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Pharmaceutical and Analytical Assessment of *Jadamayadi* Transdermal Pain Patch: An *in-vitro* Study on Development, Evaluation and optimization

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ABSTRACT

The present study focuses on the pharmaceutical and analytical assessment of Jadamayadi transdermal pain patch through an in vitro approach for its development, evaluation, and optimization. Jadamayadi choorna, a classical Ayurvedic formulation known for its analgesic and anti-inflammatory properties, was incorporated into a transdermal patch system to enhance its therapeutic efficacy and patient compliance. Preparation of the transdermal patches polymer base by dissolving the selected polymer (HPMC and PVA) in a solvent like water or ethanol, and evaluated for physicochemical parameters including thickness, weight uniformity, folding endurance, moisture content, moisture uptake, drug content, and surface pH. The in vitro anti-arthritic activity is evaluated using the protein denaturation inhibition assay, phytochemicals analysis done by LC-MS technique. The formulation was optimized based on evaluation parameters to achieve desirable mechanical properties and sustained drug release. The study shows the anti-inflammatory, antiarthritic, analgesics property and the potential of Jadamayadi transdermal pain patch as a novel, effective, and non-invasive alternative for pain management, aligning traditional medicine with modern pharmaceutical technologies.

Keywords: Anti-inflammatory, Anti-arthritic, Analgesics property, Pain patch

INTRODUCTION

Introducing patch-making technology in Ayurveda, particularly through the incorporation of herbal drugs, represents a significant advancement in the field of traditional medicine. The innovation of transdermal pain patches in Ayurveda not only addresses current healthcare challenges but also enhances the sustainability &significance of traditional healing practices within today's medical framework. With the rising prevalence of arthritis in aging populations, there is a pressing need for effective long-term pain management strategies. Transdermal pain patches are invaluable for managing arthritis offering a controlled, targeted & non-invasive method for pain relief. Patch deliver a steady release of pain-relieving &anti-inflammatory agents directly through the skin to the affected joints. This localized delivery ensures consistent relief which is crucial for arthritis patients requiring ongoing pain management to maintain mobility& quality of life. The

ease of application & sustained-release mechanism of transdermal patches enhances patient adherence by reducing the need for multiple daily doses, making them patient-friendly option for long-term arthritis care. [1] Jadamayadi Choornam as a transdermal pain patch provides novel method for managing Vataraktha revered for its potent analgesic and anti-inflammatory properties, allows the active ingredients to be absorbed directly through skin, delivering relief precisely. With its vatahara, shothahara and shoolaghna actions, the Jadamayadi patch promises enhanced patient comfort while integrating traditional systems into contemporary. The integration of patch-making technology into Ayurveda opens new avenues for research and development, facilitating the study of pharmacokinetics and pharmacodynamics. This scientific validation boosts credibility in mainstream healthcare.Considering targeted delivery, sustained release, improved absorption, enhanced convenience &compliance an in vitro study is planned to comprehensively evaluate Jadamayadi Choornam transdermal patch at pharmaceutical analytical &pharmacological levels.

OBJECTIVES

1. To formulate, develop, and evaluate Jadamayadi transdermal pain patch, with a focus on understanding drug-polymer interactions to enhance Efficacy and stability.
2. To conduct a comprehensive phytochemical analysis of the Jadamayadi Transdermal pain patch, including both the extract and final formulation, With confirmation of phytochemical retention post release.
3. To evaluate the pharmacological properties of the Jadamayadi transdermal Pain patch, specifically its anti-inflammatory and anti-arthritic activities, to establish its therapeutic potential in pain management.

MATERIALS & METHODS

Jadamayadi choornam Reference: (Sahasrayoga) [1], [2]

Ingredients and its quantity

Jada: Nardostachys grandifolia 1part
Amaya : Saussurea costus1part
Chandana: Santalum album 1part
Kundurushka: Boswellia serrata 1part
Nata: Valeriana jatamansi 1part
Aswagandha: Withania somniferera 1part
Sarala: Pinus roxburghi 1part
Rasna: Alpinia galanga 1part

REVIEW OF LITRATURE

1.Jatamansi: Nardostachys grandifolia

Family- *Caprifoliaceae*
Rasa panchaka
Rasa-Tikta, Kashaya, Madhura
Guna-Laghu Snigdha
Vipaka-Katu
Virya -Shita
Useful part – Kanda (Rhizome)
Phytoconstituents: Rhizome has essential oil which contains Jatamansic acid, aristolene, calarene, jatamansin, oroselol, orselone, dihydrojatamansin, angelic acid, jatamansinol, seselin, jatamansinone, a-pinene, β-pinene, ß-eudesmol, elmol, ß-sitosterol, jatamansinol, jatamansin and angelicin.
Pharmacological actions:
Astringent, Coolant, Antiseptic, Analgesic, Digestive, Carminative, Vermifuge, Nervine tonic, Intellect promoter, Sedative, Antipyretic, Diruretic and Emmenagogue.

2.Amaya: Saussurea costus

Family – Asteraceae
Rasa pnachaka;
Rasa -Tikta, Katu, Madhura
Guna -Laghu, Ruksha, Tikshna
Veerya - Ushna
Vipaka – Katu
Part used - root
Pharmacological action:Anticytotoxic, diuretic, antibacterial, hypolipidaemic, antiseptic, insect repellent, insecticidal, hypotensive, spasmolytic, bronchodilator, antiulcer, antimicotic, antiinflammatory, immunostimulant.

Phytoconstituents:Taraxasterol, taraxasterylacetate (leaves); saussurine, alantolactone, aplotaxene, costunolide,B-cyclocostunolide, dihydrocostuslactone, dihydrodehydrocostuslactone, B-elemene, a- and ß-ionones, (E)-9-isopropyl-6-methyl-5,9-decadiene-2-one, kushtin, 12-methoxydihydrocostunolide, cholamine, inulin (alkaloid), 12-methoxydihydrodehydrocostuslactone, saussureal, (-)- and B-selinenes, (+) selina-4,11-diene.

3.Chandna: Santalum album

Family - Santalaceae
Rasa panchaka:
Rasa - Tikta, Madhura
Guna -Ruksha, Laghu
Vipaka -Katu
Virya - Shita
Useful part: saara (heart wood)
Pharmacological action:
Antibacterial, Antiviral, Antigonorrhoeal, Antifungal, Antioxidant, Blood purifier, Intellect promoting, cooling, Diuretic, Analegesic, Antipyretic and Haemostatic.
Phytoconstituents:Sandal wood oil contains about 95% of two isomeric sesquiter-pene alcohols, a-santalol, ß-santalol, santene, santenone, teresantol, santalone and santalene.

4.Kundurushka: Boswellia serrata

Family - Burseraceae
Rasa Panchaka:
Rasa-Kashaya Tikta Madhura
Guna-Laghu Ruksha
Vipaka- Katu
Virya- Shita
Part used- Niryasa (exudate).
Pharmacological Actions:Diuretic, Emmanogogue, Aromatic, Demulcent, Stimulant.
Phytoconstituents: Bark- beta sitosterol, glucosides.

5.Nata: Valeriana jatamansi

Family - Valerianaceae
Rasa panchaka-
Rasa-Tikta, Katu, Kashaya.
Guna-Laghu, Snigdha.
Vipaka-Katu
Veerya – Ushna
Parts used- Root and rhizome with stolons
Pharmacological actions; Valerian is one of the most effective remedies in the treatment of neurosis. The plant is reported for antibiotic, antiamoebic, analgesic, antipyretic, antibacterial and mild CNS depressant activities. The root is reported as antispasmodic, diuretic, carminative (Wagner and Jurcie, 1979; Vohora et al., 1979, Yamaguchi er al, 1964) and stimulant. It has many of the properties of V officinalis and could therefore be employed as a nervine and sedative.
Phytoconstituents: Rhizomes and Root: Flavonoids: 6-methylapigenin (Wasowaski et al., 2002), hesperidin (Marder et al, 2003), naphthoic acid, acyl-linarin, linarin-0-2-methyl butyrate, acacetin-7-O-B-rutinoside, linarin isovalerate (Chari et al. 1977), valepotriates, dihydrovaltrate, linarin-isovalerianate, valeranone, nor-valeranone (Klyne et al., 1964), nardol (Sastry and Maheshwari, 1966)

6.Ashwagandha (Withania somniferera)

Family - Solanaceae
Rasa Panchaka:
Rasa: Tikta, Kashaya
Guna: Laghu, Snigdha
Vipaka: Madhura
Virya: Ushna
Useful part – Mula (Root).
Pharmacological actions:Sedative, Antimitotic, Hypnotic, Antiseptic, Antitumour
Phytoconstituents:Root-Somniferine, Somniferinine, Withanine, Nicotine, Withaniol [Steroidal lactone], Withasomnine [Pyrazole alkaloid], Tropine, Withanolide [Steroidal lactones], Analygrine, Anaferine (Alkalirid), Starch, g-sitosterol.Seed Withanolide, Withaferin A

7.Sarala (Pinus roxburghi)

Family -
Rasa Panchaka
Rasa– Katu, tikta, madhura

Guna – Laghu, Snigdha, Teekshna.
Vipaka – Katu
Veerya – Ushna.
Pharmacological action: Anthelmintic, Antiseptic, Aromatic, Carminative, Diuretic, Stimulant, Liver tonic
Part used- Stem powder, Oil, Bark, resin,
Phytoconstituents:Turpentine oil from the bark. A-and ß-pinene, abietic acid, pinosylvin, pinocembrin, pinobanskin, longifolene.

