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Original Article

# Physiochemical Characterization and *in vitro* Evaluation of Formulated Herbal Bioactive Loaded Transdermal Patches

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## ABSTRACT

**Aim:** The present research aimed to formulate a transdermal patch incorporating herbal bioactive *Vitex negundo* extract. The polymers that were used for selected sustained release of the drug are HPMC, PVP K30 and polyethylene glycol used as a plasticizer. **Materials and Methods:** Transdermal patches were prepared using the drug with two (HPMC and PVP K30) different polymers. The prepared transdermal films were evaluated for their physico-chemical characteristics such as physical appearance, weight uniformity, thickness, folding endurance, moisture content, drug content, percent moisture loss and permeation studies. The skin irritation study done on rat skin showed that the formulation does not produce irritation to the skin it showed the successful release of drug from the fabricated patch. **Results:** The *in vitro* release of formulation F1, F2, F3, F4, F5 and F6 has shown release of about 89.15%, 87.12%, 82.34%, 92.45%, 86.78% and 76.21% at 8hr. The order of drug release was found to be F4>F1>F2>F3>F6>F5. **Conclusion:** It could be concluded that it was concluded from the study, that the transdermal patch containing herbal bioactive *Vitex negundo* F4 batch showed the highest percent of drug release and other desirable properties can be developed.

**Keywords:** Transdermal, UV/Vis Spectroscopy, Physico-chemical properties, Stability, Differential Scanning Calorimetry (DSC), Fourier-Transform Infrared Spectroscopy (FTIR).

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## INTRODUCTION

The Transdermal Drug Delivery System (TDDS) is a new, painless method for delivering medicinal drugs across the circulation via the exposed skin's surface. This brand-new medicine delivery method is created to order.[1] Because the rate-controlled drug delivery system maintains a steady plasma drug level, there are fewer adverse effects and a constant output. Additionally, it demonstrates that medicinal compounds are absorbed into the circulation at a preferred rate. Researchers have created a number of transdermal patches, including anti-rheumatoid, estrogen-containing contraceptive, and anti-hypertensive ones, to achieve regulated release over a longer period of time.[2]

The current study project aimed to develop and assess a bioactive-loaded transdermal medication delivery system of herbal pharmaceuticals using *Vitex negundo* Linn. It is a large fragrant shrub from the family Verbenaceae that is sometimes referred to as the "five-leafed chaste tree" or "Nirgundi." As an anti-inflammatory, expectorant, tranquillizer, antispasmodic, anticonvulsant, rejuvenative, anti-arthritic, anthelminthic, anti-fungal, antipyretic, and antispasmodic, *Vitex negundo* Linn. is known in Indian traditional medicine as "sarvaroganivarani," which literally means "the remedy for all diseases".[3] In order to give local treatment to the afflicted tissues, such as aching joints, reduce gastrointestinal side effects, and increase patient compliance, bioavailability, and solubility by lowering the dose frequency, herbal bioactive loaded transdermal patches have been developed.

## MATERIALS AND METHODS

### Materials

Amsar Pvt. Ltd. was given a sample of the *Vitex negundo* dry extract as a gift (Indore, India). We ordered from Himedia Mumbai HPMC, PVP K30, ethanol, and oleic acid. S.D. Fine chemicals provided the isopropanol, methanol, acetone, chloroform, sodium hydroxide, sodium bicarbonate, potassium chloride, calcium chloride dihydrate, sodium chloride, potassium hydrogen phosphate, and disodium hydrogen phosphate (Mumbai, India). Throughout the investigation, double-distilled water was used.

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## Methods

### Physico-chemical characterization of *Vitex negundo* extract

#### *Organoleptic Characteristics*

The organoleptic characteristics of *Vitex negundo* like color, odour, taste and state were determined.

