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Original Article

# Evaluating the Efficacy of a Ginger Extract-Infused Mustard Oil Transdermal Patch for Arthritis Management

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## ABSTRACT

**Aim:** The aim of this study is to develop and characterize a novel herbal transdermal patch containing mustard oil and ginger extract for the management of arthritis, addressing the significant pain and reduced quality of life associated with this chronic inflammatory condition. **Materials and Methods:** Various extraction methods, including water, alcohol, and mustard oil extraction, were employed to obtain ginger extract. These extracts underwent initial evaluation and subsequent *in vitro* anti-inflammatory testing. Transdermal patch formulations were developed using the oil-based ginger extract, with naproxen incorporated as a model drug. Parameters such as weight variations, folding endurance, tensile strength, and moisture content were rigorously assessed. *In vitro* drug permeability tests and *ex vivo* permeation tests were conducted to evaluate drug release characteristics, with Rhodamine B/Oil Red O Dye used as a tracer. **Results:** Water-extracted ginger showed reduced potency on the 14$^{th}$ day with some fungal growth, while the alcohol extract exhibited lower efficacy compared to the oil extract. Six distinct transdermal patch formulations were developed, with parameters such as weight variations, folding endurance, tensile strength, and moisture content meeting specified criteria. *In vitro* drug permeability tests and *ex vivo* permeation tests revealed a drug release range of 0.1 to 0.3 mm, a moisture content of 3%, a tensile strength of 1 pascal, and a drug release rate of 90%. **Conclusion:** The study demonstrates the successful development and characterization of a novel herbal transdermal patch containing mustard oil and ginger extract for arthritis management. Despite challenges with water-extracted ginger and lower efficacy of the alcohol extract, the oil-based ginger extract showed promising results. The formulated transdermal patch exhibited favourable drug release characteristics and can potentially offer an effective therapeutic option for arthritis patients.

**Keywords:** Anti-arthritic activity, Ginger-mustard extraction, Docking study, Transdermal patches.

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## INTRODUCTION

Chronic inflammatory arthritis, which affects the joints, is still a major worldwide health issue. Despite progress in conventional drugs, novel and friendlier treatment methods are still required.[1] Transdermal drug administration has attracted interest as an appealing strategy because it is non-invasive and offers the potential for prolonged medication release.[2] Both ginger extract and mustard oil have demonstrated analgesic and anti-inflammatory characteristics, making them effective choices for the treatment of arthritis.[3] The bioactive ingredient in ginger known as gingerol has been attributed to the suppression of cytokines and enzymes involved in the development of rheumatoid arthritis.[4] It has been investigated if mustard oil, which is well recognized for its anti-irritant properties, might reduce pain and suffering.[5] In response to these factors, this study presents an innovative idea: a transdermal patch made of a mixture of ginger extract and mustard oil. We would like to capitalize on the synergistic benefits of these bioactive compounds by combining them to provide a targeted and sustained-release therapeutic treatment for arthritis. The objectives of this research are threefold: (1) to formulate a ginger extract-infused mustard oil transdermal patch with optimal drug loading and release properties, (2) to assess the *in vitro* permeation characteristics of the patch. Through a systematic investigation, we seek to address the gap in current arthritis treatments and potentially offer an improved approach that balances effectiveness and patient convenience. The outcomes of this study could hold significant implications for the field of transdermal drug delivery and arthritis management.

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## MATERIALS AND METHODS

### Materials

Naproxen was purchased from Yarrow Chem products, Mumbai, India. Mustard oil, Ginger, is purchased from local market Bellur. Red capsicum purchased from Byadgi. Liner as Coparex 75 mm and Backing membrane as Scotch pak9723 were purchased through online. Bio adhesive polymer obtained sigma Aldrich.

### Extraction process

Ginger extraction was performed on 300 g of chopped capsaicin pieces using distinct solvents: distilled water, alcohol, and mustard oil. The process entailed heating the capsaicin pieces on a heating mantle at 70°C for duration of 4-5 hr. Following this heating, the mixture was allowed to cool, subsequently subjected to filtration, and the resulting extract was reserved for further analysis and research purposes.

