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Original Article

Design and Characterization of Herbal Transdermal Patch Containing Capsaicin Extract and Mustard Oil for Arthritis

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ABSTRACT

Introduction: Arthritis, a chronic inflammatory condition causing significant pain and diminished quality of life, affects millions worldwide. This study aimed to develop and characterize a novel herbal transdermal patch using mustard oil and capsaicin extract, extracted via water, alcohol, and mustard oil. The extracts underwent preliminary examination and were assessed for *in vitro* anti-inflammatory activity. **Objectives:** The primary goal was to construct and evaluate a transdermal patch for arthritis treatment, incorporating capsaicin and mustard oil extracts. Additionally, the study aimed to assess the *in vitro* anti-inflammatory activity of the extracts, providing insights into their potential therapeutic efficacy. **Materials and Methods:** Capsaicin extraction involved water, alcohol, and mustard oil methods, with subsequent evaluation of anti-inflammatory activity. The oil extract was processed into five transdermal patch formulations, integrating naproxen as a model drug. Parameters such as weight variations, folding endurance, tensile strength, and moisture content were analysed. *In vitro* drug permeability and *ex vivo* permeation tests were conducted, employing Rhodamine B/Oil Red O dye. **Results:** The water-extracted capsaicin exhibited reduced potency on the fourteen days, with observed fungal growth. The alcohol extract showed diminished potency compared to the oil extract. The transdermal patch formulations demonstrated drug release within the range of 0.1 to 0.3 mm, 3% moisture content, 1-pascal tensile strength, and a drug release rate of 90% during *in vitro* and *ex vivo* tests, utilizing Rhodamine B/Oil Red O dye.

Keywords: Transdermal patch, Capsaicin extract, *In vitro* and *ex vivo* permeation study, Docking study, Arthritis.

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INTRODUCTION

An inflammatory disorder known as arthritis causes one or more joints to become inflamed, leading to pain, stiffness, and decreased movement.[1] It is a common and frequently disabling condition. Capsaicin has become a potential natural therapy for arthritic symptoms within the range of current treatments.[2] Chilli peppers contain a substance called capsaicin that is responsible for its unique heat.[3] Capsaicin has received a lot of interest for its possible efficacy in treating arthritis-related pain and its culinary uses. The natural vanilloid component capsaicin, which gives chili peppers their intense flavour, is now known for its ability to reduce pain.[4] It has the potential to provide long-lasting pain relief due to its interaction with the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor, which causes the desensitization of pain-sensing nerve fibres. Although there are capsaicin creams and ointments for topical pain relief, their effectiveness is occasionally diminished by inconsistent administration and variable patient reactions.[5] Capsaicin transdermal patches, which have recently become available, overcome these drawbacks by offering a regulated and consistent delivery method.[6] The patches provide a number of benefits, including specific medication administration, increased adherence to treatment plans, and less danger of unintentional cross-contamination.[7] Additionally, transdermal delivery avoids the digestive system, reducing gastrointestinal adverse effects that may be associated with oral painkillers.[8]

This study article aims to conduct a comprehensive assessment of the current body of evidence concerning the utilization of transdermal patches containing capsaicin for enhanced pain relief. The primary objectives of this article include investigating the mechanisms of capsaicin action, scrutinizing formulation and design considerations related to transdermal patches, and pinpointing potential areas for future research.

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MATERIALS AND METHODS

Chemicals

Naproxen was purchased from Yarrow Chem products, Mumbai, India. Capsaicin was purchased from Byadgi. Mustard oil was purchased from local market Bellur. DURO-TAK 387-2054 is used as bio adhesive was supplied by sigma Aldrich. Backing membrane used as scotch pak 9723 and liner as coprex was used.

Extraction

Capsaicin extraction was performed from 300 g of chopped capsaicin pieces using water, alcohol, and mustard oil as separate solvents. The capsaicin pieces were heated on a heating mantle at 70°C for 4-5 hr. After cooling, the mixture was filtered, and the resulting extract was set aside for subsequent observations and further studies.

Determination of *in vitro* Anti-Inflammatory Activity

2 mL of different sample concentrations (5 mL total) should be added to 2 mL of BSA, 2.8 mL of PBS (pH 6.4), and 0.2 mL of BSA. The mixture was heated for 5 min at 70°C after 15 min of incubation at 37±2°C. Their absorbance at 660 nm was measured after cooling, using the vehicle as a reference. distilled water as a baseline.[9]

$$\% \text{ inhibition} = 100 \times [V\,t / V\,c - 1]$$

Where,

V t - absorbance of the test sample, V c - absorbance of control.