### Determination of melting point

A little amount of *Vitex negundo* extract was put in a capillary tube that had been fused at one end, then the melting point equipment was used to measure the temperature at which the extract melted.[4]

### Partition coefficient determination

In the octanol-water system, the *Vitex negundo* partition of coefficient was calculated. The two phases included phosphate buffer pH 7.4 and pure medication 25 g/ml in octanol. In the separating funnel, the combination of octanol and phosphate buffer pH 7.4 was appropriately mixed hourly.[5] After being mixed with the medication solution, the combination was let to stand for an hour. After the mixture was separated by centrifuging it at 5000 rpm in 25°C, the absorbance was measured spectrophotometrically (UV spectrophotometer, Shimadzu 1800, India).

### Calibration curve of *Vitex negundo*

The standard stock solution of *Vitex negundo* was prepared by dissolving 2g of it into 2 ml of ethanol. Further dilution was prepared by taking 1 ml from the standard stock and diluting with 50 ml of ethanol. A calibration curve of 2µg/ml to 22 µg/ml was prepared from the sub-stock solution and absorbance was taken at $\lambda_{max}$ 276 nm.

### Drug–polymer interaction studies

#### *Fourier Transform-Infrared Spectroscopy*

In order to study the pure *Vitex negundo* drug, physical drug combination, HPMC and PVP K30, as well as the drug-loaded transdermal patches using the KBr pellets technique, Fourier Transform-Infrared Spectroscopy (FT-IR-8400S, Shimadzu, Japan) was used.

### Differential scanning calorimetry

On the chosen medication with polymer, a differential scanning calorimetric examination was carried out. The samples were first heated to eliminate the moisture, and each sample (approximately 3–7 mg) was then precisely weighed into a platinum crucible 40–aluminum pan in a hermetically sealed environment, using alpha alumina powder as a standard. Thermograms were taken from 50 to 300°C at a heating rate of 20°C/min and a flow rate of 20 ml/min of inert nitrogen gas environment. The Perkin-Elmer Pyris-1 equipment (Osaka, Japan) used for these investigations is accessible at the Department of Textile Technology, Indian Institute of Technology, in New Delhi, India.[6]

### Preparation of herbal bioactive loaded transdermal patches

Casticin-containing drug-loaded *Vitex negundo* transdermal patches were created utilizing the solvent casting technique. A petri dish with a 50.24 $cm^2$ surface area was used. In order to make HPMC and PVP K30, they were dissolved in a solution of water and ethanol (6:4). The solutions was prepared with clarity. The following phase involved using propylene glycol (30% of the total polymer) as a plasticizer and oleic acid (5% of the total polymer) as a permeation enhancer. The resulting solution was then allowed to cast inside a Petri dish that had been greased with glycerin and dried for 24 hr at room temperature. To prevent the solvent from evaporating too quickly, an inverted funnel was placed over the Petri dish and kept in a 40°C oven until the patches were completely dried. The patches were then removed from the petri dish and stored in a desiccator for further testing.[7-9] The ingredients of the *Vitex negundo* transdermal patch were listed in Table 1 and displayed in Figure 1.

### Evaluation of herbal bioactive loaded transdermal patches

#### *Organoleptic Characteristics*

Visual checks for colour, clarity, flexibility, and smoothness were made on each created patch.

### Uniformity of Weight and Thickness

By individually weighing 10 randomly chosen patches and figuring out the average weight, weight variation is explored. By utilizing a micrometre screw gauge to measure the thickness of the created polymeric film, the thickness of the film was measured, and the average thickness was calculated.[10]

### Folding endurance and Moisture Content

A short (2 cm x 2 cm) piece of film was folded repeatedly at the same location until it broke to test the film's folding durability. The difference between the beginning and final weights in relation to the final weight was used to compute the percentage of moisture content.[11]

### Drug Content and Percent Moisture Loss

100 mL of distilled water was used to make up the leftover capacity after the patch was dissolved in methanol. Then, once the solution had been filtered, the concentration was determined by measuring the solution's absorbance at 276 nm. The difference between starting and final weights with regard to final weight was used to compute the percentage of moisture content.

