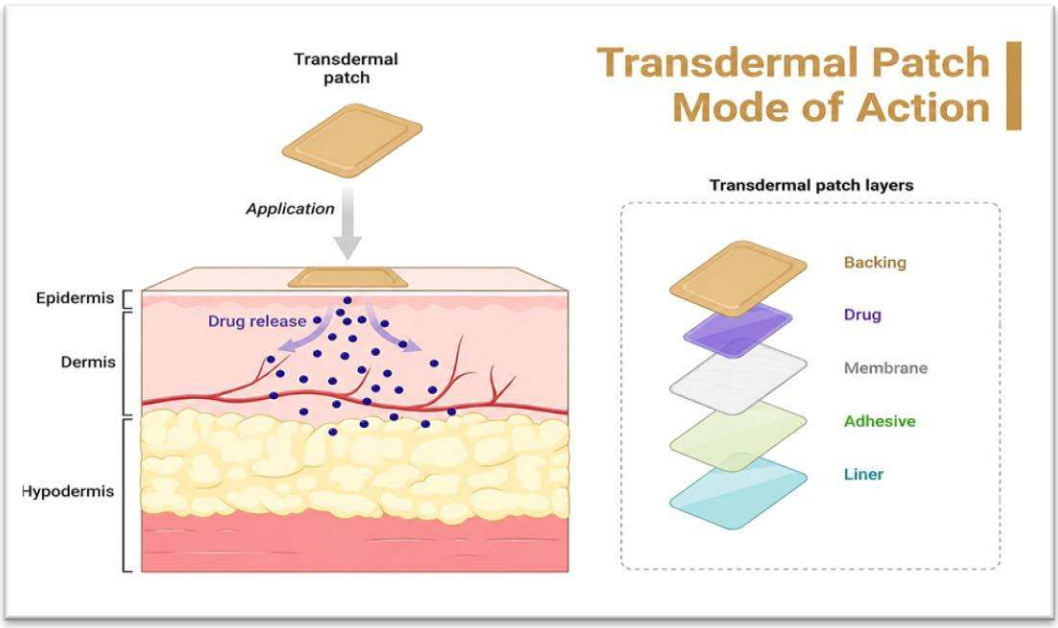


Fig no 3: Transdermal patch MOA

Introduction to Bos0wellia serrata

Fig no : 4 Boswellia serrata

Boswellia serrata is a medicinal herbal plant commonly known as Indian Frankincense or Salai Guggul.(14)

Biological Source.	Gum resin obtained from Boswellia serrata tree
Family	Burseraceae
Chemical Constituents	▪ Boswellic acids ▪ Terpenoids ▪ Essential oils ▪ Polysaccharides
Geographical Source	It is mainly found in the dry hilly regions of India, North Africa, and the Middle East
Uses	1. Boswellia serrata extract is used in tablets and capsules for joint pain and arthritis 2. It is used in topical gels, creams, and ointments for muscle pain and sprains. 3. Boswellia serrata is used in transdermal patches for prolonged anti-inflammatory action, like in your bee sting patch 4. .It is used in ayurvedic formulations for inflammatory bowel diseases like ulcerative colitis. 5. It is used in cosmetic products for its anti-aging and skin-soothing properties.

Table No: 01 drug profile

➢ **Pharmacological Actions**

- Anti-inflammatory
- Analgesic
- Antimicrobial
- Wound healing
- Anti-arthritic
- Boswellia serrata has strong anti-inflammatory activity because it inhibits 5-lipoxygenase enzyme.
- It blocks leukotriene synthesis which is responsible for inflammation, pain, and bronchospasm.
- It shows analgesic activity and helps in reducing pain in arthritis and muscle injuries.
- It has anti-arthritic activity and is used in the treatment of Osteoarthritis and Rheumatoid Arthritis.
- Boswellia serrata shows wound healing activity and helps in faster tissue repair
- It possesses anti-microbial and anti-bacterial properties against skin pathogens.