Determination of acid value

Precisely weighed 10 g of the sample, 50 mL of combination of same quantity of ethanol (95%) and ether. If the substance does not dissolve in the cold water, kept the flask with reflux condenser and slightly warm with continuous shaking. 1 mL of phenolphthalein reagent is added to the above solution, titrate with 0.1M potassium hydroxide solution, colour changes from colourless to pink constant after 30 sec.[10]

$$Acid\ value = \frac{TV\ X\ 5.61\ X\ M}{W\ X\ 0.1}$$

Determination of saponification value

Measure 25 mL of 0.5M ethanolic potassium hydroxide, add 2 g of sample taken in 200 mL flask attached with reflux condenser, boiled under water bath for 30 min. Add 1 mL of phenolphthalein reagent and titrate with 0.5M HCl acid.[11]

$$Saponification\ value = \frac{(blank - TV)X\ 28.05\ X\ M}{W\ X\ 0.5}$$

Determination of density

Density of a liquid is determined by using specific gravity bottle. Specific gravity bottle holds a definite volume of liquid when completely filled. Then,

$$Density\ of\ liquid = \frac{weight of\ liquid\ in\ S.G.\ bottle}{Volume\ of\ the\ liquid}$$

but SG bottle is not a standard apparatus. Hence, the accurate volume that it holds can be obtained using standard liquid (water) of known density.[12]

Then,

$$Volume\ of\ SG\ bottle = \frac{weight\ of\ water}{density\ of\ water}$$

$$Density\ of\ liquid = \frac{weight\ of\ the\ liquid\ X\ density\ of\ water}{weight\ of\ water}$$

Phytochemical Constituents

"In this study, mustard seeds (genus Brassica) and capsaicin were selected as the plant materials for analysis. This study was done to find out the presence and absence of constituents.[13]

Tocopherols: Ferric Chloride Test: Mix the extract with 1% alcoholic ferric chloride solution. A blue color indicates the presence of tocopherols.

Carotenoids: Salkowski Test: Mix the extract with chloroform and concentrated sulfuric acid. A red color indicates the presence of carotenoids.

Glycosylates: The Mustard Oil Test: Crush the plant material and add water. Add a few drops of dilute hydrochloric acid and filter. Mix the filtrate with ether and shake. The ether layer is separated and mixed with alcoholic silver nitrate. Formation of a yellow precipitate indicates the presence of glucosinolates.

Capsaicin: Capsaicin Test: Extract the sample with alcohol, and add a few drops of the extract to a solution of potassium hydroxide. A yellow colour indicates the presence of capsaicin.

Flavonoids: Shinoda Test: Add a few drops of concentrated hydrochloric acid to the extract, followed by a few pieces of magnesium ribbon. A pink, red, or violet colour indicates the presence of flavonoids.

Phenolics: Ferric Chloride Test: Mix the extract with 1% alcoholic ferric chloride solution. A colour changes to blue, green, or black indicates the presence of phenolic compounds.

GC-MS Investigation

Gas chromatography-mass spectrometry is known by the abbreviated form GC-MS. It is an analytical technique that uses the characteristics of mass spectrometry and gas chromatography to identify various compounds in a test sample. Oil samples were subjected to GC-MS analysis utilizing a Shimadzu 17 GC combined with a quadrapole MS (QP 5000). A fused silica

SPB'l'M-l (30 m x0.32 mm i.d., 0.25 µm coated with poly dimethyl siloxane) capillary column was employed, with helium as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1 µL and the injector temperature was set to 250°C. The initial oven temperature was 60°C, held for 2 min, then ramped to 250°C at a rate of 2°/min and finally held for 5 min.