**Table 1: Composition of *Vitex negundo* Transdermal Patch.**

| Sl. No. | Composition | Formulation | | | | | |
|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 |
| 1 | Drug (mg) | 300 | 200 | 100 | 300 | 200 | 100 |
| 2 | HPMC(mg) | 200 | 100 | 100 | 100 | 200 | 300 |
| 4 | PVP K30(mg) | 100 | 100 | 100 | 100 | 100 | 100 |
| 5 | Propylene Glycol (%) | 30 | 30 | 30 | 30 | 30 | 30 |
| 5 | Oleic Acid (%) | 5 | 5 | 5 | 5 | 5 | 5 |
| 6 | Ethanol: Water(ml) | 6:4 | 6:4 | 6:4 | 6:4 | 6:4 | 6:4 |

## *In vitro* permeation study

A cellophane membrane and Franz diffusion cell were used to conduct an *in vitro* penetration research on a synthetic *Vitex negundo* transdermal patch. Between the donor and receptor compartments of the diffusion cell was the membrane. A diametric patch was positioned in the receptor compartment with 17 ml of phosphate buffer pH 7.4 in order to make first contact with the membrane. The aluminium foil covering the backside of the patch served as a backing membrane. Throughout the experiment, a temperature of 32±0.5°C was maintained while the cell content was agitated using a magnetic stirrer. For a period of 24 hr, draw 1 ml of the sample through the sampling port at periodic intervals. For the first hour of release, samples were taken at 0, 15, 30, and 60 min. Thereafter, they were taken every hour until the sixth hour of release. The system was then left in its regular configuration for the rest of the day, and the next day's reading was obtained at the 24$^{th}$ hr while also replacing an equal volume of phosphate buffer with a pH 7.4 solution to preserve sink condition. At 276 nm, the material underwent spectrophotometric analysis.[12]

## Skin Irritation Study

According to the CPCSEA Protocol (IAEC/2019-20/RP-05), the skin irritation research was carried out at the Oriental College of Pharmacy and Research, Oriental University, Indore. The albino Wistar rats were kept in cages with access to a free supply of water and a regular laboratory food. Within 24 hr following the study's completion, the rats' dorsal abdominal skin was carefully removed from the dorsal part of their trunks by clipping, shaving, and avoiding any injury. Transdermal patches containing the herbal medication *Vitex negundo* were placed to the exposed skin and then covered with a non-sensitizing microporous tape. Over each test site, the test patch composition was fixed. When compared to a histamine solution of 1 mg/mL (control) and a drug-free blank patch, the test sites were evaluated for erythema, edoema, or any hazardous side effects 1, 24, 48, and 72 hr after application.[13]

# RESULTS

## Physico-chemical Characterization of *Vitex negundo* Extract

The drug's organoleptic properties were judged to be within acceptable bounds, as stated in Table 2. The drug sample (*Vitex negundo*) was discovered to have a melting point of 191.202°C, which, when compared to the claimed value (190°C-192°C), showed that the drug sample was pure. The drug's highest absorbance in methanol was discovered to be at λ 276 nm, which

