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Formulation, Development and In-vitro evaluation of Anti-inflammatory Polyherbal Transdermal Patch

Research Article

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Abstract

Background: Inflammation is a tissue response to any infection associated with pain, increased vascular permeability, protein denaturation & membrane alteration. Non-steroidal and steroidal anti-inflammatory drugs (NASIDs) and SAIDs are commonly used medications for inflammation but have adverse side-effects. *Dashmool*, an ayurvedic polyherbal formulation shows significant anti-inflammatory properties with no severe side-effects. Objective: This study aims to develop and evaluate a transdermal patch containing *Dashmool* extract, leveraging the benefits of transdermal drug delivery to manage inflammation and pain effectively. Methods: The aqueous extract of *Dashmool* was prepared and standardised. Anti-inflammatory activity was assessed using albumin denaturation inhibition. Various formulations of transdermal patches were created using cassava starch as a natural polymer and different ratios of hydroxypropyl methylcellulose (HPMC) and plasticisers. Physicochemical properties, including thickness, folding endurance, moisture content, and surface pH, were evaluated. Results: The aqueous extract of *Dashmool* exhibited significant anti-inflammatory activity, with the highest inhibition of protein denaturation observed at 400 µg/ml. Among the formulations, F1 (cassava starch-based) showed the best overall performance with good adhesive properties, flexibility, and moisture content. The patches demonstrated uniform thickness, acceptable folding endurance, and low moisture content, indicating stability. Conclusion: The developed *Dashmool* transdermal patch, offers a promising alternative for pain management with better stability.

Keywords: *Inflammation, Dashmool,* Transdermal patch, *Shotha*, Anti-inflammatory, Albumin denaturation.

Introduction

Inflammation is reaction of tissues towards infectious pathology involving local and systemic responses(1). Inflammation is associated with pain involving rise in vascular permeability, rise in protein denaturation and membrane alteration. Pain, heat, swelling, redness and functional loss in the damaged area are the hallmarks of this defensive reaction(2). The most commonly prescribed medications for treatment of acute inflammatory illnesses are non-steroidal and steroidal anti-inflammatory drugs (NASIDs) and SAIDs, despite their adverse effects on the kidneys and the stomach(3). These medications stop the action of the COX-1 and COX-2 enzymes. COX enzymes aid in the synthesis of prostaglandins. NSAIDs, or nonsteroidal anti-inflammatory drugs, have been around for a while. As a result, prolonged use of these medications causes unfavourable side effects that harm the gastrointestinal tract, liver, and other human biological systems. Due to unfavourable side effects such as stomach sores, heart problems, kidney failure, and intestinal damage(4), due to these drawbacks it is necessary to look for a substitute in order to get over the issues related to using NSAIDS(5,6,7).

Denaturation of a protein is the process by which it becomes denatured, or the tertiary and secondary structures of the protein get disoriented, as a result of exposure to an external stimuli(8,9). Enzymes become inactive, when substrates no longer has ability to bind to the active site, by this enzymes become inactive(10). There is consensus that medicinal plants are a vital source of novel compounds with potential medical applications. In recent years, use of herbal medicines has expanded due to cost being minimal and less side effects(11). For millennia, Ayurvedic medicine has employed numerous plant components with minimal side effects to control inflammatory pathways(12). *Dashmool*, as the name implies, is a mixture of ten different plant roots. Five of these are referred to as *brihad panchamoola*, and the other five as *laghu panchamoola*. It is a popular polyherbal ayurvedic preparation(13). While each of these roots has a variety of advantageous properties, when combined they balance the *doshas* of *Pitta*, *Kapha*, and *Vata*. This is especially helpful for *Vata vyadhi*(14). To treat inflammatory illnesses and lower accompanying pain

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and fever, this combination is a standard Ayurvedic therapy (15,16).

The ten components of *Dashamool* are thought to play a variety of functions, including those of an adjuvant, stabilizer, and carrier agent(17). Several of these substances have demonstrated their anti-inflammatory as well as analgesic properties in the in-vivo studies of inflammation and pain(18,19,20). Considering anti-inflammatory properties of *Dashmool*, transdermal patch of *Dashmool* extract was prepared.

Topical routes drug delivery method is a convenient method. It has more advantages than oral route such as ease of administration, control & sustain release of drug into plasma, avoiding first pass metabolism(21). Transdermal drug delivery (TDD) employs the skin as a drug-absorbing medium, it is a creative and enticing substitute for oral and parenteral drug administration(22). These patches are intended to be placed to the skin and work their way through the layers of the skin to release a medicinally effective amount of single or many active substances into the circulatory system. The most fundamental and significant elements of transdermal patches are natural or synthetic polymers, which serve a variety of purposes including creating the matrix, regulating the pace of medication distribution, offering protection, adhesion, flexibility, and permeation(23). Cassava starch is a polysaccharide that holds great potential as a natural biopolymer. The transdermal films made from cassava starch are non-toxic, decomposable, and odourless(21).