Molecular Docking Studies

In silico molecular docking

This study primarily used structure-based drug design as its methodology. Using the RCSB PDB, the target proteins were obtained in their three-dimensional forms, and the binding affinities of putative ligand molecules to these protein targets were assessed. Throughout the whole procedure, only the Schrödinger suite of programmes was employed.[14,15]

Ligand preparation

The intended ligand was optimised using Schrödinger's LigPrep to make sure it preserved the right stereochemistry and low-energy state. According to Brooks *et al.*, this is an important stage in virtual screening that emphasises the need to include all stereoisomers for possible lead compounds in order to reduce false negatives and prevent missing possible leads.[16] In addition, Ligand preparation makes it easier to transform a 2D created structure into three-dimensional form and then go through a sequence of procedures to produce the 3D ligand. Verifying the proper ionisation states, tautomers, ring conformations, molecular weights, and the quantity and kinds of functional groups are among these preliminary steps. LigPrep has been effectively employed in several research focusing on structure-based virtual screening for possible lead compounds addressing particular illnesses.[17]

Calibration curve of naproxen sodium

1-10 mL of the 10 µg/mL solution was pipetted into a 10 mL volumetric flask, and the volume of the flask was subsequently filled with pH 7.4 to obtain the concentration of 1-10 µg/mL. Each solution's absorbance was measured at 272 nm.[18]

Preparation of transdermal patch

The matrix type of transdermal patch was prepared. The % inhibition of anti-inflammatory activity showed best result in capsaicin extract compared with mustard oil. Five formulations were prepared using different percentage of capsaicin extract. All formulation of capsaicin extract was mixed with bio adhesive i.e., DURO TAK 387-2054. The mixture was allowed for 30 min to see whether there is a phase separation. After half an hour, it was spread on the liner i.e., coparex, which is on hot plate for 15 to 20 min at 7℃. Then the backing membrane was fixed i.e., scotch pak 9723.

Weighing the patch in the digital scale allowed us to determine the weight's uniformity. Five patches were weighed at random, and the average weight was determined.[19]

Thickness: Using digital vernier callipers, the patch's thickness was measured three times, and the average thickness was noted.[19]

Folding endurance was calculated by repeatedly folding the patch at the same location until it broke. The value of folding endurance will be determined by how many folds a patch can withstand without breaking or cracking.[19]

Moisture Content: The produced films are individually weighed and maintained at RT in a desiccator with $CaCl_2$ for 24 hr. After a certain amount of time, the films are weighed once more until they display a steady weight. in percentage.[19]

In vitro Drug Permeation Studies

The open-ended tube method was used to analyse the patch for 6 hr while utilising pH 7.4 as the diffusion medium. The cellophane membrane was placed at one end of the tube, tided, and then submerged in a receptor compartment containing methanol and 7.4 buffer solutions. It was stirred at 600 rpm and kept at a temperature of 37 ± 2°C. At regular intervals, samples were taken out and the same volume was replaced with new diffusion media. Shimadzu UV1800 UV-visible spectrophotometer was used to evaluate the samples at 272 nm. The percentage of the medication released at each time point was plotted against time to create the release profiles.[20]

Ex vivo permeation study

In this *ex vivo* permeation study, pig abdomen skin was utilized as a barrier within a Franz diffusion cell setup. The Franz diffusion cell had an exposed surface area of 1.76 cm^2 and a skin thickness ranging from 21 to 25 µm. A capsaicin extract patch was placed, facing the stratum corneum of the skin, in the donor compartment. The receptor compartment was filled with a phosphate buffer solution at pH 7.4, which also contained methanol, and a magnetic bead was positioned within the receptor compartment to ensure proper mixing. The entire Franz diffusion cell assembly was placed on a magnetic stirrer, maintaining a constant temperature of approximately 37±0.5℃ throughout the experiment. Samples were withdrawn at various time intervals, and an equal volume of phosphate buffer was added to the receptor compartment to maintain a consistent volume. These withdrawn samples were subsequently analyzed using a UV spectrophotometer at 272 nm to quantify the amount of the drug present in each sample. The resulting data were organized in tabular form, and a graph was generated to illustrate the cumulative percentage of drug permeated versus time. Permeability coefficient (Kp) and flux were calculated using the following equations:[21]

$$Kp \text{ (Permeability Coefficient)} = (dQ/dt) / (A \times C_0)$$

$$\text{Flux} = dQ / (A \times dt)$$

Where:

dQ/dt is the rate of drug permeation.

A is the surface area of the skin.

C_0 is the initial drug concentration in the donor compartment.

dQ is the quantity of drug permeated.

dt is the time interval.

These calculations help assess the ability of capsaicin to permeate through the pig abdomen skin barrier under the defined experimental conditions.

RESULTS

Chemical test of Capsaicin Extract

The chemical test were performed for ginger extract. The results are shown in Table 1.

Phytoconstituent of capsaicin and mustard

The results of phytoconstituent analysis conducted on ginger extract and mustard displayed in Table 2 and encompassing tests for flavonoids, phenolics, tocopherols, carotenoids, glycosylates, catechin, and minerals.