8.Rasna (Alpinia galanga)
Family - Zingiberaceae
Rasa panchaka:
Rasa-Tikta
Guna- Guru
Vipaka- Katu
Virya-Ushna
Useful parts – Mula (Root), Patra (Leaf) and Panchanga (whole plant).
Pharmacological action:Aromatic, Stimulant, Carminative, Antibacterial
Phytoconstituents: *Pluchea lanceolata* – Pluchine, Betain hydrochloride, Taraxasterol, í and g-sito-sterol, flavone glycoside, Quercitin and isorhamnetin.
Alpinia calcarata – Green rhizomes contain 0.6% to 1.5% of volatile oil. The oil contains methyl cinnamate (48%), Cineole (25%) Camphor and Pinene. It also contains resin, pungent galangol, alpinol, yellow crystalline substances known as galangin.

Churna Preparation
The fine powder obtained after thoroughly pounding and filtering the completely dry drugs (dried thoroughly under hot sun) is called cürna. It is also called as 'raja' and 'kşoda'. Its dosage is one karşa (12 gms). Pounding is done either manually in khalva or ulükhala yantra; or mechanically in pounding/pulverizing machines. Filtering is also done either manually using a clean cloth/a selected sieve; or mechanically through sieve shakers. Depending on the particle size, the powder is either course, fine or very fine. When no anupāna is specified, it is always better to consider 'jala' as 'anukta anupāna'. Because, the best anupāna among all is 'mähendra jala' (the rain water collected before hitting the ground).The simile given above says that, as the drop of oil put on stable water spreads swiftly, in the similar way 'anupāna' helps for 'quick absorption" and 'early assimilation' of the administered medicine. Only that quantity of 'bhāvanā dravya', which is required to soak the bhāvya dravya' (cürṇa) is used for one bhāvanā procedure. This is the opinion of experienced physician

Patch review
Transdermal drug delivery is an alternative way of delivering drugs via the skin Layer [3],[4]. The drug is carried through the skin into the bloodstream and circulates Systemically in the body before reaching the target site. The transdermal drug delivery Method has several advantages over other routes of administration. Examples include the Ability to deliver continuous doses of drugs over an extended period of time, the ability to Bypass the digestive system, and the ability to avoid first-pass metabolism in the liver [5]. Other drug administration routes, such as intravenous, can cause pain and increase the risk Of infection. Nevertheless, the oral route is inefficient, and in the inhalation method, it is Difficult in controlling the dosage. In view of its advantages over other routes, transdermal Administration is commonly used to deliver drugs for conditions such as smoking cessation, Chronic pain, and motion sickness, as well as hormone replacement therapy [6].
A transdermal patch is a medicated patch that can deliver drugs directly into the Bloodstream through the layers of the skin at a prescribed rate. In fact, patches are the most Convenient method of administration. They are non-invasive, and treatment can last for Several days and can be stopped at any time. It comes in different sizes and contains multiple ingredients. When applied to the skin, the patch can deliver active ingredient-Ents into the systemic circulation via diffusion processes. Transdermal patches may contain High doses of active

substances that remain on the skin for an extended period of time. One Of the first transdermal patches developed in 1985 was the nitroglycerin patch. The patch, Developed by Gale and Berggren, uses a rate-controlling ethylene vinyl acetate membrane.

Currently, several drugs are available as transdermal patches, including estradiol, clonidine, fentanyl, nicotine, scopolamine (hyoscine), and estradiol with norethisterone acetate. The site of application may vary depending on the therapeutic category of the drug. For example, nitroglycerin can be applied around the chest and estradiol around the buttocks or abdomen. The duration of drug release also varies depending on the usage, from the shortest (up to 9 h) to the longest (up to 9 days)

METHODOLOGY

1. Preparation of Jadamayadi choornam

Take equal amounts of Jada, Amaya, Chandana, Kundurushka, Nata, Ashwagandha, Sarala, and Rasna, finely powder each, then blend thoroughly to create a uniform Jadamayadi Choornam mixture**.**

Formulation, development and analytical evaluation of jadamayadi transdermal pain patch

2.Extraction By Solvent Method

Jadamayadi Choornam, (Nardostachys grandiflora (Jada), Saussurea costus (Amaya), Santalum album (Chandana), Boswellia serrata (Kundurushka), Valeriana jatamansi (Nata), Withania somnifera (Ashwagandha), Pinus roxburghii (Sarala), and Alpinia galanga (Rasna)) powdered extract by using the Soxhlet apparatus with selective solvent. After the extraction, evaporate the solvent to obtain a concentrated extract, either as a thick paste or a dried powder, and store it in a cool and dry place for further studies.

3.Transdermal patches

Prepare the transdermal patches polymer base by dissolving the selected polymer (HPMC and PVA) in a solvent like water or ethanol. Stir the mixture thoroughly to ensure a smooth gel without lumps. Add a plasticizer, such as glycerin, to improve the flexibility of the resulting patch. Once the polymer base is prepared, blend the Jadamayadi Choornam herbal extract into the polymer solution, ensuring a uniform extract distribution throughout the gel. To improve the penetration of the active compounds through the skin, incorporate permeation enhancers (DMSO or oleic acid) into the mixture. Spread the prepared polymer-herbal mixture evenly onto a flat, non-stick surface or into a casting mold. Underneath the solution, place a backing membrane (e.g., polyethylene) to provide structural support to the patch. Carefully distribute the mixture to ensure a uniform thickness, typically around 0.2 mm to 0.5 mm. Allow the patch to dry at room temperature, such as an oven set to 40-50°C, until a flexible film forms. After the film has dried, cut it into patches of the desired size, such as s2x2 cm or 4x4 cm, based on the intended application. To enable the patch to adhere to the skin, apply a pressure-sensitive adhesive (polyacrylate) to one side of the patch. Once the adhesive is applied, cover it with a protective release liner to shield the adhesive from contaminants before use [7],[8].

4. Evaluation of Transdermal Patches

To study physical and chemical appearance to ensure uniformity and smoothness, folding endurance to test flexibility, and weight variation to ensure consistency in patch mass. The drug content uniformity test ensures the even distribution of herbal extract across patches. An in vitro drug release study can be performed using Franz diffusion cells to determine the rate at which the active compounds are released from the patch. Finally, a skin irritation test should be conducted on human or animal skin to ensure the patch is safe and non-irritating.[9]

5. Drug polymer interaction (FT-IR)-3 sample

Three samples are typically analysed to study drug-polymer interactions in transdermal patches using Fourier Transform Infrared Spectroscopy (FT-IR):

Extract, pure polymer, and the drug-polymer patch. Each sample is prepared by finely grinding and, if necessary, mixing with potassium bromide (KBr) to form transparent pellets (unless using the ATR method). FT-IR spectra are recorded in the 4000 cm^{-1} to 400 cm^{-1} range. By comparing the spectra, interactions between the drug and polymer are identified through shifts in peak positions,

disappearance or broadening of functional group bands, or the appearance of new peaks, which may indicate molecular interactions such as hydrogen bonding or complex formation. These interactions can influence the stability and release profile of the transdermal patch [10].

6. Phytochemical analysis

6.1. Phytochemical Analysis of Extract

Analysing phytochemicals in transdermal patches is to characterise the raw herbal extract used in the formulation. About 5-10 mg of the dried extract is dissolved in a solvent such as methanol or acetonitrile, followed by filtration through a 0.22 µm syringe filter to remove particulates. The sample is injected into the LC-MS system using a C18 reverse-phase column and a mobile phase of water (0.1% formic acid) and acetonitrile (0.1% formic acid) in a gradient system. The mass spectrometer operates in either positive or negative electrospray ionisation (ESI) mode, scanning in the 100-1000 m/z range. Peaks corresponding to the phytochemicals are identified by comparing mass-to-charge ratios (m/z) and retention times with standard libraries [11].

6.2. Phytochemical Analysis of Formulation

To assess the stability and content of phytochemicals in the transdermal patch formulation, a section of the patch (typically one cm^2) is cut and placed in 10 mL of a solvent like methanol or acetonitrile. The patch is then sonicated for 30 minutes to extract the phytochemicals from the drug-polymer matrix. The extract is filtered and analysed by LC-MS using the same conditions applied to the pure extract. By comparing the spectra of the formulation with the original extract, it is possible to identify any changes in phytochemical content, degradation products, or interaction with the polymer.

