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Research article

Development and evaluation of *Morus nigra* leaves transdermal patches for their anti-inflammatory activity

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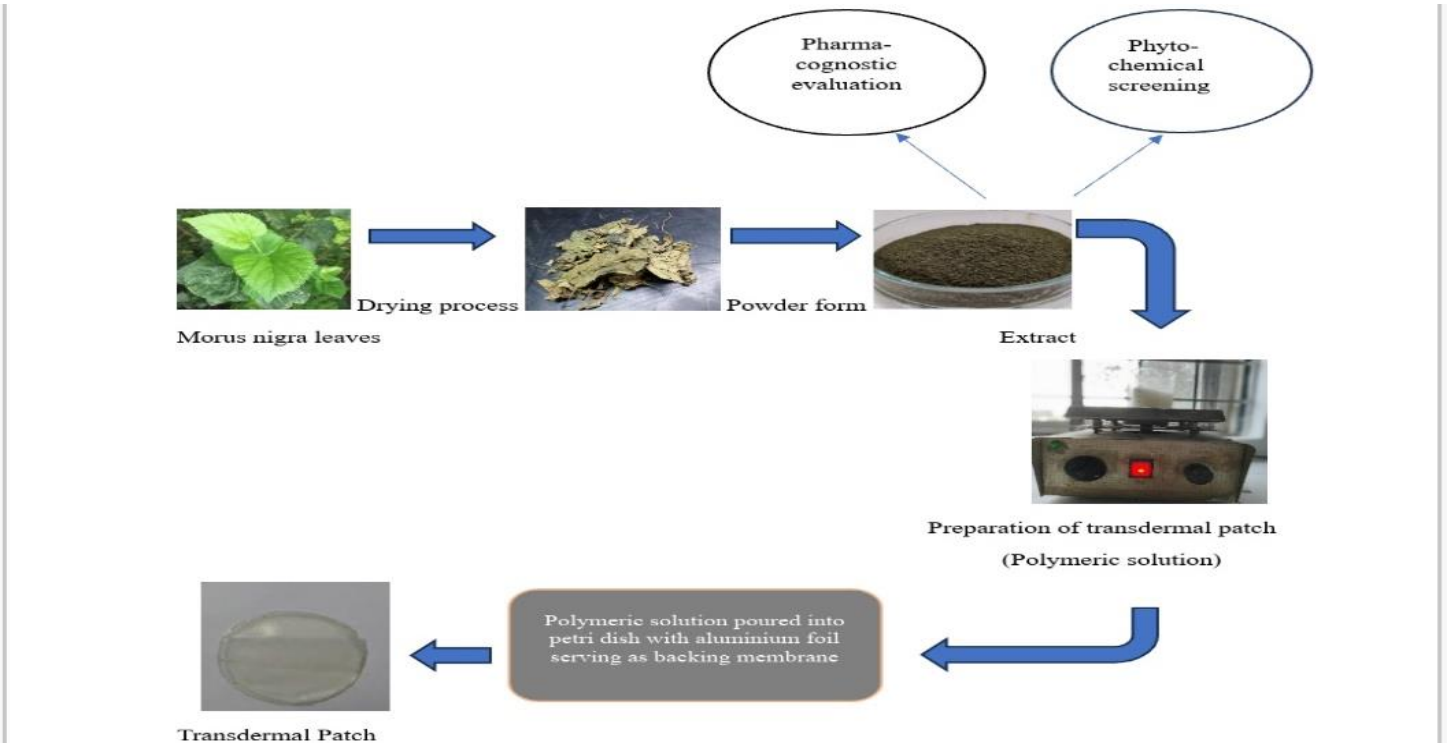
ABSTRACT

This study investigates the formulation and evaluation of transdermal patches incorporating Morus nigra leaves extract for their potential anti-inflammatory effects. Utilizing a solvent casting method, various formulations were developed, and their physicochemical properties, including thickness uniformity, moisture content, folding endurance, and drug content percentage, were systematically assessed. The patches exhibited satisfactory characteristics, with formulation F-3 showing superior drug content and folding endurance. The findings suggest that Morus nigra leaves extract can be effectively incorporated into transdermal patches, providing a promising alternative for the management of inflammatory conditions. Future research will focus on optimizing formulation parameters and exploring clinical applications to enhance therapeutic efficacy.