In vitro Anti-inflammatory activity

The study compared the *in vitro* anti-inflammatory effects of capsaicin extract with the denaturation of egg albumin. A summary of the findings is presented in the Figure 1a, 1b and 1c. The research indicates that capsaicin effectively inhibits protein (albumin) denaturation in a concentration-dependent manner. On the 1st day of Capsaicin Water extraction, promising results were observed in comparison to the 7th and 14th days, % inhibition decreased, accompanied by the emergence of some amount of fungal growth. When capsaicin was extracted with alcohol, result showed variation compared to the 1st day, while in mustard oil, % inhibition reaching up to 98%. Considering the %

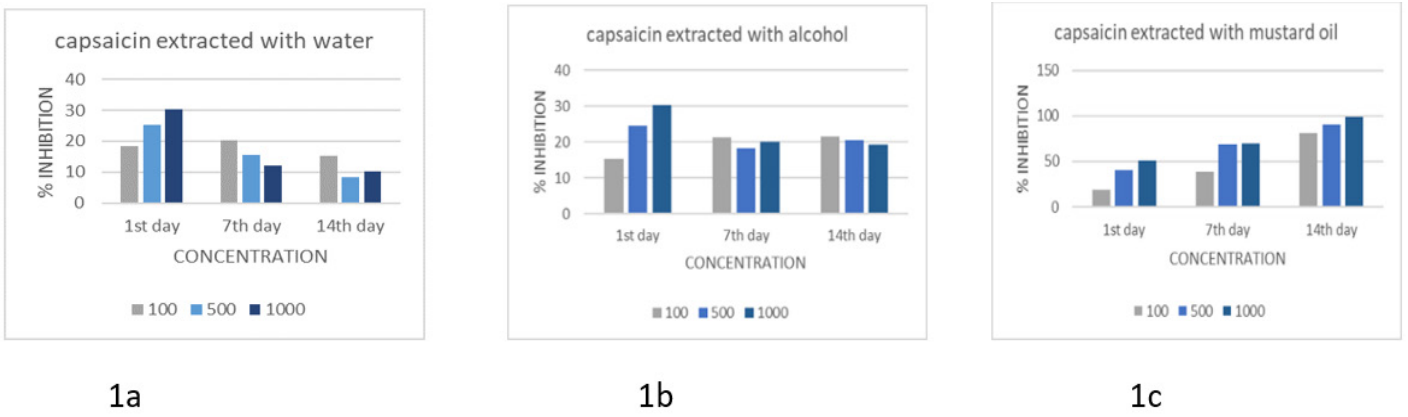


Figure 1: a. Represents %inhibition of capsaicin extracted with water, b. Represents %inhibition of capsaicin extracted with alcohol, c. Represents %inhibition of capsaicin extracted with mustard oil.

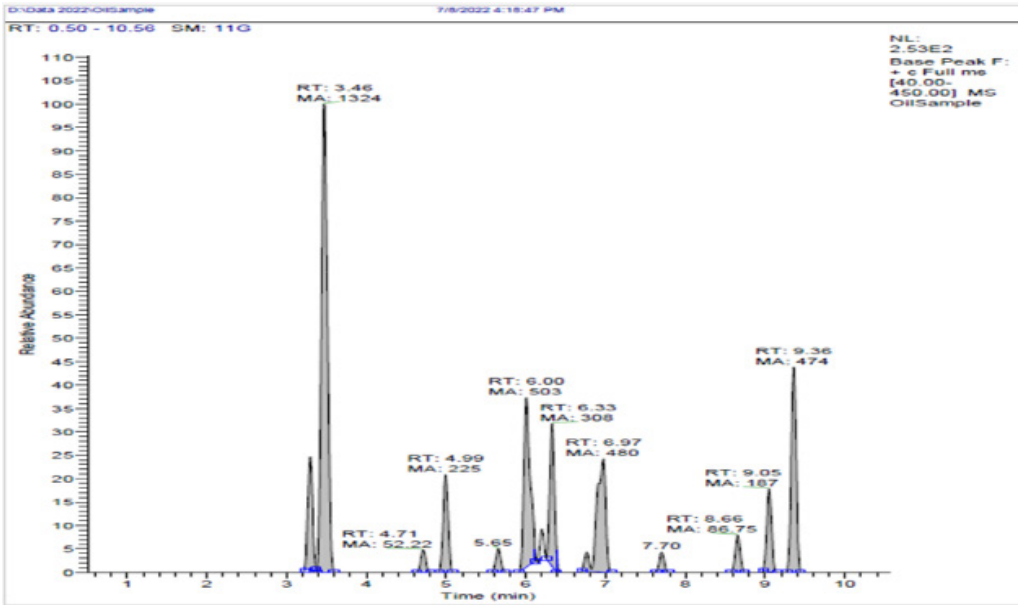


Figure 2: Represents GCMS of oil sample.