### Anti-Inflammatory Activity Procedure

The sample volume (5 mL) was made up of 2 mL of different sample concentrations, 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.2 mL of egg albumin. The combination was then heated for 5 min at 70°C after 15 min of incubation at 37°C. Their absorbance at 660 nm was measured after cooling, using the vehicle as a reference. Control by using distilled water.[6]

$$\%\ inhibition = 100 \times [\frac{Vt}{Vc} - 1]$$

Where, V t=absorbance of the test sample, V c=absorbance of control.

### Determination of an acid value

In 50 mL of a combination of equal parts of ethanol (95%) and ether, dissolve around 10 g of the chemical that has been precisely measured. In the event that the sample does not dissolve in the cold solvent, attach the flask to a reflux condenser and progressively warm while vigorously shaking the mixture until the sample dissolves. Titrate the solution with 0.1 M potassium hydroxide solution after adding 1 mL of phenolphthalein solution, until the colour of the mixture doesn't change after 30 sec of shaking.[7]

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

### Determination of saponification value

In a 200 mL borosilicate glass flask with a reflux condenser, add around 2 g of the substance being examined that has been carefully weighed. Add 25 mL of 0.5M ethanolic potassium hydroxide to it, and then boil the entire thing for 30 min at reflux on a water bath. Titrate with 0.5 M HCl acid as soon as possible after adding 1 mL of the phenolphthalein solution.[8]

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

### 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,

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

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.[9]

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 constituent

"In this study, mustard seeds (genus Brassica) and ginger (*Zingiber officinale*) were selected as the plant materials for analysis. This study was done to find out the presence and absence of constituents.[10]

### GC-MS Investigation

Gas Chromatography Mass Spectrometry (GC-MS) is an analytical method that combines the powers of mass spectrometry and gas chromatography to find and identify different types of chemicals in a given sample. Oil samples were subjected to GC-MS analysis utilising a Shimadzu 17 GC in conjunction with a quadrapole MS (QP 5000). The following are the conditions of analysis.[11] The Column-Fused silica capillary, SPB'l'M-l; Column dimension- 30 mx0.32 mm; Film thickness-0.25 µm coated with poly dimethyl siloxane; Carrier gas-Helium (l mL/min); Split ratio-1:30; Injection port temperature-250°C; Detector block temperature (FID)-250°C; Oven temperature-Programmed, 60°C (2)-2°/min 250°C (5); Sample injection-1 µL of 25 X in acetone.

### Molecular Docking Studies

#### *In silico molecular docking*

The primary approach employed in this project was structure-based drug design. This method encompassed the acquisition of target proteins in their three-dimensional configurations from RCSB PDB, followed by the assessment of binding affinities between prospective ligand molecules and these protein targets. The entire process exclusively utilized the Schrödinger suite of programs.[12,13]

### Ligand preparation

To ensure that the required ligand retained a low-energy state and proper stereochemistry, Schrödinger's LigPrep was utilised. According to 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. In addition, LigPrep makes it easier to transform a 2D sketched structure into a 3D shape 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 researches focusing on structure-based virtual screening for possible lead compounds addressing particular illnesses.[14]

### Calibration curve of naproxen sodium

For the preparation of standard calibration curve, concentration of 1-10 µg/mL was prepared by pipetting out 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 mL from the 10 µg/mL solution into a 10 mL volumetric flask and made up the volume with pH 7.4. The absorbance of each solution was measured at 272 nm.[15]

### Preparation of transdermal patch

The solvent casting method was used to prepare drug loaded adhesive transdermal patch (In this type of patch, drug is loaded or dispersed in the adhesive layer which helps the patch to bind to the skin and also assisting the drug release. This drug loaded adhesive patch may be encapsulated with the liner and backing for further support). Oil sample was mixed with the polymer thoroughly, spread over liner which is kept on a hot plate, through spreader for uniform thickness at 60-70°C for 20-30 min. Later, it is fixed with the backing membrane.