Keywords: *Morus* nigra, Herbal, Transdermal drug delivery, Transdermal patches, Anti-inflammatory effects.

INTRODUCTION

Inflammation is a fundamental biological response to injury, infection, or harmful stimuli, playing a critical role in immune defense mechanisms. However, "Chronic inflammation is linked to a wide range of pathological conditions, including arthritis, cardiovascular diseases, and autoimmune disorders. Conventional anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are effective but often associated with adverse effects such as gastrointestinal disturbances, liver toxicity, and immunosuppression when used long term." These challenges highlight the need for safer, alternative anti-inflammatory treatments derived from natural sources [1, 2].

One such natural source is *Morus nigra* (black mulberry), a plant traditionally used in herbal medicine due to its diverse therapeutic properties. *Morus nigra* leaves are known to possess a variety of bioactive compounds, "including flavonoids, alkaloids, and phenolic acids, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties. The anti-inflammatory potential of these leaves can be attributed primarily to the presence of flavonoids such as quercetin, rutin, and kaempferol, which modulate inflammatory mediators like prostaglandins and cytokines" [3].

Transdermal drug delivery systems (TDDS) have gained attention as an effective method for the administration of drugs, particularly for chronic conditions like inflammation. TDDS offers advantages over oral or injectable routes by bypassing hepatic first-pass metabolism, providing controlled and sustained drug release, reducing dosing frequency, and enhancing patient compliance. Transdermal patches deliver active substances through the skin, making them a convenient and non-invasive option for long-term therapy. Furthermore, they minimize systemic side effects, making them an attractive alternative to conventional oral medications [4].

Flavonoids and Phenolic Compounds

A study by Kang et al. (2013) demonstrated that "the flavonoid-rich fraction of *Morus nigra* leaves significantly reduced inflammatory responses in lipopolysaccharide (LPS)-induced macrophages by inhibiting the expression of nitric oxide and other pro-inflammatory markers" [5].

Similarly, Pan et al. (2015) found that "an ethanolic extract of *Morus nigra* leaves exerted anti-inflammatory effects in vitro by inhibiting the activity of cyclooxygenase (COX) enzymes, which are key mediators of inflammation. The anti-inflammatory mechanism was attributed to the suppression of nuclear factor kappa B (NF-κB) signaling pathways, which regulate the expression of inflammatory cytokines" [6].

Kim et al. (2015) demonstrated that "the antioxidant capacity of *Morus nigra* leaf extracts contributed to its protective effects against oxidative damage in cellular models of inflammation, suggesting a dual action of the plant in mitigating both oxidative and inflammatory stress" [7].

Advantages of Transdermal Delivery

Transdermal delivery bypasses the gastrointestinal tract, avoiding first-pass metabolism by the liver, which is a significant issue with oral anti-inflammatory drugs. This allows for a reduction in required dosage while maintaining therapeutic efficacy. Prausnitz and Langer (2008) highlighted the potential of TDDS for chronic diseases, where sustained drug release and enhanced patient compliance are critical factors [8].

A study by Khafagy et al. (2020) on transdermal patches for herbal anti-inflammatory compounds demonstrated that the selection of appropriate polymers (such as hydroxypropyl methylcellulose) and permeation enhancers (such as dimethyl sulfoxide) was essential for achieving the desired drug release profile and skin permeability [9].

Winter et al. (1962) established this model as a gold standard for testing the efficacy of anti-inflammatory agents, and it has since been adapted for use with various transdermal systems [10].