6.3. Confirmation of Phytochemicals After Release

The confirming the release of phytochemicals from the transdermal patch after an in vitro release study, typically using a Franz diffusion cell setup. The patch is placed on a suitable membrane, and the diffusion medium (phosphate buffer, saline, or simulated body fluid) is collected at various time points (24 hours). The medium samples are filtered and injected into the LC-MS for analysis. By comparing the LC-MS spectra with those of the extract and formulation, it is possible to confirm the presence and stability of the phytochemicals after release. This step also helps assess the release profile of active compounds over time [12]

7. PHARMACOLOGICAL EVALUATION

7.1. Sample Preparation and Extraction from Transdermal Patches for pharmacological activity.

A one cm^2 patch section is dissolved in phosphate buffer saline (PBS) and extraction solvent by methanol to assess transdermal patches anti-inflammatory and anti-arthritic activity. The solution is sonicated for 30 minutes to extract the active phytochemicals from the patch matrix. After sonication, the sample is filtered using a 0.22 µm syringe filter to remove particulates, and the resulting extract is used for further biological assays. This preparation method ensures that the bioactive compounds are released and tested under in vitro conditions similar to transdermal release

7.2. In-vitro Anti-inflammatory Activity

Transdermal patches in vitro anti-inflammatory activity is commonly evaluated using assays that measure the

inhibition of inflammatory mediators such as protein denaturation, albumin denaturation, or membrane stabilisation. For the protein denaturation assay, the patch extract or formulation is incubated with a solution of bovine serum albumin (BSA) at a pH of 6.3 and a temperature of 37°C for 20 minutes, followed by heating at 57°C for 5 minutes. The protein denaturation degree is measured spectrophotometrically at 660 nm, and the anti-inflammatory activity is expressed as a percentage inhibition of protein denaturation compared to a control sample. The formulation is effective if significant inhibition is observed [13].

7.3. In-vitro anti-arthritic activity

The anti-arthritic activity is typically assessed using bovine serum albumin (BSA) denaturation inhibition and proteinase inhibition assays. The patch extract is incubated with trypsin solution (0.06 mg/mL) and phosphate buffer (pH 7.4) for the proteinase inhibition assay for 30 minutes at 37°C. After incubation, the reaction mixture is analysed by measuring the absorbance at 210 nm. Proteinase activity inhibition is calculated, and higher inhibition percentages indicate better anti-arthritic potential. The results are compared to standard anti-inflammatory agents like diclofenac sodium to validate efficacy [14].

RESULT

Development and analytical evaluation of jadamayadi transdermal pain patch

1. Extraction By Solvent Method

Jadamayadi Choornam: (Nardostachys grandiflora (Jada), Saussurea costus (Amaya), Santalum album (Chandana), Boswellia serrata (Kundurushka), Valeriana jatamansi (Nata), Withania somnifera (Ashwagandha), Pinus roxburghii (Sarala), and Alpinia galanga (Rasna)) powdered extract by using the Soxhlet apparatus with selective solvent. After the extraction, evaporate the solvent to obtain a concentrated extract, either as a thick paste or a dried powder, and store it in a cool and dry place for further studies.

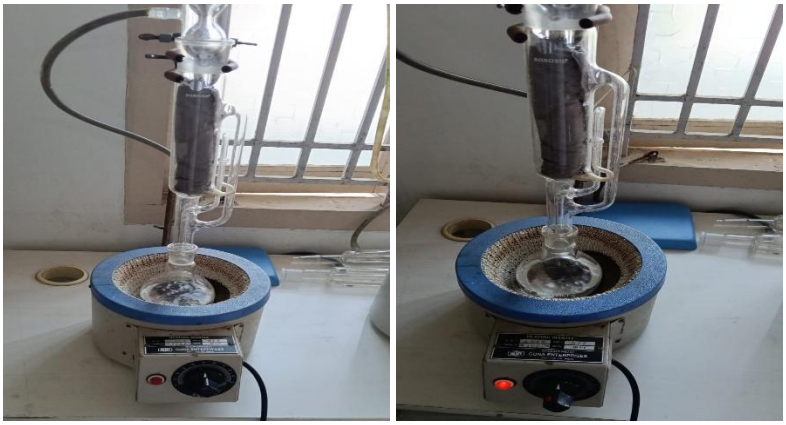

Figure 1: Soxhlet extraction

2. Phytochemical analysis of extract by LC-MS data

Analysing phytochemicals in transdermal patches is to characterise the raw herbal extract used in the formulation. About 5-10 mg of the dried extract is dissolved in a solvent such as methanol or acetonitrile, followed by filtration through a 0.22 µm syringe filter to remove particulates. The sample is injected into the LC-MS system using a C18 reverse- phase column and a mobile phase of water (0.1% formic acid) and acetonitrile (0.1% formic acid) in a gradient system. The mass spectrometer operates in either positive or negative electrospray ionisation (ESI) mode, scanning in the 100-1000 m/z range. Peaks corresponding to the phytochemicals are identified by comparing mass-to-charge ratios (m/z) and retention times with standard libraries (Kongkiatpaiboon et al., 2018; Tawfike et al., 2019).

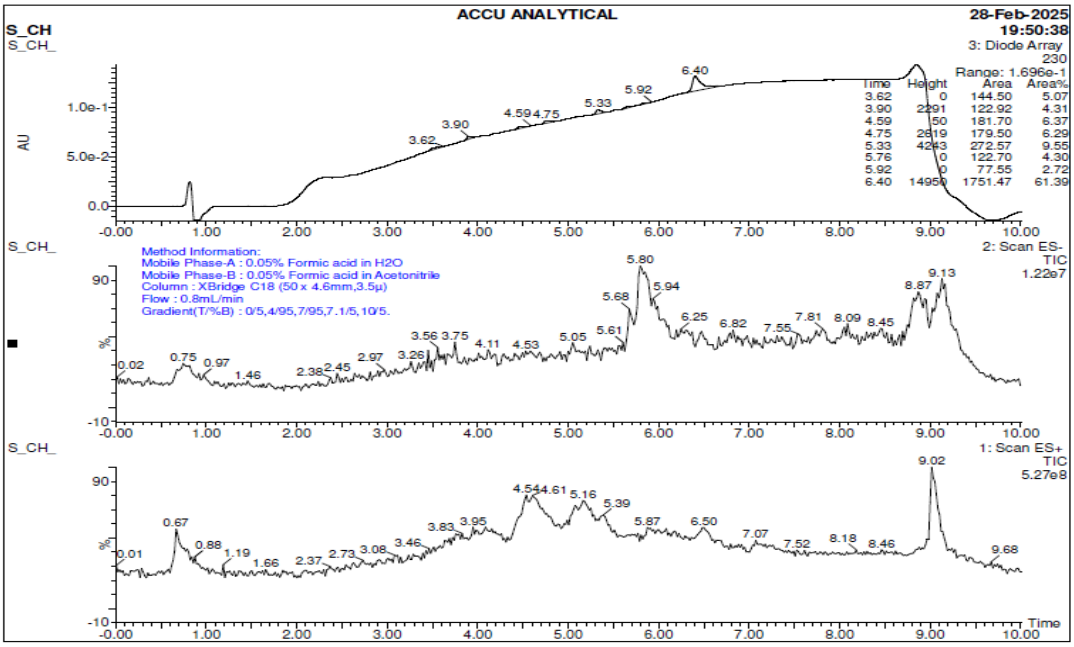

Figure 2: Jadamayadi extract LC-MS analysis chromatogram.

LC-MS analysis of Jadamayadi extract

Phytochemical Identification from LC-MS Data. Extracted Major m/z Peaks and Their Potential

Phytochemicals (Positive Mode - ES+)