- It has immuno-modulatory action and helps to regulate the immune response.
- It shows anti-asthmatic activity by reducing bronchial inflammation.(15)

➢ **Mechanism of Anti-inflammatory Action**

1. Step 1: Trigger - Bee venom contains Phospholipase A2 which releases Arachidonic Acid from cell membranes.
2. Step 2: Inflammation Pathway - Arachidonic Acid is converted by 5-LOX enzyme into Leukotrienes and by COX enzyme into Prostaglandins. These are major inflammatory mediators
3. Step 3: Symptoms - Leukotrienes & Prostaglandins cause pain, swelling, redness, burning, and itching at sting site.
4. Step 4: Boswellia Action - Boswellic acids, especially AKBA from Boswellia serrata, selectively inhibit the 5-Lipoxygenase enzyme.
5. Step 5: Result - Inhibition of 5-LOX blocks Leukotriene synthesis, thereby reducing vascular permeability, edema, and pain. This provides sustained anti-inflammatory action (16)

MATERIALS AND METHODS

➢ Requirements:

- **Active Ingredients**: Boswellia serrata extract
- **Chemicals:** HPMC, Propylene glycol, Polyethylene Glycol, Peppermint Oil, PVA,
- **Apparatus**: Beaker, Test Tube, Measuring Cylinder, Pipette, Wire Gauze, Tripod stand, stirrer (17)

SR.NO	INGREDIENT	CATEGORY	QUANTITY
1.	Boswellia serrata extract	API	0.5 gm
2.	Hydroxy polymethyl cellulose (HPMC)	gel-forming agent.	3 gm
3.	Polyvinyl Alcohol	Provides strength to patch	0.5 ml
4.	PVP K30	Enhances drug release	1 gm
5.	Glycerin	Plasticizer	0.5 ml
6.	Propylene glycol	Permeation enhancer and plasticizer	0.5ml
7	Peppermint Oil	Cooling agent	1 ml
8	Polyethylene Glycol 400 (PEG 400)	humectant and plasticizer.	0.5 ml
9	Distilled water	Vehicle	q.s to 100 ml

Table no 02: Formulation Table

EXPERIMENTAL WORK

1. Extraction of Drug

Method: Maceration Extraction method

Principle: Maceration is a cold extraction process where the powdered drug is kept in contact with the solvent for a prolonged period with occasional shaking. It allows softening and breaking of the cell

walls so that the soluble phytoconstituents dissolve into the solvent without heat degradation. (18)

Materials Required:

- Boswellia serrata gum resin
- coarsely powdered,
- Ethanol 95% as solvent
- Clean glass container with
- tight lid Muslin cloth /
- Whatman filter paper
- Funnel, beaker, water bath Weighing
- balance, mortar-pestle

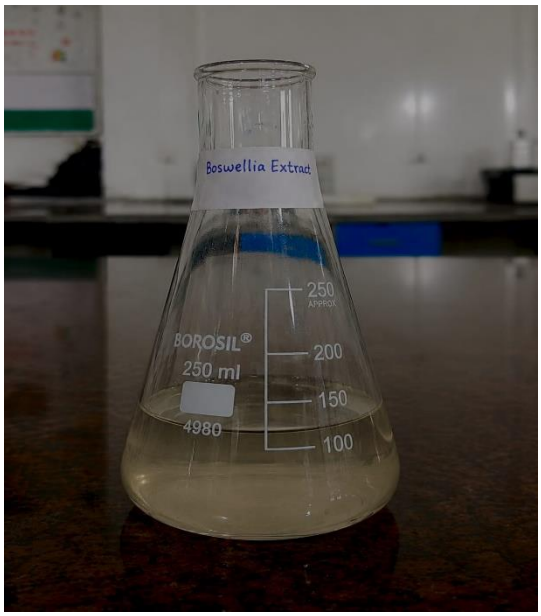


Fig no: 05 Extract

Procedure:

- **Collection:** Boswellia Serrata resin, Ethanol as a solvent
- **Size Reduction**: Dry the resin if moist. Pulverize it to a coarse powder using mortar and pestle. Pass through sieve no. 40.(19)
- **Maceration Process**

1. Weigh 1 gm of coarse powder and transfer to a clean glass container.
2. Add 100 ml of ethanol 95% in 1:1 drug to solvent ratio.
3. Close the container tightly to prevent solvent loss.
4. Keep it at room temperature for 7 days with occasional shaking 3-4 times daily.
5. The shaking helps in fresh solvent contact with plant material.

