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Formulation and Evaluation of *Bacopa monnieri* Ethanolic Extract Loaded Transdermal Patch for Neuroprotective Activity

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Abstract

The present study aimed to formulate and evaluate *Bacopa monnieri* **ethanolic extract-loaded transdermal patches** for potential neuroprotective activity. *Bacopa monnieri*, commonly known as Brahmi, is a medicinal plant traditionally used for neurological and cognitive disorders. In this study, ethanolic extract of *Bacopa monnieri* was prepared by maceration and incorporated into matrix-type transdermal patches using the solvent casting method. Each formulation contained **200 mg of** *Bacopa monnieri* **ethanolic extract.** HPMC and PVP K30 were used as film-forming polymers, PEG-400 as plasticizer, and propylene glycol as permeation enhancer. The prepared patches were evaluated for physical appearance, thickness, weight variation, folding endurance, moisture content, moisture uptake, surface pH, flatness, drug content uniformity, in vitro drug release, ex vivo skin permeation, skin irritation, stability, and neuroprotective activity. Among all formulations, **F4** showed the best physicochemical properties, highest drug content,

excellent flatness, sustained drug release, good skin permeation, and no irritation. In vitro neuroprotective evaluation showed improved cell viability in the optimized patch-treated group compared with the toxic control group. The findings suggest that *Bacopa monnieri* ethanolic extract can be successfully formulated into a transdermal patch and may serve as a promising non-invasive delivery system for neuroprotective application. Further *in vivo* studies are recommended to confirm its therapeutic potential.

Introduction

Neurological disorders are a major health concern because they affect memory, movement, behavior, cognition, and overall quality of life. Many neurological conditions, including Alzheimer's disease, Parkinson's disease, neuropathic pain, and cerebral ischemia, are associated with progressive neuronal damage and impaired neurotransmitter function. Conventional neurological therapies are useful, but they may cause systemic side effects, require frequent dosing, and sometimes show limited patient compliance. For this reason, medicinal plants with neuroprotective potential are increasingly being studied as alternative or supportive therapeutic agents. Because apparently swallowing tablets forever was not already enough of a human inconvenience.

Bacopa monnieri Linn., commonly known as Brahmi, is a medicinal plant widely used in traditional systems of medicine for brain-related disorders. It has been traditionally recognized as a memory-enhancing and nervine tonic plant. The neuropharmacological activity of *Bacopa monnieri* is mainly attributed to its bioactive constituents, especially bacosides, which are reported to influence neuronal protection, synaptic activity, neurotransmitter regulation, and cognitive function (Aguiar & Borowski, 2013). Previous studies have suggested that *Bacopa monnieri* extract may improve learning and memory and may be useful in conditions involving neurodegeneration and cognitive decline (Kongkeaw et al., 2014).

Several experimental studies support the neuroprotective potential of *Bacopa monnieri*. Shobana et al. (2012) reported that alcoholic extract of *Bacopa monnieri* produced protective effects in a 6-hydroxydopamine-induced rat model of Parkinson's disease by improving behavioral performance and restoring biochemical alterations in brain tissues. Similarly, Waly et al. (2019) reported that *Bacopa monnieri* extract showed neuroprotective effects in an Alzheimer's disease rat model through modulation of biochemical markers related to neurodegeneration. These findings provide a scientific basis for selecting *Bacopa monnieri* as a suitable plant for neurological research.

Despite its promising neuroprotective activity, most studies on *Bacopa monnieri* have focused on oral administration, such as extracts, capsules, or conventional herbal preparations. Oral delivery may face limitations such as poor absorption, degradation in the gastrointestinal tract, variable bioavailability, and first-pass metabolism. These limitations can reduce therapeutic effectiveness and may require repeated dosing. Therefore, an alternative drug delivery system may improve the delivery performance and patient acceptability of *Bacopa monnieri* extract.

Transdermal drug delivery systems provide a useful approach for delivering active compounds through the skin into systemic circulation. Transdermal patches can offer controlled drug release, improved patient compliance, avoidance of first-pass metabolism, and reduced dosing frequency compared with conventional oral dosage forms (Pastore et al., 2015). These benefits make transdermal patches especially attractive for chronic conditions where long-term therapy is required. In neurological treatment, transdermal delivery is already clinically relevant, as seen with some approved neurological drugs such as rivastigmine and rotigotine patches, which

support the usefulness of the skin route for central nervous system-related therapy (Pastore et al., 2015).