Weight variation;[16] Thickness; Folding endurance; Moisture Content.[17,18]

### *In vitro* Drug Permeation Studies

The open-ended tube method was employed to conduct a patch analysis over a 6-hr duration, employing a diffusion medium with a pH of 7.4. A cellophane membrane was affixed at one end of the tube, securely sealed, and then immersed in a receptor compartment containing a mixture of methanol and a 7.4 buffer solution. The system was maintained at a constant temperature of 37±2°C while being stirred at 600 rpm. At regular intervals, samples were withdrawn from the system and replaced with fresh diffusion medium of the same volume. Analysis of these samples was performed using a Shimadzu UV1800 UV-visible spectrophotometer at a wavelength of 272 nm. The percentage of medication released at each time point was then graphed against time to generate release profiles.[19,20]

### *Ex vivo* permeation study

In this *ex vivo* permeation study, a barrier composed of pig abdomen skin was employed within a Franz diffusion cell setup. The Franz diffusion cell featured an exposed surface area of 1.76 $cm^2$ and a skin thickness ranging from 21 to 25 µm. A ginger extract patch was positioned in the donor compartment, oriented towards the stratum corneum of the skin. In the receptor compartment, a phosphate buffer solution at pH 7.4, supplemented with methanol, was utilized. To ensure proper mixing, a magnetic bead was placed within the receptor compartment. The entire Franz diffusion cell assembly was situated on a magnetic stirrer, maintaining a constant temperature at approximately 37±0.5°C during the entire experiment. Samples were withdrawn at various time intervals, and an equivalent volume of phosphate buffer was added to the receptor compartment to maintain a consistent volume. These withdrawn samples were subsequently subjected to analysis using a UV spectrophotometer at 272 nm to quantify the drug's concentration in each sample. The resulting data were tabulated, and a graphical representation was generated to depict the cumulative percentage of drug permeation over time.

- Kp (Permeability Coefficient)=$(dQ/dt)/(A\times C_0)$

- Flux=$dQ/(A\times dt)$

Where:

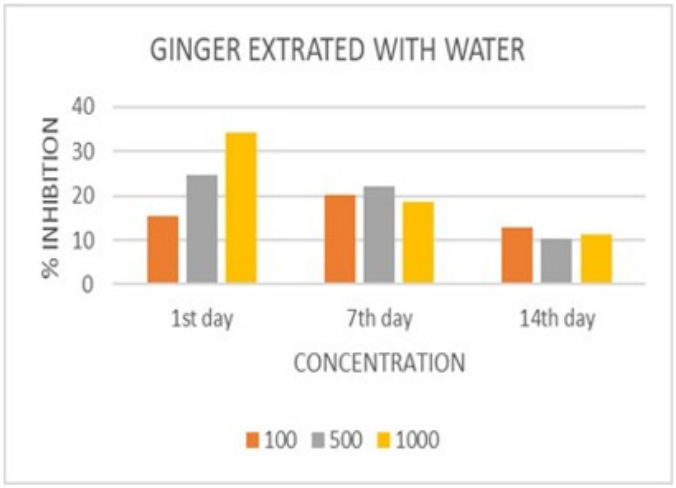
- dQ/dt is the rate of drug permeation.
- A is the surface area of the skin.
- $C_0$ is the initial drug concentration in the donor compartment.
- dQ is the quantity of drug permeated.
- dt is the time interval.

These calculations help assess the ability of ginger to permeate through the pig abdomen skin barrier under the defined experimental conditions.[21]

## RESULTS

### *In vitro* Anti-inflammatory activity

In a comparative study, the anti-inflammatory effects of ginger extract were assessed *in vitro* alongside egg albumin denaturation. The results, as depicted in the Figure 1, reveal that ginger effectively inhibits protein denaturation, specifically albumin, in a concentration-dependent manner. Notably, on the initial day of ginger Water extraction, promising outcomes were observed. However, as the study progressed to the 7$^{th}$ and 14$^{th}$ days, both the effectiveness and %inhibition decreased, accompanied by the emergence of fungal growth. Ginger's %inhibition exhibited variations when it was extracted with alcohol, diverging from the initial day's results. In contrast, when extracted with mustard oil, % inhibition remained potency and remarkably high, reaching up to 97% shown in Figure 1. Given the favourable stability characteristics of ginger, it was subsequently developed into a transdermal patch.