2. Ingredient Measurement:

Accurately weigh all ingredients using a precision balance. Common ingredients include:

- Hydroxypropyl Methylcellulose (HPMC) – gel-forming agent.
- Polyethylene Glycol 400 (PEG 400) – humectant and plasticizer.
- PVA - Provides strength to patch
- PVP K30 - Enhances drug release
- Glycerin: Plasticizer
- Peppermint oil – provides the cooling effect.
- Purified Water – solvent.(20)

3. Gel Formation:

1. Preparation of HPMC Gel Base
2. Take a clean beaker and add 70 ml of purified water.
3. Start gentle stirring using a magnetic stirrer
4. Gradually sprinkle 3 gm of HPMC into the water with continuous stirring to avoid lump formation.
5. Gentle heating at 40-50°C may be used to aid complete dispersion of HPMC
6. Continue stirring until a clear, viscous, lump-free gel is formed. Allow it to stand for 30 min for complete hydration.

4. Incorporation of Polymers and API:

1. To the prepared HPMC gel, slowly add 1 gm of PVP K30 with constant stirring to maintain uniformity.
2. Add 0.5 ml of PVA solution and mix thoroughly until a homogeneous gel is formed.
3. Accurately weigh 0.5 ml Boswellia serrata extract. And Add extract in solution to the polymer gel with continuous stirring.(21)

5. Addition of Plasticizers and Permeation Enhancers

1. Add 0.5 ml of Glycerin, 0.5 ml of Propylene Glycol, and 0.5 ml of PEG 400 to the above mixture
2. Stir the mixture continuously for 30 minutes to ensure uniform distribution of all plasticizers.

6. Incorporation of Cooling Agent

1. Carefully add 1 ml of Peppermint oil drop by drop with continuous slow stirring.
2. Ensure it is evenly dispersed throughout the gel. Peppermint oil should be added slowly to avoid localized concentration which may irritate the skin.(22)

7. Final Adjustments:

1. Check the pH of the final gel using a pH meter. Adjust the pH to 5.5-6.5 using suitable pH adjusters like triethanolamine if necessary, as this range is compatible with skin ph.
2. Assess the viscosity and consistency of the gel to ensure it is suitable for spreading and adhesion to the patch substrate.(23)

Phase 2: Sheet Formation

This phase involves applying the prepared gel onto a backing substrate to form the actual patch.

1. Substrate Preparation:

Choose a suitable backing material such as non-woven fabric, polyethylene film, or polyurethane film. This material should be breathable, skin-safe, and flexible.(24)

2. Gel Application:

Spread the gel evenly onto the backing material using a coating machine, spreader, or doctor blade. The target thickness may range from 1 to 3 mm depending on the intended duration and intensity of cooling.(25)

3. Protective Layering:

Apply a release liner or protective film (such as PET or polyethylene sheet) over the gel surface to prevent drying, contamination, and sticking during storage and transport.

Phase 3: Drying and Curing

1. Controlled Drying:

Place the gel-coated sheets in a drying chamber or tunnel oven at controlled temperature (e.g., 40–50°C).

Maintain appropriate humidity to avoid over-drying which may affect gel tackiness and flexibility.(26)

Phase 4: Cutting and Packaging

Once the sheets are ready, they are cut and packed for use.

1. Cutting:

Use manual cutters to cut the dried gel sheets into child-friendly sizes (e.g., 2 cm x 2 cm).

Ensure uniformity in size and shape for consistent application.(27)

2. Packaging:

Pack each patch individually in sterile, airtight pouches to prevent moisture loss and microbial contamination.

