RESEARCH ARTICLE

Formulation, Development, and Evaluation of Anti-Inflammatory Transdermal Patches of Essential Oil of *Amomum subulatum*

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ABSTRACT

Amomum subulatum Roxb, commonly known as *Badi Ilaichi,* is a traditional medicinal herb. The fruit of the plant is rich in essential oil content. In the present study, transdermal patches of the essential oil of *A. subulatum* seeds were prepared by solvent casting method and evaluated for various parameters, *viz.*, thickness, weight uniformity, folding endurance, % moisture content, % moisture uptake, drug content, *in vitro* and *in vivo* drug release, etc. The evaluation of transdermal patches indicated very good physico-chemical properties and significant efficacy. Formulation F4 exhibited significant results. The transdermal patches of *A. subulatum* essential oil can be utilized in case of chronic pain as it can be the alternative to give sustained relief.

Keywords: *Amomum subulatum*, Essential oil, Topical anti-inflammatory activity, Transdermal patches.

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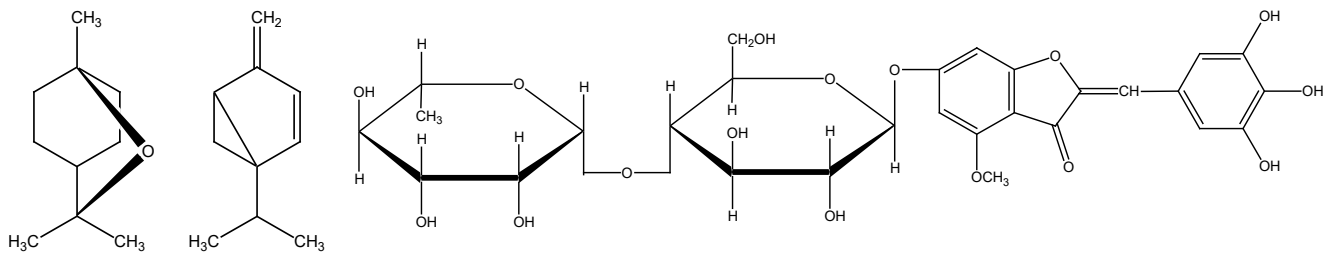
INTRODUCTION

Amomum is a genus of terrestrial, rhizomatous herb, distributed chiefly in Africa and tropical Asia, found in the eastern Himalayas and cultivated in Nepal, northern West Bengal, Sikkim, and Assam hills.[1] The herb is edible, used as a condiment, and is rich in volatile oil—terpenoids.[2]

In folk medicine, the seeds are credited with stimulant, stomachic, alexipharmic, and astringent properties and are prescribed for the treatment of indigestion, vomiting, biliousness, abdominal pains, and rectal diseases. They promote the elimination of bile and are useful in congestion of the liver; they are also used in gonorrhea. The pericarp is useful in headache and heals stomatitis. The aromatic oil extracted from the seeds is applied to the eyes to allay inflammation.[1]

The major chemical constituent of the herb is 1,8-cineole. Other minor constituents include sabinene, γ-terpinene, α-bisabolene, α-terpinyl acetate, α and β terpineol, cinnamaldehyde, linalool, cumin aldehyde, terpinen-4-ol, petudinin protocatechualdehyde, protocatechuic acid, 1, 7-bis(3,4-dihydroxyphenyl) hepta-4E, 6E-dien-3-one, and 2,3,7-trihydroxy-5-(3,4-dihydroxy-E-styryl)-6,7,8,9-tetrahydro-5H-benzocycloheptene (Figure 1). Other isolated constituents include cardamonin and alpinetin; the glycosides petunidin 3,5-diglucoside ($C_{28}H_{33}O_{17}$), leucocynidin-3-O-β-$_D$-glucopyranoside ($C_{21}H_{24}O_{12}$); a new aurone glycoside, subulin ($C_{28}H_{32}O_{16}$); chalcone, cardamonin ($C_{16}H_{14}O_4$), and a flavanone, alpinetin. Cardamonin and alpinetin have been isolated from the seeds of A. subulatum.[3-5]

The herb is useful in congestion of liver, gonorrhea, neuralgia, headache, and stomatitis.[6] It also shows the gastric anti-ulcerogenic effect,[7] and anti-oxidant activity.[8,9]



1,8-cineole Sabinene Subulin

Figure 1: Structures of major phytoconstituents of *A. subulatum*

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In our previous study, the chemical composition of essential oil isolated from fruits of *A. subulatum* was determined by GC/MS analysis, and the oil was found to contain various terpenoids. The essential oil of *A. subulatum* was also found to be effective against various strains of bacteria and fungi, as well as showed significant topical anti-inflammatory activity.[10] Hence, as an extension of our previous work, an attempt is made to formulate and evaluate anti-inflammatory transdermal patches of essential oil of the plant to provide a continuous effect of it for a longer duration of time.