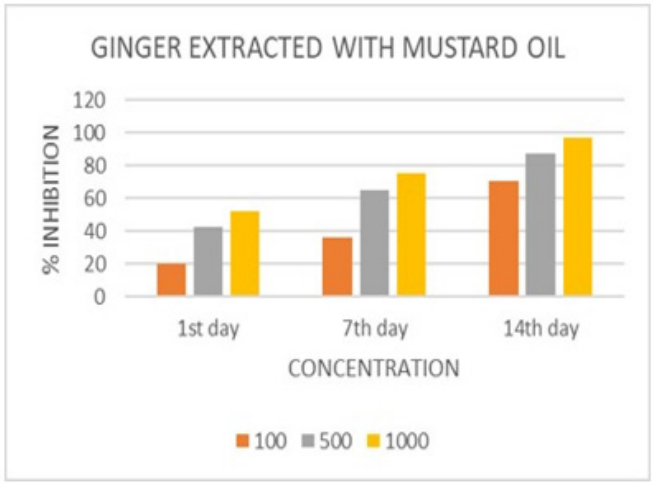




**Figure 1:** Represent % inhibition of ginger extract with water, alcohol and mustard oil.

## Chemical test of Ginger Extract

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

## Phytoconstituent of ginger 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.

## GC-MS investigation report

The GCMS stands for gas chromatography-mass spectroscopy. The graph revealed that there may be a presence of 4 compounds which is having an 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 shown in Figure 2. Based on data obtained from MS, compounds are displayed using molecular weights. The compounds may be present in oil sample are listed in Table 3.

## *In silico* studies

Base on the results obtained from docking of the isolated compounds 4-Hydroxy-3-Methoxyphenylacetone, Gingerol, and10-Gingerdione 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 shown in Figure 3.

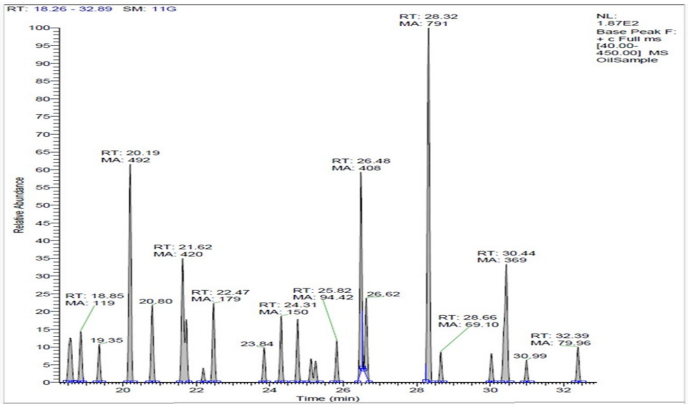



**Figure 2:** Represents GCMS investigation report.

## 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 and showed in Figure 4. 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

A transdermal patch of the matrix type was developed, with a focus on assessing its anti-inflammatory efficacy. The most promising results in terms of percent inhibition were obtained when using capsaicin extraction with mustard oil, surpassing the outcomes achieved with water and alcohol. Five different formulations were created by combining ginger extract at various concentrations (2%, 4%, 6%, 8% and 10%) with the bioadhesive