A study by Singh et al. (2017) formulated a transdermal patch containing curcumin, a natural anti-inflammatory compound, and evaluated its performance in vitro and in vivo. The results demonstrated excellent skin permeability, sustained drug release, and significant anti-inflammatory effects, providing a model for the development of *Morus nigra* transdermal patches [11].

Recent advancements in nanotechnology have also opened up new possibilities for enhancing the delivery of plant extracts through the skin. Nanoemulsion and nanoparticles have been explored to improve the stability and bioavailability of bioactive compounds in TDDS. Patel et al. (2021) investigated the use of nanoemulsion for delivering herbal extracts Trans dermally, noting that nanoemulsion enhanced skin penetration and prolonged the release of active compounds [12].

MATERIAL AND METHOD

Materials

Morus nigra leaves were sourced from a botanical garden, serving as the key active ingredient. Potassium bismuth iodide, PVP K-30, PEG-400, and Tween-80, supplied by Span Diagnostics Limited, were used as reagents, polymer matrix, plasticizer, and surfactant, respectively. Ethanol and methanol from Sigma Aldrich aided in the extraction process, while sodium bicarbonate, acetic acid, sodium chloride, and catalase from the college laboratory were used for pH adjustments and experimental monitoring. These materials ensured the formulation's stability, efficacy, and accurate evaluation of anti-inflammatory activity.

Various instruments were employed throughout the process of developing and evaluating *Morus* nigra transdermal patches, facilitating precise formulation and testing. Most of these instruments

were sourced from the college laboratory, ensuring accessibility and consistency during experimentation.

Pharmacognostic Evaluation

Ash values

Determination of Total Ash Value

A 5g pharmaceutical sample is finely powdered and placed in a pre-weighed crucible. The crucible is heated in a muffle furnace at 550-600°C until the sample is fully incinerated. After cooling in a desiccator to prevent moisture absorption, the crucible with the ash residue is re-weighed to determine the ash content.

Finally, the total ash content is calculated using the formula:

Total Ash %= (Weight of ash residue/Weight of sample) ×100

Determination of Acid Insoluble Ash Value

In this procedure, a 5g sample is finely powdered and placed into a pre-weighed crucible. The sample is then treated with hydrochloric acid (HCl) to cover it fully and gently heated until effervescence stops, indicating the decomposition of organic matter. Afterward, the crucible is transferred to a muffle furnace and heated at 550-600°C until the residue turns white or nearly white, indicating the evaporation of the acid. The acid-insoluble ash content is calculated using the formula:

Acid-insoluble Ash % = (Weight of acid-insoluble ash residue/Weight of sample) ×100

Determination of Water Soluble Ash Value

In this procedure, a 5g sample is finely powdered and placed in a pre-weighed crucible. It is treated with hydrochloric acid (HCl) until fully covered and gently heated until effervescence stops. The crucible is then placed in a muffle furnace and heated to 550-600°C until the residue turns white, indicating that the acid has evaporated. After cooling in a desiccator, the crucible is re-weighed. The acid-insoluble ash content is calculated using the formula:

Water-soluble Ash % = (Weight of water-soluble ash residue/Weight of sample) ×100

Loss on Drying

In this procedure, a 5g sample is finely powdered and placed in a pre-weighed crucible. The sample is treated with hydrochloric acid (HCl) until fully covered and gently heated until effervescence ceases. The crucible is then transferred to a muffle furnace and heated to 550-600°C until the residue turns white, indicating complete acid evaporation. After cooling in a desiccator, the crucible is re-weighed. The acid-insoluble ash content is calculated using the formula:

Loss on Drying (%) = (Initial weight - Final weight/Initial weight) ×100

Soxhlet Extraction

Soxhlet extraction is a widely used technique for extracting compounds from solid samples, particularly those soluble in organic solvents. The process begins by assembling the Soxhlet extractor and placing the solid sample, often contained in filter paper or a thimble to prevent loss, in the extraction chamber. An appropriate solvent, such as ethanol, is added to the round bottom flask, and the flask is heated to reflux, causing solvent vapor to rise into the extraction chamber. As the vapor condenses, it drips onto the solid sample, extracting soluble compounds, which then siphon back into the flask. This cycle continues until the desired concentration of compounds is achieved, which may take several hours or overnight. The process concludes when the solvent's color stabilizes, after which the round bottom flask is removed, and the solvent is evaporated using a rotary evaporator to concentrate the extract.