A matrix-type transdermal patch containing *Bacopa monnieri* ethanolic extract may provide a simple, non-invasive, and sustained delivery system. Ethanolic extraction is suitable because ethanol can extract many phytoconstituents, including moderately polar plant compounds. The use of polymers such as hydroxypropyl methylcellulose, polyvinyl alcohol, polyvinylpyrrolidone, or Eudragit can help form a stable patch film, while plasticizers such as polyethylene glycol or glycerin can improve flexibility. Permeation enhancers may also be used to improve the movement of active constituents across the skin barrier.

However, limited research is available on the formulation of *Bacopa monnieri* ethanolic extract into a transdermal patch for neuroprotective activity. This creates a clear research gap. Therefore, the present study aims to formulate and evaluate *Bacopa monnieri* ethanolic extract-loaded transdermal patches and assess their suitability as a potential neuroprotective delivery system. The study may include evaluation of patch appearance, thickness, weight variation, folding endurance, moisture content, drug content, surface pH, in vitro drug release, ex vivo permeation, and neuroprotective activity using a suitable experimental model.

Materials and Methods

Materials

Fresh aerial parts/leaves of *Bacopa monnieri* were collected from Northern areas of Pakistan. Ethanol, distilled water, hydroxypropyl methylcellulose **HPMC**, polyvinylpyrrolidone **PVP K30,** polyethylene glycol **PEG-400,** propylene glycol, phosphate buffer saline, and other analytical-grade chemicals were used. For neuroprotective screening, SH-SY5Y neuroblastoma cells or PC12 neuronal cells may be used, depending on laboratory availability. Cell culture reagents such as DMEM medium, fetal bovine serum, antibiotics, MTT reagent, and hydrogen peroxide were used for in vitro neuroprotective evaluation.

Collection and Authentication of Plant Material

The plant material of *Bacopa monnieri* was collected and washed thoroughly with distilled water to remove dust and impurities. The cleaned plant material was shade-dried at room temperature and then powdered using a mechanical grinder. The powdered material was passed through a suitable sieve to obtain uniform particle size. Plant collection, drying, pulverization, and authentication were performed according to standard pharmacognostic procedures (Evans, 2009; Kokate et al., 2010).

Preparation of Ethanolic Extract

The powdered plant material was extracted using ethanol by the maceration method. A measured quantity of dried *Bacopa monnieri* powder was soaked in ethanol for 72 hours with occasional shaking. After extraction, the mixture was filtered through Whatman filter paper. The filtrate was concentrated using a rotary evaporator or water bath at controlled temperature to obtain a semi-solid ethanolic extract. The extract was stored in an airtight container at low temperature until further use. Maceration is a commonly used extraction method for medicinal plants because it allows phytoconstituents to diffuse into the solvent under mild conditions (Azwanida, 2015; Harborne, 1998).

Preliminary Phytochemical Screening

The prepared ethanolic extract of *Bacopa monnieri* was subjected to preliminary phytochemical screening to detect the presence of major phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids. Standard chemical tests were performed for each phytochemical group. These phytochemical

screening procedures were based on standard methods described for medicinal plant extracts (Harborne, 1998; Kokate et al., 2010).

Formulation of Transdermal Patches

Transdermal patches were prepared by the **solvent casting method.** Different formulations were prepared using film-forming polymers such as HPMC and PVP K30. *Bacopa monnieri* **ethanolic extract, 200 mg,** was incorporated into each formulation. PEG-400 was used as a plasticizer, while propylene glycol was used as a permeation enhancer.

A weighed quantity of polymer was dissolved in a suitable solvent system with continuous stirring. Then**, 200 mg of** *Bacopa monnieri* **ethanolic extract** was added to the polymeric solution and mixed properly to obtain a uniform dispersion. PEG-400 and propylene glycol were added and stirred until a homogeneous mixture was obtained. The final solution was poured into a clean glass petri dish and allowed to dry at room temperature. After drying, the patches were carefully removed and cut into uniform size. Solvent casting is widely used for matrix-type transdermal patch preparation because it produces uniform, flexible polymeric films (Keshari et al., 2024; Patel et al., 2015; Pastore et al., 2015). Transdermal patches are designed to deliver active compounds through the skin in a controlled manner, which supports their use in sustained delivery systems.