**Figure 1:** Transdermal Patch of *Vitex negundo.*

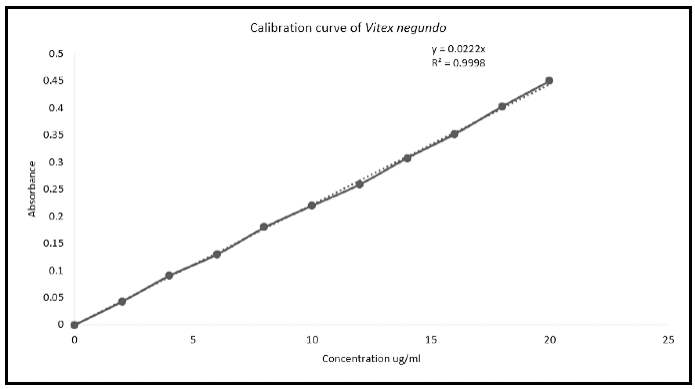


**Figure 2:** Calibration curve of *Vitex negundo.*

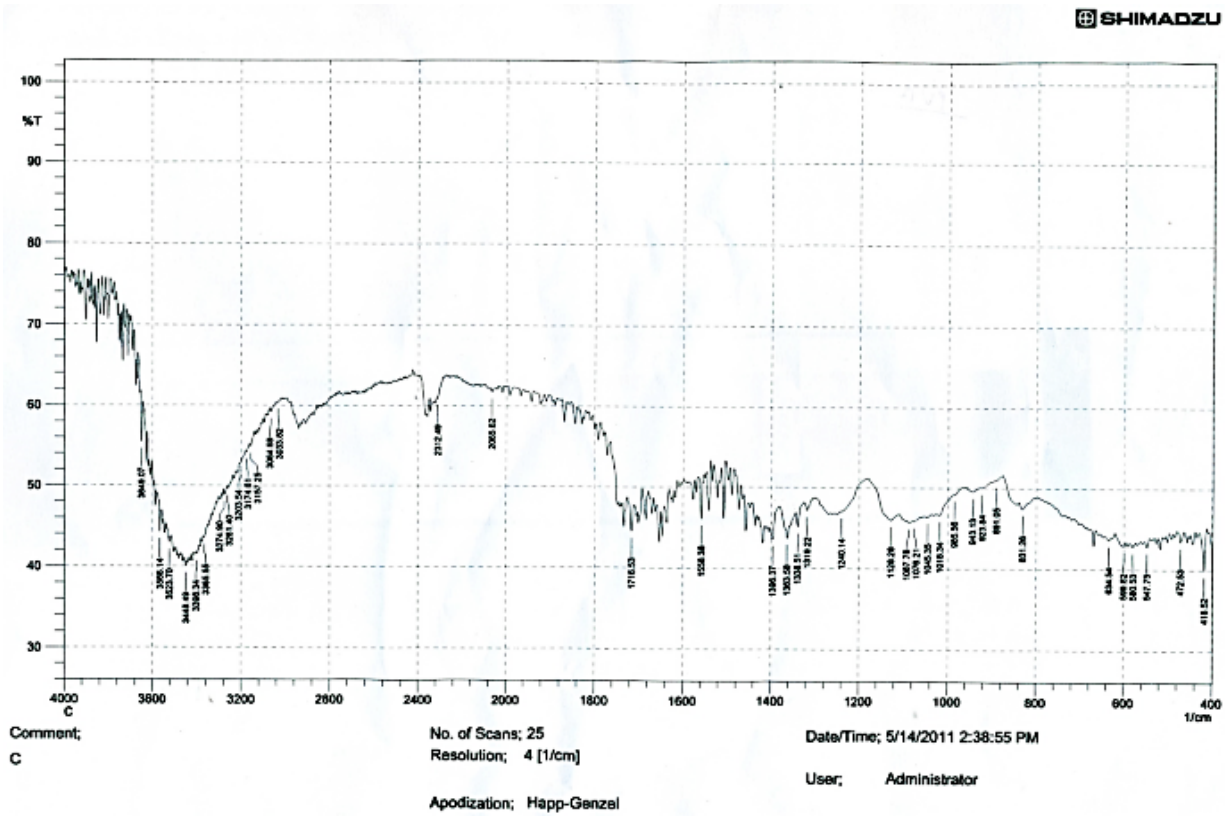


**Figure 3:** FTIR Studies of *Vitex negundo* with a polymer mixture.

**Table 2:** **Organoleptic Characteristics of *Vitex negundo*.**

| Sl. No | Characteristics | Inference |
|---|---|---|
| 1 | Color | Brownish black |
| 2 | Odor | Odorless |
| 3 | Taste | Bitter |
| 4 | State | Amorphous |