MATERIAL AND METHODS

Procurement and Authentication of Raw Material

The dried fruits of *A. subulatum* were procured from the local market of Sunder Nagar, District Mandi, Himachal Pradesh, India. The crude drug was authenticated by a senior botanist at NBPGR, New Delhi (voucher number: EP 532).

Isolation of Essential Oil

The air-dried plant material (500 g) was hydrodistilled in an all-glass apparatus according to the method recommended by the British Pharmacopoeia, 2007. The pale yellow oil was dried over anhydrous sodium sulfate and stored at 4ºC in the dark. The yield was 0.8% based on the dry weight of the sample.

Development of Topical Anti-Inflammatory Patches

As evaluated earlier, essential oil of *A. subulatum* possesses topical anti-inflammatory properties.[10] Hence, its transdermal patches were prepared (Table 1). Ethylcellulose was used for the formulation of the transdermal patch. Polyethylene glycol (PEG 400) was used as a plasticizer. Dibutyl phthalate is used as a penetration enhancer. The polymer was dissolved in chloroform:methanol (1:1) solvent. The essential oil was dispersed uniformly in the viscous solution with continuous stirring. The resulting mass was poured into leveled mercury surface in a petri dish covered with an inverted funnel. The Petri dish was left undisturbed at room temperature for one day. The patch was obtained intact by slowly lifting from the Petri dish, and transdermal patches were cut into radii of 2 cm.[11-13]

Evaluation of Transdermal Patches

Thickness of Patch

The thickness of each patch was measured by using a screw gauge at five different positions of the patch, and the average was calculated.[14]

Weight Uniformity

The patch of sizes of a 2 cm radius (4 cm diameter) was cut. The weights of five patches were taken, and the weight variation was calculated.[15]

Folding Endurance

A patch of 2 cm radius (4 cm diameter) was cut evenly and repeatedly folded at the same place till it brakes. The number of times the film was folded at the same place without breaking give the value of the folding endurance.[16,17]

Percentage Moisture Content

The prepared films were weighed individually and kept in desiccators containing fuse calcium chloride at room temperature for 24 hours. After 24 hours, the films were reweighed and determined the percentage of moisture content from the mentioned formula.[18,19]

Percentage Moisture Uptake

The weighed films were kept in desiccators at room temperature for 24 hours containing a saturated solution of potassium chloride in order to maintain 84% RH. After 24 hours, the films were reweighed and determined the percentage moisture uptake.[20,21]

Drug Content by High Performance Liquid Chromatography (HPLC)

Patches (2.8 cm^2) were cut and added to 10 mL volumetric flask and volume was made to 10 mL with methanol and kept for sonication for half an hour. Then, the subsequent dilution was made by the mobile phase. Samples were estimated by HPLC by using the standard calibration curve. The procedure was repeated in triplicate for each formulation.[22]

In vitro Release by Dissolution Studies

Herbal transdermal film measuring 2.8 cm^2 were subjected to *in vitro* diffusion testing using Keshary-Chien diffusion cell. Mesh (60) was clamped between the donor and receptor compartments, and the film was placed over the mesh. The receptor compartment contained phosphate buffer/methanol (9:1). The number of drugs diffusing into the receptor compartment across the mesh was determined by withdrawing 0.5 mL samples throughout the experiment, and an equivalent amount of diffusion medium was added to the receptor compartment to maintain a constant volume. The samples were filtered through an injection millipore filter of 0.45 µ. These samples were analyzed by HPLC. The cumulative amount of drug permeating through the mesh was then calculated.[23]

Table 1: Formulation development

Formulation	*Ethyl cellulose (mg)*	*PEG-400 (mL)*	*Dibutyl phthalate (mL)*	*Chloroform:methanol*	*A. subulatum essential oil (mg)*
F1	100	1.2	1.2	1:4	10
F2	200	1.2	1.2	1:4	10
F3	300	1.2	1.2	1:4	10
F4	400	1.2	1.2	1:4	10
F5	500	1.2	1.2	1:4	10

Primary Skin Irritation Study

Three Wistar albino rats of either sex weighing 200 to 250 grans were used for the test. The intact skin was used. The skin from the back of each rat was depilated 24 hours before the application of the patch. Two areas of the back of each rat, approximately 10 cm apart, were designated for the position of the patches. One area was used for the application of the plain polymeric patch, and the other was used for drug patches. The animals were immobilized using a rabbit holder during 24 hours of exposure. Upon removal of the patches, the resulting reaction was evaluated using weighed scores. Reading was also done after 72 hours, and the final scores represent an average of the 24 and 72-hour reading (Table 2).[24]