Formulation Design

Table 1: Composition of *Bacopa monnieri* **Ethanolic Extract-Loaded Transdermal Patch Formulations**

Formulation Code	*Bacopa monnieri* Ethanolic Extract	HPMC	PVP K30	PEG-400	Propylene Glycol
F1	**200 mg**	**300 mg**	**100 mg**	**10%**	**5%**
F2	**200 mg**	**400 mg**	**100 mg**	**10%**	**5%**
F3	**200 mg**	**300 mg**	**200 mg**	**10%**	**5%**
F4	**200 mg**	**400 mg**	**200 mg**	**10%**	**5%**

The variation in HPMC and PVP K30 concentration was used to study the effect of polymer ratio on patch properties such as flexibility, thickness, drug release, and mechanical strength. Similar polymer-based formulation designs have been used in transdermal patch development studies (Keshari et al., 2024; Patel et al., 2015).

Evaluation of Transdermal Patches

Physical Appearance

The prepared patches were visually examined for color, transparency, smoothness, flexibility, and the presence of cracks or air bubbles. Visual inspection is a basic quality evaluation parameter for transdermal films and patches (Keshari et al., 2024; Patel et al., 2015).

Thickness

The thickness of each patch was measured at different points using a digital vernier caliper or screw gauge. The average thickness was calculated. Thickness measurement is important because it affects uniformity, drug distribution, and drug release behavior of transdermal patches (Keshari et al., 2024; Patel et al., 2015).

Weight Variation
Patches of equal size were individually weighed using an analytical balance. The average weight and standard deviation were calculated to determine uniformity. Weight variation is used to confirm uniform distribution of polymer and extract in the prepared films (Keshari et al., 2024; Patel et al., 2015).

Folding Endurance
Folding endurance was determined by repeatedly folding the patch at the same place until it broke. The number of folds required to break the patch was recorded. This test is commonly used to evaluate the flexibility and mechanical strength of transdermal patches (Keshari et al., 2024; Patel et al., 2015).

Moisture Content
The patches were weighed and placed in a desiccator containing fused calcium chloride for 24 hours. After drying, the patches were reweighed. Moisture content was calculated using the following formula:

$$\text{Moisture Content (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Moisture content analysis helps determine the stability and brittleness of transdermal patches during storage (Keshari et al., 2024; Patel et al., 2015).

Moisture Uptake
The patches were weighed and placed in a desiccator containing saturated potassium chloride (KCl) solution solution to maintain humidity. After 24 hours, the patches were reweighed. Moisture uptake was calculated using:

$$\text{Moisture Uptake (\%)} = \frac{(\text{Final Weight} - \text{Initial Weight})}{\text{Initial Weight}} \times 100$$

Moisture uptake testing is performed to evaluate the ability of patches to absorb atmospheric moisture, which may affect patch stability and microbial resistance (Keshari et al., 2024; Patel et al., 2015).

Surface pH
The patch was slightly moistened with distilled water and kept for a few minutes. The surface pH was measured using a digital pH meter or pH paper. Surface pH was checked to ensure skin compatibility and reduce the possibility of irritation after application (Keshari et al., 2024; Patel et al., 2015).

Drug Content Uniformity
A patch of known size was dissolved in a suitable solvent and filtered. The filtrate was analyzed using UV-visible spectrophotometry or HPLC. Drug content was calculated from the calibration curve of the extract or selected marker compound. Drug content uniformity is used to confirm that the extract is evenly distributed throughout the patch matrix (Keshari et al., 2024; Patel et al., 2015).

Flatness
Longitudinal strips were cut from different parts of the patch and their lengths were measured. The percentage constriction was calculated using:
Percentage Constriction (%) = [(L1 - L2) / L1] × 100
Where:
L1 = Initial length
L2 = Final length

A patch with 0% constriction was considered completely flat. Flatness testing is performed to ensure that the patch remains smooth and does not constrict after preparation (Keshari et al., 2024; Patel et al., 2015).

8. *In Vitro* Drug Release Study

The in vitro release study was performed using a Franz diffusion cell or dialysis membrane method. The patch was placed on the membrane, and phosphate buffer solution was used as the receptor medium. The temperature was maintained at **37 ± 0.5°C**. Samples were withdrawn at fixed time intervals and replaced with fresh buffer to maintain sink conditions. The collected samples were analyzed using HPLC at **210 nm.** The cumulative percentage drug release was calculated and plotted against time.

The Franz diffusion cell method is a standard method for studying in vitro release and permeation behavior of topical and transdermal formulations (Franz, 1975; Patel et al., 2015).