Preliminary Phytochemical Screening of Extract

Phytochemicals, derived from the Greek word "phyto" meaning "plant," are compounds synthesized by plants that play essential roles in their protection and defense mechanisms. They are of particular interest in research due to their potential health benefits. Qualitative chemical tests are often conducted to establish the chemical composition of plant extracts.

Alkaloids (Dragendroff's Test)

Add Dragendroff's reagent to the plant extract. A positive result is indicated by an orange-brown precipitate.

Flavonoids (Shinoda Test)

Treat the extract with concentrated hydrochloric acid followed by concentrated sulfuric acid. A color change to red, purple, or blue indicates flavonoids.

Tannins (Ferric Chloride Test)

Add ferric chloride solution to the plant extract. The formation of a blue-black or greenish-black precipitate indicates tannins.

Saponins (Froth Test)

Shake the plant extract vigorously with water. A stable froth or foam layer indicates the presence of saponins.

Phenolic Compounds (Ferric Chloride Test)

Introduce ferric chloride solution to the plant extract. Color changes to green, blue, or violet indicate phenolic compounds.

Glycosides (Legal's Test)

Heat the plant extract with sulfuric acid, then add ferric chloride solution. A blue-green coloration suggests glycosides.

Terpenoids (Salkowski Test)

Add chloroform to the plant extract, followed by concentrated sulfuric acid. A red coloration in the lower chloroform layer indicates terpenoids.

Carbohydrates (Molisch Test)

Add α-naphthol solution and concentrated sulfuric acid to the plant extract. A purple or violet ring indicates carbohydrates.

Proteins (Biuret Test)

Add dilute copper sulfate and sodium hydroxide to the plant extract. A violet coloration indicates proteins.

Steroids (Liebermann-Burchard Test)

Mix the plant extract with acetic anhydride and concentrated sulfuric acid. The appearance of green, blue, or purple colors indicates steroids.

Amino Acids (Ninhydrin Test)

Apply Ninhydrin solution to the plant extract and heat. A purple or blue coloration indicates amino acids.

Glycosides (Baljet Test)

Mix the plant extract with hydrochloric acid, then add potassium permanganate. A color change to pink or purple suggests glycosides.

Development of Transdermal Patches

The chosen solvent system was utilized to dissolve the selected polymers until a clear solution was achieved, which was then mixed with a plasticizer and stirred continuously before adding the medication. This medication-containing polymeric solution was cast into an Anumbra petri dish lined with aluminum foil and left to dry at room temperature for a full day. Once dried, the patches were stored in a desiccator at 0–4°C for further assessments. The concentrations of PVP K-30 ranged from 5% to 10% w/w, serving as a film-forming agent and binder, while the drug extract concentrations varied from 5 mg to 15 mg to ensure adequate therapeutic efficacy and patch integrity. PEG-400 concentrations were set at 2.5% to 5% w/w to enhance flexibility and drug release kinetics, and Tween-80 was included at a constant 1% v/v in all formulations to improve drug solubility and skin permeation, making these concentrations suitable for optimizing patch performance.