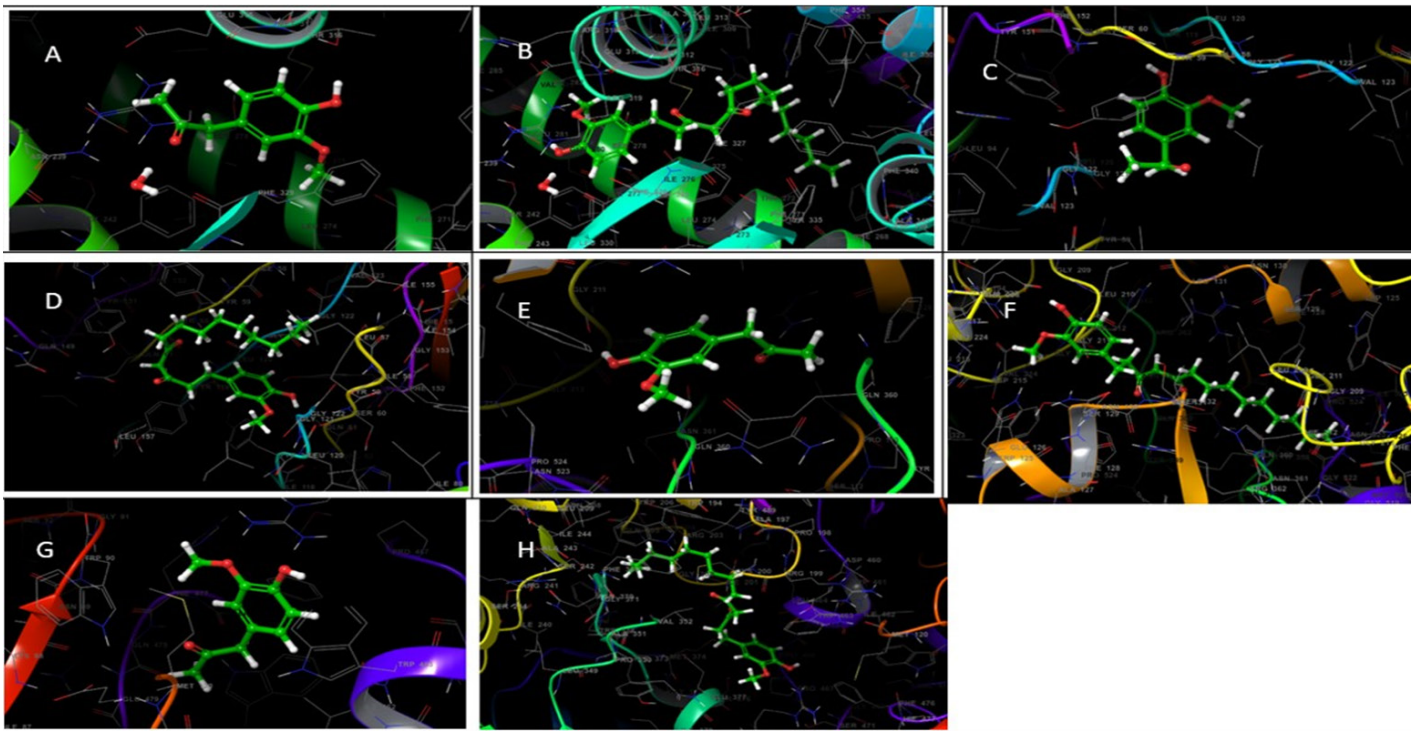



**Figure 3:** The 3D orientation of 4-Hydroxy-3-Methoxyphenylacetone with Galectin-3 (PDB ID: 1P8D), B. The 3D orientation and 2D interaction diagram of 10-Gingerdione with Galectin-3 (PDB ID: 1P8D), C. The 3D orientation of 4-Hydroxy-3-Methoxyphenylacetone with Tumour necrosis factor-a (PDB ID: 2AZ5), D. The 3D orientation of 10-Gingerdione with Tumour necrosis factor-a (PDB ID: 2AZ5), E. The 3D orientation of 4-Hydroxy-3-Methoxyphenylacetone with Cyclooxygenase-2 (PDB ID: 3LN1), F. The 3D orientation and 2D interaction diagram of 10-Gingerdione with Cyclooxygenase-2 (PDB ID: 3LN1), G. The 3D orientation of 4-Hydroxy-3-Methoxyphenylacetone with Inducible nitric oxide synthase (PDB ID: 4NOS), H. The 3D orientation of 10-Gingerdione with Inducible nitric oxide synthase (PDB ID: 4NOS).