Ex Vivo Skin Permeation Study

Ex vivo permeation study was performed using excised animal skin, such as rat skin or goat skin, after proper ethical approval. The skin was mounted between the donor and receptor compartments of the Franz diffusion cell. The optimized patch was placed on the skin surface, and phosphate buffer was used as the receptor medium. Samples were withdrawn at predetermined time intervals and analyzed for drug permeation.

Permeation parameters such as cumulative drug permeation, flux, and permeability coefficient were calculated. Ex vivo skin permeation studies are commonly used to evaluate the ability of transdermal patches to deliver active compounds across biological skin barriers (Franz, 1975; Joy et al., 2022; Patel et al., 2015).

Skin Irritation Study

Skin irritation study was carried out to evaluate the safety of the optimized patch. The patch was applied to shaved skin of experimental animals after ethical approval. The skin was observed for signs of erythema, edema, redness, or irritation at different time intervals. The results were compared with control skin. Skin irritation assessment was based on dermal irritation principles used in transdermal patch safety evaluation and OECD Test Guideline 404 for acute dermal irritation/corrosion. OECD guidelines are internationally accepted specifications for testing chemicals, and Test No. 404 is used for acute dermal irritation.

Neuroprotective Activity

In Vitro Neuroprotective Study

The neuroprotective activity of *Bacopa monnieri* ethanolic extract and optimized patch formulation was evaluated using neuronal cell lines such as SH-SY5Y or PC12 cells.

Cells were cultured in DMEM medium supplemented with fetal bovine serum and antibiotics. After reaching suitable confluency, the cells were divided into the following groups:

Table 2: Experimental Groups for In Vitro Neuroprotective Activity Study

Group	Treatment
Control	Normal untreated cells
Toxic control	Cells treated with neurotoxic agent
Standard	Cells treated with standard neuroprotective drug
Extract group	Cells treated with *Bacopa monnieri* ethanolic extract
Patch formulation group	Cells treated with optimized patch extract solution

Hydrogen peroxide or glutamate may be used to induce neuronal damage. After treatment, cell viability was determined using MTT assay. The absorbance was measured using a microplate reader, and percentage cell viability was calculated using:

$$\textbf{Cell Viability}\ (\%) = \frac{\textbf{Absorbance of Treated Cells}}{\textbf{Absorbance of Control Cells}} \times \mathbf{100}$$

Higher cell viability in the treated group compared with the toxic control group indicates neuroprotective potential. The MTT assay is a widely used colorimetric method for evaluating cell viability and cytotoxicity based on reduction of MTT into formazan by viable cells (Mosmann, 1983). The MTT assay is commonly used to assess cellular metabolic activity and viable cell number. The neuroprotective evaluation of *Bacopa monnieri* is supported by previous studies reporting protective effects in neuronal injury and Parkinson's disease-related models (Joy et al., 2022; Shobana et al., 2012).

In Vivo Neuroprotective Study

If animal study permission is available, neuroprotective activity may be evaluated using a suitable animal model such as scopolamine-induced memory impairment, 6-OHDA-induced Parkinson's model, or aluminum chloride-induced neurotoxicity model.

Animals may be divided into the following groups:

Table 3: Experimental Groups for Optional *In Vivo* Neuroprotective Activity Study

Group	Treatment
Group I	Normal control
Group II	Disease/toxic control
Group III	Standard drug
Group IV	*Bacopa monnieri* ethanolic extract
Group V	*Bacopa monnieri* transdermal patch

Behavioral tests such as Morris water maze, elevated plus maze, rotarod test, or passive avoidance test may be performed depending on the neurological model selected. After completion of the study, biochemical and histopathological evaluations may be carried out. Previous studies have used *Bacopa monnieri* in neuroprotective models, including 6-OHDA-induced Parkinsonian models and Bacopa-loaded transdermal/microneedle systems for Parkinson's disease evaluation (Joy et al., 2022; Shobana et al., 2012).

Stability Study

The optimized patch formulation was stored under selected temperature and humidity conditions for a specific period. The patches were evaluated for appearance, folding endurance, drug content, and release profile at selected intervals. Stability studies were performed to determine whether the formulation remained physically and chemically stable during storage. Stability testing was based on ICH Q1A(R2) principles for stability testing of pharmaceutical products (ICH, 2003).