Table 1: Chemical test of mustard oil and capsaicin extract.

Sl. No.	Chemical Tests	Mustard Oil	Capsaicin Extract
1	Specific gravity	0.9521	0.908
2	Density	0.9492	0.905
3	Acid value	3.21	2.32
4	Saponification value	171.80	169.05
5	Refractive index (25°C)	1.4717	1.47182

Table 2: Phytoconstituents of mustard oil and capsaicin extract.

Classes	Mustard oil	Capsaicin extract
Flavonoid	+	+
Phenolic	+	+
Tocopherols	+	+
Carotenoids	+	_
Glycosylates	+	_
Capsaicinoids	_	+

(+ presence of constituents, - absence of constituents).

inhibition of capsaicin extract, it was subsequently transformed into a transdermal patch.

GC-MS investigation report

The GCMS stands for gas chromatography-mass spectroscopy. The graph revealed that the three may be a presence of 4 compounds which is having a anti-inflammatory property. Hence, ginger mustard oil sample may be playing an important role in promoting inflammatory activity. The GC-MS chromatogram of the oil extract GC-MS analysis showed the presence of several important compounds. From the chromatogram, different peaks were obtained at different retention times. Based on data obtained from MS, compounds are displayed using molecular weights. The compounds may be present in oil sample are capsaicin and allyl isothiocyanate indicated in Figure 2.

In silico studies

Base on the results obtained from docking of the isolated Capsaicin and allyl isothiocyanate with various enzymes or proteins that are involved in treating the inflammation, it is evident that they can interact with the selected proteins. Galectin-3, Tumor necrosis factor-a, Cyclooxygenase-2 and Inducible nitric oxide synthase are selected for analyzing anti-inflammatory activity represented in Figure 3.

Standard curve of Naproxen sodium

Maximum wavelength (max), linearity, and range the analytical method's ability to provide results from tests that are directly proportional to analyte concentration in samples lying within a certain range was referred to as its linearity. A variety of aliquots of the drug's standard solution made from stock solution were created and examined in order to demonstrate the linearity of the proposed approach. The achieved linearity dilutions were then examined repeatedly by UV Spectrophotometer, and each absorbance was recorded at the maximum. A graph between the absorbance and predicted concentration was then drawn. The therapy demonstrated linearity in the 1-10 g/mL range with a correlation value of pH 7.4 at 272 nm.

Preparation of transdermal patch

The matrix type of transdermal patch was prepared. The % inhibition of anti-inflammatory activity showed best result in capsaicin extraction with mustard oil compared with water and alcohol. Five formulations were prepared using different percentages of capsaicin extract (2%, 4%, 6%, 8% and 10%) mixed with bio adhesive DURO TAK 387-2054. it was spread on the liner i.e., coparex, which is on hot plate for 15 to 20 min at 70°C. Then the backing membrane was fixed i.e., scotch pak 9723. In this approach, a number of patches were created by incorporating Rhodamine B / Oil Red O dye to facilitate the assessment of patch penetration. The oil extract was blended with a 1% Rhodamine solution, and the patch was then formulated.

Evaluation parameter of transdermal patch

The physicochemical properties of the formulated transdermal patch were evaluated for Weight variation, folding endurance and moisture content. The results are shown in Table 3.

Developed patches shown optimum thickness in the range of 0.02-0.03 mm. weight of patches was found in range of 0.249 ± 0.002 mg to 0.253 ± 0.001 mg which indicate that there is reproducibility of patches. whereas folding endurance of prepared patches was more than 500. The moisture content of prepared patches was found in the range of 2.40 to 2.53%.