m/z Value Potential Phytochemical

178.28 Cinnamic acid derivatives

233.18 Berberine.

289.41 Catechin

316.49 Epicatechin gallate

357.38 Quercetin

389.38 Kaempferol

470.53 Saponins

552.03 Procyanidin dimers

685.56 Ginsenosides

816.94 Procyanidin trimers

880.72 Tannins

1088.4 Glycosylated flavonoids

LC- MS extract of Jadamayadi choorna

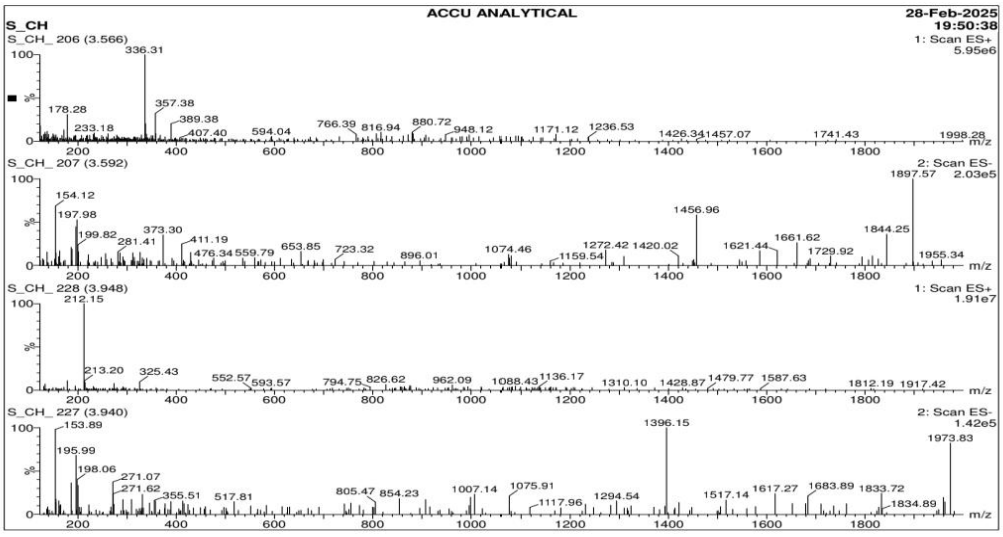

Figure :3 LC-MS of Jada

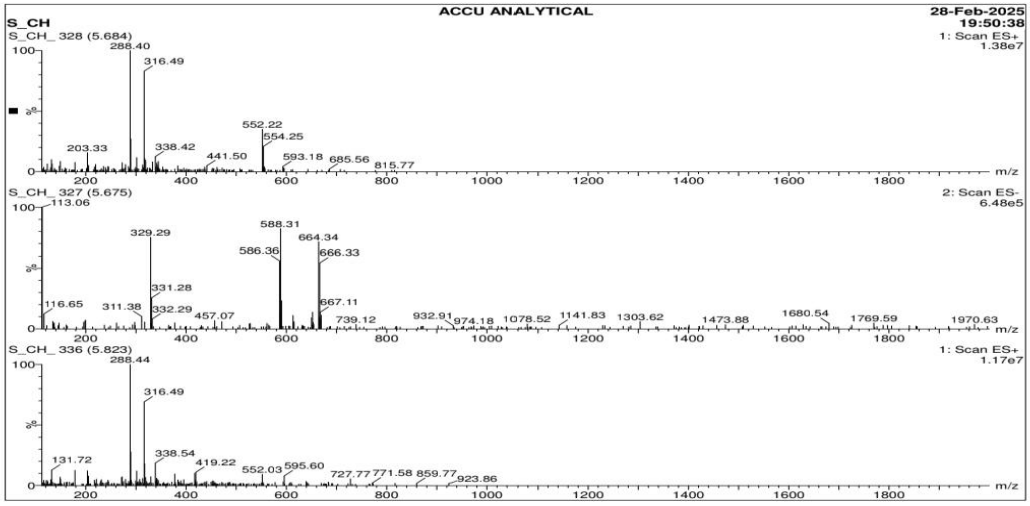

Figure :4 LC-MS of Amaya

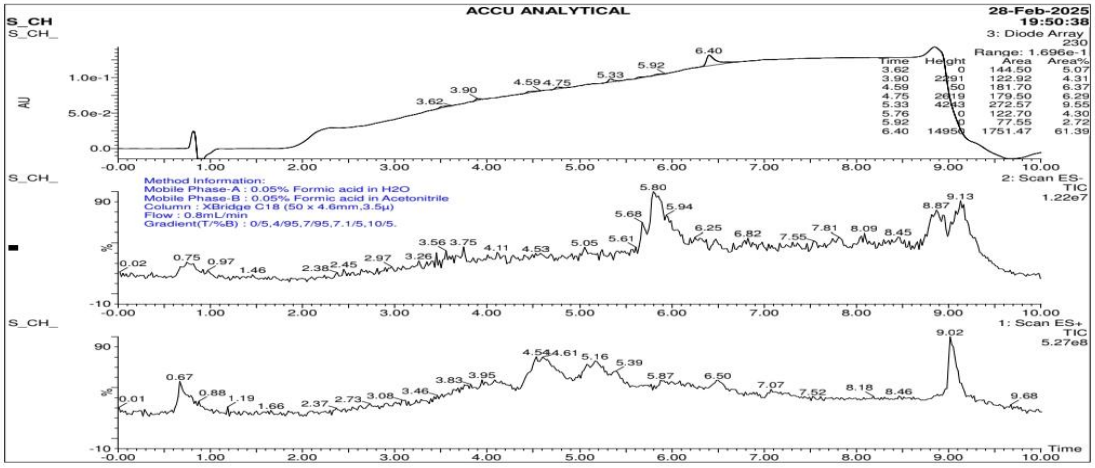

Figure :5 LC-MS of Chandana

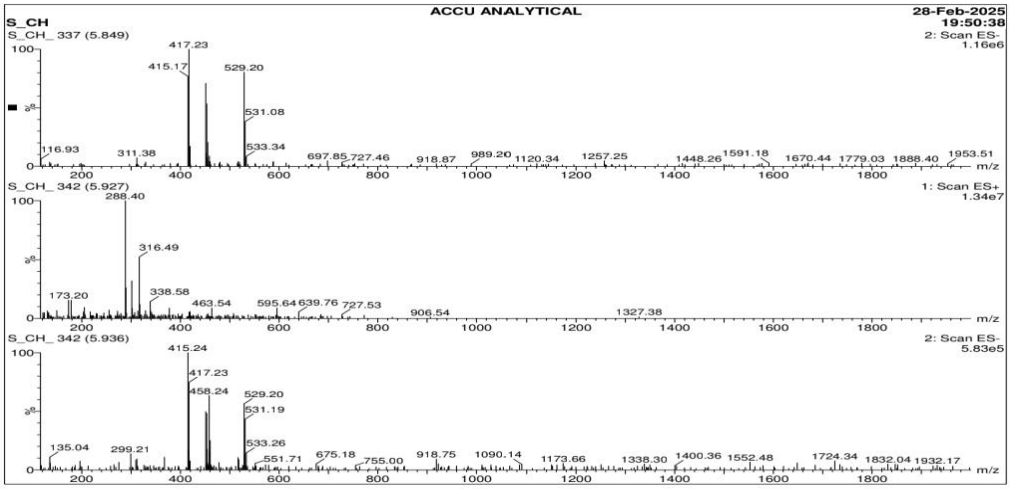

Figure :6 LC-MS of kundurushka

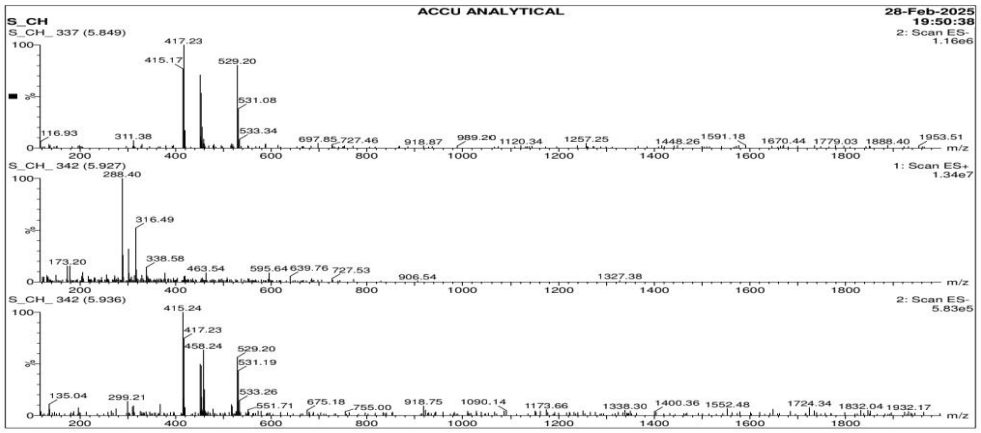

Figure 7: LC-MS of Nata

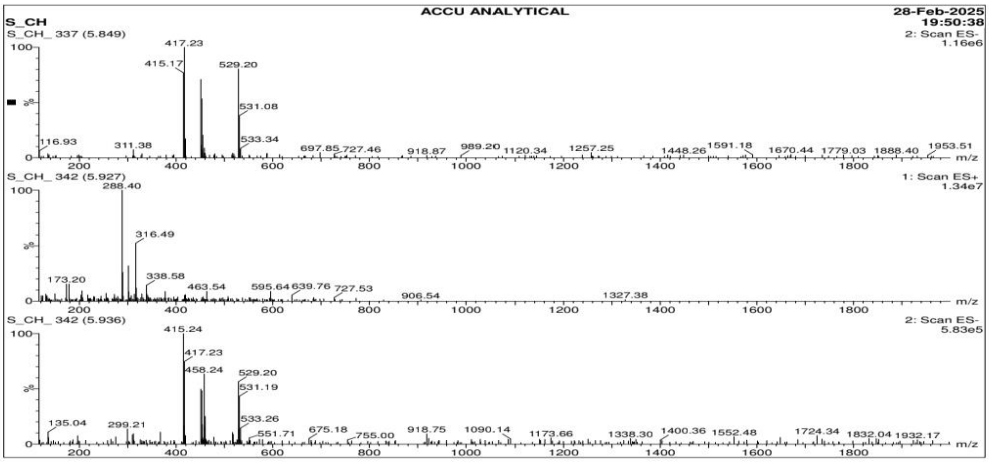

Figure 8:LC-MS of Aswagandha

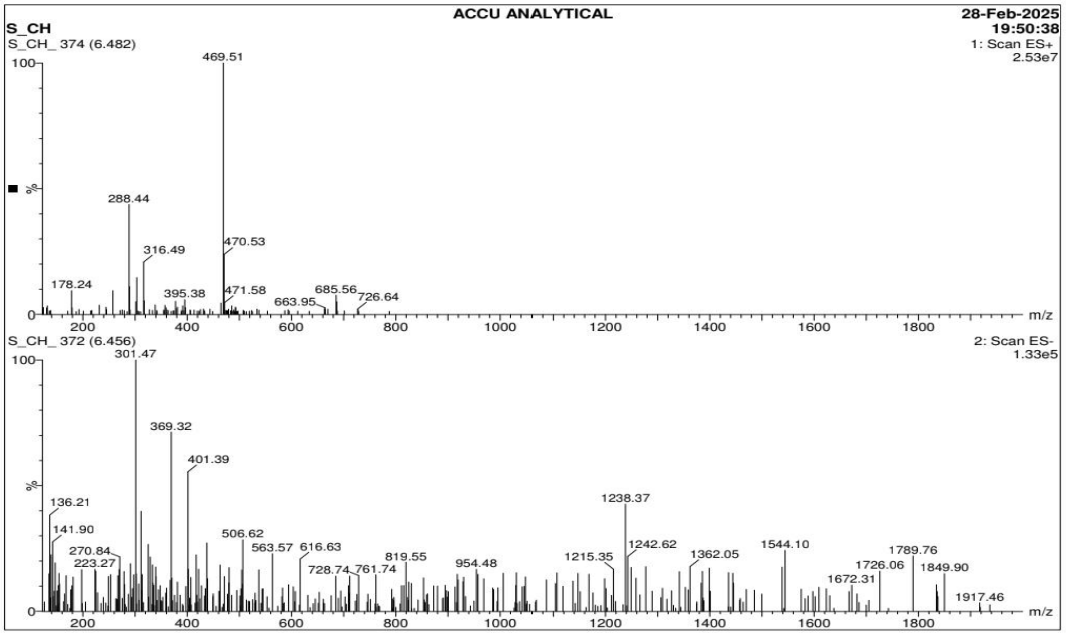

Figure 10.LC-MS of Rasna

Drug polymer interaction (FT-IR)-3 sample

Three samples are typically analysed to study drug-polymer interactions in transdermal patches using Fourier-Transform Infrared Spectroscopy (FT-IR): Extract, pure polymer, and the drug-polymer patch. Each sample is prepared by finely grinding and, if necessary, mixing with potassium bromide (KBr) to form transparent