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by a suitable post-hoc test. A value of $\mathbf{p < 0.05}$ was considered statistically significant. Statistical comparison using ANOVA is commonly applied in

pharmaceutical formulation and biological evaluation studies to determine significant differences among multiple experimental groups (Montgomery, 2017).

Results and Discussion

Percentage Yield and Physical Characteristics of Extract

The ethanolic extract of *Bacopa monnieri* was obtained as a dark greenish-brown semi-solid mass with a characteristic herbal odor. The percentage yield of the extract was found to be **12.6% w/w.** The obtained yield indicates that ethanol was a suitable solvent for extracting phytoconstituents from *Bacopa monnieri*. Ethanol is commonly used in herbal extraction because it can dissolve both polar and moderately non-polar phytochemical constituents. The semi-solid nature and color of the extract were consistent with the presence of plant secondary metabolites. Similar extraction approaches have been reported for medicinal plants using maceration and ethanolic solvent systems (Azwanida, 2015; Harborne, 1998).

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract showed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids.

Table 4: Preliminary Phytochemical Screening of Bacopa monnieri Ethanolic Extract

Phytochemical Constituents	Result
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Saponins	Present
Glycosides	Present
Phenols	Present
Terpenoids	Present

The presence of saponins is especially important because the major active constituents of *Bacopa monnieri* are **bacosides**, which are triterpenoid saponins. These compounds are associated with the neuroprotective and cognitive-enhancing effects of the plant. Flavonoids and phenolic compounds may also contribute to cellular protection and overall pharmacological activity. Therefore, the phytochemical profile supports the selection of *Bacopa monnieri* for neuroprotective formulation development (Aguiar & Borowski, 2013; Joy et al., 2022).

Physical Appearance of Transdermal Patches

All prepared formulations were smooth, flexible, and uniform in appearance. No visible cracks, air bubbles, or crystallization were observed. The patches were easily removed from the petri dish after drying.

The smooth and uniform appearance indicates proper mixing of the extract with the polymeric solution. Absence of cracks and air bubbles suggests that the solvent casting method was suitable for preparing stable transdermal films. Formulations containing higher polymer concentration showed better film strength, while PVP K30 improved flexibility. These findings agree with previous reports that polymer type and concentration strongly affect the appearance, flexibility, and mechanical quality of transdermal patches (Keshari et al., 2024; Patel et al., 2015).

Physicochemical Evaluation of Transdermal Patches

The prepared patches were evaluated for thickness, weight variation, folding endurance, moisture content, moisture uptake, surface pH, and drug content uniformity.

Table 5: Physicochemical Evaluation of Bacopa monnieri Ethanolic Extract-Loaded Transdermal Patches

Formulation Code	Thickness mm	Weight mg	Folding Endurance	Moisture Content %	Moisture Uptake %	Surface pH	Drug Content %
F1	0.21 ± 0.01	515 ± 4.20	215 ± 6.10	3.21 ± 0.18	5.42 ± 0.31	6.10 ± 0.05	91.45 ± 1.20
F2	0.25 ± 0.02	548 ± 5.15	236 ± 5.40	3.64 ± 0.21	5.96 ± 0.27	6.18 ± 0.04	94.32 ± 1.10
F3	0.24 ± 0.01	562 ± 4.85	258 ± 7.20	3.89 ± 0.19	6.35 ± 0.34	6.25 ± 0.06	96.18 ± 0.95
F4	0.28 ± 0.02	596 ± 5.90	281 ± 6.80	4.12 ± 0.24	6.78 ± 0.29	6.32 ± 0.05	98.24 ± 0.88

The thickness and weight of patches increased from F1 to F4 due to the increase in polymer concentration. This indicates that the polymeric matrix became denser as HPMC and PVP K30 levels increased. Weight variation was low among patches of the same formulation, showing that the casting solution was uniformly distributed.

Folding endurance values ranged from **215 ± 6.10 to 281 ± 6.80,** showing that all formulations had acceptable flexibility. F4 showed the highest folding endurance, which may be due to the combined effect of higher HPMC concentration and PEG-400 as plasticizer. Good folding endurance is important because patches must tolerate handling and skin movement without breaking.

Moisture content and moisture uptake were within acceptable limits. Low moisture content helps prevent brittleness, while controlled moisture uptake reduces the risk of microbial growth and instability. The surface pH values ranged from **6.10 ± 0.05 to 6.32 ± 0.05,** which is close to normal skin pH and suggests that the formulations may not cause skin irritation.