The growing interest in herbal alternatives for managing inflammation has led to promising developments in natural, less invasive therapies. Dashmool's powerful anti-inflammatory and analgesic properties, combined with the innovative approach of transdermal drug delivery, offer a potentially effective and safer solution compared to conventional NSAIDs. The use of biopolymers like cassava starch in transdermal patches not only enhances the stability, efficacy, and controlled release of active compounds but also supports environmentally sustainable practices. With continued research, such integrative approaches may redefine therapeutic options for inflammation, promoting healing with minimal side effects and supporting overall well-being. Aim of this study is formulate and develop *Dashmoool* Transdermal patch and conduct its anti-inflammatory in-vitro activity and to conduct its pharmaceutico-analytical study.

Materials and Methods

Procurement of drugs

All ten herbal drugs of *Dashmool* - *Bilva (Aegle marmelos Corr), Agnimantha (Premna mucronata Roxb), Gambhari (Gmelina arborea Linn.), Shyonak (Oroxylum indicum Vent.), Patala (Stereospermum suaveolens DC), Shalparni (Desmodium gangeticum DC.), Prishniparni (Uraria picta Desv.), Brihati (Solanum indicum Linn), Kantakari (Solanum xanthocarpum), Gokshuru (Tribulus terrestris Linn.)* were procured from authentic sources. Roots of these ten drugs were used. Additives of *Dashmool* Transdermal Patch like Cassava starch, Plasticizer (PEG 400), Glycerine were acquired of 'AR grade' (Analytical reagents) from authentic sources.

Standardisation of *Dashmool*

Standardisation of *Dashmool* was conducted using following testings like colour of aqueous extract, pH, foreign matter, loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive. Each Dashmool sample was ground, sieved, and weighed (1g) for standardisation. Aqueous extracts were prepared by heating 5g samples in distilled water, cooling, filtering, and noting colour and pH. Foreign matter was inspected, and moisture content was calculated after drying. Total and acid-insoluble ash content were determined by igniting samples and treating the ash with hydrochloric acid. Water- and alcohol-soluble extractives were measured by macerating samples in water and ethanol, filtering, evaporating, and drying. All findings were recorded for consistent quality and therapeutic potential assessment.

Preparation of aqueous extract

Course powder of mesh size 36 of all ten herbal drugs were taken for the preparation of homogeneous mixture. The aqueous extract of *Dashmool* was prepared using a Soxhlet apparatus, where the 100 g *Dashmool* mixture was placed in the thimble, and 250 ml distilled water was used as the solvent in the round-bottom flask. The Soxhlet apparatus was set up to allow the water to heat, vaporise, and condense over the herbs in the thimble, continuously extracting the active compounds. This extraction process was carried out at a temperature range of 90-100°C for a duration of 24 hours to ensure complete extraction of the soluble components from the *Dashmool* mixture.(24) The obtained liquid extract was placed over water bath for 4 hours at 80^{o}C until the entire solvent (distilled water) was evaporated. Dried *Dashmool* extract was stored in an airtight container.

Albumin denaturation Inhibition(25)

Anti-inflammatory activity of *Dashmool* extract was investigated using albumin denaturation inhibition method. Aqueous extract of *Dashmool* of 8 different concentrations ranging from 100-800 (100, 200, 300, 400, 500, 600, 700, 800) µg/ml were taken. For preparing these solutions, first the stock solution was prepared of concentration 1mg/ml and then accordingly the solution was diluted in 8 different concentrations. These concentrations were mixed with 1% bovine albumin aqueous solution. To adjust pH, 1N hydrochloric acid was added to this solution. Samples were kept in incubator for about 20 min at 37 ^{0}C. (25) After incubating the samples, they were heated in oven at 57 ^{o} C for 20 min. After cooling of samples, the turbidity produced was checked spectrophotometrically at 660 nm. The percent inhibition of protein denaturation was calculated by following equation(21) -

Percentage inhibition of protein denaturation (%)

$$= \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100\%$$

Formulation of Transdermal patch (Pilot study)

Transdermal patches containing *Dashmool* were prepared using different ratios of polymer & plasticiser as shown in following table.