In vitro Drug Permeation Studies

Since drug release affects the quantity of medication that is available for absorption, *in vitro* release studies are frequently carried out to predict how a delivery system could function under ideal conditions. The *in vitro* permeation study of the oil sample was performed using open ended cylinder. The receptor compartment contained 90 mL of phosphate buffer 7.4, patch is in between receptor and donor compartment, 10 mg of drug. Approximately 1 mL samples were withdrawn at different time intervals (0, 1, 2, 3, 4, 5 and 6 hr) and were suddenly replaced with an equal volume of receptor solution to maintain a constant

volume. The samples were analysed at 272 nm. The system is maintained at 37 ± 1°C. The receptor compartment was under constant stirring (600 rpm) by using magnetic stirrer. The drug penetrates through the membrane. F5 showed 90% drug release as shown in Figure 4a.

Ex vivo permeation study

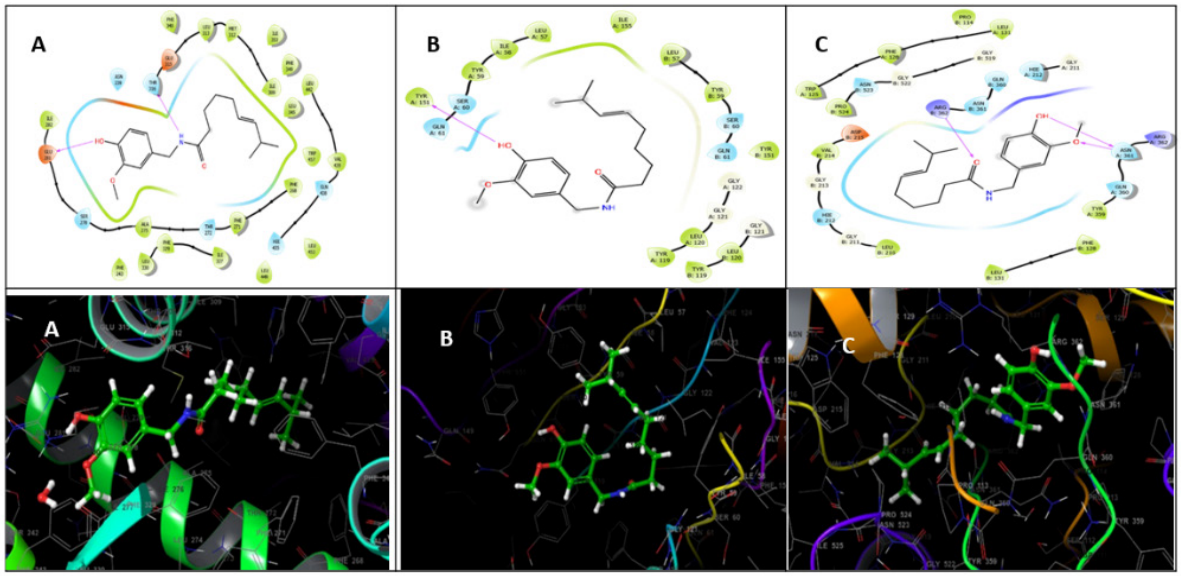
Pig skin, chosen for *ex vivo* drug permeation studies for the patch, was selected due to its similarity to human skin. The experiment was conducted using a Franz diffusion cell to assess the cumulative drug penetration through the skin. This investigation revealed an enhancement in the drug permeation rate, with the highest flux observed in formulation F5 as indicated in Figure 4b.

Fluorescence study

Fluorescence measurements offer a means to track the dispersion of dyes within the skin, facilitating the study of carrier effects and the selection of the most suitable delivery systems. In this study, formulations containing Rhodamine B (with a dye content of 0.001%) were prepared at a consistent concentration to investigate their penetration behavior, a methodology previously employed. Subsequently, 200 µL of each formulation was applied to the surface of pig skin. The pig skin samples were then sectioned into vertical slices with a thickness of 15 µm and subjected to analysis over a 24-hr period. These skin slices were examined under shortwave light, white light, and longwave light, with images captured in each of these illumination conditions. Qualitative assessment was employed to evaluate the distribution of the dye within the skin was shown in Figure 5.