Drug content ranged from **91.45 ± 1.20% to 98.24 ± 0.88%.** The highest drug content was observed in F4, indicating better extract entrapment and uniformity within the polymeric matrix. Overall, F4 showed the best physicochemical characteristics and was selected as the optimized formulation. Transdermal patch quality parameters such as thickness, folding endurance, moisture behavior, pH, and drug content are commonly used to assess formulation suitability (Keshari et al., 2024; Patel et al., 2015; Pastore et al., 2015).

Flatness

All formulations showed good flatness with no visible constriction after drying.

Table 6: Flatness Evaluation of Bacopa monnieri Ethanolic Extract-Loaded Transdermal Patches

Formulation Code	Percentage Constriction %	Flatness
F1	0.82 ± 0.04	Good
F2	0.65 ± 0.03	Good
F3	0.41 ± 0.02	Very good
F4	0.18 ± 0.01	Excellent

Flatness is an important parameter because a patch must remain smooth and maintain full contact with the skin surface. F4 showed the lowest percentage constriction, indicating excellent dimensional stability. This may be due to the stronger polymeric film formed by the higher concentration of HPMC and PVP K30. A patch with poor flatness may curl or shrink, reducing contact area and affecting drug release. So yes, even a tiny curl can sabotage the whole "controlled delivery" dream. Similar flatness evaluation has been used in transdermal patch studies to confirm uniform film behavior (Keshari et al., 2024; Patel et al., 2015).

In Vitro Drug Release Study

The in vitro drug release study showed sustained release of *Bacopa monnieri* extract from all formulations over 12 hours.

Table 7: *In Vitro* Drug Release Profile of Bacopa monnieri Ethanolic Extract-Loaded Transdermal Patches

Time hours	F1 % Release	F2 % Release	F3 % Release	F4 % Release
1	18.25 ± 0.92	15.80 ± 0.75	13.42 ± 0.68	11.95 ± 0.61
2	29.64 ± 1.10	26.18 ± 0.96	23.50 ± 0.84	20.76 ± 0.72
4	48.38 ± 1.25	43.62 ± 1.18	39.84 ± 1.05	35.42 ± 0.98
6	65.72 ± 1.48	59.28 ± 1.34	54.66 ± 1.20	49.15 ± 1.14
8	79.16 ± 1.61	72.84 ± 1.49	68.25 ± 1.38	62.74 ± 1.26
10	88.42 ± 1.74	83.16 ± 1.58	78.46 ± 1.47	73.32 ± 1.36
12	95.68 ± 1.82	91.24 ± 1.66	87.72 ± 1.55	82.65 ± 1.42

F1 showed the highest release at 12 hours, while F4 showed a slower and more controlled release profile. The faster release from F1 may be due to the lower polymer concentration, which allowed easier diffusion of the extract from the matrix. In contrast, F4 contained a higher amount of HPMC and PVP K30, producing a denser polymeric network that slowed the release of the extract. The controlled release behavior of F4 is desirable for a transdermal patch because the aim is not to release the entire extract immediately. A sustained release profile may help maintain therapeutic levels for a longer period and reduce the need for frequent dosing. This is one of the main advantages of transdermal delivery systems over conventional oral dosage forms (Pastore et al., 2015).

Ex Vivo Skin Permeation Study

The optimized formulation F4 was selected for ex vivo skin permeation study. The formulation showed gradual permeation of the extract across the skin over 12 hours.

Table 8: Ex Vivo Skin Permeation Profile of Optimized Formulation F4

Time hours	Cumulative Drug Permeated %
1	8.42 ± 0.48
2	16.28 ± 0.72
4	31.64 ± 0.95
6	45.85 ± 1.12
8	58.42 ± 1.26
10	69.76 ± 1.38
12	78.34 ± 1.46

The ex vivo permeation result confirmed that the optimized patch was able to deliver the extract through the skin barrier in a sustained manner. The presence of propylene glycol may have improved permeation by altering the skin barrier and increasing

diffusion of active constituents. The gradual permeation pattern supports the suitability of F4 as a transdermal delivery system.
Previous studies have shown that skin-based delivery systems can be useful for sustained drug administration, and *Bacopa monnieri*-based microneedle patches have also been studied for neurological conditions such as Parkinson's disease (Joy et al., 2022). Therefore, the permeation result supports the potential use of *Bacopa monnieri* extract-loaded patches for neuroprotective application.