pellets (unless using the ATR method). FT-IR spectra are recorded in the 4000 cm^{-1} to 400 cm^{-1} range. By comparing the spectra, interactions between the drug and polymer are identified through shifts in peak positions, disappearance or broadening of functional group bands, or the appearance of new peaks, which may indicate molecular interactions such as hydrogen bonding or complex formation. These interactions can influence the stability and release profile of the transdermal patch.

Drug polymer interaction (FT-IR)-3 sample

In transdermal patches, drug-polymer interactions are crucial for stability and releaseprofiles. Fourier- Transform Infrared Spectroscopy (FT-IR) analyses these interactions by comparing the extract, pure polymer, and drug-polymer patch spectra. Spectra are typically recorded between 4000 cm^{-1} to 400 cm^{-1}. There is no interaction between the polymers and the drug.

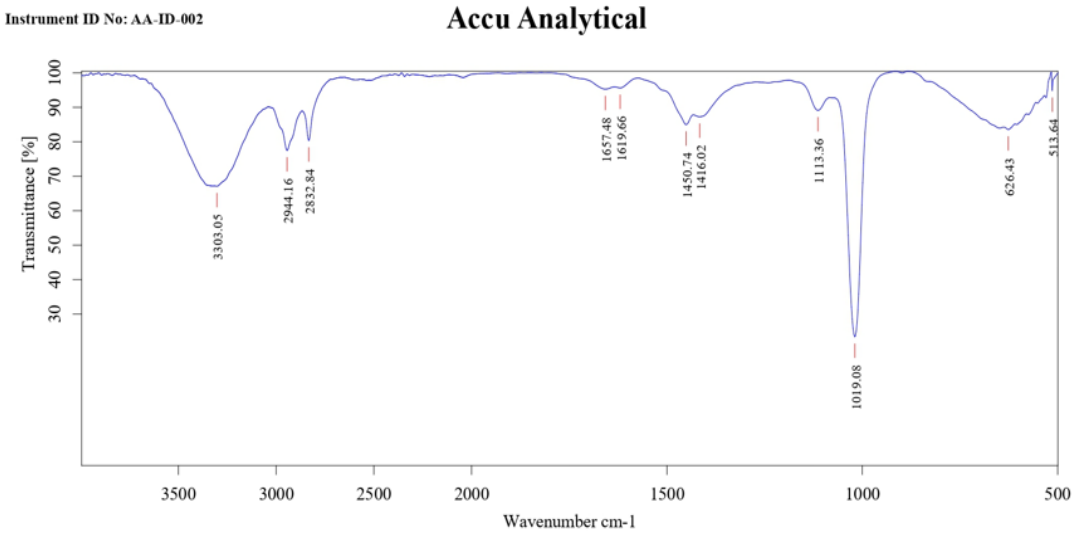

Figure 11: Jadamayadi extract FT-IR.

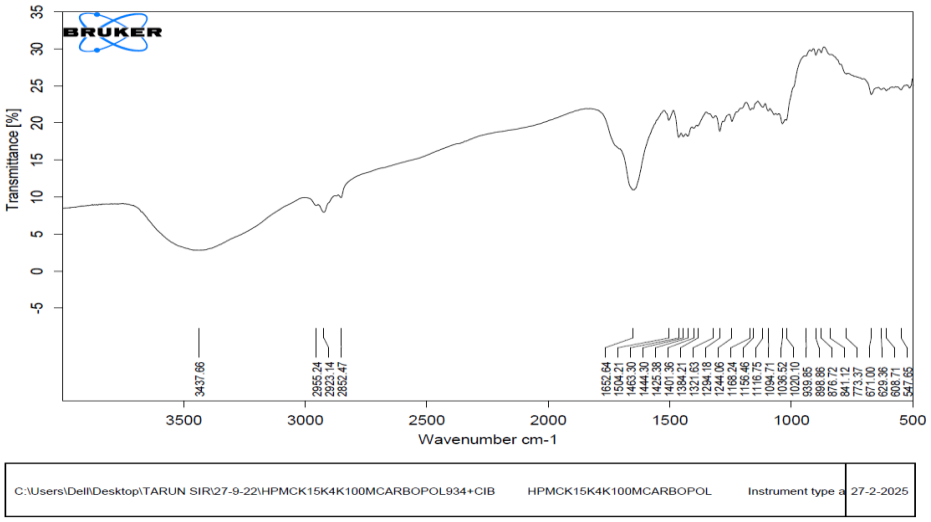

Figure 12: FT-IR HPMC.

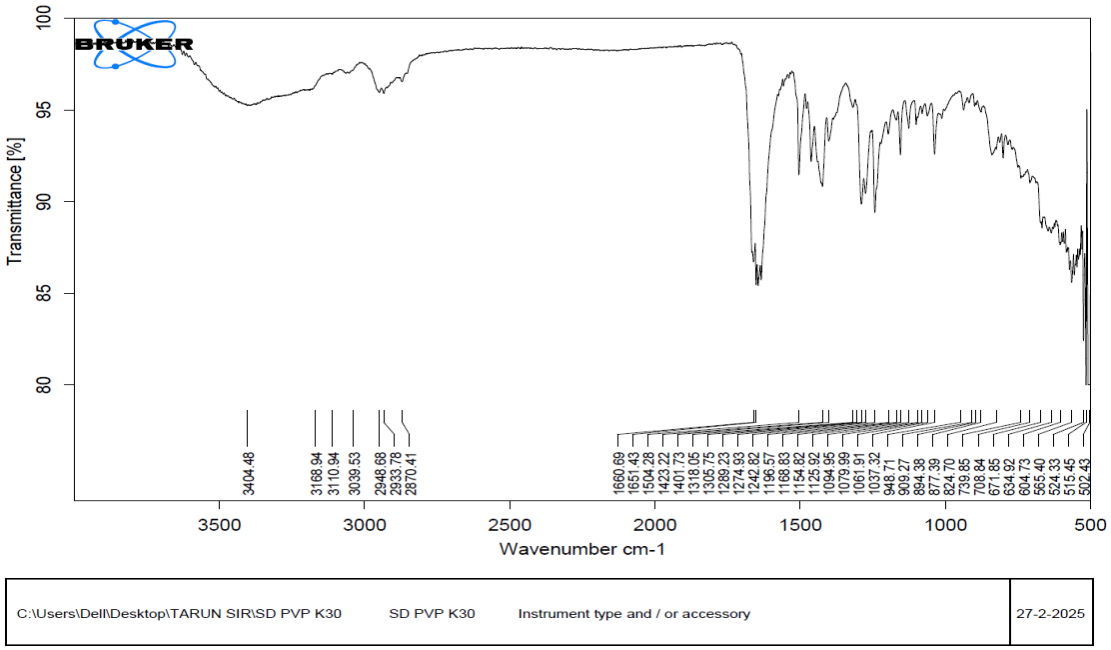

Figure 13: FT-IR PVP

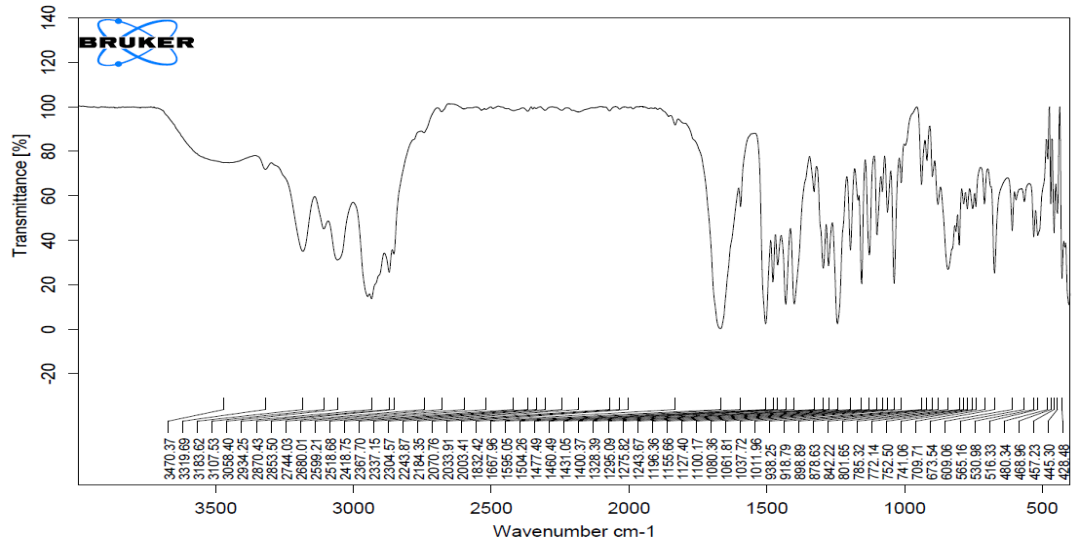

Figure 14: Extract and polymer FT-IR.