**Table 1:** **Chemical tests of ginger oil.**

| Sl. No. | Chemical tests | Ginger extract |
|---|---|---|
| 1 | Specific gravity | 0.897 |
| 2 | Density | 0.894 |
| 3 | Acid value | 0.601 |
| 4 | Saponification value | 153.93 |
| 5 | Refractive index | 1.47181 |

**Table 2:** **Phytoconstituents of ginger and mustard.**

| Classes | Mustard | Ginger |
|---|---|---|
| Flavonoid | presence | presence |
| Phenolic | presence | presence |
| Tocopherols | presence | absence |
| Carotenoids | presence | absence |
| Glycosylates | presence | presence |
| Catechin | absence | presence |
| Minerals | presence | presence |

DURO TAK 387-2054. This mixture was evenly spread onto a liner, specifically coparex, which was placed on a hot plate at 70°C for 15 to 20 min shown in Figure 5. Subsequently, a backing membrane (scotch pak 9723) was applied. In this process, multiple patches were produced with the inclusion of Rhodamine B/Oil Red O dye to enable the evaluation of patch penetration. The oil extract was blended with a 1% Rhodamine solution or Oil Red O, and the patch was then formulated accordingly. The results of evaluation of patches shown in Table 4.

## *In vitro* Drug Permeation Studies

In order to assess the impact of drug release on medication absorption, *in vitro* release studies are routinely conducted to predict the performance of a drug delivery system under ideal conditions. The *in vitro* permeation study for the oil sample employed an open-ended cylinder setup, with a receptor compartment containing 90 mL of phosphate buffer at pH 7.4, positioned between the donor compartment and the patch. This experiment involved a 10mg drug dosage. At various time points (0, 1, 2, 3, 4, 5 and 6 hr), approximately 1 mL samples were withdrawn and immediately replaced with an equal volume of receptor solution to maintain a constant volume. Analysis of the samples was performed at 272 nm, with the temperature maintained at 37±1°C, and constant stirring of the receptor compartment (at 600 rpm) was achieved using a magnetic stirrer. Notably, F5 exhibited an impressive 90% drug release in this study shown in Figure 6.

**Table 3:** List of constituents present in Ginger extract.

| Sl. No. | Compounds |
|---|---|
| 1 | Gingerol |
| 2 | 4-hydroxy-3-methoxyphenylacetone |
| 3 | Tetra hydrocurcumin |
| 4 | 10-gingerdione |
| 5 | gingerdiol |

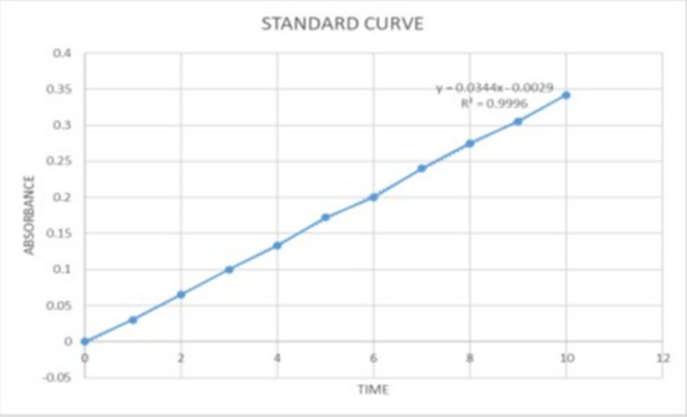



**Figure 4:** Standard calibration curve of naprozen.

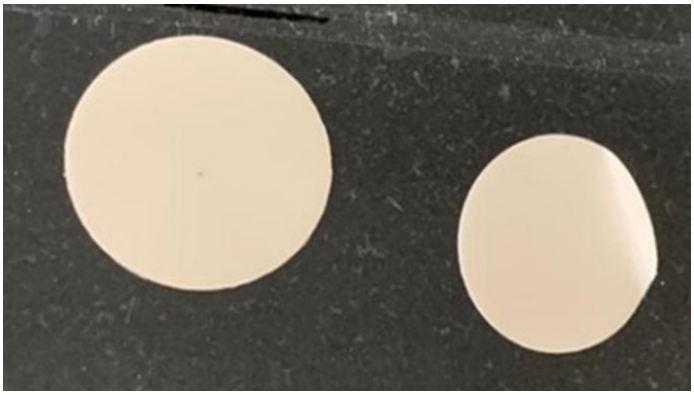


**Figure 5:** Transdermal patch.

## *Ex vivo* permeation study

Pig skin was deliberately chosen as the substrate for *ex vivo* drug permeation studies involving patches, primarily due to its close resemblance to human skin. The research utilized a Franz diffusion cell to evaluate the overall drug penetration through the skin shown in Figure 7. This investigation yielded noteworthy findings, indicating a noticeable improvement in the rate of drug permeation, with the most pronounced increase observed in the case of formulation F5.