Table 1: The development of transdermal patches

Polymer	**F-1**	**F-2**	**F-3**	**F-4**	**F-5**
PVP K-30 (%w/w)	5	10	5	10	10
Drug extract (mg)	10	15	10	10	15
PEG-400(%w/w)	2.5	5	5	2.5	2.5
Tween-80	1% (v/v)	1% (v/v)	1% (v/v)	1% (v/v)	1% (v/v)
pH Stabilizer	Citric Acid (0.1%)	Citric Acid (0.1%)	Citric Acid (0.1%)	Citric Acid (0.1%)	Citric Acid (0.1%)

Evaluation of Transdermal Patches

Thickness

Thickness is measured using, “micrometer or digital caliper by placing the patch between the instrument's anvils and recording the measurement in millimeters (mm) or micrometers (µm). To ensure reliability and consistency, at least three patches from each batch are tested.

Uniformity of Weight

The uniformity of weight in transdermal patches is essential for ensuring consistent drug content and dosing accuracy. To evaluate this, a representative sample of patches is selected and individually weighed using a sensitive balance, with at least three patches tested for reliability. The weights are recorded and compared to assess variation; ideally, they should fall within a specified range, indicating uniformity in drug content and formulation.

Moisture Content

Moisture content is a critical parameter for evaluating transdermal patches, as it influences stability, shelf-life, and performance. To ensure reliable results, a minimum of three patches from each batch are tested. The procedure involves selecting representative samples to ensure homogeneity, followed by drying the samples using methods such as oven drying or Karl Fischer titration. Oven drying heats the samples at a specified temperature for a set duration, while Karl Fischer titration offers a precise measurement of moisture content. After drying, the samples are weighed to determine their dry weight, and moisture content is calculated using the appropriate formula:

Moisture Content (%) = (Initial Weight - Dry Weight) / Initial Weight ×100%

Moisture Uptake

Moisture uptake refers to the ability of transdermal patches to absorb moisture from the environment, which can impact their stability, integrity, and performance. To ensure reliable results, a minimum of three patches from each batch are tested. The procedure includes preparing representative samples exposed to controlled conditions at a specified temperature and relative humidity for a set duration. Samples are placed in a desiccator or humidity chamber to equilibrate, and their initial weight is recorded using a sensitive balance. After exposure, “the final weights are measured, and moisture uptake is calculated by subtracting the initial weight from the final weight, indicating the amount of moisture absorbed during exposure.”

Folding Endurance

Folding endurance measures the mechanical strength and flexibility of transdermal patches, indicating their resistance to cracking or breaking under repeated bending. To ensure reliable results, at least three patches from each batch are tested. The procedure involves selecting uniform samples, setting up a folding endurance tester, and securely mounting the samples for folding along a defined line or axis. The samples undergo repeated folding at a constant rate and pressure, with the number of folds based on standard protocols. After testing, the samples are visually inspected for signs of cracking, delamination, or other damage, with any defects noted for further analysis.

Drug Content Determination

For this test, “a 100 ml solution of phosphate buffer with a pH of 7.4 was employed. A patch of one centimeter by one centimeter was cut and added to the buffer solution. Using a spectrophotometer, the solution was filtered and the drug concentration was measured by dilution at a wavelength of 240 nm for five hours while being stirred with a magnetic stirrer. Typically, to ensure the reliability and consistency of the results, a minimum of 3 patches from each batch are tested for Drug content determination.”

***In Vitro* Drug Release Study**

For the drug content determination test, "a 100 ml solution of phosphate buffer at pH 7.4 was prepared. A 1 cm² patch was cut and immersed in the buffer solution. The solution was stirred with a magnetic stirrer and filtered using a spectrophotometer to measure the drug concentration. The concentration was analyzed by dilution at a wavelength of 240 nm over five hours. To ensure reliable results, at least three patches from each batch are tested.

RESULTS

Physico-chemical Evaluation of Crude Drugs

Physical Test of Crude Drugs

The organoleptic properties of *Morus* nigra leaves extract were evaluated to characterize its appearance, color, and taste. The extract appeared as a semi-solid green substance, with a distinctly herbal odor indicating the presence of phytochemical compounds. Its taste was slightly bitter, consistent with certain bioactive constituents found in the plant. These sensory attributes provide valuable qualitative information for identifying the extract and exploring its potential applications in various industries.