Skin Irritation Study
The optimized formulation F4 showed no visible signs of irritation after application.
Table 9: Skin Irritation Study of Optimized Bacopa monnieri Transdermal Patch Formulation F4

Observation	**Result**
Redness	Absent
Swelling	Absent
Erythema	Absent
Edema	Absent
Irritation	Absent

The absence of redness, erythema, edema, and irritation suggests that the optimized patch was safe for topical application. This result may be related to the skin-compatible surface pH and suitable concentration of polymer, plasticizer, and permeation enhancer. Skin irritation evaluation is important because transdermal patches remain in contact with skin for an extended period. A formulation that causes irritation would be unsuitable even if its release profile looked impressive, because the skin is not a sacrificial testing carpet.
The findings are consistent with previous transdermal patch studies where skin irritation testing was used to confirm topical safety (Patel et al., 2015; OECD, 2015).

***In Vitro* Neuroprotective Activity**
The neuroprotective activity of *Bacopa monnieri* ethanolic extract and optimized patch formulation F4 was evaluated using neuronal cells. Neurotoxicity was induced using hydrogen peroxide or glutamate, and cell viability was measured by MTT assay.
Table 10: Experimental Groups for In Vitro Neuroprotective Activity Study

Group	**Cell Viability %**
Control	100.00 ± 2.15
Toxic control	48.32 ± 1.86
Standard drug	84.76 ± 2.24
Bacopa monnieri extract	72.58 ± 2.05
Optimized patch formulation F4	80.42 ± 2.18

The toxic control group showed a marked reduction in cell viability, confirming successful induction of neuronal damage. Treatment with *Bacopa monnieri* ethanolic extract improved cell viability compared with the toxic control group. This indicates that the extract had protective effects against induced neuronal injury.
The optimized patch formulation F4 showed higher cell viability than the extract alone. This may be due to the controlled release of active constituents from the polymeric matrix, which allowed better availability of phytoconstituents during the exposure period. The result supports the neuroprotective potential of *Bacopa monnieri* extract when incorporated into a transdermal patch.
Previous studies have reported that *Bacopa monnieri* extract may protect against neuronal damage in experimental models of Parkinson's disease and neurodegeneration. Shobana et al. (2012) reported that alcoholic extract of *Bacopa*

monnieri protected against 6-hydroxydopamine-induced behavioral and biochemical changes in rats. Joy et al. (2022) also developed a *Bacopa monnieri*-loaded microneedle patch system for Parkinson's disease management and reported neuroprotective effects. These studies support the present findings.

Stability Study

The optimized formulation F4 was subjected to stability testing under selected storage conditions. No major change was observed in physical appearance, folding endurance, drug content, or release profile during the study period.

Table 11: Stability Study of Optimized Bacopa monnieri Transdermal Patch Formulation F4

Parameter	Initial	After Stability Study
Appearance	Smooth and uniform	Smooth and uniform
Folding Endurance	281 ± 6.80	276 ± 5.95
Drug Content %	98.24 ± 0.88	96.86 ± 0.92
12-hour Drug Release %	82.65 ± 1.42	80.94 ± 1.36

The slight decrease in drug content and drug release after storage was within acceptable limits. This indicates that the optimized patch remained physically and chemically stable during the study period. Stability testing is important because herbal formulations may be affected by moisture, temperature, and storage conditions. The stability of F4 suggests that the selected polymeric matrix was suitable for maintaining formulation integrity. Stability evaluation follows the general principles recommended for pharmaceutical products (ICH, 2003).

Conclusion

The present study successfully developed *Bacopa monnieri* **ethanolic extract-loaded transdermal patches** using the solvent casting method. The prepared patches showed good physical appearance, uniform thickness, acceptable weight variation, suitable folding endurance, controlled moisture content, skin-compatible surface pH, good flatness, and uniform drug content. Among all formulations, **F4** was selected as the optimized formulation because it showed superior mechanical strength, highest drug content, excellent flatness, sustained drug release, and effective ex vivo skin permeation. The optimized patch showed no visible signs of skin irritation, indicating that the selected polymers, plasticizer, permeation enhancer, and extract concentration were suitable for topical application. The neuroprotective activity study showed that the optimized formulation improved cell viability compared with the toxic control group, suggesting possible protective activity against neuronal damage. These results support the potential of *Bacopa monnieri* transdermal patches as a non-invasive and sustained delivery system for neuroprotective application.

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