**Table 3:** **Evaluation of Herbal Loaded Transdermal Patch of *Vitex negundo*.**

| Formulation Code | Weight Variation (mg) | Thickness (µm) | Folding Endurance | Moisture Content (%) | Drug Content (%) | Percent moisture loss (%) | % Drug Release |
|---|---|---|---|---|---|---|---|
| F1 | 119.33 ± 1.53 | 0.132 ± 0.001 | 31.04±0.25 | 78.81±4.82 | 81.69 ± 3.09 | 6.16±0.12 | 89.15 ± 2.35 |
| F2 | 121.5 ± 1.0 | 0.143 ± 0.007 | 29.54±0.72 | 73.15±6.32 | 80.76 ± 0.65 | 5.85 ±0.22 | 87.12 ± 2.39 |
| F3 | 120.33 ± 1.53 | 0.137 ± 0.009 | 28.84±0.27 | 68.74±0.78 | 96.94 ± 0.12 | 5.85 ±0.22 | 82.34 ± 0.06 |
| F4 | 120.0 ± 2.64 | 0.142 ± 0.007 | 15.04±0.45 | 60.35±1.87 | 96.94 ± 0.53 | 1.24 ± 0.570 | 92.45 ± 0.06 |
| F5 | 120.0 ± 2.0 | 0.145 ± 0.007 | 22.21±1.28 | 63.54±4.42 | 80.76 ± 0.65 | 4.97 ± 0.004 | 86.78±0.94 |
| F6 | 120.66 ± 1.53 | 0.136 ± 0.012 | 24.64±0.53 | 66.98±3.83 | 80.76 ± 0.65 | 7.55 ± 0.007 | 76.21±0.16 |

**Mean ± SD (*n*=3)**

**Table 4: *In vitro* % permeation studies of *Vitex negundo* Transdermal Patch.**

| Sl. No. | Time (hrs) | % Permeation Drug Release |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 1 | 7.03 ± 0.44 |
| 3 | 2 | 12.15± 0.95 |
| 4 | 3 | 19.27± 0.21 |
| 5 | 4 | 25.26 ± 0.60 |
| 6 | 5 | 34.37± 5.57 |
| 7 | 6 | 41.97 ± 0.81 |
| 8 | 7 | 48.44 ±0.72 |
| 9 | 8 | 56.70 ± 0.64 |

is consistent with the notion that the drug sample was pure (Figure 2).

By using FTIR investigations (Figure 3), it was possible to establish if a medication and a polymer were compatible. Both pure drugs and mixtures of polymers were submitted for the tests, and the findings showed that there were no interactions between the two. The melting point of the drug was shown to peak at 191.202°C on the DSC Thermogram (Figure 4), IR Spectroscopy, and physical observation[13] during the compatibility analysis. The results of the FTIR analysis confirmed the presence of the chemicals benzene, bromo alkanes, alcohol, carboxylic acid, aromatic compound, nitro compound, and phenol. These compounds had strong peaks at 3446.49, 2312.49.92, 1716.53,

**Table 5: *In vitro* % Cumulative Drug Release studies of *Vitex negundo* Transdermal Patches.**

| Sl. No. | (hrs) | % Cumulative Drug Release | | | | | |
|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 2 | 13.22 | 19.23 | 18.23 | 10.21 | 18.00 | 7.23 |
| 4 | 3 | 30.23 | 25.24 | 27.89 | 29.44 | 25.24 | 24.23 |
| 5 | 4 | 49.58 | 55.22 | 46.34 | 48.15 | 53.22 | 42.13 |
| 6 | 5 | 68.45 | 68.98 | 60.23 | 74.28 | 73.44 | 59.99 |
| 7 | 6 | 77.23 | 72.54 | 69.34 | 84.12 | 77.80 | 67.11 |
| 8 | 7 | 83.23 | 75.65 | 73.24 | 8.30 | 81.42 | 72.73 |
| 9 | 8 | 89.15 | 87.12 | 82.34 | 92.45 | 86.78 | 76.21 |