Consider aluminum foil pouches with a resealable option for convenience.

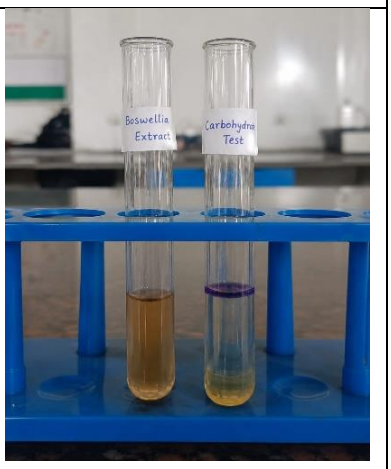
3. Labeling and Storage:

Label each pack with product name, batch number, manufacturing and expiry dates, and usage instructions. Store in a cool, dry place away from direct sunlight.(28)

EVALUATION RESULT

Preliminary Phytochemical Analysis

Prepared extracts of each drug were used for preliminary phytochemical analysis. In this, chemical tests for the detection of some primary metabolites (carbohydrate, amino acid, protein, lipid and starch) and secondary metabolites (alkaloids, flavonoids, tannins, saponins, glycosides) were done.(29)

Test for	Test	Observation	Inference	Pic of the test
Primary Metabolites -				
Carbohydrate	Molisch's test (General test) To 2-3 ml aqueous extract, add few drops of alpha naphthol solution in alcohol, shake and add conc. H2SO4 from side of the test tube.	Violet ring is formed at the junction of two liquids.	Present	
Amino Acid	**Ninhydrin test (General test)** Heat 3 ml T.S. and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min	Purple / bluish colour appears.	Present	
Protein	**Biuret test (General test**) To 3 ml T.S. add 4% NaOH and few drops of 1% CuSO4 solution.	Violet or pink colour not appeared.	Absent	

Starch	**Iodine test**: Mix 3 ml test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling.	Blue colour does not appear	Absent	
Lipid	Extract dropout on filter paper drought comes then lipids are present	No permanent oily stain on filter paper	Absent	
Secondary Metabolites **Alkaloids:** Evaporated the aqueous, alcoholic and chloroform extracts separately. To residue, dilute HCI added. After shaking well and filtration, using filtrate, following test was performed.				
	Wagner's test 2-3 ml filtrate with few drops Wagner's reagent gives reddish brown ppt Alpha naphthol solution in alcohol, shake and add conc. H2SO4 from side of the test tube.	Reddish brown ppt	Present	

Flavonoid	Shinoda Test To dry powder or extract, add 5 ml 95% ethanol few drops conc. HCI and 0.5 g magnesium turnings. Orange, pink, red to purple colour appears.	Orange red colour observed	Present	
Tannins	% FeCl3 solution	Deep blue-black colour	Present	
Saponins	Add water into sample and shake for 15 sec.	Foam is observed	Present	
Glycosides				

Legal's test (Test for cardenoloids)	To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Alpha naphthol solution in alcohol, shake and add conc. H2SO4 from side of the test tube.	Pink to red colour appeared	Present	
Test for deoxy sugar (Keller-Killiani test)	To 2 ml extract, add glacial acetic acid, one drop 5% FeCl3 and conc. H2SO4. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green.	No reddish brown colour appears at junction of the two liquid layers.	Absent	

Table no : 03 Preliminary Phytochemical Analysis

- **PHYSICAL APPARANCE**

Fig no 6: Transdermal patch

Method:

Visual inspection of a representative number of patches under good light for continuity, brittleness, translucency, clarity, tackiness and any visible defects (cracks, bubbles, lumps). Record observations.

Colour: Transparent to slightly translucent, whitish appearance

Texture: Smooth and glossy surface, no visible cracks or wrinkles

Odor: Slight characteristic aromatic odor of Boswellia resin (30)

- **FOLDING ENDURANCE (FLEXIBILITY):**

Method: Hold a strip between thumb and forefinger and fold repeatedly at the same place until it breaks; count number of folds. Perform in triplicate and report mean. Acceptance criteria: Folding endurance ≥200 folds (≥100 acceptable for brittle formulations). No crack or rupture during normal handling.