## Fluorescence study

Fluorescence measurements offer a means to track the dispersion of dyes within the skin, facilitating the study of carrier effects and

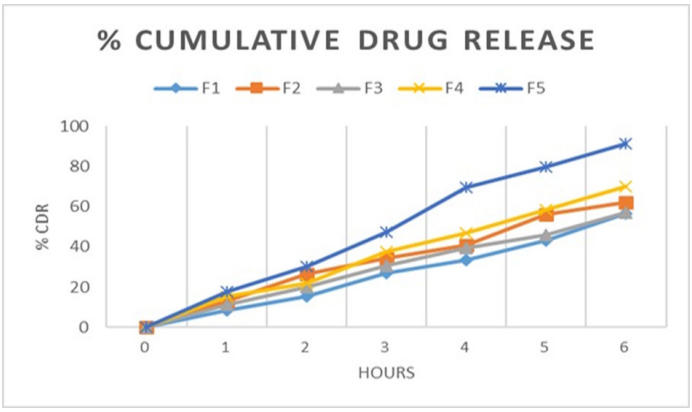



**Figure 6:** Represents cumulative amount of drug release.

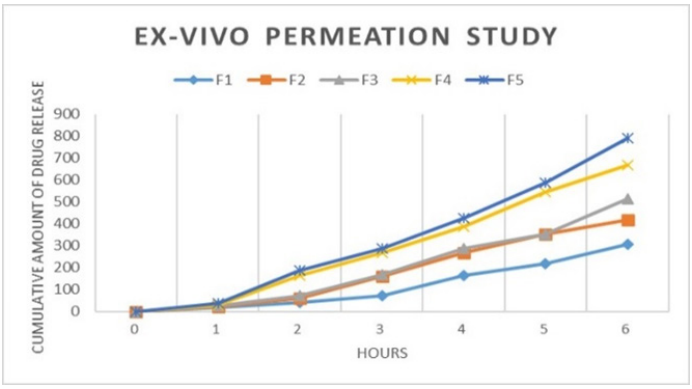



**Figure 7:** Represents drug release using pig skin.

the selection of the most suitable delivery systems. In this study, formulations containing Rhodamine B/Oil Red O (with a dye content of 0.001%) were prepared at a consistent concentration to investigate their penetration behaviour, 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 shown in Figure 8. Qualitative assessment was employed to evaluate the distribution of the dye within the skin.

## DISCUSSION

The study's primary goal, "Evaluating the Efficacy of a Ginger Extract-Infused Mustard Oil Transdermal Patch for Arthritis Management," focuses on whether a novel therapeutic intervention may effectively treat arthritic symptoms. Our goal is to determine whether employing a transdermal patch enhanced with mustard oil and ginger extract could alleviate arthritis-related pain and suffering. After ginger oil was extracted, we evaluated it for factors like saponification value, acid value, specific gravity, and additional variables, and all of the tests worked well. A different concentration of extracted oil was used for anti-inflammatory