In vivo Study

The anti-inflammatory activity and sustaining action of the drug-loaded matrix patches were evaluated using the "carrageenan" induced hind paw edema method developed by Winter. Young male rats, weighing approximately 175 grams, were taken for the experiment, divided into four groups, each containing four rats. The rats were kept on fasting overnight. The backsides of rats were shaved 12 hours before starting the experiment. Patches were applied on the backs of all the animals (except control group) half an hour before sub plantar injection of carrageenan in the right paw. Paw edema was induced by injecting 0.1 mL of a 2% w/v homogenous suspension of carrageenan in double-distilled water. The volume of injected paws measured immediately (0 hours) and 1, 2, 3, 4, and 5 hours after injection using plethysometer. The amount of paw swelling was determined from time to time, and expressed as percent edema relative to the initial (0 minutes) hind paw volume. Percentage inhibition of edema produced by each patch treated group was calculated against the respective control group using the following formula.[25]

$$\% \text{ inhibition} = \frac{\text{Edema (control) - Edema (drug)}}{\text{Edema (control)}} \times 100$$

Table 2: Evaluation of skin reactions

Skin reaction	*Score*
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema	4
Total possible erythema score	4

Statistical Analysis

The data were analyzed using one-way ANOVA followed by Post Hoc Scheffe's test using SPSS computer software version 7.5. The level of significance was fixed at 0.05.

Stability Studies

The prepared films were wrapped in aluminum foil. The aluminum foils were placed in a stability chamber whose temperature was maintained at 40 ± 2°C at 75% RH for 45 days. Then, films were withdrawn and evaluated for physical parameters like color, thickness, and weight of the films. All storage conditions were maintained as per ICH guidelines.

RESULTS AND DISCUSSION

The hydrodistillation of fruits of *A. subulatum* gave golden yellow colored oil, having a strong odor with a yield of 0.8 mL per 100 g.

Formulation Development

Ethyl cellulose and polyethylene glycol 4000 were used for the preparation of the polymer. The films obtained were smooth and flexible.

Evaluation of Formulation

The formulated films were characterized for various parameters such as weight variation, thickness, and drug content (Table 3). These are essential parameters for the evaluation of the dosage form to achieve a formulation with uniformity and consistency within a batch. The results of weight variation, thickness, and drug content, as shown in Table 4.

The films exhibited uniform weight and thickness among the various batches. The uniformity of weight and thickness indicates that the polymeric solution of the drug is well dispersed on a flat surface. However, little variation in weight and thickness observed in a different formulation may attribute to the variation in polymeric content. All formulation exhibited a slight variation in drug content ranging from 80.86 to 98.29%.

The moisture content and moisture uptake were found to be decreasing with the increasing proportion of PEG, which can attribute the non-hygroscopic nature of PEG. The small moisture content in the formulation helps them to remain stable and prevent from being completely dried and brittle. A low moisture uptake protects the material from microbial contamination and bulkiness of the patch.[25]

Table 3: Evaluation of various parameters of transdermal patches

	Formulations				
Parameters	*F1*	*F2*	*F3*	*F4*	*F5*
Thickness (mm)	0.534 ± 0.02	0.522 ± 0.04	0.528 ± 0.03	0.52 ± 0.01	0.536 ± 0.02
Weight uniformity (g)	0.302 ± 0.02	0.349 ± 0.02	0.404 ± 0.03	0.402 ± 0.02	0.354 ± 0.04
Folding endurance	118 ± 2.6	112 ± 2.7	108 ± 2.2	102 ± 2.0	94 ± 2.6
% moisture content	6.26 ± 0.06	5.76 ± 0.02	5.28 ± 0.12	5.11 ± 0.06	4.34 ± 0.42
% moisture uptake	5.62 ± 0.2	5.22 ± 0.12	4.92 ± 0.11	4.74 ± 0.22	4.65 ± 0.28
Drug content (%)	96.94 ± 0.53	86.75 ± 0.64	81.69 ± 3.09	98.29 ± 0.42	80.76 ± 0.65

To check the folding endurance, results show that the plasticity of formulation increases with the increasing proportion of PEG.

In vitro Dissolution Studies

Dissolution studies are important for ensuring the sustained release performance and the reproducibility of rate and duration of drug release (Table 4). Release studies are required for predicting the reproducibility of rate and duration of drug release. The importance of polymer dissolution on drug release from matrices has been known for ensuring the sustained release performance.[26] Dissolution studies for different formulation were performed using Keshary-Chien diffusion cell using 10% methanolic phosphate buffer, pH 7.4, as dissolution medium at 32°C.[25]

A maximum percentage of drug release was found for the formulation F4, and the minimum percentage of drug release was observed for the formulation F5.