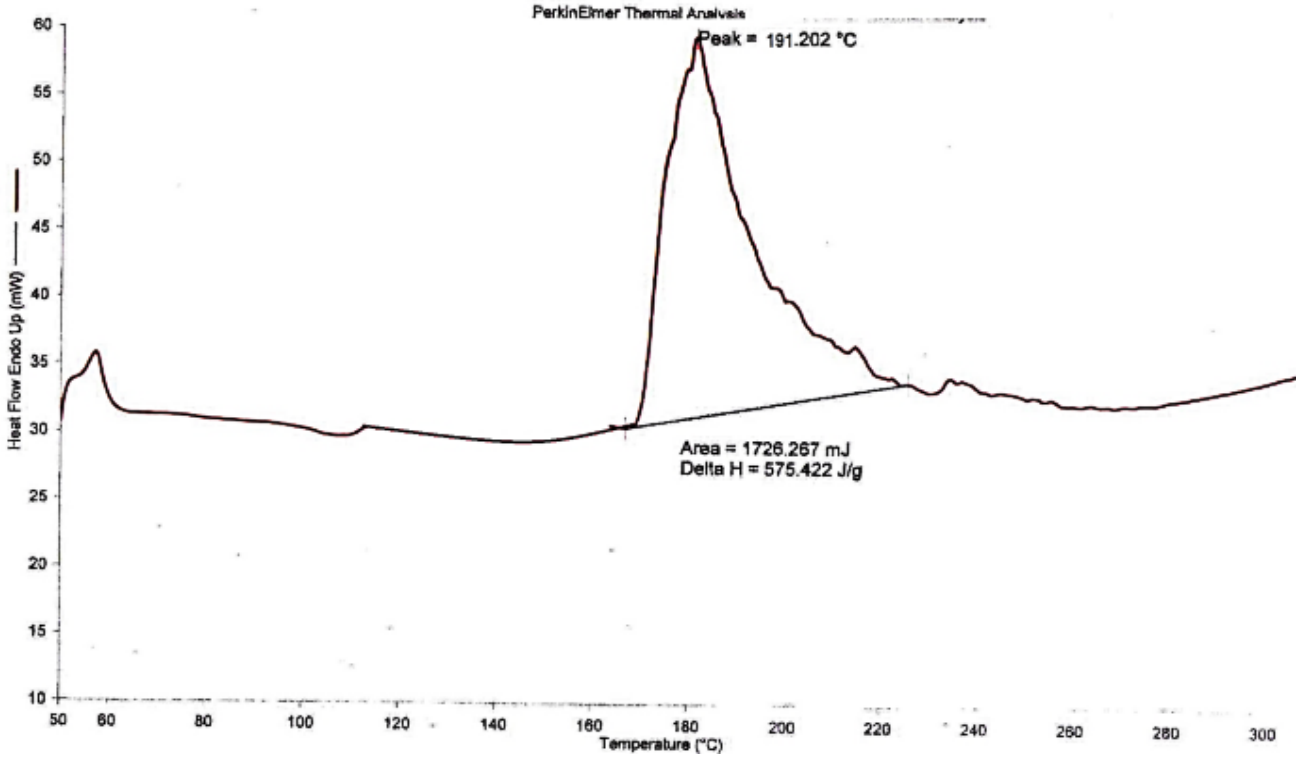


**Figure 4:** Differential scanning colorimetric Drug *Vitex negundo*+ Polymer Mixture.

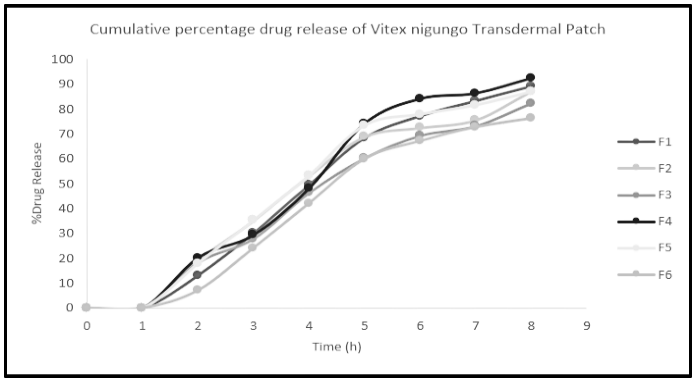


**Figure 5:** Cumulative percentage drug release of *Vitex negundo* Transdermal Patches.

1558.38, 1388.51, 1087.78, and 599.82, respectively. According to the findings, there is no conflict between the medicine and particular excipients. As a result, the chosen excipients can be utilised with the medications since they are compatible.

### Evaluation Transdermal Patches

The most popular approach to achieve, smooth and with excellent thickness, was the solvent casting method for transdermal preparation. Numerous batch optimised batches were discovered to be F4 based on medication content and release. Polymers like HPMC and PVP K30 were used to create patches. The patches were smooth, translucent, and adaptable.

Table 3 shows the effects of weight fluctuation, thickness, moisture content, moisture absorption, folding endurance, and medication content. The patch displays constant weight and thickness. The patch was best suited for high medication release at a regulated pace because of its reduced thickness. It was discovered that the skin irritation score (erythema and edoema) was less than 2. As a result, there was no skin sensitivity in the *Vitex negundo* transdermal herbal patches. Employing modified diffusion cells, *in vitro* research on membrane barrier permeation and patch permeation using cellophane were conducted. The outcome of the permeation investigation of the improved formulation F4 in 8 hr is presented in Table 4 as 56.70 0.64. It was discovered that F4 had a cumulative drug release percentage of 92.450.06%. (Table 5, Figure 5). The patch's consistent thickness suggests that the drug's polymeric solution is evenly distributed throughout it.