Transdermal patches

Prepare the transdermal patches polymer base by dissolving the selected polymer (HPMC and PVA) in a solvent like water or ethanol. Stir the mixture thoroughly to ensure a smooth gel without lumps. Add a plasticizer, such as glycerine, to improve the flexibility of the resulting patch. Once the polymer base is prepared, blend the Jadamayadi Choornam herbal extract into the polymer solution, ensuring a uniform extract distribution throughout the gel. To improve the penetration of the active compounds through the skin, incorporate permeation enhancers (DMSO or oleic acid) into the mixture. Spread the prepared polymer-herbal mixture evenly onto a flat, non-stick surface or into a casting mold. Underneath the solution, place a backing membrane (e.g., polyethylene) to provide structural support to the patch. Carefully distribute the mixture to ensure a uniform thickness, typically around 0.2 mm to 0.5 mm. Allow the patch to dry at room temperature, such as in an oven set to 40-50°C, until a flexible film forms. After the film has dried, cut it into patches of the desired size, such as 2x2 cm or 4x4 cm,

based on the intended application. To enable the patch to adhere to the skin, apply a pressure-sensitive adhesive (polyacrylate) to one side of the patch. Once the adhesive is applied, cover it with a protective release liner to shield the adhesive from contaminants before use.

Table 1. Formulation of jadamayadi extract transdermal patches

Ingredients	Formulation (F1)	Formulation (F2)	Formulation (F3)
Extract	150 mg	200 mg	250 mg
Polyvinyl alcohol	0.17 ml	0.17 ml	0.17 ml
Polyvinyl pyrrolidine	170 mg	170 mg	170 mg
Polyethene glycol 400	0.1 ml	0.1 ml	0.1 ml
Dimethyl sulfoxide	0.1 ml	0.1 ml	0.1 ml

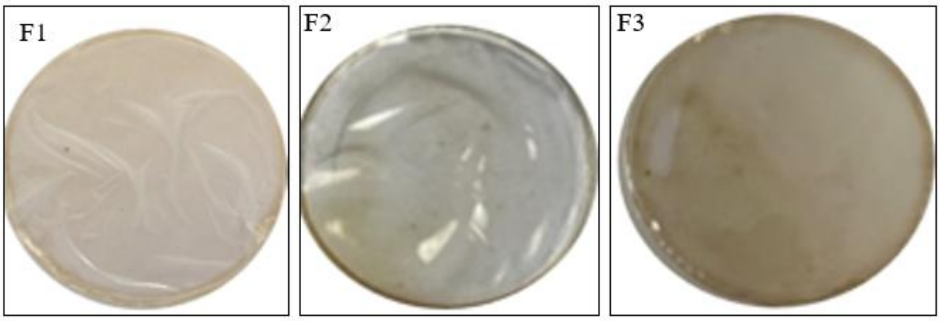

Figure 15: Transdermal patches of jadamayadi extract

Evaluation of Transdermal Patches

To study physical and chemical appearance to ensure uniformity and smoothness, folding endurance to test flexibility, and weight variation to ensure consistency in patch mass. The drug content uniformity test ensures the even distribution of herbal extract across patches. An in vitro drug release study can be performed using Franz diffusion cells to determine the rate at which the active compounds are released from the patch. Finally, a skin irritation test should be conducted on human or animal skin to ensure the patch is safe and non-irritating.

Evaluation of jadamayadi extract transdermal patches

Based on the optimization data, transdermal patches containing jadamayadi extract were formulated F1, F2 and F3. The physical characterization of these patches revealed favorable quality attributes. The formulation displayed optimal physical properties, including a colorless appearance, smooth surface texture, flexibility, and clarity (Figure 9). The thickness of the patches was consistently measured at 0.7 mm using a Vernier caliper, indicating uniform formation. Weight analysis showed a mass of **0.07 g** with a variation of ± **0.04 g**, suggesting acceptable batch-to-batch uniformity. The F2 formulation demonstrated superior folding endurance, reflecting excellent mechanical strength and flexibility, which is crucial for ensuring patch durability during handling and application. These characteristics indicate that the F2 formulation meets the desired physical parameters for an effective transdermal delivery system.

Drug release kinetics

Standard calibration curve of F2

The F2 formulation was calibrated using a UV spectrophotometer, which measured absorbance at 287 nm across various concentrations. The absorbance values for 2, 4, 6, 8, and 10 µg/ml concentrations were 0.344, 0.688, 1.032, 1.376, and 1.72, respectively. This data shows a direct linear relationship between the concentration of the extract and its absorbance at 287 nm. This linear relationship indicates that UV spectrophotometry can quantify

phytochemicals precisely in experimental setups with different drug concentrations. The calibration curve (Table 5) further supports this linear relationship, providing a reliable method for determining concentrations based on absorbance measurements. The calibration demonstrates the potential for accurate and efficient drug content analysis using UV spectrophotometry.

Table 2: Standard calibration curve of F2

Concentration (µg/ml)	Absorbance at 287 nm
0	0.00
2	0.34
4	0.68
6	1.03
8	1.37
10	1.72

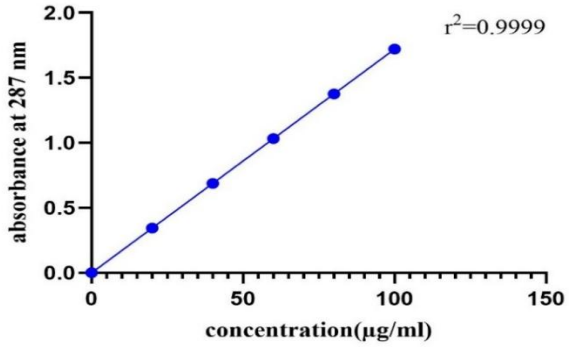

Figure 16: Standard calibration curve of Jadamayadi extract.

In vitro drug release studies of jadamayadi extract TDS

To assess how the drug is released from the gel, a phosphate buffer (pH 6.8) medium was used for testing over time. The results showed a gradual and sustained release of the active compound. Among the formulations, F2 had the highest drug release (68% after 480 minutes), followed by F3 (65%) and F1 (64%). This sustained release suggests that the gel could provide long-lasting therapeutic effects.

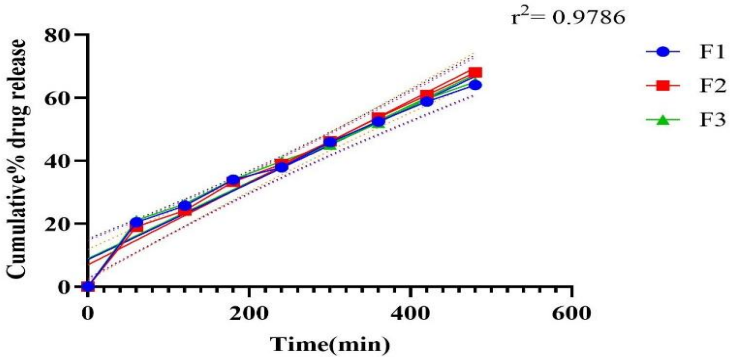

Figure17: *In-vitro* drug release studies of F1, F2 and F3 TDS

Table 3: *In-vitro* drug release F1, F2 and F3 TDS.

Time (min)	Cumulative % drug release		
	F1	F2	F3
0	0	0	0
60	20.42	19	21.08
120	25.66	24.14	26.29

180	33.94	33.28	34.01
240	37.96	38.87	40.81
300	45.85	46.19	46.18
360	52.44	53.70	53.02
420	59.78	61.84	58.65
480	62.03	69	65

The *in-vitro* drug release studies of jadamayadi extract transdermal delivery systems (TDS) were conducted using a phosphate buffer medium. The results showed the active compound's gradual and sustained release over time. Among the formulations, F2 demonstrated the highest drug release at 68% after 480 minutes, followed by F3 at 65% and F1 at 64%. This sustained-release pattern indicates that the gel formulation could provide long-lasting therapeutic effects, making it suitable for prolonged treatment regimens. The consistent release profiles suggest potential benefits for maintaining therapeutic levels without frequent reapplication.

Phytochemical analysis of jadamayadi extract transdermal patche (F2) by LC-MS

Analysing phytochemicals in transdermal patches is to characterise the raw herbal extract used in the formulation. About 5-10 mg of the dried F2 patch is dissolved in a solvent such as ethanol, followed by filtration through a 0.22 µm syringe filter to remove particulates. The sample is injected into the LC-MS system using a C_{18} reverse-phase column and a mobile phase of water (0.1% formic acid) and acetonitrile (0.1% formic acid) in a gradient system. The mass spectrometer operates in either positive or negative electrospray ionisation (ESI) mode, scanning in the 100-1000 m/z range. Peaks corresponding to the phytochemicals are identified by comparing mass-to-charge ratios (m/z) and retention times with standard libraries.