Table 1 : Formulations of Dashmool transdermal patch

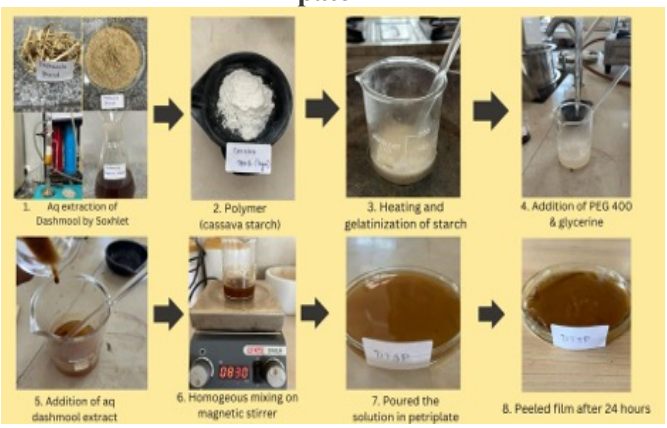
Formulation	Drug	Cassava starch	HPMC (Hydroxypropyl Methylcellulose)	Plasticizer (PEG 400)	Glycerine
F1	400ug	4 gm	0 gm	30% (w/v)	2 ml
F2	400ug	4 gm	1 gm	30% (w/v)	2 ml
F3	400ug	4 gm	2 gm	30% (w/v)	2 ml
F4	400ug	4 gm	3 gm	30% (w/v)	2 ml
F5	400ug	0 gm	4 gm	30% (w/v)	2 ml

Preparation of *Dashmoola* transdermal patch

F1 formulation was selected standard from the pilot study by proper evaluating all the formulations.

4 g cassava starch was added to 50 ml distilled water. This mixture was heated with double boiling method (stainless steel vessel containing water was taken. Mixture of Cassava starch and HPMC was taken in a beaker and that beaker was placed in water containing stainless steel vessel and this was placed on gas stove and heated) till 90° C and stirred continuously until the stage of gelatinisation was obtained. 2 ml glycerine and PEG 400 was added to this mixture. After cooling the mixture till 40-45° C, 400 ug drug solution was added to the mixture. The mixture was placed on magnetic stirrer for 20 mins at 680 rpm to achieve homogenous mixing and to reduce the lumps of polymer. The mixture was poured in petri plate and dried at room temperature for 24 h. After drying, the film was peeled(26).

Figure 1 : Preparation of Dashmool transdermal patch



Total 3 batches were prepared of the above mentioned formulation and evaluated.

Evaluation of Transdermal patches

The following techniques for physicochemical characterisation of the formed film were assessed.

Thickness

Thickness of the film was determined using digital micrometre screw gauge at four different sides and the mean value was computed(21).

Folding endurance

The film was folded over and over at a same location for multiple times until it was broken. Folding endurance was determined by the number of folds done until the film break(21).

Moisture content

The prepared films were weighed and dried in oven at temperature 105° C for 24 hours. After removing the film from oven, the film was weighed again and weight was recorded. Percentage of the moisture content was determined by following formula –(21)

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

Results and Discussion

Standardisation of Dashmool

Colour of Dashmool was faint brown, odour characteristic, taste bitter, Average weight content - 50 gm, Colour of aqueous extract (10%)- Dark brown, pH of aqueous extract (10%) - 8.22, Foreign matter - 0.3 %, Loss on drying - 2.25 %, Total ash- 0.70 %, Acid insoluble ash - 0.42 %, Water soluble extractive - 7.09 %, Alcohol soluble extractive - 1.88 %. Three samples were taken and average was calculated.

Table 2 : Standardisation of contents of *Dashmoola*

Sr. No.	Drugs	Description	pH	Loss on Drying	Total ash	Acid insoluble ash	Water soluble extractive	Alcohol soluble extractive
1	Bilva	Colour – yellowish Odour – characteristic Taste - bitter	5.22	4.75%	0.10%	0.14%	19.35%	8.41%

2	Agnimantha	Colour – yellowish brown Odour – characteristic Taste – slightly astringent	5.23	2.65%	0.63%	0.03%	9.55%	3.17%
3	Shyonaka	Colour – Greyish brown Odour – odourless Taste – slightly sweet	5.64	9.75%	0.05%	0.17%	43.69%	21.18%
4	Patala	Colour – brown Odour – not distinct Taste - bitter	6.36	0.89%	1.78%	0.54%	31.45%	17.47%
5	Gambhari	Colour – yellowish Odour – Mucilaginous Taste - sweetish	6.22	1.86%	0.01%	0.13%	20.92%	9.81%
6	Kantakari	Colour – greenish brown Odour characteristic Taste – bitter and acrid	6.68	6.5%	0.64%	0.73%	19.40%	7.41%
7	Brihati	Colour – yellowish brown Odour – odourless Taste - tasteless	6.04	5.94%	0.25%	0.02%	12.5%	3.9%
8	Shalparni	Colour – light brown Odour – characteristic Taste - sweet	6.09	4.38%	3.51%	0.67%	4.92%	5.40%
9	Prishniparni	Colour – greenish brown Odour – odourless Taste – slightly acrid	6.12	7.17%	0.23%	0.14%	11.98%	10.57%
10	Gokshura	Colour – greenish yellow Odour – characteristic Taste - astringent	5.97	8.56%	0.26%	0.24%	11.32%	6.54%

Inhibition of albumin denaturation

Aqueous extract of *Dashmool* showed anti-inflammatory activity at the dose of 400 ug/ml as presented in Table 3. Denaturation of protein is an indicator of inflammatory condition. Agents which prevents denaturation of protein acts as anti-inflammatory agents. As shown in the table, among various doses of *Dashmool*, the highest anti-inflammatory activity of *Dashmool* was shown at 400 ug/ml.