## DISCUSSION

*Vitex negundo* extract is employed in the current work to make transdermal patches utilising the solvent casting process and polymers such HPMC, PVP K30, and polyethylene glycol. *Vitex negundo* is used to treat a number of illnesses, including diabetes, hormonal problems in women, and inflammation. Therefore, the patch created here passes all tests for consistency in weight and thickness across all patches. Additionally, it passes the test for skin irritability and has acceptable drug release kinetics. Therefore, we can utilise it to treat female hormonal disorders.

## CONCLUSION

The solvent casting method was used to create the *Vitex negundo* transdermal patches. The homogeneous weight and thickness of the produced patches showed that the medication was distributed well throughout the polymeric solution. The produced product can be used transdermally to treat a variety of illnesses.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**HPMC:** Hydroxypropyl Methylcellulose; **PVP:** Polyvinylpyrrolidone; **DSC:** Differential Scanning Calorimetry; **FTIR:** Fourier Transform Infrared; **TDDS:** Transdermal Drug Delivery System; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institute Animal Ethics Committee.

## SUMMARY

The goal of the current research is to create a transdermal patch using herbal *Vitex negundo* extract that is bioactive by employing HPMC, PVP K30, and polyethylene glycol as a polymer. *Vitex negundo* transdermal patches were created using the solvent casting technique. Physical and chemical properties of the produced patches were assessed using *in-vitro* drug release and skin irritation tests. FTIR and DSC techniques were used to study the interaction between a medication and a polymer. The FTIR research shows that the medication and polymer have high compatibility. The formulations F1, F2, F3, F4, F5, and F6 released approximately 89.15%, 87.12%, 82.34%, 92.45%, 86.78%, and 76.21% at 8 hr, according to *in vitro* testing. Drugs were discovered to release in the following order: F4>F1>F2>F3>F6>F5. The transdermal patch containing herbal bioactive *Vitex negundo* F4 batch demonstrated the highest percentage of drug release, and additional desired features can be produced, according to the study's findings.

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