DISCUSSION

The main objective of the study, "Design and Characterization of Herbal Transdermal Patch Containing Capsaicin Extract and Mustard Oil for Arthritis," is to determine if arthritic symptoms may be effectively managed by a new therapeutic intervention. We want to know if using a transdermal patch supplemented with mustard oil and capsaicin extract might reduce pain and suffering associated with arthritis. Capsaicin was extracted using water, alcohol, and mustard oil. Anti-inflammatory tests were conducted on the 1st, 7th, and 14th days of extraction. The stability results for capsaicin extracted with water and alcohol exhibited a decreasing trend, with reductions of 30%, 10%, and 12% and 30%, 20%, and 22%, respectively. In contrast, capsaicin extracted with mustard oil displayed remarkable stability and potency, maintaining levels



A:The 3D orientation and 2D interaction diagram of Capsaicin with Galectin-3 (PDB ID: 1P8D), B:The 3D orientation and 2D interaction diagram of Capsaicin with Tumour necrosis factor-a (PDB ID: 2AZ5), C:The 3D orientation and 2D interaction diagram of Capsaicin with Cyclooxygenase-2 (PDB ID: 3LN1)

Figure 3: represents *in silico* study of oil sample.

Table 3: represents evaluation of patches.

Formulations	Weight Variation (mg)	Thickness (mm)	Moisture Content (%)
F1	0.249	0.03	2.53
F2	0.251	0.02	2.45
F3	0.253	0.03	2.40
F4	0.249	0.03	2.40
F5	0.251	0.02	2.45

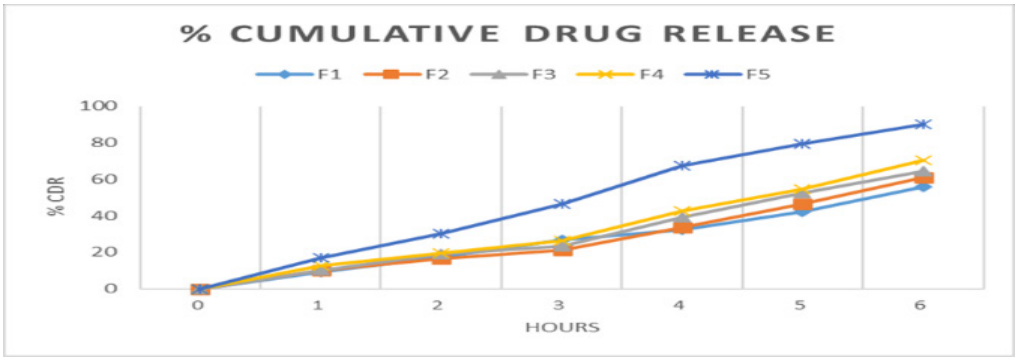


Figure 4a: represents the cumulative amount of drug release in oil sample.

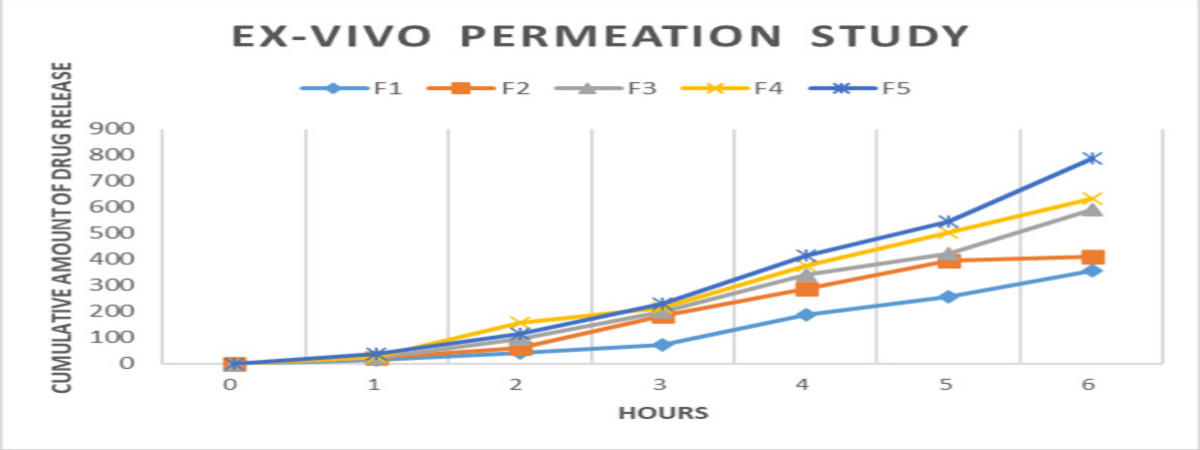


Figure 4b: Represents the permeation study by using pig skin.