Table 3: Percent inhibition of protein denaturation

Concentration (ug/	Percentage of albumin
100	50%
200	52%
300	45%
400	63%
500	48%
600	57%
700	46%
800	44%

Physico-chemical evaluation of DTP

Thickness and weight uniformity

Dashmool Transdermal patch films were uniform in thickness with smooth homogenous surface. No cracks in films were seen. The average thickness were observed to be in the range of 0.223 mm. Weight of the film was in range of 2.165-2.488 mg.

Folding endurance

Good flexibility with few mechanical properties were exhibited by the films. The film was folded multiple times at a same place. Without cracks the folding endurance of the film was 110, thus indicating good folding endurance.

Moisture content

The produced formulation had a low moisture content, which contributes to its stability during long-term dry storage and keeps the film from drying out and breaking.

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Table 4: Physico-chemical evaluation of DTP formulations

Formulation	Thickness (mm)	Flatness (%)	Weight (mg)	Folding endurance	Moisture content (%)	Surface pH
F1	0.223mm	90%	2.165 mg	110	2.30	7.56
F2	0.230mm	92%	2.170 mg	106	2.29	7.62
F3	0.227mm	96%	2.488 mg	113	2.24	7.61
F4	0.149mm	97%	2.263 mg	108	2.20	6.89
F5	0.134 mm	98%	2. 190 mg	120	2.21	6.85

Considering all the prepared formulations, F1 was prepared from natural polymer (Cassava starch). In further formulations from F2 – F4, concentration of synthetic polymer (HPMC) was increased. Final formulation F5 was prepared using plain synthetic polymer (HPMC). F1 showed good adhesive property and showed more moisture content with good flexibility of the film. F2 showed perfect adhesive property and good moisture content and perfect flexibility of film. F3 & F4 showed less adhesive property and had less flexibility and low moisture content. F3 & F4 patches were harder in consistency compared to F1 &F2. F5 (with plain HPMC) showed least adhesion amongst all and less flexibility with very low moisture content. F5 was hardest in consistency amongst all formulations. All the prepared *Dashmool* transdermal patches were uniform in thickness. The variation in thickness was found to range from 0.134 to 0.230 mm. F2 showed highest range of thickness and F5 showed the lowest thickness. This showed that thickness of transdermal patch is depended on concentration & solubility of polymer. Therefore rise in concentration and increase in solubility results in thick patch(27). All the formulations of transdermal patches weighed from 2.165 to 2.488. The folding endurance of the patches was 110 - 120, which concludes that the patches can withstand and pressure with good flexibility. F5 showed maximum folding endurance concluding that transdermal patch prepared from plain synthetic polymer (HPMC) can withstand maximum pressure. Concentration of polymer plays vital role in folding endurance. Flatness of patches were found to be 90-98%. All three films showed low moisture content from 2.21 – 2.30 %. This concludes the long term stability of the film. The surface pH of the films were found to range from 6.85 – 7.62.

Table 5: Physico-chemical evaluation of 3 batches of DTP – F1 formulation

Batch (F1)	Thickness (mm)	Flatness (%)	Weight (mg)	Folding endurance	Moisture content (%)	Surface pH
1st	0.223 mm	90%	2.164mg	110	2.30	7.55
2nd	0.221mm	90%	2.165mg	111	2.29	7.56
3rd	0.222 mm	91%	2.165mg	110	2.30	7.56

F1 formulation prepared in 3 batches were evaluated. Thickness on F1 formulation was in the range of 0.221-0.223 mm, flatness 90-91%, weight ranging from 2.164-2.165 mg, folding endurance 110-111, moisture content 2.29-2.30, surface pH 7.55-7.56 with good adhesive property.

Conclusion

Dashmool Transdermal patch an innovative formulation was developed for pain management using herbal drugs mentioned in classical texts of Ayurveda. Drug dosage was decided by performing inhibition of albumin denaturation percentage. The developed formulation exhibited moisture content, thickness, folding endurance. The herbal transdermal patch developed is considered a better medicine for pain than conventional dosage forms.

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