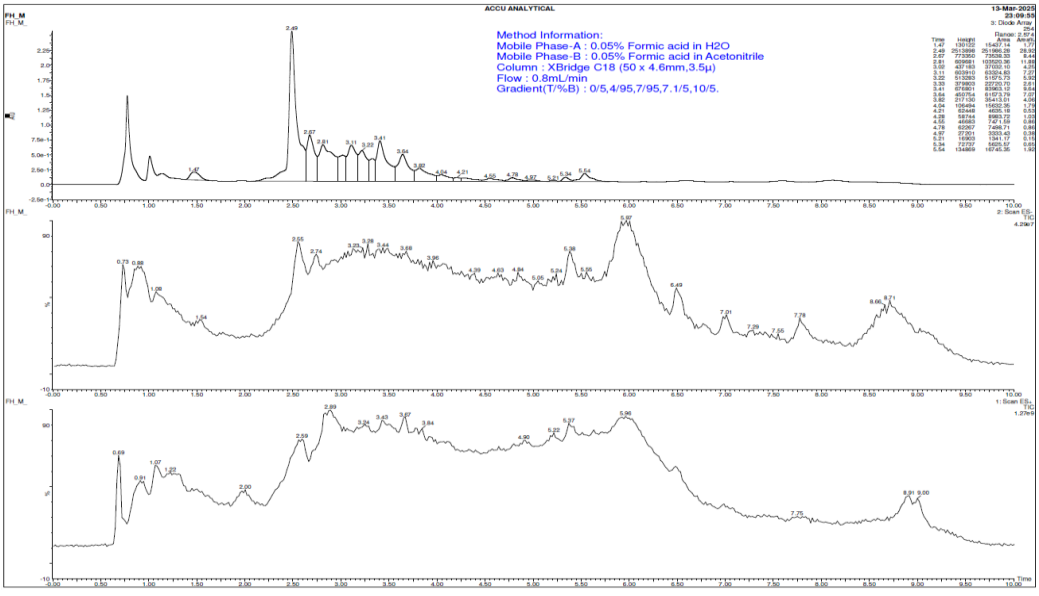

Figure 18: Phytochemical analysis of jadamayadi extract transdermal patch (F2) by LC-MS.

6A: Phytochemical analysis of jadamayadi extract transdermal patches by LC-MS

Using LC-MS, the phytochemical identification of jadamayadi extract transdermal patches revealed several significant peaks corresponding to potential phytochemicals. In positive mode, peaks at m/z values of 233.18, 289.41, 357.38, 470.53, 552.03, 685.56, 880.72, and

1088.43 were identified as berberine, catechin, quercetin, saponins, procyanidin dimers, ginsenosides, tannins, and glycosylated flavonoids, respectively. These compounds are known for their diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. These phytochemicals suggest that jadamayadi extract could offer therapeutic benefits when delivered transdermally.

Pharmacological Evaluation

Sample Preparation and Extraction from Transdermal Patches for Pharmacological Activity.

A one cm^2 patch section is dissolved in phosphate buffer saline (PBS) and extraction solvent by methanol to assess transdermal patches anti-inflammatory and anti-arthritic activity. The solution is sonicated for 30 minutes to extract the active phytochemicals from the patch matrix. After sonication, the sample is filtered using a 0.22 µm syringe filter to remove particulates, and the resulting extract is used for further biological assays. This preparation method ensures that the bioactive compounds are released and tested under *in vitro* conditions similar to transdermal release.

7A. *In-Vitro Anti-Inflammatory* activity (Protein denaturation assay)

The proteinase inhibitory activity of F2 extracts was assessed using a method adapted from Gunathilake KDPP et al., 2018. A 2 mL reaction mixture containing 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 1 mL of a test solution consisting of 0.02 mL F2 extract and 0.98 mL methanol. This mixture was incubated at 37°C for 5 minutes, followed by adding 1 mL of 0.8% (w/v) casein solution and further incubation for 20 minutes. The reaction was terminated with 2 mL of 70% perchloric acid, and after centrifugation, the absorbance of the supernatant was measured at 210 nm against a phosphate buffer control. The percentage inhibition of protein denaturation was calculated using the formula % inhibition of denaturation = 100 × (1 − A2/A1), A1 = absorption of the control sample, and A2 = absorption of the test sample.

Table 4: *In-Vitro Anti-Inflammatory* activity (Protein denaturation assay) F1 and extract

Concentration (mg/mL)	% Inhibition of Protein Denaturation (F2)	% Inhibition of Protein Denaturation Extract
0	0	0
25	64	65
50	67	67
75	69	70
100	71	72

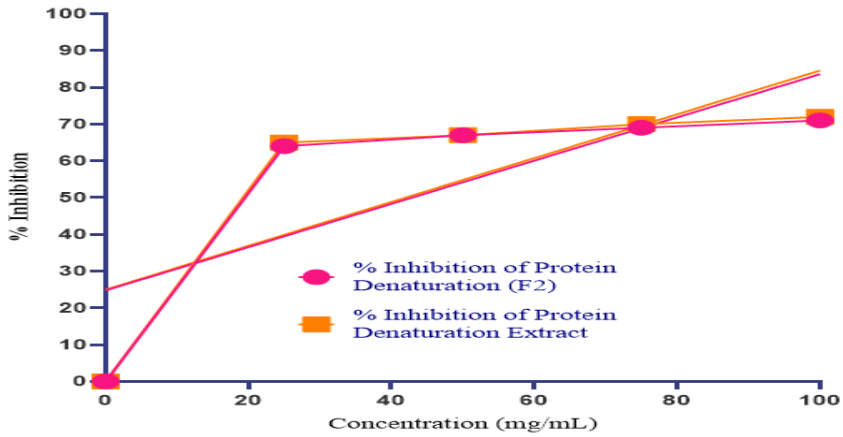

Figure 19: inhibition and concentration

The protein denaturation inhibition assay results reveal a consistent and dose-dependent inhibitory effect of both the F2 extract and the extract. At concentrations ranging from 25 to 100 µg/mL, both extracts demonstrated significant inhibition of protein denaturation, with the F2 extract showing slightly lower inhibition rates than the overall extract. The highest inhibition observed was approximately 71% for the F2 extract and 72% for the overall extract at 100 µg/mL. These findings suggest that both extracts possess notable anti-denaturant properties, which could benefit protein stabilisation applications. The similarity in inhibitory effects between the F2 extract and the extract indicates that the F2 extract is a key contributor to the extract's anti-denaturant activity. This suggests potential therapeutic applications in conditions where protein denaturation plays a role.

7B. *In-vitro* anti-arthritic activity

The in vitro anti-arthritic activity is evaluated using the protein denaturation inhibition assay, where test samples 100 µg/ml to 1000 µg/ml (F1 extract and Diclofenac) are mixed with a 1% bovine serum albumin (BSA) solution and incubated at 37°C. After heating at 70°C for 5 minutes, absorbance is measured at 660 nm. The percentage inhibition of protein denaturation is calculated. The percentage inhibition of protein denaturation is calculated using the formula: % Inhibition= (A control−A test/A control) × 100.

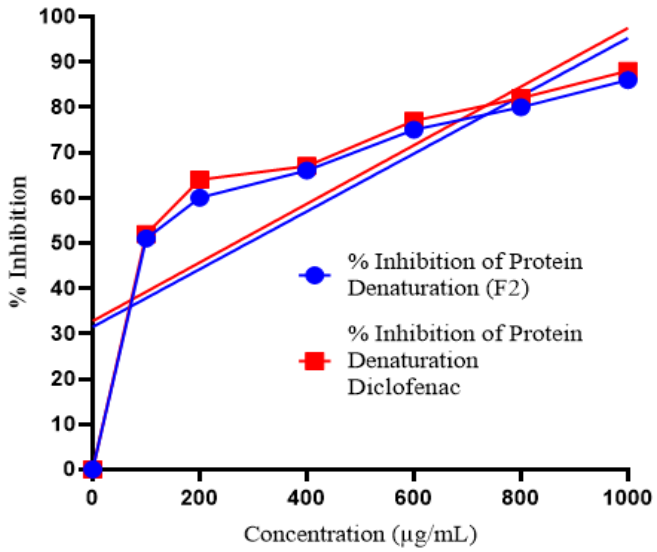

Figure 20: inhibition and concentration

Table 5: *In-vitro* anti-arthritic activity

Concentration (µg/mL)	% Inhibition of Protein Denaturation (F2)	% Inhibition of Protein Denaturation Diclofenac
0	0	0
100	51	52
200	60	64
400	66	67
600	75	77
800	80	82
1000	86	88

The protein denaturation inhibition assay results show a concentration-dependent increase in inhibitory activity for the F2 extract and diclofenac. At concentrations ranging from 100 to 1000 µg/mL, both exhibited significant inhibition of protein

denaturation. The F2 extract achieved up to 86% inhibition while diclofenac reached 88%. The close similarity in their inhibitory effects suggests that the F2 extract has comparable anti-denaturant properties to diclofenac, a known anti-inflammatory drug. This indicates potential therapeutic applications for the F2 extract in protein denaturation conditions. The consistent increase in inhibition with concentration highlights the efficacy of both agents.