Table 2: Phytochemical screening for extract of leaves extract

Chemical test	*Morus* nigra leaves extract
	Ethanol
Test for steroids for triterpenoids	
Liebermann's Burchard test	_
Salkowski test	_
Test for saponnins	
Foam test	_
Test for alkaloids	
Hager's test	+
Mayer's test	+
Test for glycosides	
Borntrager's test	+
Keller killiani test	+
Test for tannins and phenolic compounds	
Gelatine test	+
Ferric chloride test	+
Test for flavonoids	
Ferric chloride test	+
Alkaline reagent test	+
Test for proteins	
Biuret test	+
Xanthoproteic test	+
Test for carbohydrates	
Fehling test	+

Evaluation of Transdermal Patches

Thickness of patches

The evaluation of transdermal patches included assessing thickness uniformity. The average thicknesses for each formulation code were as follows: F-1 at 0.50 mm, F-2 at 0.45 mm, F-3 at 0.39 mm, F-4 at 0.47 mm, and F-5 at 0.51 mm. In comparison, the marketed formulation had a thickness of 0.142 mm.

Weight Variation

The evaluation of transdermal patches assessed thickness uniformity, with average thicknesses of F-1 at 0.50 mm, F-2 at 0.45 mm, F-3 at 0.39 mm, F-4 at 0.47 mm, and F-5 at 0.51 mm. The marketed formulation had a thickness of 0.142 mm.

% Moisture Content

The percentage moisture content of transdermal patches is essential for their stability and effectiveness. Testing revealed the following moisture contents: F-1 at 3.05%, F-2 at 3.55%, F-3 at 4.01%, F-4 at 3.46%, and F-5 at 3.75%. The marketed formulation had a moisture content of 3.24%.

% Moisture Uptake

The moisture content of transdermal patches is crucial for stability and effectiveness. Testing showed moisture contents of F-1 at 3.05%, F-2 at 3.55%, F-3 at 4.01%, F-4 at 3.46%, and F-5 at 3.75%, with the marketed formulation at 3.24%.

Folding Endurance

The folding endurance of transdermal patches, important for assessing mechanical strength and resilience, was evaluated across various formulations. F-1 showed a folding endurance of 20.05, indicating moderate durability, while F-2 had a higher value of 24.55, suggesting increased strength. F-3 displayed 22.05, reflecting good resilience, and F-4 had a slightly lower endurance of 21.35, indicating robust properties. F-5 showed a consistent performance with 20.93. In comparison, the marketed formulation had a folding endurance of 47.66.

Drug Content (%)

The drug content percentage, indicating the concentration of the active pharmaceutical ingredient (API) in transdermal patches, was evaluated across various formulations. F-1 had a drug content of 94.05%, while F-2 showed a slightly higher concentration at 95.65%. F-3 displayed a lower content of 93.42%, and F-4 recorded 95.06%. The marketed formulation had a maximum drug content of 98.29%.