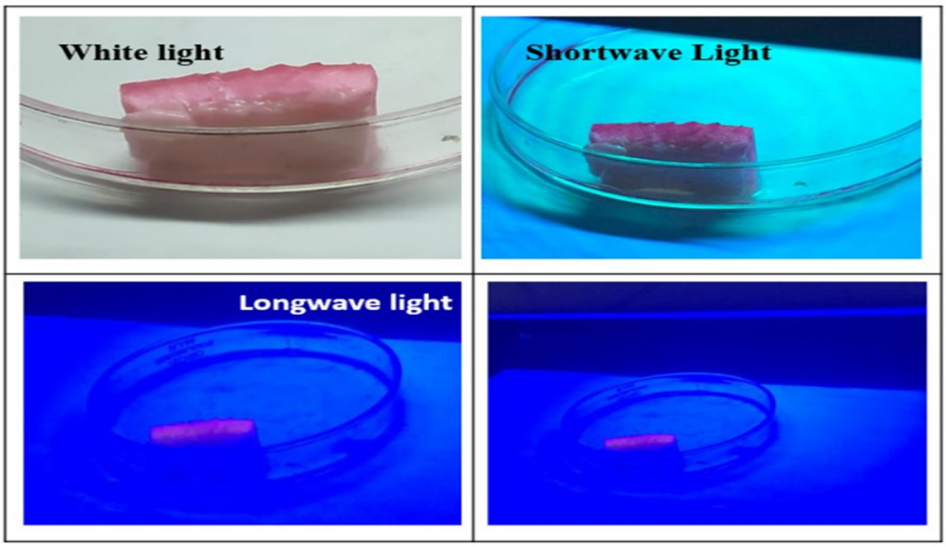



**Figure 8:** Fluorescence study. Chemical test of Ginger Extract.

**Table 4: Evaluation of transdermal patch.**

| Formulations | Weight variation (mg) | Thickness (mm) | Moisture content (%) |
|---|---|---|---|
| F1 | 0.249 | 0.02 | 2.52 |
| F2 | 0.250 | 0.01 | 2.41 |
| F3 | 0.252 | 0.02 | 2.40 |
| F4 | 0.249 | 0.02 | 2.40 |
| F5 | 0.251 | 0.02 | 2.43 |

action, and the results were 50% positive. The preparation of the drug-loaded adhesive transdermal patch was done using the solvent casting technique. For patches, we measured things like thickness, moisture content and folding resistance which showed 0.1 to 0.2 mm, <3% to prevent degradation of product, smooth patch tensile strength is <1 pascals. The outcome of the patches in permeation research demonstrated a peak penetration of up to 90%, with the highest observed flux. By analyzing the docking scores (Glide Score), 4-Hydroxy-3-Methoxyphenylacetone, and 10-Gingerdione shown good binding affinity to words the selected enzymes. The 3D conformation and 2D interaction diagram were shown in Figure 3. With this *in silco* studies we can estimate that the 4-Hydroxy-3-Methoxyphenylacetone, and 10-Gingerdione may be showing anti-inflammatory activity through multiple pathways. Researching new, efficient treatment options for arthritis, a chronic and debilitating ailment that affects millions of people worldwide, is crucial to improving the quality of life for those who have it. As a result, our research aims to provide insight into the possible advantages of this novel strategy for managing arthritis.

## SUMMARY

This research aimed to develop and characterize a novel herbal transdermal patch containing mustard oil and ginger extract for arthritis management, a condition causing significant pain and decreased quality of life worldwide. Ginger extract was obtained using various methods, including water, alcohol, and mustard oil extraction. Initial evaluation and *in vitro* anti-inflammatory testing were performed on these extracts. While water-extracted ginger showed reduced potency and fungal growth on the 14$^{th}$ day, the alcohol extract had lower efficacy compared to the oil extract. Six transdermal patch formulations were created using the oil-based ginger extract, incorporating naproxen as a model drug. Parameters such as weight variations, folding endurance, tensile strength, and moisture content were rigorously assessed. *In vitro* drug permeability tests and *ex vivo* permeation tests revealed a drug release range of 0.1 to 0.3 mm, a moisture content of 3%, a tensile strength of 1 pascal, and a drug release rate of 90%. Rhodamine B/Oil Red O Dye served as a tracer in these assessments.

## CONCLUSION

In conclusion, our study evaluating the efficacy of a Ginger Extract-Infused Mustard Oil Transdermal Patch for Arthritis Management has provided valuable insights into a promising alternative for arthritis symptom relief. Through rigorous testing and analysis, we have found that the transdermal patch offers a notable reduction in pain and discomfort for individuals living with arthritis. This innovative approach not only provides a potential solution for managing arthritis symptoms but also

underscores the significance of exploring natural remedies in the field of medical research.

## ACKNOWLEDGEMENT

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## ABBREVIATIONS

**GC MS:** Gas Chromatography Mass Spectroscopy; **RCSB PDB:** Research Collaboratory for Structural Bioinformatics Protein Data Bank; **UV Spectroscopy:** Ultra Violet spectroscopy.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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