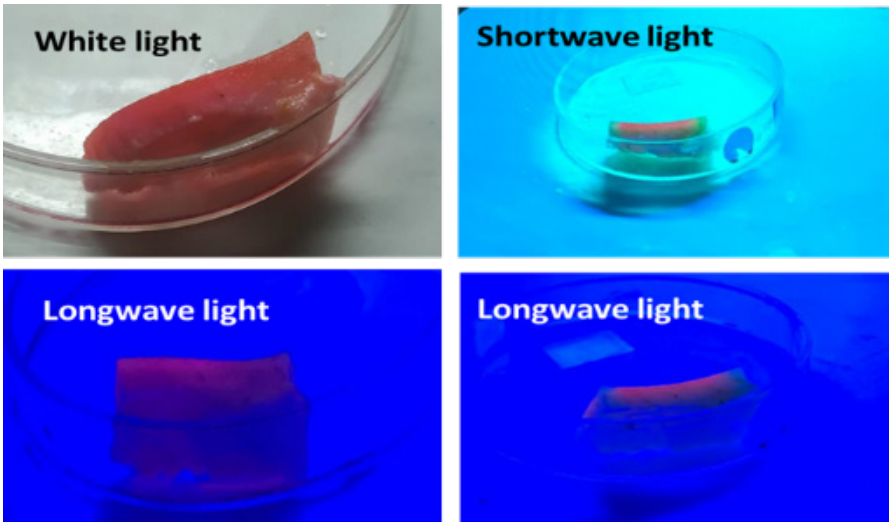


Figure 5: Represents fluorescence study.

of 50.87%, 70.03%, and 98.66% from the 1st day until the 14th day. Due to its superior stability, the capsaicin extract from mustard oil was chosen to formulate transdermal patches. 5 different patches were prepared by using various concentrations of this mustard oil-extracted capsaicin. The characteristics of capsaicin extracted with mustard oil, such as saponification value, acid value, specific gravity, and others, were found to meet acceptable standards. The drug-loaded adhesive transdermal patches were manufactured using a solvent-based casting process. The patches exhibited a thickness ranging from 0.1 to 0.2 mm, a moisture content of 2.4% to minimize product damage, and a smooth patch with a tensile strength of 1 pascal. In formulation F5, the highest drug release, reaching 90%, coincides with the maximum observed flux. The transdermal patch, which incorporated Rhodamine B/Oil Red O dye, demonstrated effective penetration when applied to pig skin. By analysing the docking study, with this Insilco studies we can

estimate that the capsaicin may be showing anti-inflammatory activity through multiple pathways. To improve the quality of life for those who suffer from arthritis, a chronic and crippling disease that affects millions of people worldwide, new, effective treatment alternatives must be investigated. Our investigation thus indicates to provide light on potential benefits of this innovative arthritis management approach.

CONCLUSION

Our research suggests that a transdermal patch containing mustard oil and capsaicin extract might be an effective treatment for arthritic symptoms. The results of this study imply that this novel therapeutic approach can dramatically lessen pain, increase joint mobility, and improve the quality of life for arthritis sufferers. A potential route for treating arthritis is suggested by the mechanisms of action involving anti-inflammatory characteristics, improved blood flow, and pain regulation.

Transdermal patches containing mustard oil and capsaicin extract have a number of benefits over oral drugs, including portability and maybe a reduction in systemic adverse effects. The therapy might not be appropriate for all individuals, and we are aware that individual reactions can vary. Therefore, it should be viewed as one of the possibilities in a comprehensive strategy for managing arthritis that is suited to the unique requirements and preferences of each patient.

In summary, the transdermal patch containing mustard oil and capsaicin extract offers a viable means of tackling the problems associated with arthritis alleviation. This study adds to the expanding body of information in the field of managing arthritis and emphasises the value of creative strategies that put patients' comfort and wellbeing first. The entire potential of this therapy to enhance the lives of arthritis sufferers will be determined by more research and clinical trials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC MS: Gas Chromatography Mass Spectroscopy; **RCSB PDB:** Research Collaboratory for Structural Bioinformatics Protein Data Bank; **UV Spectroscopy:** Ultra Violet spectroscopy; **PBS:** Phosphate Buffer Solution; **BSA:** Bovine Serum Albumin; **TV:** Titrant Volume.

SUMMARY

This study focuses on designing and characterizing a herbal transdermal patch containing capsaicin extract and mustard oil for arthritis management. The patch aims to provide pain relief through enhanced skin permeability and sustained drug release, offering a non-invasive and effective alternative for arthritis treatment with natural anti-inflammatory properties.

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