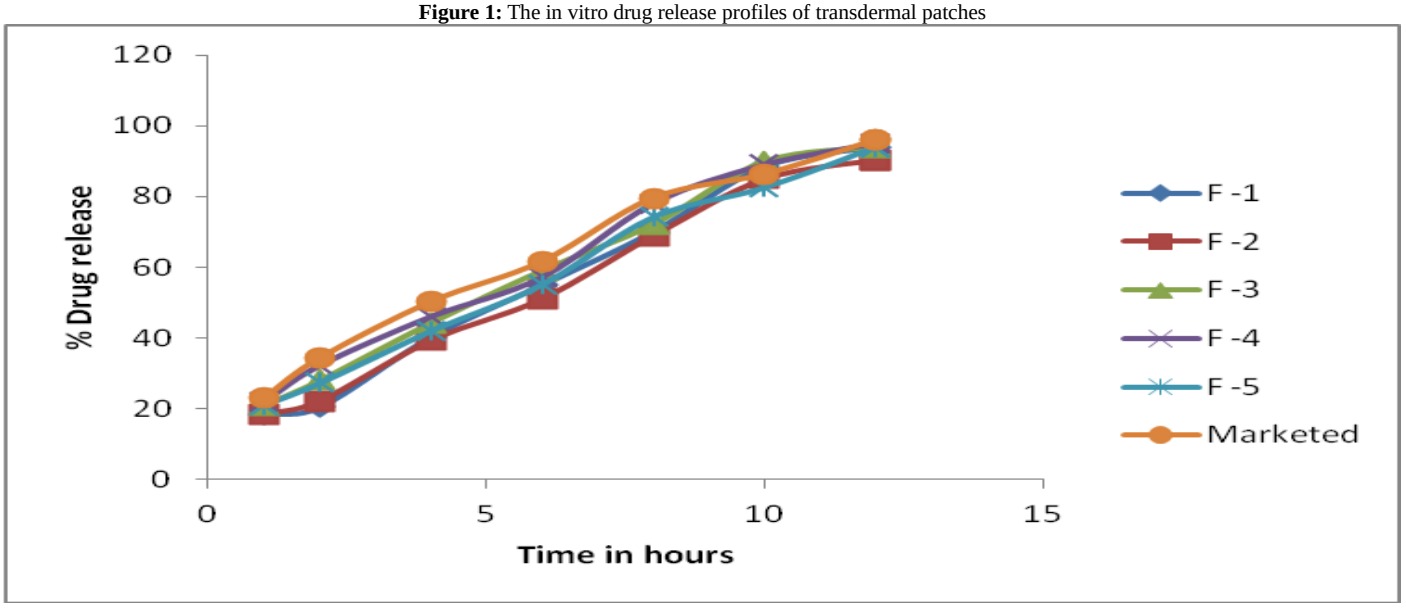
In Vitro Drug Release Profile

The drug content percentage of transdermal patches was evaluated across various formulations, revealing the following results: F-1 at 94.05%, F-2 at 95.65%, F-3 at 93.42%, and F-4 at 95.06%. The marketed formulation exhibited a maximum drug content of 98.29%.

Table 3: The in vitro drug release profiles of transdermal patches

Time(Hrs)	Cumulative % drug release					
Time(Hrs)	F -1	F -2	F -3	F -4	F -5	Marketed
1	18.55	18.25	21.05	22.05	21.08	23.11
2	20.52	22.05	28.05	32.25	27.34	34.36
4	40.30	39.28	44.24	46.05	42.05	50.24
6	55.06	51.05	59.06	57.22	55.17	61.61
8	70.11	68.99	72.05	78.06	74.25	79.52
10	88.56	85.05	89.77	89.06	82.66	86.25
12	94.02	90.45	93.74	95.05	94.05	95.92

Figure 1: The in vitro drug release profiles of transdermal patches



CONCLUSION

In conclusion, this study successfully developed and evaluated transdermal patches formulated with Morus nigra leaves extract for their anti-inflammatory properties. The comprehensive assessment of the patches, including their physical characteristics, drug content, moisture content, and mechanical properties, demonstrated the formulations' consistency and stability. Notably, formulations F-3 and F-4 exhibited significant cumulative drug release, moisture content, and folding endurance, supporting their potential as effective transdermal delivery systems. These findings highlight the potential of *Morus* nigra leaves extract as a therapeutic agent in transdermal formulations, paving the way for further research into its clinical applications in pain management and inflammatory conditions.

The future scope of this study on transdermal patches formulated with *Morus* nigra leaves extract includes conducting extended pharmacological studies to elucidate the anti-inflammatory mechanisms at the molecular level, followed by clinical trials to assess the efficacy and safety in human subjects. Additionally, further optimization of the patch formulations by investigating different ratios of polymers and plasticizers could enhance drug release profiles, mechanical properties, and skin permeability.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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