7C: Analgesic activity - Cyclooxygenase (COX-2) Inhibition Assay

The COX-2 enzyme assay evaluates analgesic activity by measuring the inhibition of cyclooxygenase-2, a key enzyme in prostaglandin synthesis. Test F1 extract incubated with COX-2 enzyme and arachidonic acid in a buffered solution at 37°C, followed by adding a chromogenic reagent like TMPD. Absorbance is measured at 590-610 nm, and the percentage inhibition is calculated using the formula: % Inhibition = (A control−A test /A control) × 100. Higher inhibition suggests strong COX-2 inhibitory activity, indicating potential analgesic properties.

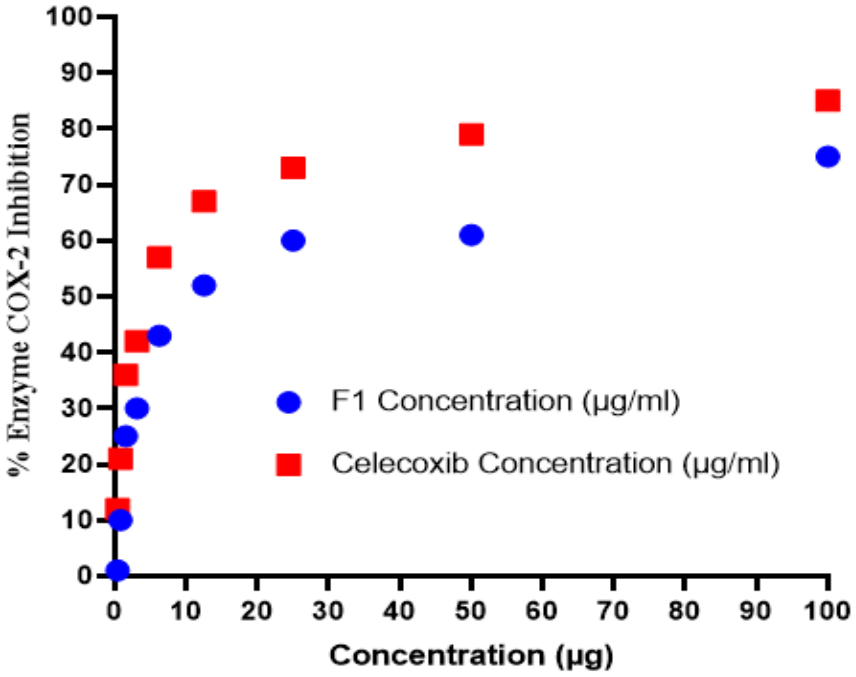

Figure 21: %enzyme COX-2 inhibition

Table 6: COX-2 enzyme assay evaluates analgesic activity

Concentration (µg)	F1 Concentration (µg/ml)	Celecoxib Concentration (µg/ml)
100	75	85
50	61	79
25	60	73
12.5	52	67
6.25	43	57
3.125	30	42
1.5625	25	36
0.78125	10	21
0.390625	1	12

The COX-2 enzyme assay results indicate that both F1 extract and celecoxib exhibit concentration-dependent inhibition of COX-2 activity, which indicates analgesic potential. At higher concentrations, celecoxib showed slightly higher inhibitory activity than the F1 extract. For instance, at 100 µg/mL, celecoxib achieved 85% inhibition, while the F1 extract reached 75%. However, as concentrations decreased, the difference in inhibitory activity between the two became less pronounced. This suggests that the F1 extract could be a viable alternative or complementary agent to celecoxib for pain management, given its notable COX-2 inhibitory activity. The findings support further investigation into the therapeutic potential of F1 extract as an analgesic agent. Overall, the results highlight the potential of natural extracts like F1 in contributing to pain relief strategies.

DISCUSSION

The present study focused on the development and evaluation of a transdermal patch incorporating Jadamayadi Choornam extract. Soxhlet extraction using selective solvents effectively yielded a concentrated extract rich in bioactive compounds, as confirmed by LC-MS analysis. Key phytoconstituents such as quercetin, kaempferol, catechin, and ginsenosides were identified compounds known for their anti-inflammatory, antioxidant, and analgesic properties. A comparison of LC-MS profiles between the raw extract and the formulated patch confirmed the retention of these phytochemicals, indicating that the formulation process preserved their integrity.

FT-IR analysis demonstrated no significant shifts or the formation of new peaks, indicating no chemical interactions between the extract and the polymers (HPMC and PVP). This suggests a physically embedded system, where the drug is released through polymer swelling and diffusion, thereby maintaining chemical stability and therapeutic efficacy.

Among the tested formulations, F2 showed optimal mechanical properties including a smooth surface, high flexibility, consistent thickness (0.7 mm), and strong folding endurance—all essential for effective application and user compliance. The incorporation of DMSO as a permeation enhancer facilitated sustained drug release, with in-vitro studies showing 69% release of active compounds over 8 hours.

Drug release evaluation using UV spectrophotometry showed a highly linear calibration curve, validating the quantification method. The F2 patch exhibited the highest release rate, likely due to an ideal combination of polymer concentration, extract load, and enhancer effectiveness. This sustained release profile supports its potential for long-term therapeutic use, reducing the dosing frequency and improving patient adherence.

Post-formulation LC-MS analysis of F2 patches confirmed the preservation of key phytochemicals such as berberine, saponins, and flavonoids, highlighting the formulation's stability and the continued presence of therapeutic agents.

Pharmacological evaluations further reinforced the efficacy of the formulation. Protein denaturation assays demonstrated dose-dependent anti-inflammatory activity, with the F2 patch showing similar inhibition to the raw extract. Anti-arthritic activity tests revealed that F2 patches performed comparably to Diclofenac, suggesting potential as a natural alternative to conventional NSAIDs. Additionally, COX-2 inhibition assays showed the extract inhibited, slightly below the standard celecoxib, confirming a significant analgesic effect suitable for chronic pain management.

CONCLUSION

In conclusion, the transdermal delivery of Jadamayadi extract offers a non-invasive method for sustained drug release, circumventing gastrointestinal issues

associated with oral administration. This formulation is scientifically validated through comprehensive in vitro analyses, and a comprehensive phytochemical analysis of the Jadamayadi Transdermal pain patch, including both the extract and final formulation, with confirmation of phytochemical retention post releases, and also the COX-2 enzyme assay results indicate that both F1 extract and celecoxib exhibit concentration-dependent inhibition of COX-2 activity, which indicates analgesic potential. The protein denaturation inhibition assay results show a concentration-dependent increase in inhibitory activity for the F2 extract and diclofenac. The F2 extract achieved up to 86% inhibition while diclofenac reached 88%. The close similarity in their inhibitory effects suggests that the F2 extract has comparable anti-denaturant properties to diclofenac, a known anti-inflammatory drug. The in vitro anti-arthritic activity is evaluated using the protein denaturation inhibition assay, where test samples 100 µg/ml to 1000 µg/ml (F1 extract and Diclofenac) are mixed with a 1% bovine serum albumin (BSA) solution and incubated at 37°C. After heating at 70°C for 5 minutes, absorbance is measured at 660 nm

Future studies focusing on in vivo pharmacokinetics, skin permeation, clinical efficacy, long-term stability, bioavailability, and toxicity are required to fully realize the formulation's potential as a therapeutic and marketable product.

OUTCOMES AND SIGNIFICANCE OF THE STUDY

The Soxhlet extraction method effectively yielded a bioactive-rich extract from Jadamayadi Choornam. LC-MS analysis confirmed the presence of therapeutic phytoconstituents such as quercetin, kaempferol, catechin, ginsenosides, berberine, flavonoids, and saponins, validating the traditional formulation's medicinal potential. A comparison of LC-MS profiles between the extract and transdermal patches confirmed the retention of key phytochemicals post-formulation, indicating that the formulation process preserved the chemical integrity of active constituents.

FT-IR analysis showed no chemical interactions between the extract and polymers (HPMC and PVP), establishing compatibility and supporting the stability and safety of the drug-polymer matrix for transdermal delivery. Among the tested formulations, F2 demonstrated optimal mechanical properties—smooth surface, flexibility, appropriate thickness, and high folding endurance—along with the best drug release profile, achieving 69% release over 8 hours, aided by DMSO as a permeation enhancer.

UV spectrophotometric analysis showed high linearity, validating the drug quantification method. The sustained drug release from the F2 patch suggests its potential for long-term therapy with reduced dosing frequency, thereby improving patient adherence. In vitro pharmacological evaluations revealed that the F2 patch retained significant anti-inflammatory activity (71% inhibition), anti-arthritic effects comparable to Diclofenac, and potent COX-2 inhibition (up to 75%), affirming its therapeutic efficacy.

The study offers strong scientific support for the traditional use of Jadamayadi Choornam and highlights its potential in modern phytomedicine through an effective, stable, and patient-friendly transdermal delivery system. Furthermore, the outcomes lay a solid foundation for future in vivo, clinical, and toxicological studies to advance the development and commercialization of this natural alternative to conventional anti-inflammatory and analgesic drugs.

This research bridges traditional knowledge and modern pharmaceutical technology, providing a scientific basis for the therapeutic use of Jadamayadi Choornam in a novel dosage form. It supports the integration of Ayurvedic principles into contemporary medicine and opens avenues for further clinical and toxicological studies.

The findings have the potential to lead to the development of marketable, safe, and effective natural transdermal therapies for pain and inflammation management.

Declaration by Authors

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Conflict of Interest: No conflict of interest.

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