The drug release from all the films was rapid in the initial hours (up to 6 hours), which could be due to the presence of drugs on the surface of films. Later, the drug was released slowly from the patches.

It was observed that as the concentration of hydrophilic polymer, PEG increases in the formulation, the rate of dissolution decreases subsequently. This may be due to the film-forming property of PEG, which may form on the surface of the film and delay the drug release.

Primary Skin Irritation Study

The results of skin irritation studies showed negligible erythema with prepared films when compared with control. The absence of edema indicated that the polymeric patches are compatible with the skin and hence, can be used for the transdermal application.

Table 4: *In vitro* dissolution studies of transdermal patches of essential oil of *A. subulatum*

	Cumulative % of drug release				
Hour	*F1*	*F2*	*F3*	*F4*	*F5*
0	0	0	0	0	0
1	9.241	12.117	8.722	10.125	8.59
2	15.64	15.348	14.689	17.284	10.431
3	22.951	26.723	20.506	27.225	16.423
4	28.257	30.142	27.27	33.057	23.522
5	32.404	33.156	31.184	37.531	27.732
6	40.433	41.298	36.422	43.847	31.222
7	43.213	45.545	41.027	48.692	35.549
8	49.711	50.509	45.233	52.129	38.262
9	53.834	54.182	48.812	55.332	40.015
10	55.802	56.542	50.951	58.742	44.328
11	57.902	58.187	53.209	60.342	42.906
12	58.222	60.253	54.627	62.132	44.236

Table 5: Paw edema and % inhibition obtained on carrageenan challenge in male rats

Treatment (dose mg/kg)	*Edema ΔV mL (% inhibition)*					
	0.5 hr	*1 hr*	*2 hr*	*3 hr*	*5 hr*	*10 hr*
Control	0.51 ± 0.16	0.7 ± 0.21	0.88 ± 0.5	1.11 ± 0.16	0.92 ± 0.07	0.85 ± 0.16
Standard (10)	0.32 ± 0.06*	0.42 ± 0.03*	0.45 ± 0.07*	0.53 ± 0.02*	0.49 ± 0.11*	0.46 ± 0.06*
	37.24	40.0	48.86	52.25	46.68	45.91
F1	0.45 ± 0.05	0.62 ± 0.06	0.67 ± 0.04	0.64 ±0.05	0.62 ± 0.07	0.58 ± 0.08
	11.76	11.43	23.86	42.34	32.61	31.77
F2	0.44 ± 0.08	0.6 ± 0.03	0.63 ± 0.04	0.61 ± 0.08*	0.57 ± 0.03	0.53 ± 0.07
	13.73	14.29	28.41	45.05	38.04	37.65
F3	0.39 ± 0.03	0.53 ± 0.06*	0.64 ± 0.03*	0.58 ± 0.04*	0.55 ± 0.02	0.50 ± 0.07
	23.53	24.29	27.27	47.75	40.22	39.76
F4	0.33 ± 0.06	0.41 ± 0.03*	0.46 ± 0.07*	0.55 ± 0.07*	0.49 ± 0.06*	0.46 ± 0.02
	35.29	41.43	47.73	50.05	46.74	45.88
F5	0.47 ± 0.05	0.63 ± 0.04	0.7 ± 0.06*	0.68 ± 0.06*	0.65 ± 0.07	0.61 ± 0.12
	7.84	10	20.45	38.74	29.35	28.24

*ap < 0.05 when compared with control (one-way ANOVA followed by post hoc Scheffe's test); all values are expressed as mean ± SD; n = 4

In vivo Study

Carrageenan-induced rat paw edema has been considered as a useful model for studying the anti-inflammatory effect of profile. Table 5 depicts a comparison of mean paw volume and percentage inhibition of edema with duration after the application of patches. The formulation F4 was found to provide the maximum protective effect as compared to remaining other formulations. The results are compared at 3rd-hour of induction of inflammation as at this point, it shows a maximum response.

Stability Studies

The results of stability studies indicate that all formulations were stable with respect to physical properties for up to 45 days. However, extensive stability studies up to 6 months require confirming these results.

CONCLUSION

In conclusion, the transdermal patches of essential oil of *A. subulatum* were prepared successfully by using different proportions of ethyl cellulose and PEG by the solvent casting method. Amongst the prepared formulations, F4 exhibited the best results as compared to others. The present work can be further proceeded to evaluate the pharmacokinetic profile.

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