Fig no 07: Folding endurance of patch

- **PH:**

Soak patch in distilled water and measure pH using litmus or pH meter. pH should be close to skin pH (4.5–6.5).

Fig no 08: Ph test

- **ADHESION TEST:** Checks the sticking ability on skin without causing irritation. Apply patch on clean, dry skin for 8 hours. Observe if it stays adhered.(32)

Fig no 09: Adhesion test

- **PHOTOSENSITIVITY TEST:** Determines if the patch causes skin reaction when exposed to sunlight. Apply patch on one arm and expose to UV/sunlight for 30 mins; compare with a protected/control area

Fig no 10: Photosensitivity test

Test	Description	Methodology	Result
1. Adhesion Test	Checks the sticking ability on skin without causing irritation.	Apply patch on clean, dry skin for 8 hours. Observe if it stays adhered.	Patch should remain in place without peeling or causing discomfort.
2. Durability Test	Measures performance over a set duration.	Keep patch on for recommended time (e.g., 8 hrs.) and observe physical condition and cooling effect.	Cooling sensation should last as indicated (4–8 hrs.).
3. Skin Irritation Test	Assesses potential for skin irritation	Apply patch on forearm or back for 24 hours. Check for redness, itching.	No signs of irritation or sensitization.
4. Photosensitivity Test	Determines if the patch causes skin reaction when exposed to sunlight.	Apply patch on one arm and expose to UV/sunlight for 30 mins; compare with a protected/control area.	No erythema (redness) or pigmentation in exposed area.
5. pH Stability Test	Checks the pH balance to ensure compatibility with skin.	Soak patch in distilled water and measure pH using litmus or pH meter.	pH should be close to skin pH (4.5–6.5).
6. Microbial Limit Test	Ensures product is free from harmful microbes.	Swab patch surface and culture on agar media. Check for bacterial/fungal growth.	Should comply with pharmacopeial microbial limits.

Table no 5.2 Evaluation test

Summary:

The present research work entitled Formulation and Evaluation of an Anti-Inflammatory Transdermal Patch for Honey Bee Stings was carried out to develop a natural and effective topical delivery system

The transdermal patch was formulated by solvent casting method using Boswellia serrata extract (0.5 gm) as API, HPMC (3 gm) as gel-forming agent, PVA (0.5 ml) to provide strength to patch, PVP K30 (1 gm) to enhance drug release, Glycerin (0.5 ml) as plasticizer, Propylene glycol (0.5 ml) as permeation enhancer, PEG 400 as humectant, Peppermint oil (1 ml) for cooling effect, and Purified water as solvent. The pH of the final formulation was maintained at 5.5-6.5 to match skin pH

The prepared patch was evaluated for various parameters and the following results were obtained:

- Physical Evaluation: The patch was smooth, flexible, transparent, and uniform in appearance
- Adhesion Test: The patch showed excellent adhesion and remained firmly attached to the skin for 8 hours without any signs of erythema, edema, or itching.
- Durability Test: The patch maintained its physical integrity for 8 hours. Due to peppermint oil, it provided immediate cooling which was sustained for 6-8 hours

- pH Test: The surface pH of the patch was 5.5-6.5, confirming it is non-irritant and compatible with skin

The results confirmed that the formulated patch is stable, non-irritant, and effective for topical delivery

CONCLUSION

The present research work entitled "Formulation and Evaluation of an Anti-Inflammatory Transdermal Patch of Boswellia serrata Resin for the Treatment of Inflammation Caused by Honey Bee Sting" was successfully completed. In conclusion, the developed transdermal patch is a promising, safe, cost-effective, and patient-compliant product for the first-aid management of honey bee stings. The formulation successfully overcomes the drawbacks of existing market products like poor adhesion and short duration of action. It has high potential for development as an over-the-counter herbal remedy

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